THERAPEUTIC DRUG MONITORING OF ANTIPSYCHOTICS

HPLC ANALYTICAL METHOD DEVELOPMENT
AND
PHARMACEUTICAL ADVICES TO THE WARD CLINICIANS

FOR SUCCESSFUL INDIVIDUALIZED THERAPY

Therapeutisches Drug Monitoring von Antipsychotika – HPLC Analytische Methodenentwicklung und Pharmazeutische Beratung der Stationsärzte- für Erfolgreiche, Individualisierte Therapie

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List of content

List of figures

List of tables

List of abbreviation

Abbreviation	German	English
t1/2	Eliminationshalbwertszeit	Elimination half-life
ABDA	Bundesvereinigung Deutscher Apothekerverbände	Federal Union of German Associations of Pharmacists
ACN	Acetonitril	Acetonitrile
ADHS	AufmerksamkeitsDefizit-/Hyperaktivitätsstörung	Attention Deficit Hyperactivity Disorders
ADR	Unerwünschte Arzneimittelwirkung	Adverse Drug Reaction
AGATE	Arbeitsgemeinschaft Arzneimitteltherapie bei Psychiatrischen Erkrankungen	Association of Drug Therapy in psychiatric Disorders
AGNP	Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie	The Association of Neuropsychopharmacology and Pharmacopsychiatry
AKdÄ	Arzneimittelkommission der Deutschen Ärzteschaft	Drug Commission of the German Medical Association
AMÜP	Arzneimittelüberwachung in der Psychiatrie	Drug Monitoring in Psychiatry
Aqua dem.	Destilliertes Wasser	Demineralized water (destilled water)
BPD	Benperidol	Benperidol
BRP	Bromperidol	Bromperidol
BZD	Benzodiazepine	Benzodiazepines
Cl	Clearance	Clearance
CN	Cynonitril	Cyanonitrile
CNS	Zentralnervensystem	Central Nervous System
Conc	Konzentration	Concentration
CV	Variationskoeffizient	Coefficient of Variation

D2 receptors	Dopaminrezeptoren	Dopamine 2-receptors
DA	Dopamin	Dopamine
De	Dosis	Maintenance Dose
DGPPN	Deutsche Gesellschaft für Psychiatrie und Psychotherapie, Psychosomatik und Nervenheilkunde	German Society for Psychiatry, Psychotherapy and Psychosomatics
DIN	Deutsch Institut für Normung	German Institute for Standardization
DMC	Desmethylcitalopram	Desmethylcitalopram
DOPAC	Dihydroxyphenylessigsäure	Dihydroxyphenylacetic acid
DOX	Doxepin	Doxepine
DRR	Dosisbezogener Referenzbereich	Dose-Related Reference Range
EEG	Elektroenzephalographie	Electroencephalography
EPMS	Extrapyramidale Motorisches System	Extra Pyramidal Motor System
F	Bioverfugbarkeit	Bioavailability
FDA	US Food and Drug Administration	US Food and Drug Administration
Fig	Abbildung	figure
FLT	Flupentixol	Flupentixol
GABA	Gamma-Aminobuttersäure	Gamma-aminobutyric acid
GTFCh	Gesellschaft für Toxikologische und Forensische Chemie	Society of Toxicological and Forensic Chemistry
H1-Receptors	Histaminrezeptoren	Histamine-1 receptor
HBr	Hydrogenbromid	Hydrogen bromide
HCl	Hydrogenchlorid	Hydrogen chloride
HLP	Haloperidol	Haloperidol
HPLC	Hochleistungsflüssigkeitschromatographie	High-Performance Liquid Chromato-

		graphy
ICD	Internationale Klassifikation von Krankheiten	International Classification of Diseases
ICH	Internationale Konferenz zur Harmonisierung	International Conference on Harmonization
Ind.	Induktion	Induction
Inh.	Hemmung	Inhibition
Inject. Vol	Injektionsvolumen	Injection volume
ISO	Internationale Organisation für Normung	International Organization for Standardization
LOD	Nachweisgrenze	Limit of Detection
LOQ	Bestimmungsgrenze	Limit of Quantification
mAU	Molecular Absorption Unit (Peakhöhe)	Molecular Absorption Unit (peak height)
mAU x min	Molecular Absorption Unit per min (Peakfläche)	Molecular Absorption Unit per min (Peak area)
MeOH	Methanol	Methanol
Min	Minute	Minute
MLP	Melperon	Melperone
MT	Methoxytyramin	Methoxytyramine
MW	Durchschnittlich	Mean
ND	Nicht erfasst	Not detected
p.o	Peroral	Perorale
PTSD	Posttraumatische Belastungsstörung	Posttraumatic Stress Disorder
r^2	Bestimmitheitsmaß (Determinationskoeffizient)	Coefficient of Determination
RP	Umkehrphase	Reversed Phase
Ret. Time	Retentionszeit	Retention time
SD	Standardabweichung	Standard Deviation
SSRI	Selektive Serotonin Wiederaufnahmehemmer	Selective serotonine Reuptake Inhibitor

TCA	Trizykische Antidepressiva	Tricyclic antide-pressants
TDM	Therapeutische Drug Monitoring	Therapeutic Drug Monitoring
TEMED	Tetramethylethylendiamin	Tetramethylethyl-enediamine
Temp	Temperatur	Temperature
TRR	Therapeutischer Referenzbereich	Therapeutic Refe-rence Range
UV	Ultraviolett	Ultraviolet
WHO	Weltgesundheitsorganisation	world health or-ganization
WL	Wellenlänge	Wave lenght

1 Introduction

The goal of treatment of schizophrenic patients with antipsychotic drugs is to effectively control positive and negative symptoms at a lowest possible dosage. Therapeutic drug monitoring (TDM) is one of the applicable means to achieve this goal by measuring serum concentrations of the antipsychotic drugs. A pre-requisite for TDM is the availability of a laboratory method with sufficient sensitivity and specificity.

1.1 General Description of Schizophrenia and Symptoms

The German Society for Psychiatry, Psychotherapy and Neurology characterized schizophrenia as a disorder with a characteristic pattern of different psychological areas such as perception, ego functions, affective and psychomotor disturbance [DGPPN, 2006]. It is a disorder that must be present for at least six months, including at least one month of delusions (erroneous beliefs that usually involves a misinterpretation of perceptions or experiences), hallucinations (for example, visual and olfactory), disorganized speech, catatonic behavior or negative symptoms, which denote functional impairments [Benkert and Hippius, 2007]. The acute phase of schizophrenia may last several weeks before subsiding and the symptoms may recur after several years. During the chronic schizophrenic phase, the symptoms progress insidiously with persistent residual symptoms [Benkert and Hippius, 2007].

The two main types of schizophrenic symptoms are the positive symptoms and the negative symptoms. The positive symptoms are considered as an excess of normal functions of the brain. They are attributed to over-activity of dopamine neurons specifically in the mesolimbic pathway. Examples of positive symptoms are

delusions, hallucination and disorganized speech [Stahl, 1996]. The negative symptoms are diminutions or loss of normal functions of the brain. They involve other regions of the brain such as the dorsolateral prefrontal cortex and other neurotransmitter systems that might attribute to under-activity of distinct neuronal systems. Examples of negative symptoms are affective flattening (restriction in range and intensity of emotional expression), alogia (restrictions in the fluency of thoughts and speech), avolition (restrictions in the initiation of goal-directed behavior), anhedonia (lack of pleasure) and attention impairment. [Stahl, 1996].

One of the recognized diagnostic classification systems of schizophrenic diseases is the ICD system (see table 1) released by the World Health Organization (WHO). Table 1 shows the current version of ICD-10 with eight different codes (F20-F29), assigned to certain clinical categories.

Table 1. ICD-10 Classification of schizophrenia and schizophrenia-related sickness.

ICD-10 Code	ICD-10 Classification	Clinical Categories
F20	Schizophrenia	Paranoid schizophrenia
F21	Schizotypal disorder	Schizophrenia simplex
F22	Persistent delusional disorder	Post-Schizophrenic depression
F23	Acute and transient psychotic disorder	Residual schizophrenia
F24	Induced delusional disorder	Catatonic schizophrenia
F25	Schizoaffective disorder	Hebephrenic schizophrenia
F28	Other non-organic psychotic disorder	Non-organic psychotic disorder
F29	Unspecified non-organic psychosis	Undifferentiated schizophrenia

Note: The chapter five of the ICD-10 system describes the blocks F00-F99 mental and behavioral disorders, whereby F20-F29 belong to the group of schizophrenia, schizotypal and delusional disorders [World Health Organisation, 1993].

1.2 Transmission of Neurotransmitters and Development of Schizophrenia

The onset and the course of the schizophrenic sickness is regarded as a result of pathophysiological abnormalities during neurodevelopment [Liebermann, 1998]. Temporary changes in the neurochemical steady state due to environmental cues are suggested to trigger the symptoms. The onset of the schizophrenic sickness and the time for the manifestation of the symptoms can be both, in an early age as well as in adult age. If neurotransmission is interrupted early in the development, the brain may not reach its full potential, such as in the case of a mental retardation. If neurotransmission is interrupted later in life, the brain may regress from the potential it had earlier on, as in the case of various dementias [Stahl, 1996]. Hence, education

in the language and principles of chemical neurotransmission is crucial for a better understanding of the impact of neurological and neuropsychological diseases on the central nervous system and to interpret the behavioral consequences of antipsychotics [Stahl, 1996]. This can be achieved through knowledge of the mechanism of neurotransmission in pre- and postsynaptic neurons.

Neurotransmission includes transmission of signals from the presynaptic neuron through the synaptic gap into the postsynaptic neuron (see figure 1). The synapses are dynamic and constantly changing in the brain. The signals from the presynaptic neuron cause a rush of calcium ions into the synaptic bouton which bind to receptors on the inside. The calcium binding to calcium-sensitive proteins in the presynaptic membrane stimulates the vesicles carrying the neurotransmitters in the presynaptic neuron, which thereupon merge with the presynaptic membrane to release their neurotransmitters into the synaptic cleft. Released neurotransmitters bind to receptors in the postsynaptic neuron and cause the activation of specific pathways. Connecting neurons can send and receive synaptic information from other neurons with the help of a neurotransmitter [Stärker, 2001]. Since the synapse is the site of chemical neurotransmission, information transfer in the brain is vitally dependent on these processes.

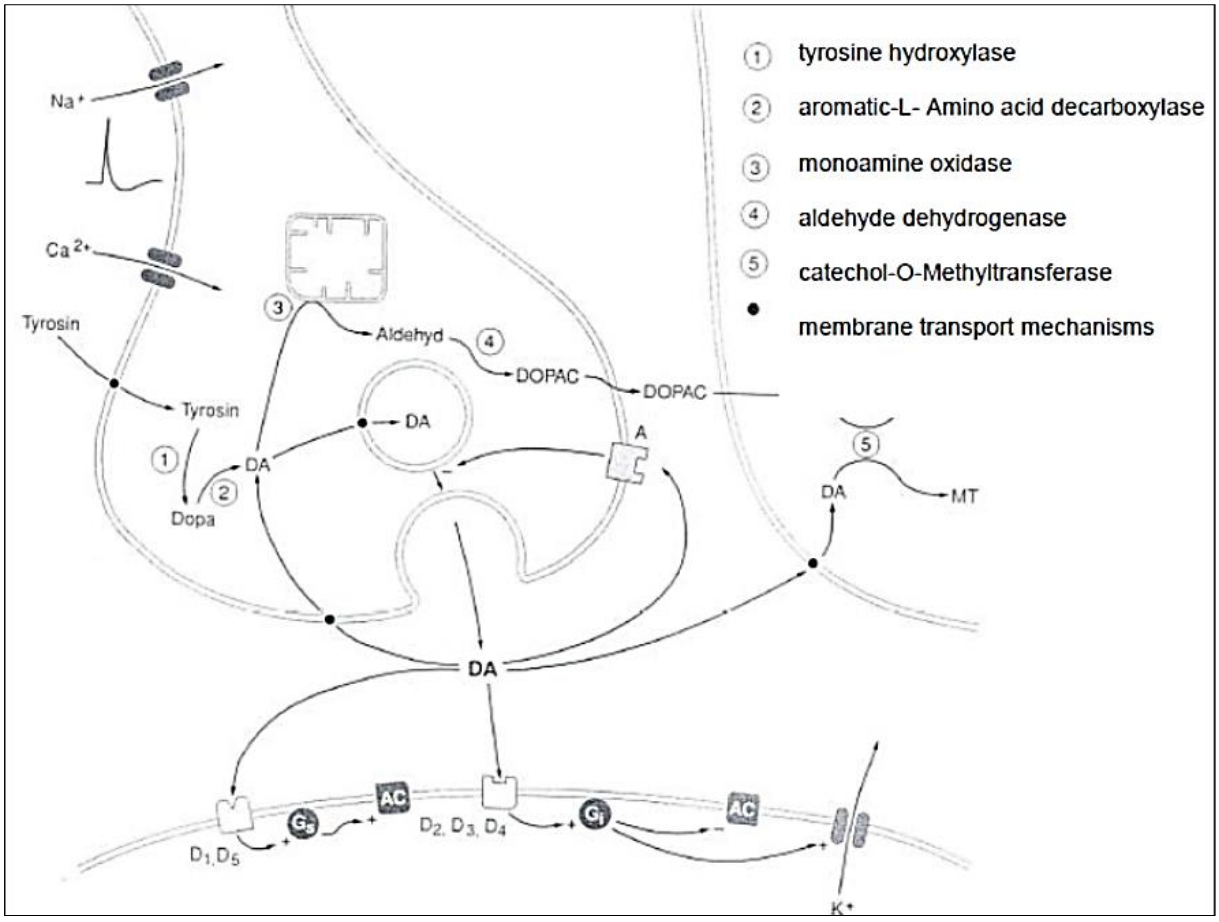


Figure 1. The synapses and the neuronal receptors of antipsychotic drugs [Stärker, 2001].

Note. D1-D5 represent different dopamine receptors where especially antipsychotic medications bind. After the release of dopamine (DA) from the vesicle, it can either bind on its receptor and/or be received back to the vesicle through the autorecetor (A). Dopamin D2 autoreceptors modulate DA neuron firing, DA release, and DA synthesis through a negative feedback mechanism [Calipari et al., 2014].

Neurotransmitters are chemicals synthesized in the presynaptic cell and stored in the vesicles until latter are stimulated. Examples of neurotransmitters are dopamine, serotonin, GABA, adrenaline, noradrenaline, acetylcholine and histamine. They are biochemical substances that transmit, enhance, or modulate chemical impulses from one cell to another (see figure 1). Once the neurotransmitter has been released from the presynaptic neuron, it diffuses across the synapse where it hits target sites on

receptors with high affinity for that specific neurotransmitter. The vast majority of drugs such as antipsychotics known to work in the central nervous system (CNS), act upon the process of neurotransmission [Stahl, 1996] such as the mesolimbic, the nigrostriatal, mesocortical and the tuberoinfundibular dopamine pathways.

1.3 Pathways of Dopaminergic Neurotransmission

Dopamine and its activity in different neuronal pathways (see figure 2) play an important role in the genesis of schizophrenic illness. The neuroanatomy of dopamine neuronal pathways in the brain can explain both the therapeutic effects and the undesired effects of antipsychotic agents. Changes in the rate of receptor or enzyme synthesis can affect the amount of neurotransmitter available for neurotransmission, and can thereby alter the neurotransmission process [Stahl, 1996].

The mesolimbic dopamine pathway is thought to control behavior and produce delusions and hallucinations when overactive. The nigrostriatal dopamine pathway controls movement. When dopamine receptors are blocked by more than 80% in the postsynaptic projections of this dopamine system, it produces disorders of movement that can appear very much like those in Parkinson’s disease. Since the nigrostriatal pathway extends to basal ganglia, a part of the extrapyramidal neuronal system of the central nervous system, undesired effects such as extra pyramidal motor symptoms (EPMS) can occur. The mesocortical dopamine pathway is related to the mesolimbic pathway and its role lies in mediating positive and negative psychotic symptoms. The blockade of this pathway will help to reduce negative symptoms. The

tuberoinfundibular dopamine pathway controls prolactin secretion. When the dopamine receptors in this pathway are blocked, prolactin levels rise [Stahl, 1996].

Knowing the molecular causes of what leads to abnormal neurotransmission can lead to a rationale for developing an antipsychotic therapy for the treatment of schizophrenia [Stärker, 2001]. All antipsychotic drugs capable of treating positive psychotic symptoms of schizophrenia are blockers of dopamine receptors, particularly D2 dopamine receptors. This is because scientific studies have proved that dysregulation of DA at D2 receptors are most intimately associated with the positive symptoms of schizophrenia [Ginovart and Kapur, 2012].

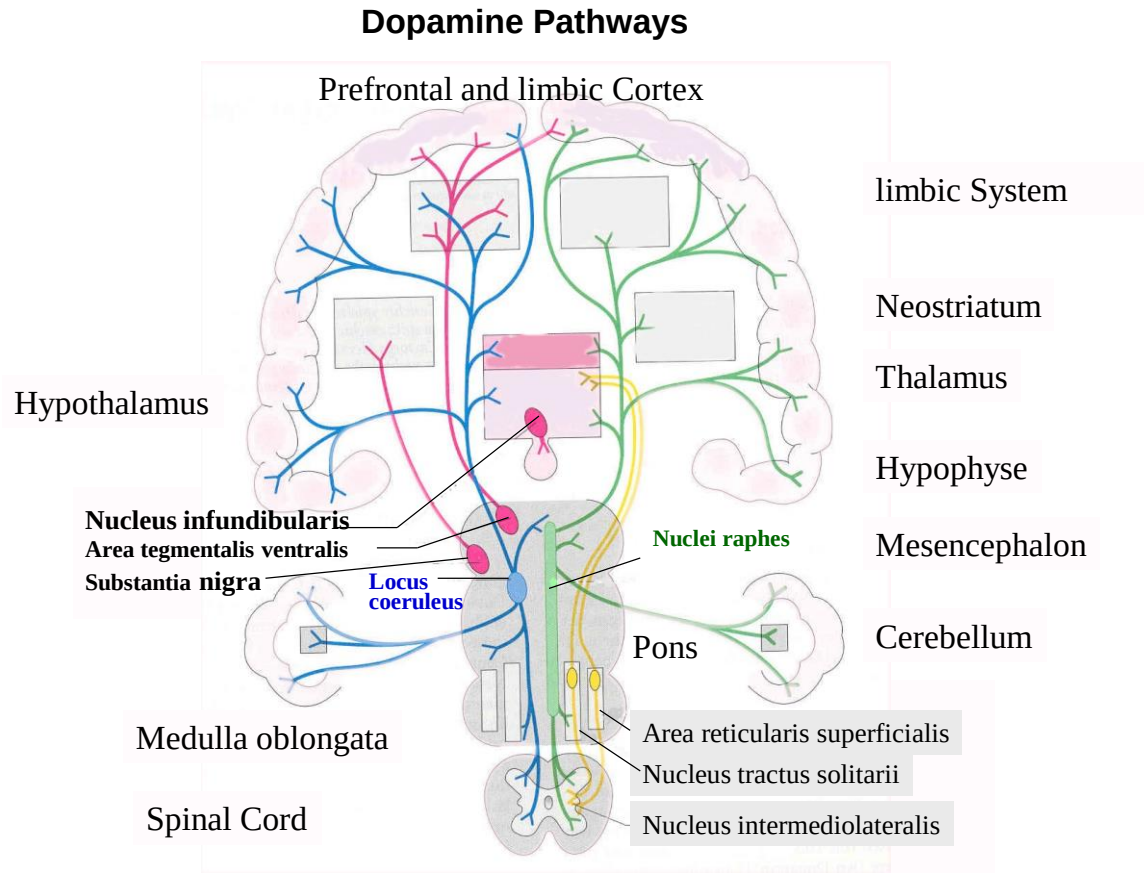


Figure 2. Neuronal pathways of dopamine neurotransmission in CNS [Haen, 2012].

1.4 Phases of Schizophrenia and Therapeutic Goals

The aim of modern pharmacotherapy is to attain the highest possible level of functioning of patients by using effective drugs with doses that are as low as possible and as high as necessary [Haen, 2002]. In order to achieve this, the Drug Commission of the German Medical Association (AKdÄ) and the German Society for Psychiatry, Psychotherapy and Neurology (DGPPN) have compiled guidelines for the treatment of schizophrenic disorders with therapeutic recommendations that reflect the current state of science. According to these guidelines, schizophrenia is indicated when there is evidence of the existence of acute psychotic states and when chronic disease progression is associated with cognitive and social impairment [DGPPN, 2006]. To attain the therapeutic goal in the acute phase a therapeutic relationship between the therapist and the patient must be established. Clarification of the sickness and treatment concepts, taking measures to eliminate or reduce the symptoms and the disease-related impairments are all aimed to preventing harm to self and society.

The long-term psychotic phase occurs mostly when there is a relapse in the sickness. Early detection of an impending relapse and relapse prevention are very important in order to implement a proper stabilization concept. For psychological symptoms that are in remission or have subsided, stabilization measures such as treatment of cognitive and social deficits and other negative symptoms are initiated. Harmonization and stabilization of environmental conflicts such as social contacts, as well as preparation and maintenance of rehabilitative measures are some other significant factors for successful therapy. These are aimed at the promotion of

compliance, preventing suicides and improving the patients' quality of life [Stärker, 2001].

Individual therapeutic responses and the occurrence of antipsychotic undesired effects require careful selection of substances, concomitant medications, dose, and application procedures. An optimal dose can be assumed if a good effect on the whole spectrum of psychotic symptoms with differential focus in each phase (see figure 3) of the sickness is achieved with minimal undesired effects. Antipsychotics should be preferred that show less dose-dependent extra pyramidal motor syndrome (EPMS) and at the same time do not exhibit effects on positive symptoms, but demonstrate better efficacy against the negative symptoms [Advokat et al., 2000].

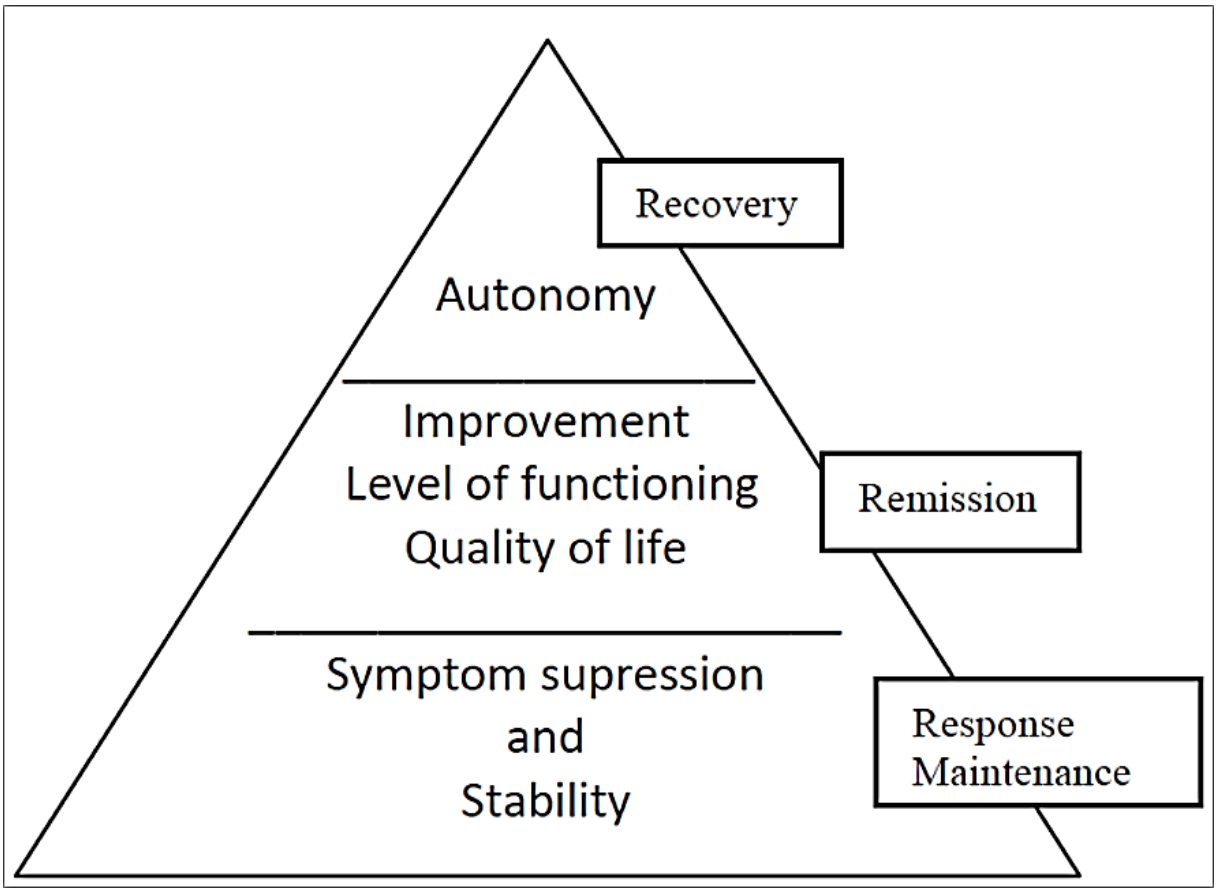


Figure 3. Stages of schizophrenic treatment [Falkai et al., 2007].

A risk-benefit assessment of different antipsychotics as depicted in figure 3 is necessary and in case of serious undesired drug effects, a switch to a more tolerable antipsychotic (based on individual patients) is more advisable. After remission of symptom, the dose can gradually be reduced and adjusted to a lower maintenance dose in long-term treatment.

The key criterion for a better integration of the patients is not necessarily complete symptom remission [Falkai et al., 2007], but how patients cope with demands made on them at work and in social life despite possibly still experiencing symptoms. The goal of the therapy should therefore go far beyond a response to treatment on the symptom level [Kasper, 1999; Gabel et al., 2002]. Objectives of psychological treatments in schizophrenia are the reduction of individual vulnerability, reduction of undesired effects of external stressors, promoting compliance, and increasing the quality life of the patients. Examples of psychological treatments are (1) psycho-education for optimizing the process of preventing symptom-recurrence, and (2) cognitive behavioral therapy to reduce the intensity of positive psychotic symptoms and to encourage flexibility in the process of abstract thinking (alogia) [Stahl, 1996].

1.4.1 Therapy with Antipsychotics – Differentiation of schizophrenia from Psychosis

Antipsychotics are a group of medication indicated for the treatment of psychosis in general and schizophrenia in particular. Though schizophrenia is one of the best known types of psychotic sickness and the most common psychotic disorder, it is not synonymous with psychosis, but is just one of the many causes of psychosis [Cannon et al., 1997; Howard et al., 2000]. The integration of genetic vulnerability in the expression of a disease, life event stressors (such as divorce and financial

problems), the individual's personality, coping skills, and social support available from others and other environmental influences are important factors involved in the formation of psychiatric disorders including psychosis and schizophrenia [Stahl, 2000].

Psychosis can be considered as a set of symptoms in which a person's mental capacity, affective response, and capacity to recognize reality, communicate, and relate to others is impaired. Psychosis can be paranoid, disorganized and/or depressive. Paranoid psychosis is accompanied with preoccupations of delusional beliefs (paranoid projections), expression of feelings of hostility (hostile belligerence) and hearing voices that praise and extol (grandiose expansiveness). Disorganized psychosis consists of a conceptual disorganization (giving answers that are irrelevant, drifting off the subject), disorientation, and excitement. Depressive psychosis is characterized by retardation, apathy (for example, the manifestation of unusually slow speech and fixed facial expression) and anxious self-punishment and blame (for example, the tendency to blame or condemn oneself and preoccupation with suicidal thoughts) [Stahl, 1996].

1.4.2 The Use of Antipsychotics for the Treatment of Schizophrenia

Basic treatments of schizophrenia are antipsychotic drugs, which block primarily dopamine receptors. Antipsychotic treatment can vary notably in terms of specific antipsychotic drug, dose, duration of treatment, and combinations with additional psychotropic drugs and drugs used in internal medicine. Some antipsychotic drugs such as melperone (MLP), benperidol (BPD), haloperidol (HLP), bromperidol (BRP), flupentixol (FLT), and zuclopenthixol (ZLT) act on different neurotransmitter receptors that mediate their undesired effects but not their therapeutic effects, namely the

antihistamine properties (weight gain), alpha-adrenergic-blocking properties (undesired cardiovascular effects), and muscarinic-cholinergic-blocking properties (dry mouth) [Stahl, 1996]. Antipsychotics differ in their undesired effect profiles, but not in their overall therapeutic profiles, because the various antipsychotic agents differ in terms of their ability to block the receptors. Some antipsychotics are for example more sedating than others and some are more prone to cause undesired cardiovascular effects than others. However, all antipsychotics reduce psychotic symptoms, especially positive psychotic symptoms in the group of schizophrenic patients [Voruganti et al., 2000].

In contrast to oral antipsychotics, depot antipsychotics are poorly soluble in water and are released from the depot form into the blood stream very slowly (depot effect). They therefore have a longer onset and duration of action compared with oral administration. Examples of some depot antipsychotics are, among others, haloperidol decanoate, flupentixol decanoate, zuclopenthixol decanoate and risperidone decanoate. The choice of antipsychotic agent for the treatment of schizophrenia is mostly carried out under the consideration of present symptoms and the therapeutic goals set by the attending clinicians [Jauhar et al., 2012].

Long-term undesired effects have lead to the pursuit of antipsychotic treatments that would reduce or eliminate such problems yet still be powerful antipsychotic agents against positive symptoms. Troublesome undesired drug effects also lead to noncompliance, since patients frequently wish to discontinue their medications to rid themselves of the undesired effects despite a high risk of relapse of psychotic symptoms. Therefore, therapy success critically depends on stable medication, medication dose, and appropriately advicing the patients and attending clinicians,

which will allow for an adequate control of the serum concentrations to avoid undesired drug effects [Fleischhacker et al., 1994].

1.5 Indication for the Therapeutic Drug Monitoring of Antipsychotics

Therapeutic Drug Monitoring (TDM) is a clinical laboratory determination of specific drugs in human serum or plasma at stipulated intervals that, with appropriate clinic-pharmacological interpretations, aims to optimize individual therapies. TDM is generally used for drugs with narrow therapeutic ranges (such as anticonvulsants, antipsychotics, immunosuppressants, and other psychotropic medications), drugs with marked pharmacokinetic variability, drugs for which target concentrations are difficult to monitor, and drugs known to cause therapeutic and severe undesired drug effects despite the usual clinical dose. TDM can also be used for case-specific indications such as suspected drug interaction, suspected intoxication, no or insufficient therapeutic response, recurrence under maintenance dose, prevention of relapse in long-term therapy, suspected noncompliance and expensive pharmacovigilance, children and young people, patients aged over 65 years, comorbidity, and forensic indications [Greiner, 2008]. TDM of antipsychotic drugs in the blood of pregnant or breastfeeding women can, for example, help to limit drug exposure to the fetus or the newborn [American Academy of Pediatrics, 2000].

The practical indication for the correct TDM of antipsychotics in the treatment of schizophrenia can be derived from the guidelines of the association for neuro-psychopharmacology and pharmacopsychiatry (AGNP). It is aimed at guiding clinicians in choosing correct therapeutic doses of different medications for individual patients. It is also a useful tool to optimize and illustrate the psycho-pharmacotherapy and the analytical principles of pharmacokinetic, pharmacogenetic and the present

scientific state of knowledge concerning the relationship between the plasma concentration and the clinical effect [Greiner, 2008].

In practice however, the clinical use of TDM is rather limited. Moreover, clinical studies about the relationship between the given dose and drug concentration in blood and evaluation of therapeutically effective concentrations are rare.

1.5.1 The AGNP Guidelines for the Application of TDM

The TDM working group of the association for neuro-psychopharmacology and pharmacopsychiatry (AGNP) published guidelines on the best practical use of TDM in psychiatry. Reported therapeutic concentration values are obtained through the determination of serum antipsychotic concentrations of different patients. Reported recommendations were obtained after a detailed review of reliable scientific sources [Baumann et al, 2004]. Criteria for the evaluation of the sources were the presence of the established therapeutic reference ranges (TRR) and the presence of controlled clinical trials, which supports the usefulness of TDM and also gives hints of increased toxicity at higher therapeutic concentrations. The TDM Rating Levels according to the AGNP Guidelines for the use of antipsychotics are listed in table 2.

Table 2. Application of TDM in antipsychotic therapy according to the guidelines of the AGNP [Baumann et al. 2004].

Level	Characteristic	Advantage	Antipsychotics
1. Highly recommended	Established TRR, presence of controlled clinical trials	Prevention of toxic concentrations and thereby an enhancement in patients'compliance, the	Amisulpride Clozapine Fluphenazine Haloperidol

		efficacy and safety of drug therapy	Olanzapine Perazine Perphenazin Thioridazin
2. Recommended	Presence of therapeutic guidelines by at least one prospective study with defined improvement criteria	Improvement of the therapeutic concept through the use of TDM	Aripiprazole Bromperidol Chlorpromazine Flupentixol Fluspirilen Paliperidone Quetiapine Risperidon (plus 9-OH-Risperidon) Sertindole Sulpiride Ziprasidone
3. Beneficial	Presence of therapeutic guidelines derived from pharmacokinetic studies in pharmacokinetic steady states with retrospective analysis of TDM data.	Testing the plausibility of the measured concentrations at a given dose and to check if clinical improvement can be achieved by non-responders with very low serum concentration through dose increase.	Benperidol Chlorprothixene Iloperidole Levopromazine Melperone Pimozide Pipamperon Zotepine Zuclopenthixol
4. Potentially	Presence of	TDM is not recommended for	Asenapine

beneficial	therapeutic values derived from pharmacokinetic studies in pharmacokinetic steady states in the absence of valid clinical data	dose- finding but it can be potentially useful for specific indications or specific problems. It should therefore be limited for specific issues.	Prothipendyl

Note: The different TDM rating levels for the measurement of medications in human serum give general information on the recommended medications for the practice of TDM in order to achieve an expected therapeutic goal.

The DRR data for study antipsychotic medications are derived from studies of drug concentrations of healthy individuals or patients treated with a constant dose (see table 3). The calculation of the dose-related reference range is the calculation of the dose of a medication, which will lead to a steady state concentration. $D = D \times F/\tau = c \times Clt$ (D = constant dose per day at steady state, c = blood concentration, Clt = total clearance of the drug. [Haen et al., 2008; Hiemke et. al., 2011].

Table 3. Consensus data for DRR and TRR of the applied substances.

Substances	Consensus (C/D-low)	Consensus (C/D-high)	Consensus (TRR-low)	Consensus (TRR-high)
Melperone	0.14	0.28	30	100
Benperidol	0.15	0.31	1	10
Haloperidol	0.61 (oral)	0.99	1	10
	0.07 (decano-ate)	0.12 (decanoate)		
Bromperidol	0.09	0.19	1	10
Flupentixol*	0.78	0.87	1	10
Zuclopenthixol	0.13	0.35	4	50

Note: C/D-low and C/D-high are factors for calculating dose- related reference range, TRR = therapeutic reference range.

* The flupentixol-DRR factor applied in this work was derived from the pharmacokinetic data of flupentixol applicable at the TDM laboratory (C/D for flupentixol oral = 0.66 – 1.74 and C/D flupentixol decanoate = 1.65 – 4.35), which is difference from that of consensus.

1.5.2 Chemical Groups of Antipsychotic Drugs Analysed in this Thesis

The chemical groups of antipsychotics studied here in this thesis are butyrophenones and thioxanthenes, subgroups of phenothiazines (see figure 4–figure 7) for the structures of these antipsychotics. The “R” in these figures denotes possible substituents. The chemical differences in the active groups of these substituents are one of the explanations for their different pharmacological effects, and at times demand different analytical methods for their therapeutic drug monitoring (TDM). The explanation of TDM to ward clinicians will help in clinical decisions and in the enhancement of individualized therapy.

Phenothiazines and thioxanthenes have basic side chains of different derivates in position 10 of their ring system (see figures 4 and 5). The tertiary amino group of the side chain is separated from the tricyclic system by three carbon atoms. The antipsychotic effect of the derivates with aliphatic side chains is in relation to the dose of the given medication relatively small but slightly higher in piperidine-substituted and most pronounced in piperazine-substituted derivates [Gothert et al., 2009].

Thioxanthenes are classified as a sub group of phenothiazines. The difference from phenothiazines is that the N-atom in position 10 of the ring system is replaced with a carbon atom (see figure 4). The side chain in Thioxanthenes is connected by a double bond to the ring system (see figure 5). An additional substitution in position 2 leads to cis-isomers. The cis-isomers have higher antipsychotic potency than the trans-isomers. Flupentixol and zuclopenthixol belong to the thioxanthenes with a piperazine side chain. They are high-potency antipsychotics used in the treatment of different schizophrenic and psychotic symptoms. Their side effect profile and mode of action are similar to that of butyrophenones despite the differences in chemical

structure and dopamine receptor affinity. This is because they have a common site of drug action at the dopamine receptor subtype-D2 [Gothert et al., 2009].

The potency of antipsychotic drugs is based on the ability of the drug to bind to the receptors. High-potency antipsychotics have for example a high affinity for the dopamine-2 (D2) family of dopamine receptors [Gothert et al., 2009].

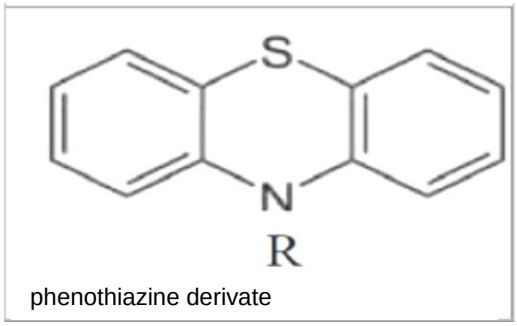


Figure 4. The substitution on the N-atom of the phenothiazine ring.

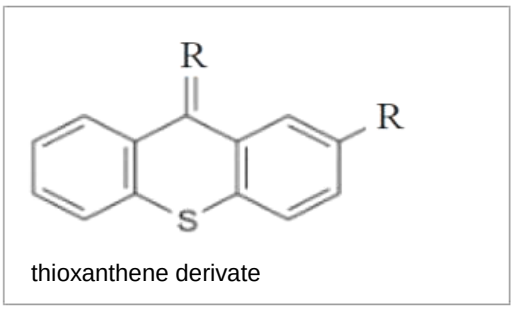


Figure 5. The thioxanthene ring.

Note: Substitution connected by a double bond to the ring system and the substitution of the H-atom at position 2.

Butyrophenones and diphenylbutylpiperidenes have as a common structural feature a quadrinomial alkyl chain which closes with a piperidine ring and in some butyrophenones with a piperazine ring (see figure 6 and 7). The intensity of their antipsychotic effect can be compared to that of phenothiazines with piperazine side chain [Gothert et al., 2009]. Butyrophenones exhibit their pharmacological action on

the D2 receptors in the dopamine pathways, and thus allow the treatment of different psychotic disorders.

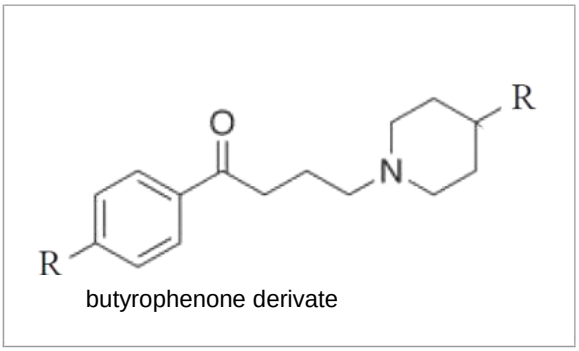


Figure 6. Quadrinomial alkyl chain closes with a piperazine ring.

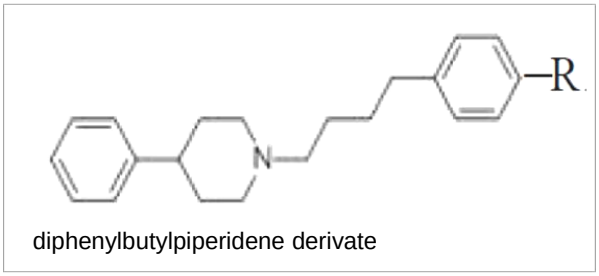


Figure 7. The piperazine ring is substituted with a phenyl ring.

Common undesired drug effects of butyrophenones and phenothiazines are EPMS, tremors and dry mouth. A discontinuation of antipsychotic drug treatment can be associated with patients' vulnerability to relapse and the development of induced undesired drug effects which varies based on several factors such as gender, age, and pharmacokinetic interactions of the medication and/or co-medications taken by the patients [Kanner and Frey, 2000].

1.5.3 Determination of Serum Antipsychotic Concentrations

The contribution of pharmacokinetic variability to differences in dose requirements of antipsychotics can be identified by measuring the serum concentration at a steady-state (after at least 5 elimination half-lives by a constant dose) and modifying the

dose to attain a desired therapeutic concentration. Melperone has a mean elimination half-life of 6 ± 2 hours, benperidol 7.65 hours, haloperidol 22 ± 1 hours, bromperidol 36 hours, flupentixol 29 ±1 hours [Jorgensen, 1980], and zuclopenthixol 20 hours. The information regarding the elimination half-life of the drugs mentioned was taken from their respective summary of product characteristics.

An appropriate evaluation of the measured concentration requires the acquisition of correct timed blood samples. It is therefore recommended to draw blood samples at trough or just before the next dose, for example, in the morning before the first medication-intake, in the case of two or three daily administrations, and after the elapse of 24 hours since the last dose, in the case of once-a-day morning administration. In this way, the lowest concentration during the steady state, which is not strongly subjected to inter-individual variation, absorption and distribution problems, is less likely to be influenced. If the serum sample is drawn before distribution of antipsychotics into tissues is complete, the obtained concentration after measurement may be higher than the expected dose-related reference range and will lead to a false laboratory result and its clinical interpretation [Hiemke et al., 2011].

The dose-related reference range (DRR), the therapeutic reference range (TRR), the maintenance dose, co-medications and individual factors, like illness and age are some TDM tools for the control of serum antipsychotic concentrations. Significant numbers of patients who do not respond effectively to antipsychotic therapy, because of some pharmacokinetic and pharmacodynamics reasons [Hiemke et al., 2005] will thus be helped through TDM.

A dose-related reference range (DRR) is a concentration range in which serum concentrations of a drug can be calculated with a given dose of the drug. It indicates

whether the measured concentration is within the expected range and if the administered dose is enough to yield the expected therapeutic concentration. There is a proportional relationship between the dose and drug concentration in pharmacokinetic balance (see table 3). The calculation is done based on the direct correlation between the drug dose D (maintenance dose in mg) to its concentration in serum c (ng/ml), and with the total clearance of the drug Cl_t (l/h) being the correlation coefficient $D = D/\tau = c \times Clt$ [Haen and Greiner, 2009]. The concentration of antipsychotics in human serum can however be influenced by the biological activities of some known metabolites [Calligaro et al., 1997; Sharma et al., 2005].

The therapeutic reference range (TRR) is an optimum concentration of a particular drug in blood that gives the intended benefit, while minimizing or avoiding undesired effects. “It assumes that there is a plasma concentration range of the drug which is characterised by maximal effectiveness and maximal safety” [Hiemke et al., 2011]. The lower limit of the therapeutic reference range is the one derived from the mean plus one standard deviation (MW + 1SD) of the drug concentration evaluation of patients, who responded to the therapy after two weeks of drug administration. The therapeutic response is assessed according to the corresponding disease scale for schizophrenia when 84.14% of the measured value can be found on the scale [Sachs and Hedderich, 2006]. The upper limit of the therapeutic reference range indicates that at a particular concentration, 15.87% of the patients suffered from undesired drug effects. This upper limit should, however, be separately measured depending on the expected undesired effects such as EPMS und hypotension.

The therapeutic reference range gives a population-based value that is applicable to all patients. Individual patients can show an optimal therapeutic response at a drug

concentration outside the given therapeutic reference range. Eventually, an "individual therapeutic concentration" of each patient in which an optimal response to the psycho-pharmacotherapy exists has to be identified [Hiemke et al., 2011]. The therapeutic reference ranges of antipsychotic drugs are supported by studies that have shown correlations between the serum concentrations and therapeutic effects [Hiemke et al., 2011].

In the TDM laboratory in Regensburg, the active metabolites of antipsychotics are measured and considered as well during the calculation of the measured concentrations. Drugs with inactive metabolites are calculated without the consideration of such metabolites. Examples of pharmacokinetic parameters for the calculation of an orally administered antipsychotic are listed in table 4.

Table 4. Pharmacokinetic parameters of the orally administered study substances

Substances	Number of patient **(n)**	Total clearance **(Clt)** **ml/min**	Daily dose* **mg**	Elimination half-life **($t_{1/2}$)** **hour**	Bioavailability **(F)** **%**	Excretion
MLP	6	1484-2898	25-400 mg /1-3 times per day	4-6	60	Faeces*
BPD	14	1073-2240	1-40 mg /1-3 times per day	Ca. 5	50	Faeces*
HLP	6	420-680	3-15 mg/day	12-36	60	Faeces and urine*
BRP	14	3570-7938	1-10 mg/ day	20-36	30*	Faeces
FLT	3	440-490	5-20 mg/ day	20-40	60	Faeces*

ZLT	8	867-2300	2-50 mg distributed 1-3 times/ day	15-25	40	Faeces*

Note: According to TDM consensus, 2011, pharmacokinetic parameters of melperone [Borgstrom et al., 1982], pharmacokinetic parameters of benperidol [Seiler et al., 1994], pharmacokinetic parameters of haloperidol [Cheng et al., 1987], pharmacokinetic parameters of bromperidol [Lee et al., 2006], pharmacokinetic parameters of flupentixol [Jorgensen, 1980], and Pharmacokinetic parameters of zuclopenthixol [Jerling et al., 1996].

* = information from the summary of product characteristics (SPC) for Melperon, Glianimon, Haldol, Impromen, Fluanxol, Ciatyl-Z. Substance excretion of MLP, BPD, BRP, FLT and ZLT through the urine is less than 10 %. 60% HLP is excreted through the faeces and 40 % through the urine. Individual dosing of all the study substances was recommended in their respective SPC.

According to table 3, Jorgensen, 1980 expressed the value of the total clearance (0.44-0.49) in l/min which corresponds to 440-490 ml/min. Total clearance is the elimination of the drug from all the metabolizing/eliminating organs of the body, mainly liver and kidney, by scaling the drug elimination rate by the corresponding plasma concentration. Irrespective of the intra-individual variation observed during the estimation of the elimination half-life ($t_{1/2}$), Jorgensen, (1980) gave 24 hours as a reasonable dose interval (τ) for an oral flupentixol medication. The reason for the low bioavailability (F) was a result of the first part metabolism in the liver or an incomplete absorption of flupentixol [Jorgensen, 1980]. How long it will take to reach the steady-state concentration was not mentioned in his work, but applying the given $t_{1/2}$, it could be assumed that the steady-state concentration of orally administered flupentixol will be reached between 4-8 days. Steady-state concentration (C_{ss}) is the serum concentration reached after five elimination half-life. That is when the rate of absorption of a medication is equal to the rate of elimination of the same medication.

Css is dependent on the dose, the dose interval, the bioavailability and the clearance {(C=D x F)/(τx C_{lt} ±SD)}.

The use of pharmacokinetic equations enables a mathematical calculation of the dose-related reference range of flupentixol. The proportionality factor (the reciprocal of the clearance and its variation) must be multiplied by the daily dose of the patient to obtain a defined range where 68.27% of the drug concentration is located. The upper value of the dose-related reference range is obtained by subtracting the standard deviation from the clearance, and the lower value of the dose-related reference range is obtained by adding the values of the standard deviation and the clearance (see table 5). The drug concentrations must correspond to the population in which the drug constant for the proportional relationship between dose and substance concentration was measured [Haen et al., 2008].

Table 5. Example of calculating dose related reference range using available pharmacokinetic parameters for flupentixol.

Parameter	Equation
Maintainance dose (D)	$D = \frac{D}{\tau} = c\ x\ Clt$
Concentration (c)	$\mathrm{C} = \frac{D\ x\ F}{\tau \text{ x } \mathrm{Clt}}$
Concentration (c_{ss})	$\mathrm{Css} = D\ x\ \frac{F}{\tau \text{ x } \mathrm{Clt}\ \pm \mathrm{SD}}$
Concentration (c) by F = 54%	$\mathrm{C} = D\ x\ \frac{0.54}{24\mathrm{h} \text{ x } (\mathrm{Clt}\ \pm \mathrm{SD})}$

Concentration (c) by lower value of the dose-related reference range	$\mathrm{C} = D\ x\ \frac{0.54}{\tau \text{ x } \mathrm{Clt} + \ \mathrm{SD}}$
Concentration (c) by upper value of the dose-related reference range	$\mathrm{C} = D\ x\ \frac{0.54}{\tau \text{ x } (\mathrm{Clt} - \ \mathrm{SD})}$

Note: D: dose, Clt: total clearance, t: time, F: bioavailability, τ: dose interval, SD: standard deviation.

1.5.4 Description of the Nine-Fold Table for the Interpretation of Antipsychotic TDM

A detailed overview of the relationship between the dose-related reference range and the therapeutic reference range is shown in the nine-fold table (see table 6).

Drug substance can be defined by dose-related reference ranges and therapeutic reference range as “too low”, “expected” or “too high”, rated in relation to the administered dose and in relation to the therapeutic reference range. With the help of a nine-fold table, these 3 x 3 different ratings can be plotted against each other. The column shows the three possibilities for rating the therapeutic reference range (column A–C) and the horizontal line shows the three ratings of the dose-related reference range (lines 1–3). The nine-fold table enables a possible interpretation of the laboratory measurement under the consideration of both reference ranges. Nine interpretation constellations are possible for a single measurement.

With the information shown on table 6, assuming that there are no concomitant medications and that the blood sample was taken before the next medication intake, the patient can be considered compliant, and the possibility of developing undesired effects is lower at this point, because the concentration is within the therapeutic reference range. Nevertheless, it cannot be considered a successfull therapy

because of high interindividuality of antipsychotic medications. The dosing of this group of medication strictly demands individual therapy for it to be as successful as expected.

Table 6. An overview of the nine-fold table.

Concentration in Relation to Dose-Related Reference Range		Concentration in Relation to Therapeutic Reference Range		
		Too low	expected	Too high
		A	B	C
Too low	1			
expected	2		**X**	
Too high	3			

Note: The letter "X" in B-2 of the nine-fold table shows that the measured concentration is within the DRR and also within the TRR.

1.5.5 Serum Antipsychotic Concentration and Clinical Pharmacological Report

To properly interpret a serum concentration, the TDM team must be informed about the maintenance dose, when the serum sample was taken in relation to the last administered dose, and when the therapy was initiated. To meet individual variations at the TDM laboratory, the response of the drug concentration is ideally supplemented by commentaries in the form of clinical pharmacological reports that provide clues to the cause of either “too high” or “too low” concentration in relation to

the dose administered to the patients. In this ways, the attending clinicians can therefore select suitable drugs for their patients at optimal applicable doses while taking into consideration the overall context of the pharmacotherapy [Greiner and Haen, 2007].

The existence of individual biological differences in the level of absorption such as food absorption, the differences in the metabolism due to polymorphisms of cytochrome P450 isoenzymes, xenobiotic transporters, drug interactions and the differences in the elimination activities of the body due to pathological changes of the excretory organs [Trenton et al., 2003] are always considered during the therapeutic drug monitoring. The above-mentioned difference makes it difficult to predict the exact concentration of the administered drugs in individual patients. Therefore the measurement of serum antipsychotic concentrations and its clinic-pharmacological interpretation for special groups of patients such as children and elderly patients is of particular importance.

The metabolic systems of children are not fully developed, and may lead to higher concentrations when an adult dose is given. Older people are often affected by functional limitations of the excretory organs, such as the kidney and liver. Therefore a reduction of the clearance and an increase in the elimination half-life of the ingested drug are applied. The retention time of the drug in the body grows, and the concentrations of the active substance increase at a constant dose. A vast number of drug interactions caused by polymedications [Cozza et al., 2003] can lead to ineffectiveness of the drug in question as well as increase or decrease the serum drug concentrations through the inhibiting and inducing effects of the co-medications on the metabolizing enzymes.

Smoking is one of the factors that has been proven to affect the effectiveness of certain antipsychotic medications such as clozapine due to common metabolic pathways of nicotine and clozapine on CYP 1A of the cytochrome P450 system. The influence of narcotics [Haen and Wodarz, 1999], on the antipsychotic medications can be observed in the low serum concentrations often reached by smokers who are treated with clozapine. The explained relevant factors are summarized in the clinic-pharmacological reports depending on individual case and measured drugs.

1.6 Different Analytical Methods

Medical professionals and researchers use different methods to predict patients' responses to targeted therapies. Seiler et al. (1994) reported the pharmacokinetics and bioavailability of benperidol in schizophrenic patients. Their focus was on the pharmacokinetic and bioavailability differences of the various application forms (oral and intravenous) of benperidol [Seiler et al., 1994]. In their study, the plasma concentrations were determined by using high performance liquid chromatography and electrochemical detection (HPLC/EC). HPLC/EC is sensitive because it entails the use of high voltage that can be problematic in monitoring antipsychotics in plasma. Food and concomitant medications, however, can interfere with the target medication. Different plasma materials such as plasma proteins can influence the sensitivity and stability of the electrodes and cause false measurements of the targeted medication, and will thereby lead to false results.

Furlant et al. (1987) also reported the determination of benperidol in human serum using an HPLC/ EC method. Although only a small volume of serum (1 ml) was required to obtain a desirable detection limit of 0.2 ng/ ml for benperdiol, the above-mentioned disadvantages of HPLC/EC still remain a problem [Furlant et al., 1987].

Furlant et al. (1987) and Seiler et al. (1994) applied similar analytical method (HPLC/EC) for their research, using the same substance (benperidol). Furlant et al. (1987) laid emphasis on the serum determination of benperidol, whereas Seiler et al. (1994) laid emphasis on the bioavailability of the different pahrmaceutical forms of benperidol in serum.

Hiroshi et al. (2000), analyzed various butyrophenones and their analogues in whole blood by means of HPLC/MS. They succeeded to detect five butyrophenones and two analogues at a detection limit of 0.1 ng/ml [Hiroshi et al., 2000]. The use of HPLC/MS, however is complex. It is expensive and requires great expertise in order to operate. Many TDM laboratories cannot afford to operate under these conditions. They need simple analytical equipment which they can afford, for the measurment of drugs in human serum.

The application of HPLC/MS for the simultaneous determination of clozapine, olanzapine, risperidone and quetiapine in plasma was also reported by Zhou et al. (2004). The samples were alkalinized and extracted twice before the HPLC preparation and measurement could take place [Zhou et al., 2004]. This is a delay in the analytical preparation, which will be avoided if the double extraction procedures are not applied.

Zhu et al. (1998) reported the use of gas chromatography-mass spectrometry (GC/MS) with selected ion monitoring to determine clozapine (CLP) and its N-demethylated metabolite in serum. The paper shows that the expected results were obtained and successfully used in clinical pharmacokinetic study of CLP [Zhu et al., 1998]. The described GC/MS method in this paper requires an intensive preparation of the samples before measurement. GC is limited to volatile samples and it is not

suitable for temperature sensitive substances. The intensive preparation applied in this method is not time-saving and the limitation to volatile and temperature sensitive substances is a disadvantage to the robustness of the method, especially when other co-medications and unknown materials are present in the serum. MS as already explained above is expensive and operated by experts.

The use of reversed-phase high performance liquid chromatography with ultra-violate detection (HPLC/UV) for determination of phenothiazines in human serum was described by Tanaka et al. (2007). He simultaneously separated twelve phenothiazines in human serum, with detection limits of 3.2 – 5.5 ng/ml. However, the authors needed a pre-step procedure before they could measure the substances [Tanaka et al., 2007]. Also, the high detection limits are not suitable for the serum determination of antipsychotics with low therapeutic refence range [Hiemke et al., 2011] such as benperidol, haloperidol, and flupentixol (1– 10 ng/ml) substances.

Other methods reported in the literature such as gas chromatography with nitrogen-phosphorus [Bianchetti and Morselli, 1978] and the use of immunoassay for the determination of carbamazepine and phenytoin [Krasniqi et al., 2010] are time-consuming and are not common methods for routine measurements in many therapeutic drug monitoring laboratories.

There is a need for a new method to overcome the disadvantages of the currently available methods.

2 Aims and Objectives

The objective of this thesis was the development of a method for the simultaneous, qualitative and quantitative measurement of melperone (MLP), benperidol (BPD), haloperidol (HLP), bromperidol (BRP), flupentixol (FLT) and zuclopenthixol (ZLT) in human serum. In order to meet the needs of clinicians and patients the method should be less expensive, more precise, faster, and also more reliable than existing methods. It should have the potential to be used in small-scale TDM laboratories.

MLP, BPD, HLP, BRP, FLT, and ZLT are frequently encountered in clinical toxicology, clinical pharmacology and forensic chemistry, generally for the treatment of schizophrenia and psychotic disorders. They have narrow therapeutic ranges and the quantity required to be effective is near the quantity that causes significant undesired effects such as EPMS. The low therapeutic reference ranges, including the inter-individuality in their metabolism, lead to the necessity of monitoring their therapeutic dosages and serum concentrations. Maintaining their steady state and determining an accurate individual therapeutic dose therefore requires an effective and practicable analytical method in measuring their concentrations in human serum.

The application of TDM to antipsychotic drugs requires a valid analytical procedure for the measurement of drugs in human serum. This is also the condition for an effective TDM service [Hiemke et al., 2011].

A method is necessary for the serum determination of these substances without interference to known and unknown impurities or other substances in human serum. The method should allow that many substances can be reliably measured at the same time. This will save time and also meet the needs of the clinicians and patients. TDM laboratories, especially the small-scale laboratories require a simple, precise

and less expensive method for the individual monitoring of antipsychotics in human serum. It should be a simple procedure without a pre-extraction phase, because this will enable a faster measurement and interpretation of the results for the benefits of the patients, waiting for the outcome of the measurement for continuation of their therapy.

Summarizing the requirements, it was aimed to develop and validate a simple, rapid, inexpensive and robust method for determination of MLP, BPD, HLP, BRP, FLT, and ZLT in human serum. The method should also ensure measurements of low concentrations, especially for antipsychotics with narrow therapeutic ranges.

MLP, BPD, HLP, BRP, FLT, and ZLT (with the exception of haloperidol) could not reliably and simultaneously be detected till date, with the already existing methods (see section 1.6) in small scale TDM laboratory such as the TDM laboratory in Regensburg. At the TDM laboratory in Regensburg, for example, haloperidol can be measured simultaneously with risperidone and paliperidone Therapeutic drug monitoring also includes clinical interpretation of the result. This requires expert knowledge to ensure that TDM promotes individualized therapy.

This thesis presents an approach to solve these problems. The majority of established methods are either expensive, time-consuming, not sufficiently robust, limited to particular substance group (such as volatile substances by GC), very sensitive and /or they require special experts for the operation. This is a problem that should be resolved, so that especially small-scale TDM laboratories can operate with a simple, faster and precise method in measuring drug concentrations in human serum with the financial means available to them.

The research work for the development of a new analytical method, as well as relating the received laboratory results to daily clinical application of drugs, in order to help clinicians in promoting individualized antipsychotic therapy will be described.

Questions Raised

Based on the presented problems, the following questions were formulated:

- Which HPLC method that uses isocratic or gradient elution is appropriate under the available laboratory condition for the separation of antipsychotics?
- How can the given acceptance criteria for the validation of analytical methods be achieved?
- How can these substances simultaneously be measured without interference?
- How can this method provide reliable measurements for antipsychotics with low therapeutic concentrations and ranges expecially melperone, benperidol, haloperidol, bromperidol, flupentixol and zuclopenthixol?
- How can users of this method in small-scale TDM laboratories be enabled to operate it without the involvement of specially trained experts?

3 Materials and Methods

This secton is focused on the HPLC instrumentation applicable for the method development and the experimental works. At first, the applicable instruments and chemicals were introduced and subsequently the HPLC methods already in use and the different tests carried out for the method development are described. The last part of this section deals with the method for the analytical determination of the study substances in human serum through TDM and its clinical application.

3.1 Materials

The already existing materials at the TDM laboratory Regensburg and the materials to be tested for the new HPLC method development are described in the following sections.

3.1.1 Laboratory Instrumentation

A vortex device from Heidolph REAX TOP (Schwabach, Germany) and a Megafuge 2.OR from Heraeus GmbH (Osterode, Germany) were applied for shaking and centrifugation. The HPLC system (Dionex GmbH, Idstein, Germany) consists of a P680LPG pump and a P680 series binary pump, an Ultimate 3000 auto sampler, a Rheodyne 6 port valve for automated column switching, a Lichrospher ADS-RP-4 column for online sample preparation, a C18 reversed phase analytical column (Phenomenex Luna Phenyl-hexyl, 150 x 3.0 mm; 3 µm particle diameter) with a C18 reversed phase guard column (Phenomenex Luna Phenyl-hexyl, 4 x 2.0 mm) from Phenomenex LTD (Aschaffenburg, Germany), a C18 reversed phase analytical column (Nucleodur 100-3 CN-RP, 150 x 4.6 mm; 3 µm particle diameter) with a C18 reversed phase guard column (EC guard column 4 x 3 mm), a TCC 100 column

oven, and a photodiode array detector (DAD-320S) for photometric detection at variable wavelengths. The list of the analytical columns applied during the method development can be found in table 7. The column properties are reported according to the manufacturers' information.

Table 7. Test analytical columns for the HPLC method development.

Analytical Column	Dimension (mm)	Particle Size (µm)	Column Manufacturer
BetaBasic C4	150 x 4.6 mm	3 µm	Thermo (Dreieich, Germany)
BetaBasic C4	150 x 4.6 mm	5 µm	Thermo (Dreieich, Germany)
Betasil C8	250 x 4.6 mm	5 µm	Thermo (Dreieich, Germany)
Gemini C6	150 x 4.6 mm	5 µm	Phenomenex (Aschaffenburg, Germany)
Gemini-Nx C18	150 x 4.6 mm	5 µm	Phenomenex (Aschaffenburg, Germany)
Hypersil ODS	250 x 4.6 mm	5 µm	Thermo (Dreieich, Germany)
LiChrospher CN	250 x 4.6 mm	5 µm	Mainz-AT (Mainz, Germany)
Luna CN	250 x 4.6 mm	5 µm	Phenomenex (Aschaffenburg, Germany)
Luna Phenyl-Hexyl	150 x 3.00 mm	3 µm	Phenomenex (Aschaffenburg, Germany)
Luna Phenyl-Hexyl	150 x 3.00 mm	5 µm	Phenomenex (Aschaffenburg, Germany)
Nucleodur 100-3, CN-RP	150 x 4.6 mm	3 µm	Macherey Nagel (Düren, Germany)
Nucleosil C_8 EC	150 x 4.6 mm	3 µm	Macherey Nagel (Düren, Germany)
PerfectSil 120 ODS-L	250 x 4.6 mm	5 µm	Mainz-AT (Mainz, Germany)
SphereClone ODS (2)	150 x 4.6 mm	5 µm	Phenomenex (Aschaffenburg, Germany)
Thermo Betasil C6	250 x 4.6 mm	5 µm	Thermo (Dreieich, Germany)

Xterra Schild C18	100 x 4.6 mm	3. 5 µm	Waters (Eschborn, Germany)

Note: Though column materials from different manufacturer possess the same material, they do not exhibit the same characteristics during analytical procedures.

3.1.2 Reagents and Chemicals

Melperone hydrochloride (MLP) was obtained from Nordmark (Uetersen, Germany); desmethylcitalopram (DMC) from Lundbeck (Kopenhagen, Denmark), benperidol (free base-BPD) from Abbott Company (Wiesbaden, Germany); Haloperidol (HLP) and Doxepin (free base-DOX) were both obtained from Sigma-Aldrich (Steinheim, Germany), Bromperidol (BRP) from Janssen Cilag (Neuss, Germany), flupentixol-dihydrochloride (FLT) and zuclopenthixol dihydrogenchloride (ZLT) from Bayer HealthCare AG (Leverkusen, Germany) respectively. Drug-free pool serum was obtained from healthy volunteers at the university hospital Regensburg, Germany. Oasis HLB cartridges was from Waters Corporation (Eschborn, Germany). Methanol, acetonitrile, TEMED (tetramethylethylendiamine), and phosphoric acid (all of HPLC grade) were purchased from Merck GmbH (Darmstadt, Germany). Demineralized water (Aqua dem.) used throughout the method development was produced by a Milli-Q Gradient A10 water purification system from Millipore (Bedford, MA, USA). The list of other chemicals already applicable in the TDM laboratory, which also served for experimental purposes during the method development, is shown in table 8.

Table 8. Active substances found in the antipsychotic co-medications.

Substance	Molecular Weight (g/mol)	Manufacturer
10-hydroxy-carbazepine	254.3	Novartis (Basel, CH)
9-OH-Risperidone	420.5	Bio Trend (Köln)

Acetylsalicylic acid	180.2	Sigma-Aldrich (Steinheim)
Alprazolam	308.8	Sigma-Aldrich (Steinheim)
Amantadine (HCl)	187.8	Sigma-Aldrich (Steinheim)
Amisulpride	369.5	Sanofi Aventis (Frankfurt a. M.)
Amitriptyline (HCl)	313.9	Sigma-Aldrich (Steinheim)
Amitriptylinoxide dihydrate	329.4	Sanofi Aventis (Frankfurt a. M.)
Amlodipine besilate	567.1	Pfizer (Freiburg)
Aripiprazole	448.4	Bristol Meyers Squibb (Princeton, USA)
Ascorbic acid	176.1	Sigma-Aldrich (Steinheim)
Benperidol	381.4	Abbott (Wiesbaden)
Biperiden (HCl)	347.9	Abbott (Wiesbaden)
Bisoprolol hemifumarate	767.0	Merck KG (Darmstadt)
Bromazepam	316.2	Sigma-Aldrich (Steinheim)
Bromperidol	420.3	Janssen Cilag (Neuss)
Bupropion (HCl)	276.2	Glaxo Smith Kline (Durheim, UK)
Buspirone (HCl)	385.5	Sigma-Aldrich (Steinheim)
Caffeine	194.2	Sigma-Aldrich (Steinheim)
Carbamazepine	236.3	Sigma-Aldrich (Steinheim)
Carbamazepine-10,11-epoxide	252.3	Sigma-Aldrich (Steinheim)
Chlordiazepoxide (HCl)	336.2	Sigma-Aldrich (Steinheim)
Chlorprothixene (HCl)	352.3	Sigma-Aldrich (Steinheim)
Cisaprid	466.0	Sigma-Aldrich (Steinheim)
Citalopram	324.4	Sigma-Aldrich (Steinheim)
Citalopram (HBr)	405.3	Sigma-Aldrich (Steinheim)
Clobazam	300.7	Sigma-Aldrich (Steinheim)
Clomipramine (HCl)	351.3	Sigma-Aldrich (Steinheim)
Clozapine	326.8	Sigma-Aldrich (Steinheim)
Clozapine-N-oxide	342.8	Sigma-Aldrich (Steinheim)
Dehydroaripiprazole	447.4	Labor Dr. Mark (Worms)
Desipramine (HCl)	302.9	Sigma-Aldrich (Steinheim)
Desmethylcitalopram (HCl)	347.0	Lundbeck (Kopenhagen, DK)
Dextrane	162.1	Sigma-Aldrich (Steinheim)
Diclofenac-Natrium	318.1	Sigma-Aldrich (Steinheim)

Dihydrocodeine hydrogentartrate	451.5	Sigma-Aldrich (Steinheim)
Dimethylbiguanide (HCl)	165.6	Sigma-Aldrich (Steinheim)
Doxepin (HCl)	315.8	Sigma-Aldrich (Steinheim)
Duloxetine (HCl)	333.9	Lilly (Indianapolis, USA)
Enalapril maleate	492.5	LKT Laboratories (St. Paul, USA)
Estradiol	272.4	Sigma-Aldrich (Steinheim)
Fluoxetine (HCl)	345.8	Sigma-Aldrich (Steinheim)
Flupentixol (x 2HCl)	503.1	Bayer HealthCare AG (Leverkusen)
Fluperlapine	309.4	Biomol (Hamburg)
Fluphenazine decanoate	591.8	FineChemicals (Eppindust, ZA)
Fluvoxamine maleate	434.4	Solvay Pharma (Weesp, NL)
Furosemide	330.7	Sigma-Aldrich (Steinheim)
Gabapentin	171.2	Pfizer (Freiburg)
Galantamine (HBr)	368.3	Alexis Biochem (Lausen, CH)
Glimepiride	490.6	Sanofi Aventis (Frankfurt a. M.)
Haloperidol	375.9	Sigma-Aldrich (Steinheim)
Hydrochlorothiazide	297.7	Sigma-Aldrich (Steinheim)
Imipramine (HCl)	316.9	Sigma-Aldrich (Steinheim)
Lamotrigine	256.1	Glaxo Smith Kline (Durham, UK)
Levodopa	197.2	LKT Laboratories (St. Paul, USA)
Levomepromazine (HCl)	364.9	Tropon (Köln)
Lorazepam	321.2	Sigma-Aldrich (Steinheim)
Maprotillin (HCl)	313.9	Sigma-Aldrich (Steinheim)
Melperone (HCl)	299.8	Nordmark(Uetersen)
Memantine (HCl)	215.8	Enzo Life Science (Lausen, CH)
Methylrisperidone	420.5	Sigma-Aldrich (Steinheim)
Metoprolol tartrate	684.8	Sigma-Aldrich (Steinheim)
Mianserin (HCl)	300.8	Sigma-Aldrich (Steinheim)
Mirtazapine	265.4	Organon (Oberschleißheim)
Monohydroxycarbamazepine	254.3	Novartis (Basel, CH)
Nateglinide	317.4	Novartis (Basel, CH)
N-Desmethylclozapine	312.8	Sigma-Aldrich (Steinheim)
N-Desmethylolanzapine	298.4	Labor Dr. Mark (Worms)

Nitrazepam	281.3	Sigma-Aldrich (Steinheim)
Norclomipramine (HCl)	337.3	Sigma-Aldrich (Steinheim)
Nordiazepam	270.7	Sigma-Aldrich (Steinheim)
Nordoxepine (HCl)	301.8	Sigma-Aldrich (Steinheim)
Nortriptyline (HCl)	299.8	Sigma-Aldrich (Steinheim)
O-Desmethylvenlafaxine	263.4	Wyeth (Münster)
Olanzapine	312.4	Lilly (Indianapolis, USA)
Omeprazole	345.4	LKT Laboratories (St. Paul, USA)
Oxazepam	286.7	Sigma-Aldrich (Steinheim)
Oxcarbazepine	252.3	Novartis (Basel, CH)
Pantoprazole-Natriumsalt	406.4	Altana (Konstanz)
Paroxetine(HCl)	365.8	Glaxo Smith Kline (Durheim, UK)
Perazinbishydrogenmalonate	571.7	Altana (Konstanz)
Phenytoin	252.3	Sigma-Aldrich (Steinheim)
Pipamperone (x 2HCl)	448.4	Janssen Cilag (Neuss)
Pirenzepin (HCl)	424.3	Sigma-Aldrich (Steinheim)
Pregabalin	159.2	Pfizer (Freiburg)
Primidone	218.3	Sigma-Aldrich (Steinheim)
Promethazine (HCl)	320.9	Sigma-Aldrich (Steinheim)
Propranolole (HCl)	295.8	Sigma-Aldrich (Steinheim)
Quetiapine	383.5	Astra Zeneca (Wedel)
Quetiapine fumarate	883.1	Astra Zeneca (Wedel)
Ramipril	416.5	LKT Laboratories (St. Paul, USA)
Reboxetine	313.4	Pfizer (Freiburg)
Risperidone	426.5	Sigma-Aldrich (Steinheim)
Rivastigmine hydrogentartrate	400.4	Novartis (Basel, CH)
Sertraline	306.2	Pfizer (Freiburg)
Sultiame	290.4	Desitin (Hamburg)
Sumatriptan	295.4	Glaxo Smith Kline (Durham, UK)
Testosterone	288.4	Sigma-Aldrich (Steinheim)
Theobromine	180.2	Sigma-Aldrich (Steinheim)
Theophylline	180.2	Sigma-Aldrich (Steinheim)
Topiramate	339.4	Janssen Cilag (Schaffhausen, CH)
Triamcinolone acetonide	434.5	Sigma-Aldrich (Steinheim)

Triamterene	253.3	Sigma-Aldrich (Steinheim)
Triazolam	343.2	Sigma-Aldrich (Steinheim)
Trimipramine maleate	410.5	Sigma-Aldrich (Steinheim)
Valproic acid	144.2	Sigma-Aldrich (Steinheim)
Venlafaxine (HCl)	313.9	Sigma-Aldrich (Steinheim)
Ziprasidone	449.4	Pfizer (Freiburg)
Zopiclone	388.8	Sanofi Aventis (Frankfurt a. M.)
Zuclopenthixol (x 2HCl)	473.9	Bayer HealthCare AG (Leverkusen)

Note: These active substances were evaluated from the AGATE pharmacovigilance program.

3.1.3 Human Serum for HPLC Analysis

Drug-free serum was pooled from the employees of the clinic and polyclinic for psychiatry and psychotherapy, university of Regensburg at the district hospital Regensburg. In the absence of suitable material, drug free serum was also obtained commercially from PAA Company (Colbe, Germany). The suitable serum material was sent to TDM laboratory for the method development and for daily routine applications. The patient drug serum was obtained from whole blood by using serum monovette supplied from Sarstedt Company (Nümbrecht, Germany). The serum monovette contains synthetic granulate for progranulation.

3.1.4 Study Antipsychotics

The study antipsychotics MLP, BPD, HLP, BRP, FLT, and ZLT exhibit different physicochemical properties (see table 9). These differences will be considered in the selection of column materials, the choice of the adequate mobile phase and the applicable HPLC method development. Their chemical structures are displayed in figure 8.

Table 9. Differences in the physicochemical properties of the applied antipsychotics for use in TDM.

Properties	Butyrophenones	Phenothiazines (Thioxanthene class)
Molar mass (g/mol)	263.35–420.3	473.89-507.44
Appearance	White powder	White powder
PKa	5.4–9.1	6.18–7.6
Soluble	Water, methanol	Water, methanol
Melting point (^{o}C)	151.5-211	194-216
Protein binding (%)	≤ 50 > 90	95 ≥ 98

Note: The values were derived from the summary of product characteristics of the drugs, the reports of Venkatesh et al. (2010), Bundesanzeiger Nr. 5 from 09.01.1992, Brittain (2007), Vincent et al. (1980), and Moffat et al. (2003).

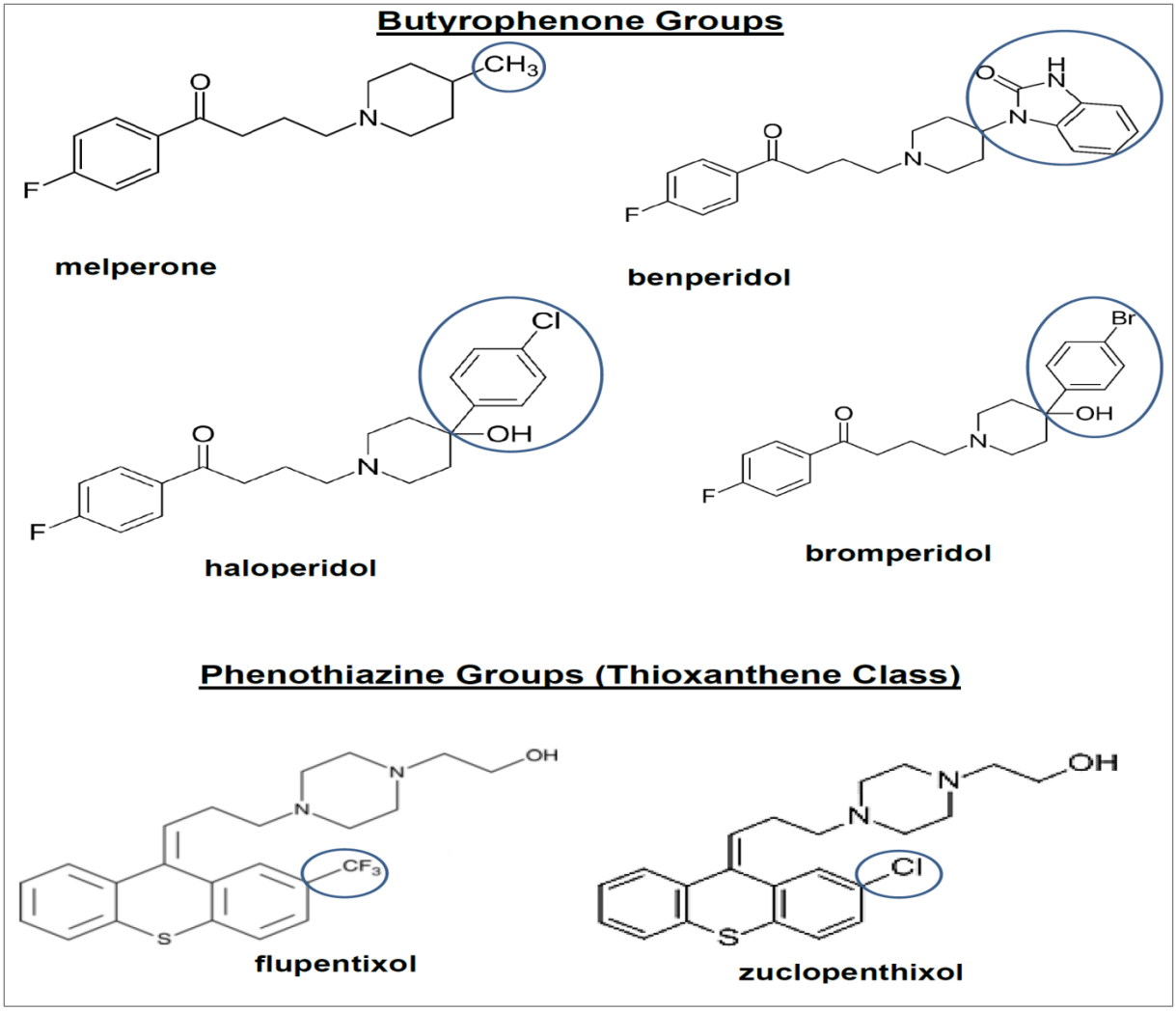


Figure 8. Chemical structures of antipsychotic groups for the method development.

Note: Target drug substances for TDM in human serum.

3.1.5 The Hospital and the Clinic Ward

The district hospital at the University of Regensburg is equipped with 611 beds. It consists of several neurologic and psychiatric clinics and polyclinics for children, adults and forensic. Attached to the clinic are nursing homes, nursing training school and an educational institute for human development. The clinic also operates outpatient clinics for children, youth and adult psychiatric patients. The clinic staff is

comprised of medical doctors with different specializations, psychologist, nurses, social workers and trainees in various departments [Zentrum für allgemeinpsychiatrie II der Klinik und Poliklinik für Psychiatrie und Psychotherapie der Universität Regensburg am Bezirksklinikum Regensburg, 2012.].

Station 1b belongs to the socio-therapeutic department of the hospital with 22 beds. It is specifically equipped to allow a certain connection between a stationary treatment and outpatient treatment. The working groups in this station are made up of medical doctors, nurses, psychologists, ergo-therapists, social workers, and intern students (medical students, and psychology students). Patients in ward 1b are patients with predominant schizophrenic and schizoaffective disorders. Outpatients visit the station for routine check-ups after an appointment with their attending clinicians and psychotherapist [Zentrum für allgemeinpsychiatrie II der Klinik und Poliklinik für Psychiatrie und Psychotherapie der Universität Regensburg am Bezirksklinikum Regensburg, 2012].

3.1.6 Materials for the Antipsychotics Data Analysis

The TDM request form and the Konbest program will be applied to evaluate the TDM of antipsychotics and the influence of smoking on haloperidol and flupentixol serum concentration. The database of AGATE (Arbeitsgemeinschaft Arzneimitteltherapie bei psychiatrischen Erkrankungen), an independent and interdisciplinary quality assurance program for the pharmacotherapy will be applied for the evaluation of MLP-BPD data analysis on undesired drug effects [AGATE Pharmacovigilance Programm, 2012]. The frequency of haloperidol request and the impact on the medication cost was evaluated with ABDA data. The AGNP consensus C/D data will

be applied to monitor the comparison between the value of the measured concentration and the expected DRR.

3.2 Method

This section is divided into three sections. The frst section contains the description of the analytic method already applied in the TDM laboratory Regensburg and subsequently deals with the development and validation of a new chromatographic method suitable for analytical determination of melperone, benperidol, haloperidol, bromperidol, flupentixol and zuclopenthixol in human serum. The second section is focused on the routine measurements of patients' samples with the validated analytical method and its application in TDM and at the clinic ward. The third section will deal with the method of data analysis.

3.2.1 Current HPLC Method in TDM Laboratory Regensburg

The TDM laboratory in Regensburg applies already some methods for the determination of varieties of drugs in human serum (see table 10). There is however, the need to support these methods with a new method in order to enable a measurement in cases where confirmations of the obtained results are requested and for the determination of antipsychotics that have not yet been tested in the TDM laboratory Regensburg.

Table 10. The existing methods and parameters in the TDM laboratory Regensburg.

Method	Mobile phase (ml)	pH	Temp. (^{o}C)	Column
TDM-1a	ACN/KH2PO4-buffer (300 /700)	3.0	25	Thermo-Betasil C6, 5 µm 250 x 4.6 mm
TDM-1b	ACN/KH2PO4-buffer (300 /700)	3.0	25	Thermo-Betasil C6, 5µm 250 x 4.6 mm
TDM-2	ACN/NH4-formiat-buffer	3.0	30	Thermo-Betasil C6, 5µm

	(300/700)			250 x 4.6 mm
TDM-3a	ACN/MeoH/TEMED/H2O (660/200/8/1132)	6.5	30	PerfectSil 120 ODS-L 5 µm 250 x4.6mm
TDM-3b	ACN/MeoH/TEMED/H2O (500/200/8/1229)	7.0	30	PerfectSil 120 ODS-L 5 µm 250 x4.6 mm

Note: Temp.: temperature, ACN: acetonitrile, NH4-formiat: ammonium formiate, MeOH: methanol, TEMED: tetramethylethylendiamine, KH2PO4: potassium dihydrogenphosphate.

The routine measurements in the laboratory are carried out on different days of the week. If necessary, an exceptional measurement is always available. The measurement of serum concentration of some drugs can be carried out with different methods (see table 11), and can therefore be measured at different days within the week.

Table 11. Substances grouped according to their routine methods 1-3.

Methods	TDM-1a	TDM-1b	TDM-2	TDM-3a	TDM-3b
Substance	Lamotrigine Aripiprazole Citalopram Escitalopram Venlafaxine	Oxcarbazapine	Citalopram Escitalopram Venlafaxine Amisulprid Mirtazapine	Olanzapine Clozapine Quetiapin Duloxetine Ziprasidon Perazin	Risperidon Paliperidon Haloperidol

Note. Medications are grouped and measured at different days of the week. TDM 1a-3b represent different analytical methods.TDM: therapeutic drug monitoring.

A separate method is applied in the TDM laboratory Regensburg for the determination of the serum concentration of tricyclic antidepressants (TCA) and benzodiazepines (BZD: see table 12). The test instructions for this method are supplied as a complete kit to the laboratory.

Table 12. The TCA method and the substances applicable during the routine measurement.

Parameter	Method	TCAs	BZD
Mobile phase	NA	Amitriptyline	Alprazolam

PH value	NA	Amitriptylinoxid	Bromazepam
Temp.	NA	Clomipramine	Chlordiazepoxide
Column	NA	Desipramine	Clobazepam
		Doxepine	Diazepam
		Imipramine	Flunitrazepam
		Trimipramine	Flurazepam
		Maprotiline	Nitrazepam
		Nortriptyline	Nordiazepam
		Imipramine	Oxazepam

Note: NA: not available, Temp: temperature, TCA: Tricyclic antidepressants, BZD: Benzodiazepines.

3.2.2 New Method Development by Automated Column Switching HPLC

The target chromatographic method for the new development is the reversed phase chromatograpy (RPC). The following chapters describe the preparations' steps to be taken before commencing with the separation of the substances. Then the measurements and the validation method are discussed.

a) Treatment of Drug-Free Human Serum for HPLC Analysis

The purification of drug free serum was carried out through a solid phase extraction (SPE) on Oasis HLB® cartridges 500 mg obtained from Waters GmbH (Eschborn, Germany). The Oasis HLB® cartridges 500 mg were washed with methanol and then with demineralised water (aqua dem.). After washing with aqua dem., the openings of the oasis were closed and then the drug free serum was applied to Oasis HLB® cartridges 500 mg for purification. After the solid phase extraction, the washed serum is collected in a test tube with the help of a vacuum pump. The serum is then transferred in a sterile 50 ml tube and stored at -20 ^{o}C to avoid degradation. When

needed, sterile tube containing 50 ml of the freshly treated drug free serum was used immediately.

b) Preparation of Stock Solutions

Two stock solutions were prepared for this work. The substances were first weighed accurately and transferred to a 25 ml round bottom flask, respectively. The stock solution A was obtained after completely dissolving 2.5 mg of each substance, respectively (melperone, benperidol, haloperidol, bromperidol, flupentixol, zuclopenthixol) in 25 ml methanol to give a concentration of 0.1 mg/ml. To prepare stock solution B, 5 µl was taken from stock solution A and diluted with 995 µl Aqua dem. The prepared solutions were filled in Eppendorf caps and stored frozen at -20 ^{o}C for three months.

c) Preparation of Calibrators

Drug-free serum preserved at -20 ^{o}C was thawed prior to use and centrifuged. The stock solutions were allowed to adjust to room temperature before they were used. Three concentrations of the calibrators (L: low, M: middle, H: high) were prepared by spiking drug-free serum (final volume 2000 µl) with appropriate volumes of stock solution B of the six substances (see table 13).

Table 13. The volume concentrations of butyrophenones and phenothiazines.

Substances	**Low (volume in µl) conc. in ng/ml**	**Middle (volume in µl) conc. in ng/ml**	**High (volume in µl) conc. in ng/ml**
Butyrophenones			
Melperone (MLP)	(100) 25	(228) 57	(460) 115

Benperidol (BPD)	(8) 2	(40) 10	(120) 30
Haloperidol (HLP)	(8) 2	(40) 10	(120) 30
Bromperidol (BRP)	(12) 3	(60) 15	(120) 30
Phenothiazines			
Flupentixol (FLT)	(24) 6	(70) 17.5	(140) 35
Zuclopenthixol (ZLT)	(24) 6	(240) 60	(480) 120

Note: The calibrators were prepared of through reconstitution of the stock solutions. The values in brackets are the volume of stock solutions spiked in a drug-free serum. Conc: concentration.

d) Preparation of Test Specimens

Test specimens in drug-free serum were prepared by spiking different volumes of stock solution B of each substance to a drug-free serum to obtain the desired concentration for the measurement. The mixtures were vortexed and centrifuged for 5 min. The prepared 20 ml drug solutions served for further separation processes.

e) Preparation of the Mobile Phases

An accurately weighed 5.44 g potassium dihydrogenphosphate was added to 2,000 ml Erlenmeyer flask and dissolved first in 500 ml aqua dem. and stirred. While the solution was still stirring on a magnetic stirrer, the remaining 1,500 ml aqua dem. was added and the pH value was adjusted to pH 6 with orthophosphoric acid. The prepared buffer solution is the mobile phase A.

Mobile phase B was prepared by adding an accurately weighed 300 ml acetonitrile (ACN) and 130 ml methanol (MeOH) in a 1,000 ml Erlenmeyer flask and stirred. After mixing the two organic phases, 570 ml aqua dem. was added to the mixture. The pH

was set to 4.3 and later to 5.0 with orthophosphoric acid. The mobile phase B do not contain tetramethylethylenediamin (TEMED).

Mobile phase C was prepared by adding an accurately weighed 300 ml acetonitrile (ACN) and 130 ml methanol (MeOH) in a 1.000 ml Erlenmeyer flask and stirred. After mixing the two organic phases, 100 ml Aqua dem. was added to the mixture and stirred for 2 min. after which 4 ml TEMED was added, and while still stirring, another 450 ml Aqua dem. was added to the solution. After the adjustment of the pH to 5.0, the Erlenmeyer flask was filled up to the 1,000 ml mark with Aqua dem.

f) Column Selection

The selection of the suitable column for the determination of MLP, BPD, HLP, BRP, FLT and ZLT was carried out with 16 different columns of different physico-chemical properties. The mobile phases were each allowed to equilibrate for 60 min with different analytical columns. Thereafter, aliquots à 500 µl drug solutions were injected into the HPLC-System and analysed at temperatures between 20 ^{o}C to 40 ^{o}C. The flow rate was adjusted between 0.2 and 0.8 ml/min according to the particle size of the columns. Table 14 shows the application of different methods to examine the available columns in the laboratory, in order to choose the suitable columns for the method development.

Table 14. Analytical columns and parameters for selection.

Analytical Column	**Mobile Phase (ml)**				**pH Value**	**Inject. Vol (µl)**	**Temp (^{o}C)**	**Conc. of Drug Solutions (ng/ml)**
	ACN	MeOH	Buffer (B)	H_2O				

			TEMED (T)					
Thermo Betasil C6 5 µm 250 x 4.6 mm	300	100	600 (B)	-	4.3	100	25	100-1000
Luna Phenyl-hexyl 3 µm 150 x 3.00 mm	300	100	600 (B)	-	4.0	100-750	20-30	2-10
	300	130	4 (T)	566	5.0			
Hypersil ODS 5 µm 250 x 4.6 mm	300	100	600 (B)	-	4.3	100	30	100
SphereClone ODS (2) 5 µm 150 x 4.6 mm	300	100	600 (B)	-	4.3	100	30	100-1000
BetaBasic C4 3 µm 150 x 4.6 mm	300	100	600 (B)	-	4.3	100	25	100-1000
Betasil C8 5µm 250 x 4.6 mm	300	100	600 (B)	-	4.3	100	25	100-1000
Xterra Schild C18 5 µm 100 x 4.6 mm	300	100	600 (B)	-	4.0	100	30	1000
PerfectSil 120 ODS-L 5 µm 250 x 4.6 mm	330	100	4 (T)	566	6.5	100	30	100-200
Gemini-Nx C18 5 µm 150 x 4.6 mm	330	100	4 (T)	566	4.3	100	30-40	10-100
Gemini C6 5 µm 150 x 4.6 mm	330	100	4 (T)	566	4.3	100	30-40	10-500
Luna Phenyl-hexyl 5 µm 150 x 3.00 mm	300	100	600 (B)	-	4.3-5.0	100-750	20-30	2-10
	330	130	4 (T)	566				
Luna CN	330	130	4 (T)	566	4.3	750	30	100

5 µm 250 x 4.6 mm								
LiChrospher CN 5µm 250 x 4.6 mm	330	130	4 (T)	566	4.3	100	30	100
BetaBasic C4 5 µm 150 x 4.6 mm	330	130	4 (T)	566	4.3	100	30	10
Nucleosil C_8 EC 3µm 250 x 4.6 mm	300	100	600 (B)	-	4.0-5.0	100-750	20-30	2-100
	300	130	4 (T)	566				
Nucleodur CN-RP 100-3 150 x 4.6 mm	300	100	600 (B)	-	4.0-5.0	100	30	10
	300	130	4 (T)	566				

Note: The measurement time was programmed to 120 min for each column, respectively. The buffer used was potassium dihydrogenphosphate. Inject. Vol: injection volume, Temp: temperature; Conc: concentration, TEMED: tetramethylethylendiamine, MeOH: methanol, ACN: acetonitrile.

3.2.2.1 HPLC Separation Method for the Serum Determination of Antipsychotics

The selected column was then applied for the separation of the study substances with HPLC. Two separation methods were tested, the isocratic and the gradient separation. An isocratic HPLC separation was carried out under a constant mobile phase composition. The gradient separation method was carried out with mobile phase C under a variable mobile phase composition.

Isocratic HPLC Separation

The frozen substance solutions were thawed prior to use and centrifuged. The reconstitution of the stock solutions was carried out with the drug-free serum. The mobile phase C (30% ACN, 13% MeOH, 0.4% TEMED and 56.6% aqua dem) was used for the analysis. The columns Luna phenyl-hexyl 150 x 3 mm, 3 µm for

butyrophenone and Nucleodur CN-RP 150 x 4.6 mm, 3 µm for phenothiazines were fixed into the HPLC equipment and equilibrate for 30 - 60 mins, respectively. Separate aliquots à 750 µl and 1250 µl of butyrophenone and phenothiazine specimens were injected into the HPLC-System, respectively. The flow rate for the HPLC analysis was set to 0.2 ml/min at the temperature of 20 ^{o}C for butyrophenones and 0.6 ml/min at the temperature of 30 ^{o}C for phenothiazines. The diod array detector set to record at WL 220, 230, 240, 245, and 248 nm was used to detect the substances. The correction factor of 0.88 by MLP, 0.86 by FLT and 0.85 by ZLT were considered in all the measurements. For the simultaneous determination of the substances, aliquot of each of the substances solution was added into the 1.5 ml HPLC glass tube and measured as already described.

The acidity of the mobile phase for the simultaneous separation of butyrophenones was adjusted from pH 4.3 to pH 5 to obtain the expected resolution between melperone and benperidol. The chromatogram for all measurements was recorded and analysed by the software Chromeleon® Version 6.8 SP2 Build 2284 on the basis of peak height. The total chromatographic run time was 25 minutes and 30 minutes for butyrophenones and phenothiazines, respectively.

Gradient HPLC Separation

The gradient test was carried out by an intermixture of mobile phase C and a solution of hundred percent acetonitrile (100% ACN). The 100% ACN of HPLC purity grade was added in portions between 5-95% to the mobile phase C at different time intervals between 16 and 20 min. The percentage of the solvents and the time for the admixture are depicted in table 15. The mixing processes were carried out at different temperatures with the multiple step gradient method. The column applied for the

gradient measurement was Luna phenyl-hexyl 150 x 3 mm, 3 µm. The aliquots of the substance solutions, the injection volumn and the wave length were the same as described for iscocratic measurements.

Table 15. The percentage volume of the solvents used during gradient measurements.

Sample Number	Mobile Phase A (Portion in %)	Acetonitrile (Portion in %)	Time for the Admixture (min.)
A	85	15	20.0
B	85	15	16.0
C	80	20	16.0
D	85	15	18.0
E	90	10	16.0
F	**95**	**5**	**15.8**
G	90	10	16.2
H	75	25	16.0

Note: Each sample number has a different mixing process during the gradient HPLC measurement.

3.2.2.2 Validation of the Newly Developed HPLC Method

The validation steps and specifications applied were according to the guidelines of GTFCh (Society of Toxicology and Forensic Chemistry) [Paul and Musshof, 2009] in consideration of ISO 5725 (International Organization for Standardization), FDA (US Food and Drug Administration), guidance for industry on biomedical method validation (2001), set norms by the German institute of standardization [DIN 32645, 1994], and the requirements of ICH (International Conference on Harmonization) [ICH-harmonised tripartite guideline-validation of analytical procedures, 1996]. The information received from the principles and practices of method validation [Fajgeli A, Ambrus A, 2000], and from the Kromidas' book of analytical validation [Kromidas, 1999] were equally considered during the validation process.

a) Calibration

The calibration curve describes the linear relationship between measured values and/or measuring signal and the analyte concentration. The correlation coefficient provides information about the variation of the measured values to the calibration curve [Söfftge, 2000]. Calibration curves for the validation of this method were documented over a concentration range of 2, 4, 5, 6, 10, 25, 30, 50, 60, 90, 100, 120, 150, and 200 ng/ml. The linear regression equations and the coefficient of certainty r^2 were obtained for all the substances by calculating a linear regression analysis.

b) Limit of Quantification (LOQ) and the Limit of Detection (LOD)

The limit of detection (LQD) is the lowest concentration in a sample that can be detected while the limit of quantification (LOQ) is the lowest concentration in a sample that be quantified. The lowest concentrations of analyte that can be detected in a sample can vary from the quantification limit of the samples depending on the applicable experimental conditions [Guidance for Industry on biomedical method validation, 2001]. The LOQ and the LOD of the measured antipsychotics were based on a signal-to-noise ratio of 10:1 and 3:1, respectively. Six different concentrations (5- 120 ng/ml for melperone, 1-10 ng/ml for benperidol, 1-10 ng/ml for haloperidol, 3-20 ng/ml for bromperidol, 1-10 ng/ml for flupentixol and 2-16 ng/ml for zuclopenthixol) were analysed, starting with the lowest estimated concentration (n=4). The values

were verified by linear regression analysis over the data points. The calculation of LOD and LOQ was carried out according to DIN 32645 [DIN 32645, 1994] in consideration of $p \leq 0.01$ (significance level) and $k = 3$ (analytical uncertainty ≤ 33%).

c) Recovery

The recovery is a measure of the efficacy of the method in detecting all the analyte present in a sample [Braggio et al., 1990]. Through multiple measurements of different substance concentrations, the relationship between the mean value of the respective concentrations and the target value were analyzed [Söfftge, 2000]. The absolute recovery for the study substances in serum was determined at concentrations 22, 51, and 102 ng/ml for melperone; 2, 10, and 30 ng/ml for benperidol and haloperidol; 3, 15, and 30 ng/ml for bromperidol; 5, 15, and 30 ng/ml for flupentixol; 5.1, 51 and 102 ng/ml for zuclopenthixol. All the measured substances were compared to aqueous drug solutions, set to 100% (n=6) of the spiked drug serum samples.

d) Precision

Precision is the closeness of the measured data between series of measurements obtained from multiple sampling under the same analytical conditions [Shah et al., 1992]. The coefficient of variation in percent was used to determine the variation limit of the analysis [Guidance for Industry on biomedical method validation, 2001]. The precision of serum samples containing 25, 57, 115 ng/ml of MLP; 2, 10 and 30 ng/ml of BPD; 2, 10 and 30 ng/ml of HLP; 3, 15 and 30 ng/ml of BRP; 6, 17.5, and 35 ng/ml of FLT; 5.1, 51, and 102 ng/ml of ZLT were assessed by repeated analyses on the

same day (intra-assay) and on six different days (inter-assay). The intra-assay variation was measured six consecutive times for each concentration, and the inter-assay variation was measured twenty-four times for each concentration. The assays were expressed as the coefficient of variation (CV in %).

e) Accuracy

The accuracy describes the nearness of the experimental value to the expected value. The expected value was derived from the quality control of each chromatographic run. Deviations of the mean from the expected target value were expressed as % of the target value (bias). Accuracy was calculated as 100%-bias. The accuracy of the method was assessed by repeated analyses of self prepared quality control samples (concentration low: 25 ng/ml, middle: 57 ng/ml, high: 115 ng/ml for MLP; low: 2 ng/ml, middle: 10 ng/ml, high: 30 ng/ml for BPD; low: 2 ng/ml, middle: 10 ng/ml, high: 30 ng/ml for HLP; low: 3 ng/ml, middle: 15 ng/ml, high: 30 ng/ml for BRP; low: 6 ng/ml, middle: 17.5 ng/ml, high: 35 ng/ml for FLT; low: 5.1 ng/ml, middle: 51 ng/ml, high: 102 ng/ml for ZLT).

f) Stability

The verification of the stability of the analytes in solvent and serum solution after a long storage period and after repeated freeze/thaw processes was carried out through the long-term stability and the freeze/thaw stability measurements. The solutions of MLP, BPD, HLP, BRP, FLT, and ZLT were determined by measuring the concentrations of 25 ng/ml and 115 ng/ml MLP, 2 ng/ml and 30 ng/ml BPD, 2 ng/ml and 30 ng/ml HLP, 3 ng/ml and 30 ng/ml BRP, 17.5 ng/ml and 35 ng/ml FLT, and 51

ng/ml and 102 ng/ml ZLT after 3 months, after 6 months and after 12 months [Shah et al.,1992]. Freshly self prepared standard solutions were measured alongside with the solutions for the stability test control. The freeze/thaw stability test was carried out with the same concentrations as in the long-term stability. The solutions were thawn/freezed and measured four times for each concentration (n = 12 for each substance). The mean stability data was calculated by setting the corresponding control value between 90 -100% according to DIN 32645.

g) Robustness

The reliability of the developed method when exposed to different parameters other than the ones used during the method development was analysed for butyrophenones and phenothiazines through the application of realistic changes. The test on robustness of the method was carried out through changes on the acidity of the mobile phase, composition of the mobile phase, temperature, chromatographic run time, as well as measurement with other available HPLC equipment.

h) Selectivity

The selectivity test is the measurement carried out to verify the interference of the test substances with other substances [Kromidas, 1999], such as substances that can be found in different co-medications or possible impurities in different solutions during the method development. The selectivity of this method was assessed with interference tests of 109 different substance solutions identified as the medications prescribed to schizophrenic patients. Then the retention time of the susbtances were compared with that of the study substances.

3.2.2.3 Patients' Test Samples and Measurement

The validated method was tested for the routine analysis of the patients' samples. Test specimens from patients' serum were received from clinic ward 1b and from the stored rest patients' serum from the routine analysis in the TDM laboratory. Prior measurement, they were centrifuged for 3 min before analysis, transferred to HPLC vials. The standard solutions were thawed, centrifuged and filled in the HPLC vials. The same operational step was applied for the measurement of patients' samples and the standard samples. The total chromatographic run was set at 30 minutes. Example chromatograms of all the study substances and the standard concentrations were collected.

3.2.3 TDM Routine Analysis with the Validated Method

The daily analysis of the patients' samples containing the study medications were carried out with the validated method in the TDM laboratory.

3.2.3.1 Type of Study Patients

Patients were eligible for this retrospective study if they receive any of the study substances based on the diagnoses according to ICD-10 and/or suffering from psychological illness treated with any psychotropic medications. The attendance for the pharmacist discussion was directed to patients admitted in clinic ward 1B and out-patients of the same ward. Patients outside the clinic ward 1B were not forseen for the discussion. All the study patients were informed about the study and they voluntarily gave their consent. The patients' name were not disclosed in this study.

3.2.3.2 TDM Request for the Study Substances

The set criteria for the request of TDM in this study were suspect of undesired drug effect, drug interactions, lack of response to treatment, patients' compliance, and change in dosage regime. The set time appropriate for the sample collection was the collection at steady-state concentration. The calculation of the dose required to reach a steady-state concentration (c) of a drug in serum is based on the direct correlation of the drug constant dose (D) per day, with the total clearance of the drug (Clt) being the correlation coefficient (c = D / Clt). With this information, the expected dose-related serum concentration of a drug in a patients specimen can be calculated [Hiemke et al., 2011].

3.2.3.3 Laboratory Measurments and Data Analysis

Patients' serum samples were sent for qualification and quantification from various hospitals to the TDM laboratories. The samples were centrifuged at 20.000 rounds/ min for 10 min. 2 ml of each subordinate was transferred in sterile caps, stored at 5 ±3 °C for a maximum of two days. Prior measurement, they were centrifuged for 3 min before analysis. 1.6 ml of each prepared patients' sample and the standard samples were transferred to HPLC vials and analyzed.

The graph of dose-concentration relationship of each substance was plotted using the values of measured serum concentration, the dose-related reference range DRR) and the therapeutic reference range (TRR). The DRR was calculated by multiplying the given C/D low and C/D high of the respective substances with the maintenance dose. The values of C/D low and C/D high and the TRR were obtained from the data

of AGNP consensus guideline: update 2011. Boxplots were plotted as well to compare the relationship between the measured concentration and the DRR.

Method of Data Collection

The patients' demographics and all the required information for the interpretation of the measured concentration such as patients age, gender, health status, diagnoses, treatment onset, co-medications, undesired drug effect and time of sample collection were recorded in the TDM request form. The records were transferred manually into Konbest. Konbest is a web-based laboratory information management system (LIMS) for TDM-laboratories [Koestlbacher and Haen, 2008]. The konbest data of the study substances were grouped in dose, measured concentration, dose-related reference range, undesired drug effects, co-medications, and frequency of request. The review of the administered co-medications was carried out by a sequential listing of all noted co-medications. The listed medications were alphabetically arranged according to their product names. The evaluation was carried out with only the active substances contained in the co-medications. The number of co-medications administered per patients, the rate of drug administration and the co-medication with common metabolic pathways with HLP and FLT were evaluated.

The ABDA (ABDA = The Federal Union of German Association of Pharmacists) data analysis for HLP administration and Cost was analysed in two steps. First, the data for the frequency of administration in konbest was arranged. They were then sorted according to year. The number and frequency of administration each year were grouped in a table. Secondly, the medication costs documented in the database of the Federal Union of German Association of Pharmacists (ABDA) were assessed. The frequency of the dose administration for HLP was multiplied with the

documented HLP price in ABDA database to estimate the treatment cost for HLP. A graph was plotted for the illustrated cost-administration of HLP.

AGATE Data Collection for Melperone and Benperidol Polymedication were derived from the pharmacovigilance data of the AGNP-group for the documentation of psychiatric medications and co-medications. Since 1995, the following five items have been recorded for each patient hospitalized in the AGATE hospitals at two particular days per year called index days (one in April, the other one in October): age, gender, diagnoses, medication, and dosage. The diagnoses for which melperone and benperidol were administered and the data documentation were classified according to the International Statistical Classification of Disease and Related Health Problems-9 and 10 (ICD-9 and ICD-10) [International Statistical Classification of Disease and Related Health Problems-9, 1975; International Statistical Classification of Disease and Related Health Problems-10, 2010]. The number of drug and the rate of drug administration per patients and the rate of TDM request were evaluated.

Smoking and the Serum Concentration of HLP and FLT

The patients' data were arranged according to smokers and non-smokers. The data for smokers were further grouped into those that received co-medication and those who underwent monotherapy. The serum concentration reached by smokers and nonsmokers were compared for haloperidol and flupentixol, respectively. Furthermore, the serum concentrations reached by only patients who smoked during haloperidol and flupentixol therapy were compared. For the comparison, graphs of dose-concentration relationships and boxplots were plotted. C/D low x D and C/D high x D were used to calculate the dose-related reference range of each sample.

3.2.4 Clinical Application of Laboratory Values through TDM

The measured drug concentrations in patients serum were analyzed, and with an accompanied clinical reports the values were sent to the respective clinics and attending clinicians.

Five factors were applied for the interpretation of the laboratory values in the TDM laboratory: the dose-related reference range, the therapeutic reference range, the nine-fold table, the co-medications, and the CYP-Enzyme table [Haen et al., 2008], under consideration of the given patients' and therapy information in the TDM request form. The drug-drug interaction from the CYP-Enzyme table, as authomatically selected by konbest for a particular sample was reviewed in connection with the measured concentration if applicable, and interpreted in terms of enzyme substract, enzyme induction or enzyme inbition and the possible impact in the serum concentrations. The contact channels to the attending clinicians were fax, telephone or email. Telephone contact was used in cases of a suspect intoxication.

3.2.4.1 Therapeutic Drug Monitorin at the Clinic Ward

At the clinic, the patients' samples from ward 1B at the clinic and polyclinic for psychiatry and psychotherapy at the University of Regensburg were further discussed by the pharmacist during the clinic ward visitation and different cases were treated. Serum samples of the ward patients and the newly admitted inmates were accepted in TDM laboratory for the purpose of therapeutic drug monitoring using the developed method. The determined drug concentrations were returned together with a written clinical pharmacological report [Haen and Laux, 2011; Haen, 2011]. The test results were discussed under the consideration of two reference ranges: the

therapeutic reference range (TRR) and the dose-related reference range (DRR). The reason for either undesired drug effect, lack of effect, too high drug or too low drug concentration was discussed. These discussions also took place outside the ward visitations whenever the need arose.

3.2.4.2 Routine Clinic Ward Visitation

The participation at the daily ward meetings and the pharmaceutical advices to ward clinicians were carried out at several days in the week. The team discussion with all the team members of the station took place once a week. The documentation of the reported undesired drug effects and drug abuse took place two times in a week. The Triple-Team discussion between a Patient, a psychiatrist and the pharmacist took place once a week. The activities of the pharmacist at the station were pharmaceutical advises before and during the drug therapy, the documentation of the reported adverse drug reactions and the presentation of TDM results accompanied with advices on further drug therapy after literature researches. The methods of communication applied were written communication (emails) before and/or after the team discussion and oral communication, mostly during the team discussion. Request and questions regarding problematic therapies were forwarded to the pharmacist by the attending clinicians. Existing scientific studies regarding the possible intervention of a pharmacist at the clinic and its outcome were reviewed during the visitation period.

Triple- Team Discussion (Patient-Clinician-Pharmacist)

The discussion of the triple team was carried out in the office of the attending clinicians. Present during the discussion were the concerned patient, the psychiatrist

(attending clinicians) and the pharmacist. The concerned patients were always informed before each visit of the pharmacist and their consent were seeked. During this meeting, the present state of patients' health conditions, the experienced undesired drug effects, compliance problem and possible drug abuse and/or over-dose, especially when their expectations on the efficacy of the drugs were not reached, were discussed.

The role of the pharmacist during the discussion consisted of giving pharmaceutical advices to the clinicians and the patients on the reported cases, documenting the reported undesired drug effects from the patient, remedying possible compliance problems due to the reported undesired drug effects, suggesting a better drug administration with the help of the clinician to reduce possible drug-drug interactions, and applying the therapeutic drug monitoring to optimize the patients' individual therapy.

3.2.4.3 Preparation of Clinic Ward Questionnaires

Two questionnaires were developed: a questionnaire for the patients and a questionnaire for the attending clinicians. The questionnaire for the patients contained 13 questions with annonymised information about the patients, patients' understanding about the therapeutic measures, and their knowledge about the administered drugs and possible undesired drug effects.

The questionnaire for the attending clinicians contained 15 questions about the group of patients (based on diagnosis) treated during this research work, the improvement of the clinicians' therapeutic decisions through the pharmacist advice and contribution, and the evaluation of the pharmacist's activities in the clinic ward.

4 Results

The results section describes the outcome of the different laboratory preparations and measurements including validation of the newly developed HPLC method. The method was applied in clinical practice. Moreover, an intervention of the pharmacist at the clinic ward was designed to guide antipsychotic therapy by TDM. The merge of the laboratory results with daily clinical practices and therapeutic decisions was considered as well. This section ends with data findings from Konbest, ABDA, and AGATE to support the goal of this research project.

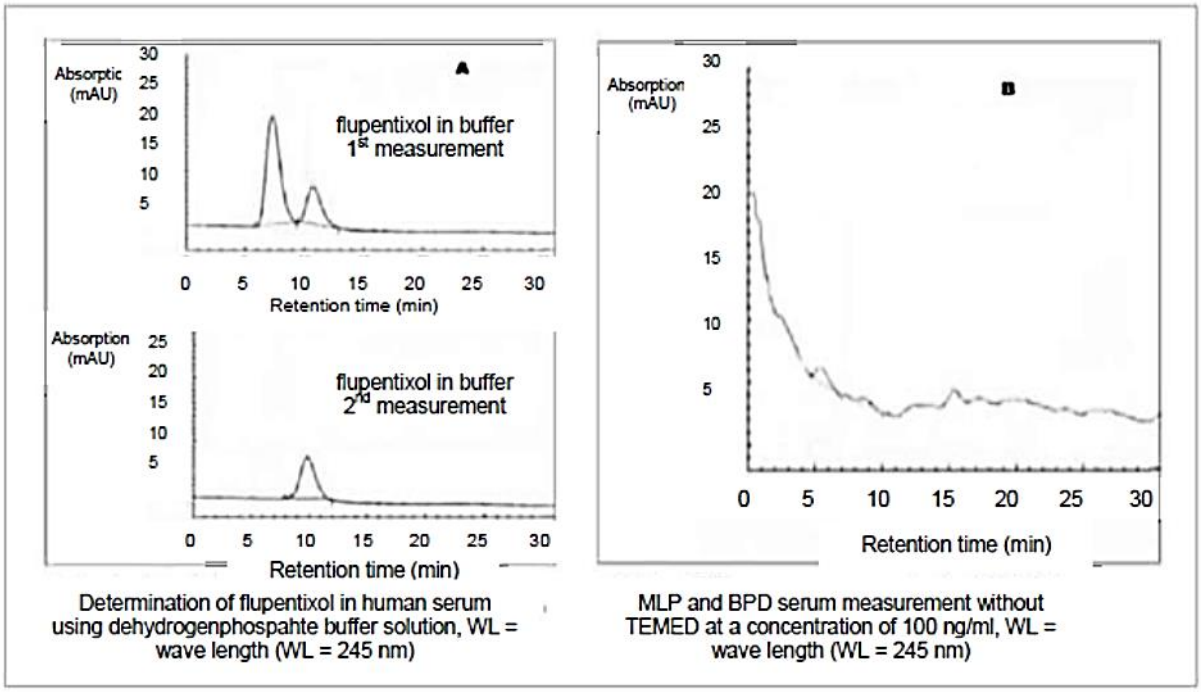
4.1 Method Development

Several analytical columns, mobile phases and separation methods such as isocratic and gradient method were tested for suitability. At the end, the result of the validated applicable separation method will be demonstrated.

4.1.1 Choice of Mobile Phase and Analytical Column

Three mobile phases were prepared. Mobile phase A contains a dihydrogenphosphate buffer solution. Mobile phase B contains neither buffer nor TEMED, but only acetonitrile and methanol, while mobile phase C contains a TEMED solution. Nine columns were tested along with the prepared mobile phase at different analytical conditions. The peaks observed after the measurement with the solution of dihydrogenphosphate buffer (mobile phase A) were irregular. Double peaks were observed after the first measurement. Further measurements showed one peak and eventually no peak. (see figure 9 A). Though the measurements carried out with the pure drug solutions under the application of mobile phase B (mobile phase without TEMED and without buffer) yielded a good result, they, nevertheless did not show

any absorption in human serum even at a concentration of 100 ng/ml (see figure 9 B). This corresponds with the expectation of irregular peaks by measurements of pH-stable solutions without buffer. The measurement carried out in TEMED mobile phase (mobile phase C) showed reliable results at wavelengths of 245 nm and 230 nm, respectively. Significant and reliable peaks were observed both in serum solutions as well as in pure drug solutions.



Determination of flupentixol in human serum using dehydrogenphospahte buffer solution, WL = wave length (WL = 245 nm)

MLP and BPD serum measurement without TEMED at a concentration of 100 ng/ml, WL = wave length (WL = 245 nm)

Figure 9. Determination of antipsychotics in buffer solution and solution without TEMED.

Note: First measurement and repeated measurement in human serum with dihydrogenphosphate buffer at concentration 100 ng/ml (A); measurement in human serum at 100 ng/ ml with neither TEMED nor buffer in the mobile phase (B). mAU = peak height.

4.1.2 Comparison of TEMED and Dihydrogenphosphate Buffer Solutions

The measurements carried out with the solution of dihydrogenphosphate buffer were not reliable. Inconsistent retention times and peak forms were observed. No

qualitative and quantitative analysis of the measured substances could therefore be conducted.

The results obtained from the measurements carried out with different mobile phases showed the TEMED mobile phase (mobile phase C) to be more reliable. Good elution of the substances with baseline separation were observed at 20°C and 30°C. The qualification and quantification of all the applied antipsychotics were reliably analyzed at low concentrations between 2-10 ng/ml. The reliable peak height was observed at a molecular absorption unit of 245 nm for butyrophenones and 230 nm for phenothiazines (see figure 10).

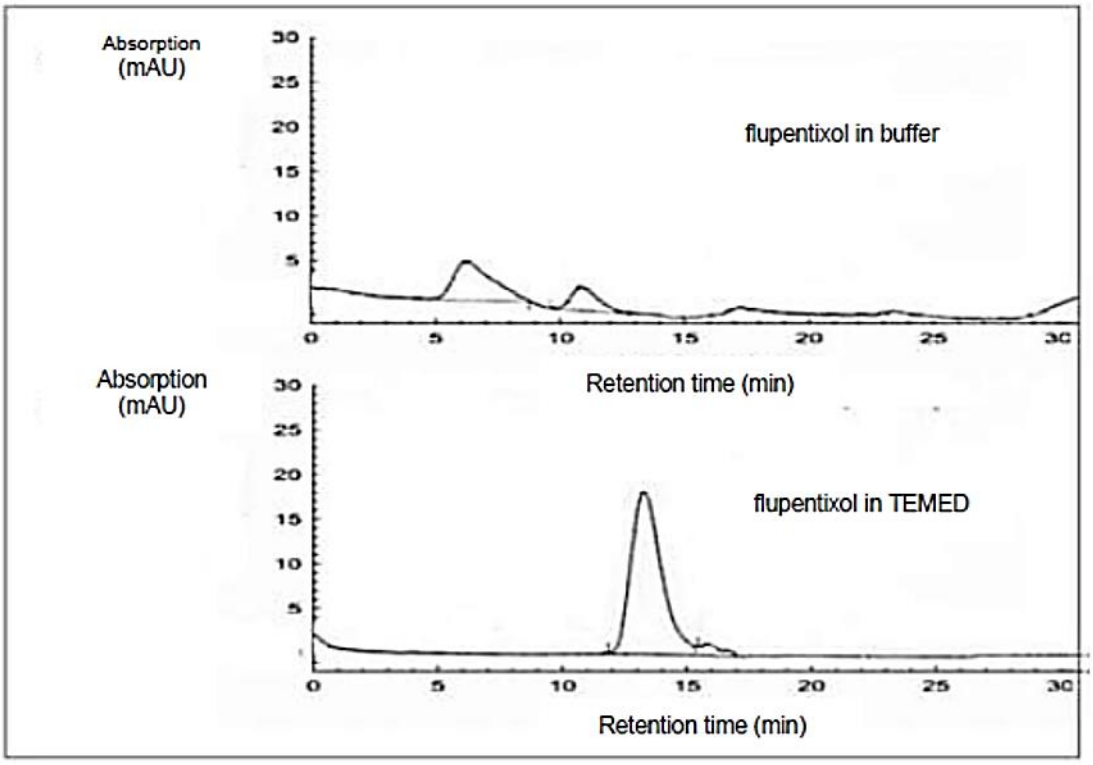


Figure 10. Comparing the measurement with dihydrogenphosphate buffer and TEMED mobile phase.

Note: Measurement of flupentixol with dihydrogenphosphate buffer at conc. 100 ng/ml, WL 230 nm (upper chromatogramm); Measurement with TEMED in the mobile phase at conc. 100 ng/ml, WL 230 nm (lower chromatogram). mAU = peak height.

4.1.3 Column Selection and Measurements with Buffer Mobile Phase

Nucleodur CN-RP 150 x 4.6 100-3

The measurements with the analytical Nucleodur column (Nucleodur CN-RP 150 x 4.6, 100-3) in buffer showed chromatographic irregularities. Therefore, this method must be considered unreliable. In the first measurement, two peaks were observed. After the repetition of this measurement, one of the peaks was observed to have significantly reduced and no peak was seen in further measurements (see figure 9A). The parameters applied during the measurement are listed in appendix 1a

Thermo Betasil C6 250 x 4.6 mm 5 µm

The measurement at a concentration of 2000 ng/ml gave a molecular absorption unit of 14.8 mAU (see table 16). The reduction of the concentration to 400 ng/ml gave a molecular absorption unit of 2.7 mAU and the reduction to 200 ng/ml gave 1.2 mAU. The irregular chromatographic base lines observed at concentrations of 400 ng/ml and 200 ng/ml mean that the measurement is qualitatively and quantitatively unreliable (see figure 11A to 11C). The obtained results did not give any indication of the possibility for further reduction of the concentration to 2 ng/ml while applying the parameters listed in appendix 1b.

Luna Phenyl-hexyl 150 x 3.0 mm 3 µm (and 5 µm)

Sharp peaks were observed with Luna Phenyl-hexyl analytical column 3 µm. The obtained peak heights (see figure 11D) at the concentration of 10 ng/ml for each of the substances were qualitatively and quantitatively reliable and showed the possibility of measurements in lower concentrations. However, the columns were

easily blocked during the measurement with buffer mobile phase. The washing of the column for 5 minutes after each run did not prevent the blockage of the column. The column regeneration carried out with different isopropanole concentrations showed no better result either. The application of 5 µm particle size of Luna Phenyl-hexyl column with the chosen parameters (see appendix 1c) to prevent column blockage did not yield the expected results.

Hypersil ODS 5 µm 250 x 4.6 mm

The result received after the measurement at a concentration of 100 ng/ml was less than 1.0 mAU (see figure 11E). A reliable peak at 5 ng/ml was not obtained using this column and the applied parameters (see appendix 1d).

SphereClone ODS (2) 150 x 4.6 mm 5 µm

Different drug concentrations were tested with this column. None of them produced a reliable result. The peak height obtained after measuring the concentration of 1,000 ng/ml was 20.2 mAU (see figure 11F). The reduction of the concentration by factor 10 to100 ng/ml gave the peak height of 3.5 mAU (see figure 11G). Further reduction of the concentration to arrive at the lowest expected therapeutic concentration of the substance did not yield reliable results within the given parameters (see appendix 1e). The obtained results were measured at a very low wavelength (WL) of 205 nm.

Table 16. Sample Chromatograms measured with buffer in mobile phase.

Chromatogram	Substance concentration (ng/ml)	pH value – mobile phase	Wave length (WL)	Column
A	2000	4.3	230	Thermo Betasil C6, 5 µm, 250 x 4.6 mm
B	400	4.3	230	Thermo Betasil C6, 5 µm, 250 x 4.6 mm
C	200	4.3	230	Thermo Betasil C6, 5 µm, 250 x 4.6 mm
D	10	4.0	230	Luna Phenyl hexyl, 3 µm, 150 x 3.0 mm
E	100	4.3	230	Hypersil ODS, 5 µm, 250 x 4.6 mm
F	1000	4.3	205	Sphere Clone ODS(2), 5 µm, 150 x 4.6 mm
G	100	4.3	205	Sphere Clone ODS(2), 5 µm, 150 x 4.6 mm

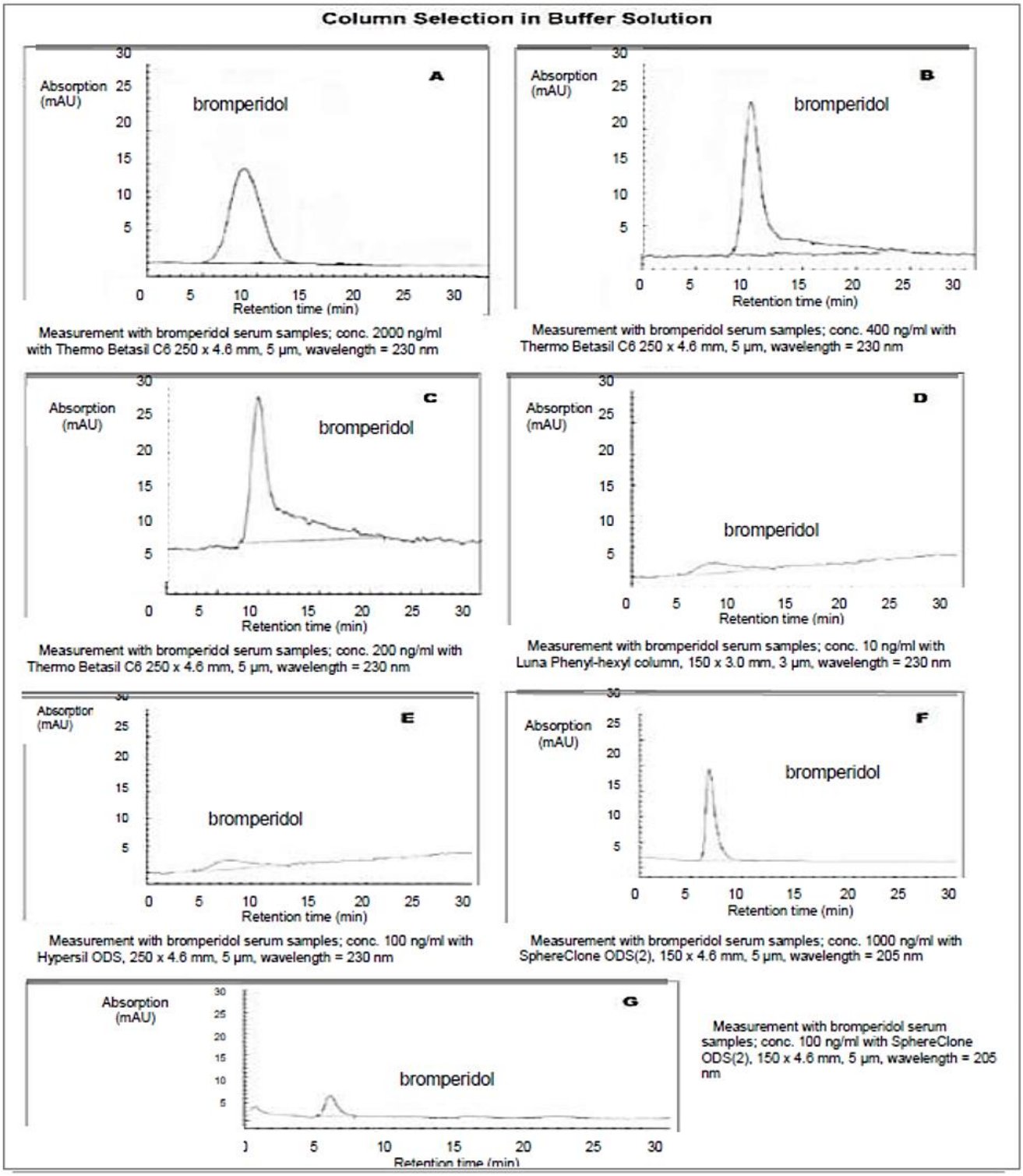


Figure 11. The application of buffer solution in mobile phase.

Note: Buffer solution = (ACN 300 ml, MeoH 100 ml, buffer 600 ml); mAU = peak height.The column separation of antipsychotics at different concentrations (D = 10 ng/ml, E = 100 ng/ml) with different columns, sample substance is bromperidol (see table 16).

4.1.4 Column Selection with TEMED Mobile Phase

PerfectSil 120 ODS-L 250 x 4.6 mm 5 µm

The measurements in low concentrations were unreliable due to the observed irregular baseline. The base line was observed to be so irregular that the peaks of the concentration at 100 ng/ml could not be identified (see figure 12A, appendix 2a). The concentration of 200 ng/ml gave a peak height of 4.3 mAU (see figure 12B and table 17). The reduction of the noise and the peak resolution were observed by an increase of the concentration to 1000 ng/ml.

Gemini-Nx C18 5 µm 150 x 4.6 mm and 250 x 4.6 mm

Gemini-Nx 5 µm, 150 x 4.6 mm and Gemini-Nx 5µm, 250 x 4.6 mm are the same column of different length. At the detection wavelength of 230 nm, a retention time of 10.7 min (see figure 12C) was observed for the shorter column Gemini-Nx 5 µm 150 x 4.6 mm, while 17. 8 min were observed for the longer column Gemini-Nx 5 µm, 250 x 4.6 mm. The total chromatographic run for Gemini-Nx 5 µm 150 x 4.6 mm was 15.0 min and 30 min for Gemini-Nx 5 µm 250 x 4.6 mm. The peak height obtained at the concentration of 200 ng/ml was 4.7 mAU. Concentrations of 2 and 5 ng/ml could not be identified with the applied parameters (see appendix 2b).

Gemini C6 5 µm 150 x 4.6 mm

The peak height reached at 2000 ng/ml was 14.7 mAU (see figure 12D). The detection wavelength was 230 nm and the retention time was 11.3 min. There was no peak observed at 10 ng/ml. The applied parameters for the measurement are listed in appendix 2c.

Nucleodur CN-RP 150 x 4.6 mm 100-3

The measurement with TEMED mobile phase and the Nucleodur column showed a reliable result at a detection wavelength of 230 nm with a diode array detector, the retention time being 15.6 min for FLT and 14.4 min for ZLT (see figure 12E). These results were obtained after a 3% increase of the methanol portion of the mobile phase from 100 ml to 130 ml, 3% reduction of the acetonitrile portion from 330 ml to 300 ml and an increase in the injection volume from 100 ml to 1250 ml (see appendix 2d). The measurements at lower serum concentrations of phenothiazines were precise, reliable and satisfactory. Nucleodur column CN-RP with 3 µm particle size and column size of 150 x 4.6 mm was chosen for the serum determination of phenothiazines under the application of TEMED mobile phase.

Luna Phenyl-hexyl 3 µm 150 x 3.0 mm

The simultaneous determination of serum concentrations of BPD, HLP and BRP at 2.0 ng/ml was carried out successfully (see figure12F), after a change in the mobile phase constituents and an increase of the injection volume to 750 µl (see appendix 2e). The optimal wavelength obtained with a diode array detector was 245 nm. A pH value of 4.3 was observed to be optimal for the respective detection of MLP, BPD, HLP and BRP, but not for a simultaneous measurement of MLP and BPD. With a change of the pH value to 5.0, optimal and reliable simultaneous separations of all the applied butyrophenones were possible.

Luna Phenyl-hexyl 5 µm 150 x 3.0 mm

The Luna phenyl-hexyl analytical column with 5 µm particle size was applied to resolve the column blockage. The result was poorer than expected. There was no chromatogram observed when the Luna Phenyl-hexyl column was applied in order to determine phenothiazines in human serum. The measurements carried out for butyrophenones could not be applied for lower concentrations.

Table 17. Sample Chromatograms measured with TEMED in mobile phase.

Chromatogram	Substance concentration (ng/ml)	pH value- Mobile phase	Analyte	Wave length (WL)	Column
A	100	6.5	bromperidol	254	PerfectSil 120 ODS -L, 5 µm, 250 x 4.6 mm
B	200	6.5	bromperidol	254	PerfectSil 120 ODS -L, 5 µm, 250 x 4.6 mm
C	200	4.3	bromperidol	230	Gemini-NX, 5 µm, 150 x 4.6 mm
D	2000	4.3	bromperidol	230	Gemini C6, 5 µm, 150 x 4.6 mm
E	100	5.0	zuclopenthixol	210	Nucleodur CN-RP, 150 x 4.6 mm, 3 µm
F	100	5.0	Benperidol, haloperidol, bromperidol,	245	Luna Phenyl hexyl, 3 µm, 150 x 3.0 mm

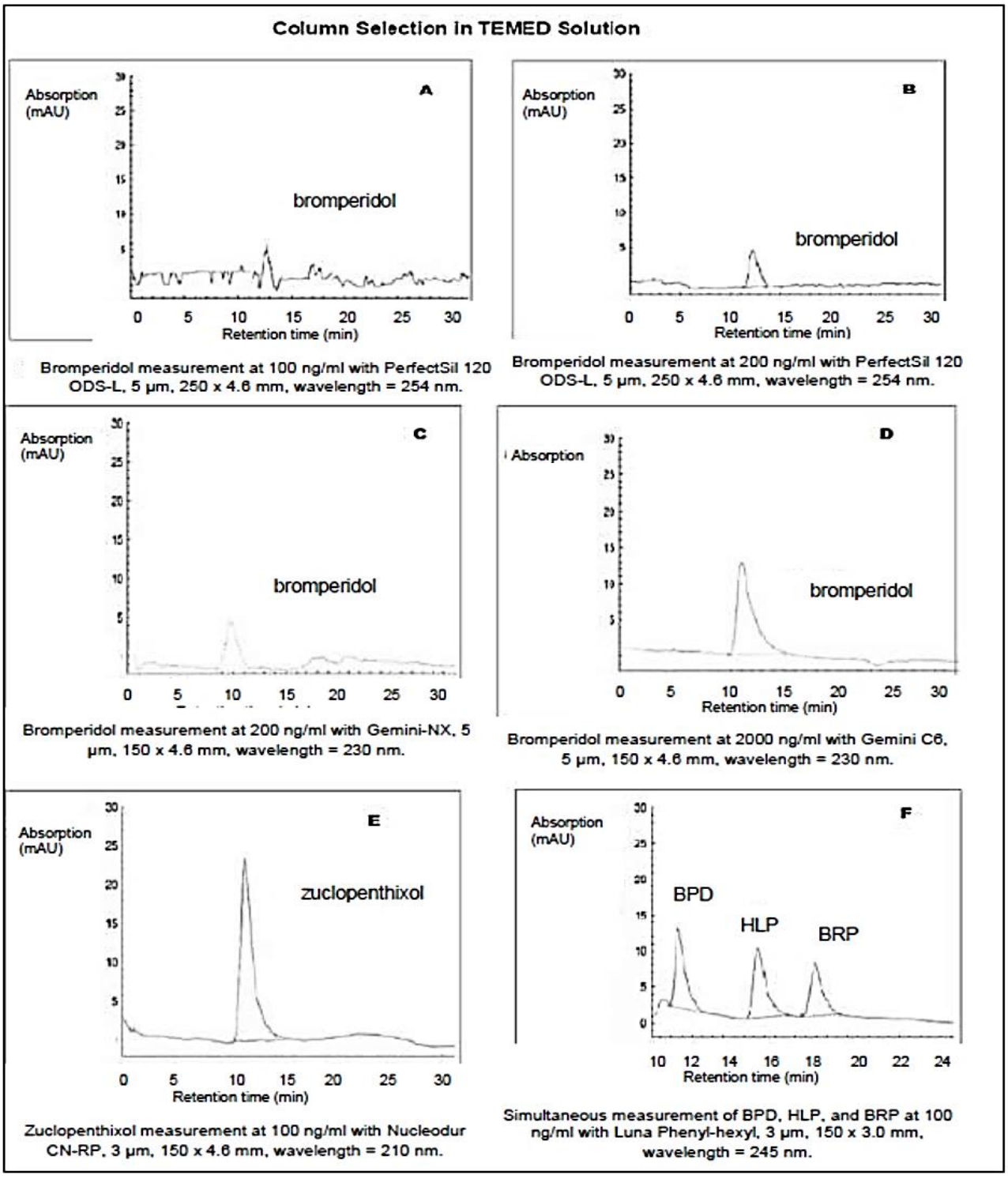


Figure 12. The selection of analytical column using TEMED solution.

Note: TEMED solution = (ACN 330 ml, MeOH 100 ml, TEMED 4 ml, H2O 566 ml).The column separation of antipsychotics at different concentrations with different columns and substances (see table 17). Substance A – D = bromperidol, substance E = zuclopenthixol, substance F = benperidol, haloperidol and bromperidol. The concentration of Chromatogram A is 100 ng/ml, Chromatogram B = 100 ng/ml, Chromatogram C = 200 ng/ml, Chromatogram D = 2000 ng/ml, Chromatogram E = 100 ng/ml, Chromatogram F = 100 ng/ml. mAU = peak height.

4.1.5 Influence of Column Particle Sizes on the Measurement

It was observed that the two different particle sizes (3 µm and 5 µm) of the Luna Phenyl-hexyl analytical column affect the obtained results in different ways. The highest flow rate was 0.2 ml/min. At a flow rate of 0.3 ml/min, the pressure reached 290 bars and led to the shutdown of the HPLC equipment.

The results obtained with the column particle size of 3 µm were more precise and reliable (see table 18). A flow rate of 0.2 ml/min and a temperature of 20 ^{o}C gave an optimal result at a wavelength (WL) of 245 nm (see figure 13). The result of the peak area (see table 19) showed higher values for the 5 µm particle size (see figure 14). The peaks were so wide that the results of the lower concentrations from 2 to 5 ng/ml could not be interpreted (see figure 15).

Table 18. Differences in peak height of Luna Phenyl-hexyl particle sizes at 100 ng/ml.

Substance	**5 µm (mAU ± SD)**	**3 µm (mAU ± SD)**
Melperone	14.9 ± 0.03	18.7 ± 0.01
Benperidol	11.5 ± 0.01	13.5 ± 0.02
Haloperidol	6.6 ± 0.03	9.6 ± 0.05
Bromperidol	3.5 ± 0.02	6.1 ± 0.01

Note. SD:standard deviation, mAU: peak height.

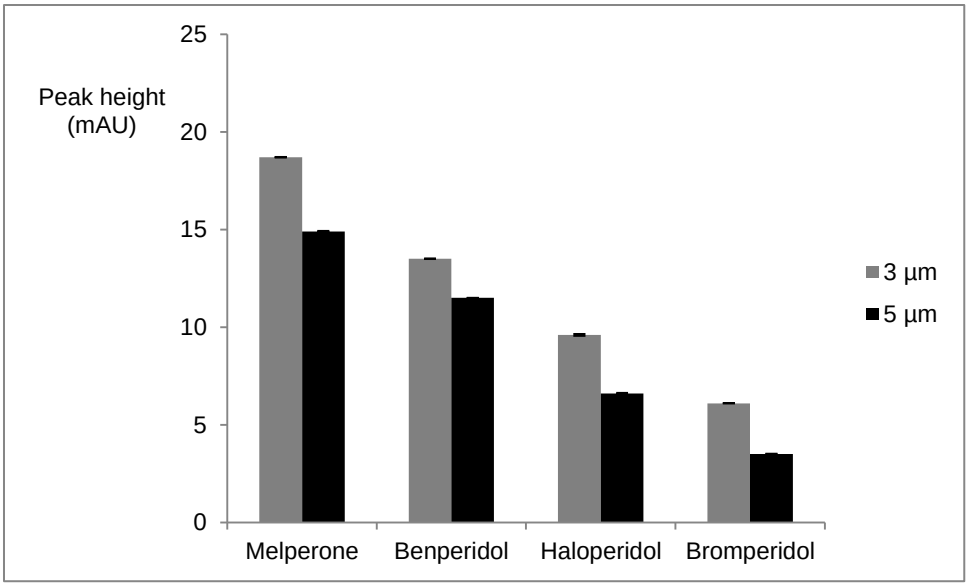


Figure 13. Differences between the peak height of Luna Phenyl-hexyl columns.

Note: 3 µm and 5 µm are the tested particle sizes, conc.: 100 ng/ml. The columns are the peak heights obtained at wave length 245 nm.

Table 19. Differences in peak area of Luna Phenyl-hexyl particle sizes measured at 100 ng/ml.

Substance	**5 µm (mAU x min ± SD)**	**3 µm (mAU x min ± SD)**
Melperone	7.7 ± 0.01	5.4 ± 0.03
Benperidol	8.6 ± 0.04	4.3 ± 0.02
Haloperidol	6.7 ± 0.02	4.6 ± 0.02
Bromperidol	3.7 ± 0.01	3.1 ± 0.03

Note: SD: standard deviation, mAU x min: peak area.

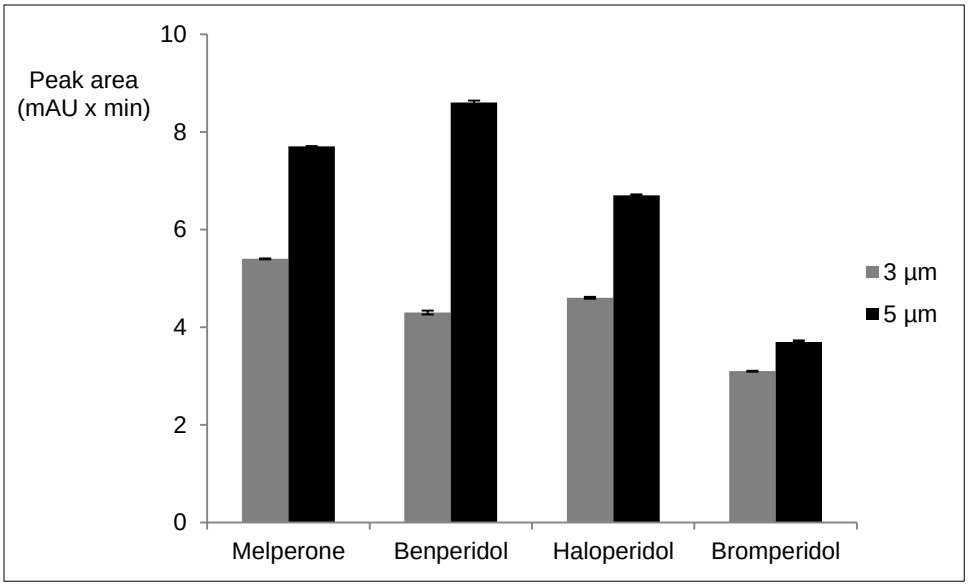


Figure 14. Differences between the peak area of Luna Phenyl-hexyl columns.

Note: 3 µm and 5 µm are the tested particle sizes, conc.: 100 ng/ml. The columns are the peak areas obtained at wave length 245 nm.

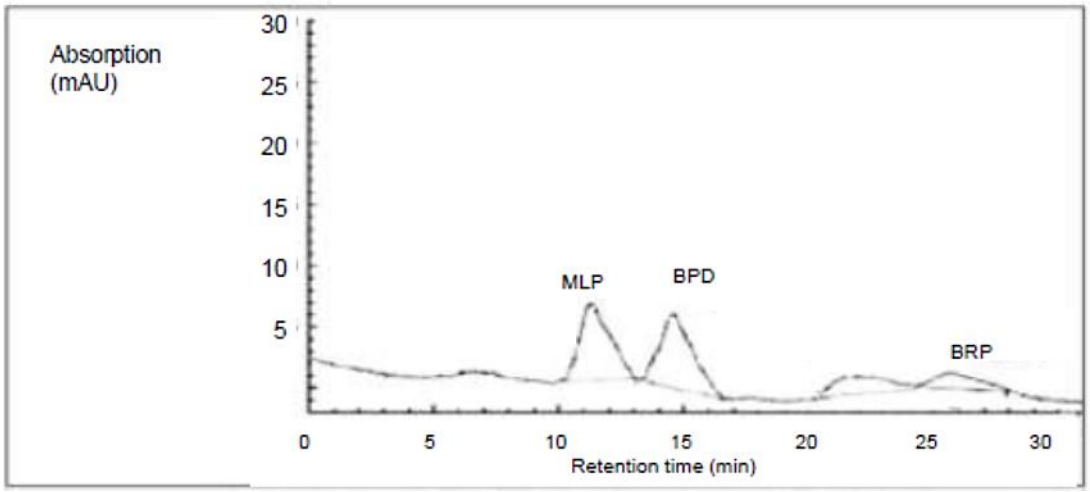


Figure 15. Example of chromatogram with Luna Phenyl-hexyl 5 µm 150 x 3.0 mm.

Measurement at conc.: 5 ng/ ml, WL 245 nm with TEMED in mobile phase; mAU = peak height. The tested substances are melperone, benperidol, haloperidol and bromperidol, measured with TEMED mobile phase. The observed wider peaks proved problematic for the measurements in lower concentrations.

4.1.6 Summary of Results of the Column Selection

The tested columns were made of different materials and they gave different results during the test (see table 20). For the selected columns, pressure and column-clogging problems were handled by cleaning intervals after each run of 2 to 5 min and washing the column (including the inverted position) once a week for at least 30 minutes.

Table 20. Characteristics of the applied analytical columns.

Analytical Column	Properties of the Package Material
Thermo-Betasil C6 5 µm 250 x 4.6 mm	Hexylsilane chemically bonded to totally porous silica particles [HPLC column selection, 2010]. It is designed for small molecules, and gives good results with acids, bases and neutral bonding.
Luna Phenyl-hexyl 3 µm 150 x 3.00 mm	The phenyl bonded phase uses a phenyl ring with a hexyl linker and is densely bonded to Luna silica surface. Dense bonding and the hexyl linker reduce bonded hydrolysis and increase chemical stability, which can lead to an optimal retention of aromatic and polar, amine compounds [HPLC column guide-Phenomenex, 2012]
Hypersil ODS 5 µm 250 x 4.6 mm	Octadecyl silane (C_{18}) ligand is chemically bounded to silica [HPLC column selection, 2010]. It is applied for the separation of wide range of compoubds including nonpolar, moderately polar, and lipophilic compounds such as triglycerides [HPLC column guide-Thermo, 2012].
SphereClone ODS (2) 5 µm 150 x 4.6 mm	Octadecyl silane (C_{18}) ligand is chemically bounded to silica [HPLC column guide-Phenomenex, 2012]. It can be applied for the separation of diverse analytes.
BetaBasic C4 3 µm 150 x 4.6 mm	Butyl silane is chemically bonded to porous silica particles [HPLC column guide-Phenomenex, 2012]. It is used for small molecules, peptides and protein digets.
BetaBasic C4 5 µm 150 x 4.6 mm	Butyl silane is chemically bonded to porous silica particles [HPLC column selection, 2010]. The difference with number 5 is based on the particle size.
Betasil C8 5µm 250 x 4.6 mm	Octasilane is chemically bonded to porous silica particles [HPLC column guide-Phenomenex, 2012; HPLC column selection, 2012]. This type of column is designed for small molecules, and gives good results with acids, bases and neutral bonding.

Xterra Schild C18 5 µm 100 x 4.6 mm	The function is based on the first generation of hybrid particle technology; they are mearnt to deliver sharp symmetrical peaks for basic compounds [HPLC column guide-Waters, 2012].
PerfectSil 120 ODS-L 5 µm 250 x 4.6 mm	Octasilane (C18) is chemically bonded to porous silica particles [HPLC Säulen-high lights, 2012; HPLC column guide-Phenomenex, 2012; HPLC column selection, 2010]. It can be applied for the separation of diverse analytes.
Gemini-Nx C18 5 µm 150 x 4.6 mm	Rugged Gemini column that controls the selectivity of ionizable compounds for optimized methods. They are meant to have consistent performance in both volatile and non-volatile buffers [HPLC column guide-Phenomenex, 2012].
Gemini C6 5 µm 150 x 4.6 mm	Phenyl group is bonded to the organo-silica surface using a 6-carbon chain for a low bleed aromatic selective phase [HPLC column guide-Phenomenex, 2012].
Luna phenyl-hexyl 5 µm 150 x 3.00 mm	Descriptions correspond to Luna Phenyl-hexyl 3 µm 150 x 3.00 mm with the exception of the particle size of 5 µm.
Luna CN 5 µm 250 x 4.6 mm	Contains smooth silica, which allows for a more uniform bonding with improved resistance to bonded phase hydrolysis to produce a stable CN phase, applicable for carboxyl, carbonyl and amine containing compounds [HPLC column guide-Phenomenex, 2012].
LiChrospher CN 5 µm 250 x 4.6 mm	Particles of silica with gamma-cyanopropyl function, LiChrosphere ® 100 CN has both weak polar properties and weak hydrophobic properties, which is expected to enable the separation of complex samples. It is applicable both for normal phase chromatography and reverse phase chromatography [LiChrospher® HPLC columns, 2012].
Nucleosil CN-RP 3 µm 150 x 3.0 mm	Polar to mid-polar cyano-(nitrile)-modified silica for reversed phase, normal phase and molecules containing pi electron systems (e.g. analytes with double bonds) chromatography. Nucleosil ® CN-RP columns are based on robust silica [MN application database, 2012].
Nucleodur 100-3 CN-RP 150 x 4.6 mm	Cyano nitrile modification is for reverse phase separations. It possesses a high retention capacity especially for very polar and unsaturated compounds and its retention characteristic is different compared with C8 and C18 types. This is because the materials are based on high purity and very pressure stable silica. [Instruction leaflet Nucleodur® CN / CN-RP].

Note: The descriptions of the applied columns were according to the manufacturers.

The selected columns for the determination of antipsychotic serum concentrations were Luna Phenyl-hexyl 3 µm 150 x 3.0 mm and Nucleodur CN-RP 3 µm 150 x 4.6

mm under the application of TEMED mobile phase (mobile phase C). The difference in the silica bonding of Luna Phenyl-Hexyl column and Nucleodur CN-RP column is represented in figure 16. The Luna Phenyl-hexyl 3 µm 150 x 3.0 mm was applied for the determination of butyphenones (MLP, BPD, HLP, BRP) in serum. The Nucleodur CN-RP 3 µm 150 x 4.6 mm was used for the determination of phenothiazines (FLT, ZLT) in serum.

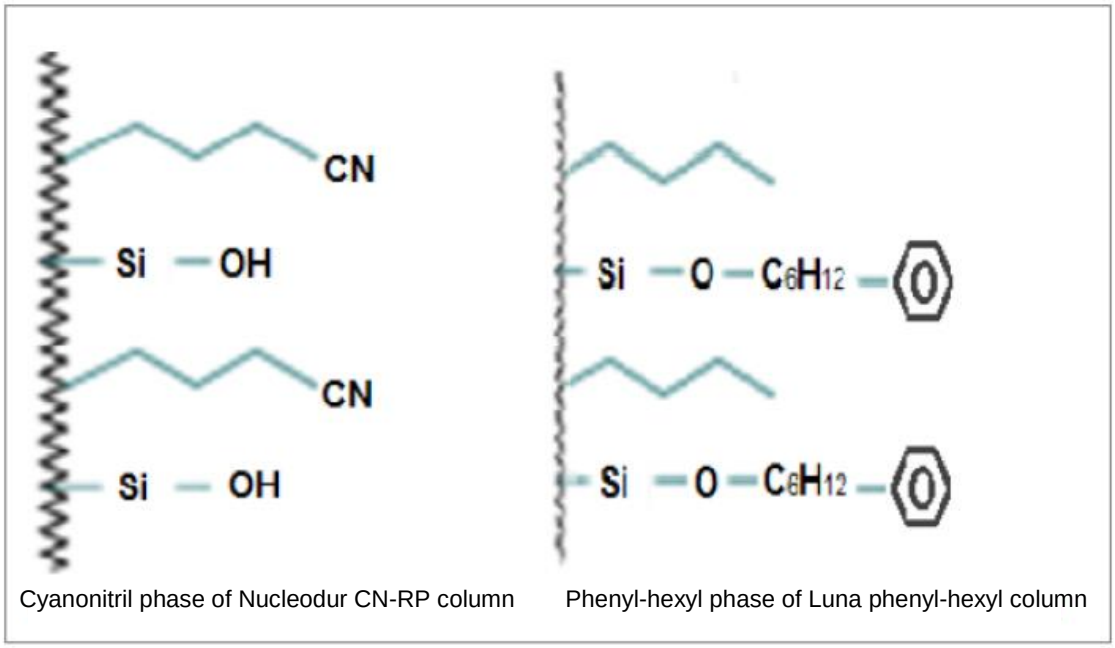


Figure 16. The difference in the silica bonding of Luna Phenyl-Hexyl column and Nucleodur CN-RP column.

Illustrations on the practical criteria for the selection of the appropriate columns are depicted in table 21 and 22. The columns were tested in mobile phase with buffer soluion and mobile phase containing TEMED. The mobile phase with buffer consists of 30 vol% ACN, 10 vol% MeOH, and 60 vol% buffer. The TEMED mobile phase consists of 30 vol% ACN, 13 vol% MeOH, 0.4 vol% TEMED, and 56.6 vol% demineralized water.

Table 21. Practical selection of analytical columns through the test with buffer solution.

Column	Result	
	Advantage	**Disadvantage**
Nucleodur CN-RP 150 x 4.6 100-3	-	The measurements showed chromatographic irregularities and therefore an unreliable column.
Thermo Betasil C6 250 x 4.6 mm 5 µm	-	The measurement at a concentration of 2000 ng/ml gave a molecular absorption unit of 14.8 mAU. The reduction of the concentration to 400 ng/ml gave a molecular absorption unit of 2.7 mAU and the reduction to 200 ng/ml gave 1.2 mAU. The irregular chromatographic base lines observed at concentrations of 400 ng/ml and 200 ng/ml made the measurement qualitatively and quantitatively unreliable
Luna Phenyl-hexyl 150 x 3.0 mm 3 µm	Sharp peaks were observed with Luna Phenyl-hexyl analytical column 3 µm at 10 ng/ml. The qualitative and quantitative measurement in lower concentrations can be obtained with this column.	A slight blockage of the column during the measurement with buffer mobile phase was observed. The washing of the column for 5 minutes and more after each run did not prevent blockage of the column. The column regeneration carried out with different isopropanole concentrations showed no better result.
Luna Phenyl-hexyl 150 x 3.0 mm 5 µm	Lower back pressure	The application of 5 µm particle size of a Luna Phenyl-hexyl column within the chosen parameters to prevent column blockage was not successful, and quantitative measurements of lower serum concentrations were however not realized.
Hypersil ODS 5 µm 250 x 4.6 mm	-	The result received after the measurement in serum at a concentration of 100 ng/ml gave a molecular absorption unit of less than 1.0 mAU. It showed that a reliable peak by 5 ng/ml cannot be obtained with this column.
SphereClone ODS(2) 150 x 4.6 mm 5 µm	-	Different concentrations tested with this column did not give a reliable result. The peak height obtained after measuring the concentration of 1000 ng/ml was 20.2 mAU. Reduction of the concentration by factor 10 to 100 ng/ml gave the peak height at 3.5 mAU. Further reduction of the concentration to arrive at the lowest expected therapeutic concentration of the substance did not yield reliable results.

PerfectSil 120 ODS-L 5 µm 250 x 4.6 mm	-	No detection
Gemini-Nx 5 µm 150 x 4.6 mm,	-	No detection
Gemini-Nx 5 µm 250 x 4.6 mm,	-	No detection
Gemini C6, 5 µm 150 x 4.6 mm.	-	No detection
BetaBasic C4 150 x 4.6 mm 3 µm	Good for measuring substances in clear solutions without particles.	Production of high back pressure, which lead to an inefficient measurement in human serum.
BetaBasic C4 150 x 4.6 mm 5 µm	Large particle size, good for measuring substances in serum.	Measurement of the applied antipsychotics in low concentrations (2-10 ng/ml) was not possible.
Betasil C8 250 x 4.6 mm 5 µm	Longer column length and large particle size enables the measurement	A longer retention time, longer measurement period and no detection at low concentrations (2-10 ng/ml)

Note: Column selection using buffer mobile phase (Mobile Phase A). The advantage and disadvantage refer to the results obtained after measurement with HPLC device.

Table 22. The use of TEMED mobile phase for the selection of column.

Column	**Result**	
	Advantage	**Disadvantage**
PerfectSil 120 ODS-L 250 x 4.6 mm 5 µm	The improvement of the noise and the peak resolution were observed at an increase of the concentration to 1000 ng/ml. This showed that this column is better used for measurements at high substance concentrations.	The measurements in low concentrations were very unreliable because of the observed noise. The base line was observed to be so irregular that the peaks of the concentration at 100 ng/ml could not be identified. The concentration of 200 ng/ml gave a peak height of 4.3 mAU.
Gemini-Nx C18 5 µm 150 x 4.6 mm and 250 x 4.6 mm	The total chromatographic run was 15.0 min.	The peak height obtained at the concentration of 200 ng/ml was 4.7 mAU. The concentrations at 2 and 5 ng/ml could not be identified because of the low peak height and the noise observed.

Gemini-Nx C18 5 µm 250 x 4.6 mm	Low back pressure	The total chromatographic run was 30 min. Measurement in low concentrations was not reliable.
Gemini C6 5 µm 150 x 4.6 mm	-	There was no peak observed at 10 ng/ml. The peak height received at 2000 ng/ml was 14.7 mAU. Detection wavelength was 230 nm and retention time was 11.3 min.
Nucleodur CN-RP 150 x 4.6 mm 100-3	The measurement showed a reliable and satisfactory result at a detection wavelength of 230 nm with diode array detector.	5 min column wash after each run to avoid blockage
Luna Phenyl-hexyl 5 µm 150 x 3.0 mm	-	There was no peak observed for measurements carried out in low concentrations
Luna Phenyl-hexyl 3 µm 150 x 3.0 mm	The determination of low serum concentrations at 2.0 ng/ml was carried out successfully. The optimal wavelength obtained with a diode array detector was 245 nm and pH at 5.0.	5 min column wash after each run to avoid blockage
Thermo Betasil C6 5 µm 250 x 4.6 mm	-	No absorption
Hypersil ODS 250 x 4.6 5 µm	-	No absorption
SphereClone 5 µm ODS(2) 150 x 4.6 mm.	-	No absorption
Xterra Schild C18 100 x 4.6 mm 3. 5 µm	A very short column, possibility of measuremnt in a very short time.	No detection observed, built up a very high back pressure during the measurement.
LiChrospher CN 250 x 4.6 mm 5 µm	-	Detection was only observed at higher concentrations from 100 ng/ml upwards, longer measurement period.
Luna CN 250 x 4.6 mm 5 µm	-	No detection observed

Note: Column Selection using TEMED Mobile Phase (Mobile Phase C). The advantage and disadvantage refer to the results obtained after measurement with HPLC device.

4.1.7 Isocratic Separation of Butyrophenones

The simultaneous separation of melperone and benperidol at pH 4.3 was not reliable. During column selection, it was observed that both substances have a tendency to overlap. Haloperidol and bromperidol gave good results for this pH value. The chosen mobile phase for this initial separation was the mobile phase C containing TEMED, and the chosen column was the Luna Phenyl-hexyl 150 x 3.0 mm 3 µm. Wavelength, temperature, flow rate, and the acidity of the mobile phase were the factors considered for the analytic separation.

4.1.7.1 Wavelengths and Retention Times

The measurements were successfully carried out at wavelengths of 230 nm and 245 nm. The measurement at 230 nm gave smaller molecular absorption units compared with the measurement at 245 nm. The result obtained at a wavelength of 245 nm (see table 23) was maintained because of its reliability and precision for the qualitative and quantitative evaluation of MLP and BPD in human serum.

Table 23. The simultaneous measurement of MLP and BPD.

Substances	Concentration (ng/ml)	Retention Time (min)	Standard Deviation (SD)
melperone (MLP)	25	15.84	0.02
	57	15.74	0.02
	115	15.69	0.14
benperidol (BPD)	2	17.3	0.09
	10	17.16	0.03
	30	17.06	0.03

Note: The retention times of melperone and benperidol were measured four times (n=4) at the wavelength of 245 nm, n: number of measurements for each concentration.

4.1.7.2 Influence of the Temperature

The observation was made that the lower the temperature, the better the separation of the peaks. The result of a measurement carried out at a temperature of 30 ^{o}C showed a poor resolution of melperone and benperidol peaks (see figure 17). Well-distinguished melperone and benperidol peaks were observed after a reduction of the temperature to 20 ^{o}C.

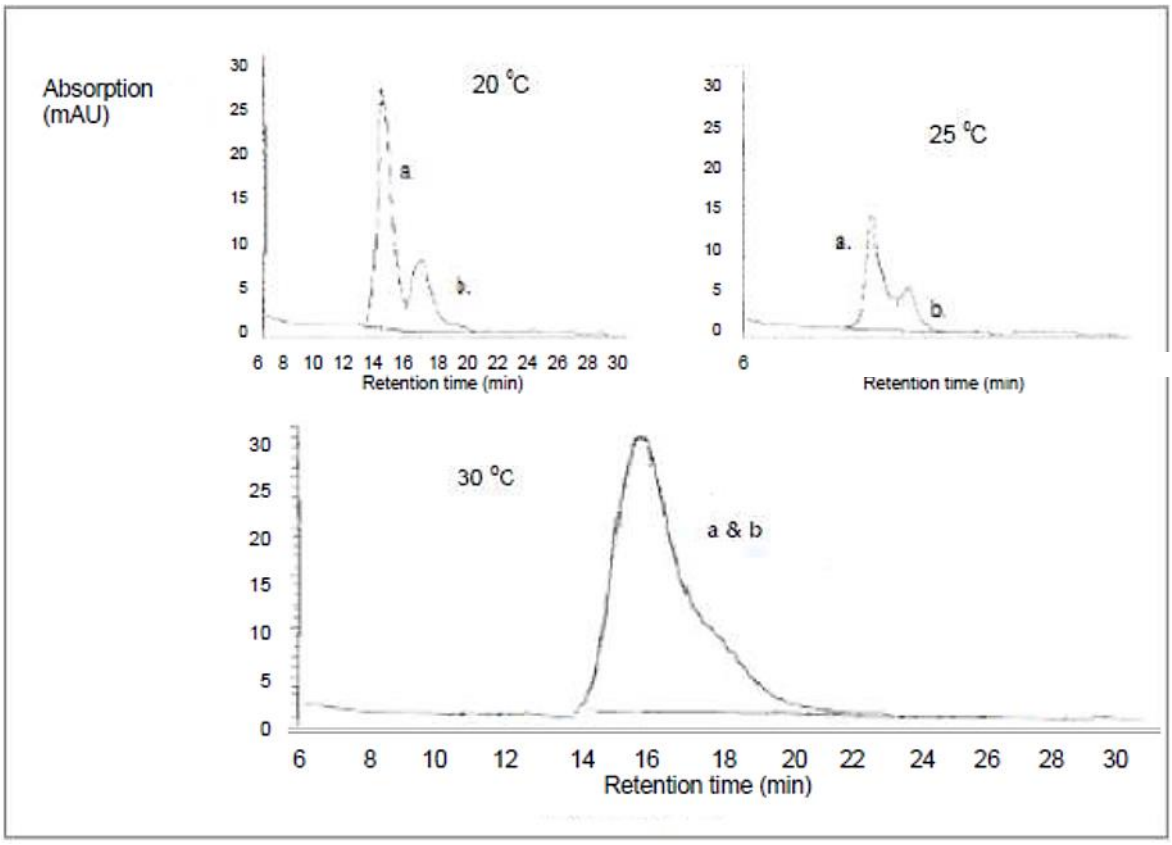


Figure 17. Effect of different temperatures during the HPLC separation of MLP (a) and BPD (b).
Note: Tested temperatures were 20 ^{o}C, 25 ^{o}C, and 30 ^{o}C, at conc. 100 ng/ml.Measurement with TEMED mobile phase (30 vol% ACN, 13 vol% MeoH, 0.4 vol% TEMED, and 56.6 vol% demineralized water). mAU = peak height.

4.1.7.3 Influence of the Flow Rate

Best separation of MLP and BPD was achieved after a reduction of the flow rate to 0.2 ml/min. Measurements carried out with lower flow rates of 0.15 ml/min and 0.1 ml/min showed prolonged retention times and poor resolution of both substances (see figure 18). The flow rate of 0.2 ml/min was therefore set for further measurements.

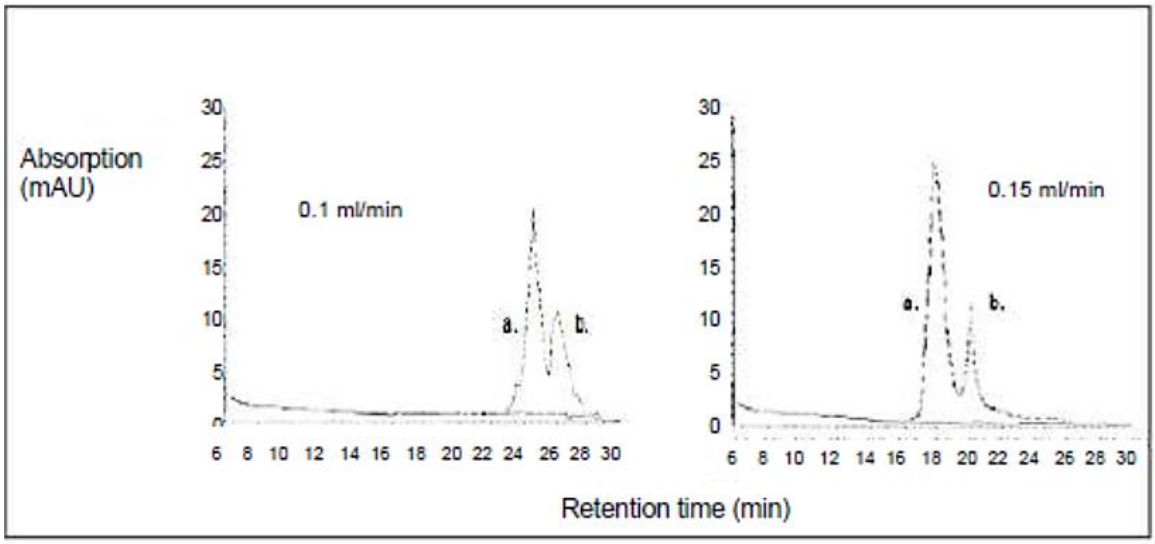


Figure 18. The variation of the flow rate at 0.1 ml/min and 0.15 ml/ min.

Note: Tested during the separation of melperone and benperidol. at conc. 100 ng/ml. Mobile phase (30 vol% ACN, 13 vol% MeoH, 0.4 vol% TEMED, and 56.6 vol% demineralized water). mAU = peak height.

4.1.7.4 Influence of pH

The TEMED gave the mobile phase the expected stability at an optimal pH value of 5.0. The pH value of the TEMED mobile phase prepared between pH 4.0 and pH 4.3 did not show any improvement on the separation of melperone and benperidol. An

increase in the acidity to pH 5.0 showed a reliable and precise separation (see figure 19).

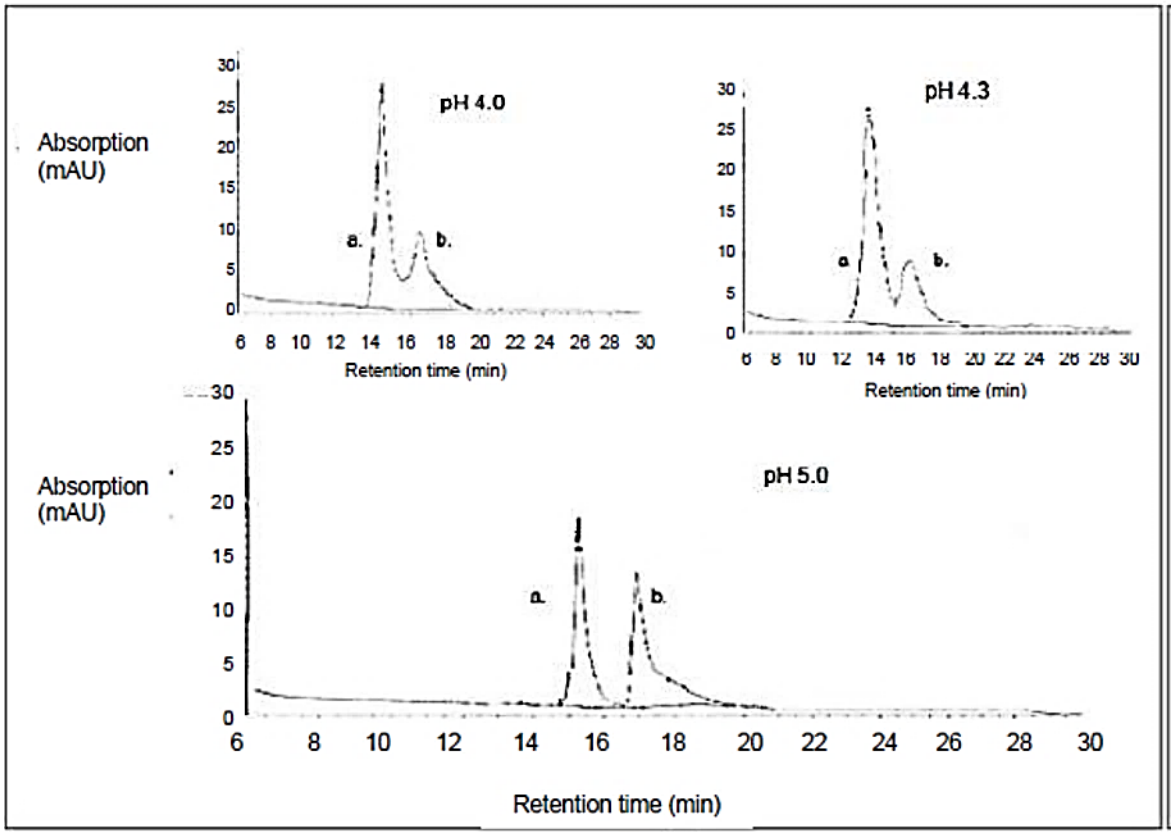


Figure 19. A reliable separation of MLP (a) and BPD (b) at an increase of the pH value of the mobile phase to pH 5.0.

Note: Conc. 100 ng/ml. Mobile phase (30 vol% ACN, 13 vol% MeoH, 0.4 vol% TEMED, and 56.6 vol% demineralized water). mAU = peak height.

Summary of Results of the Qualitative Separation of MLP and BPD

An observation was made during the measurement that the wavelength necessary for the reliable identification and separation of melperone and benperidol was 245 nm. A temperature above 20 ^{o}C showed an irregular base line and a flow rate above 0.2 ml/min caused a high back pressure. The set parameters for further qualification and quantification of both substances are listed in table 24. In general, baseline separation of melperone and benperidol was successfully achieved at pH 5.0, a

constant temperature at 20 ^{o}C, and a flow rate of 0.2 ml/min. These parameters were used for the simultaneous separation of all the butyrophenones for this study.

Table 24. Parameters for the separation of melperone and benperidol.

Parameters	Set Values
Wavelength	245 nm
Temperature	20 ^{o}C
Flow rate	0.2 ml/min
Mobile phase acidity	pH 5.0
Column	Luna Phenyl-hexyl 150 x 3.0 mm, 3 µm
Mobile phase	(30 vol% ACN, 13 vol% MeOH, 0.4 vol% TEMED, and 56.6 vol% demineralized water).

Note: These are fixed parameters for further measurements of melperone and benperdiol.

4.1.8 Application of the Gradient HPLC Separation Method

The result of the simultaneous test carried out for the determination of antipsychotics in human serum showed a gap on the chromatographic scale during the evaluation. The multistep gradient applied to close the gap (see figure 20) and reduce the total chromatographic run did not yield the expected result.

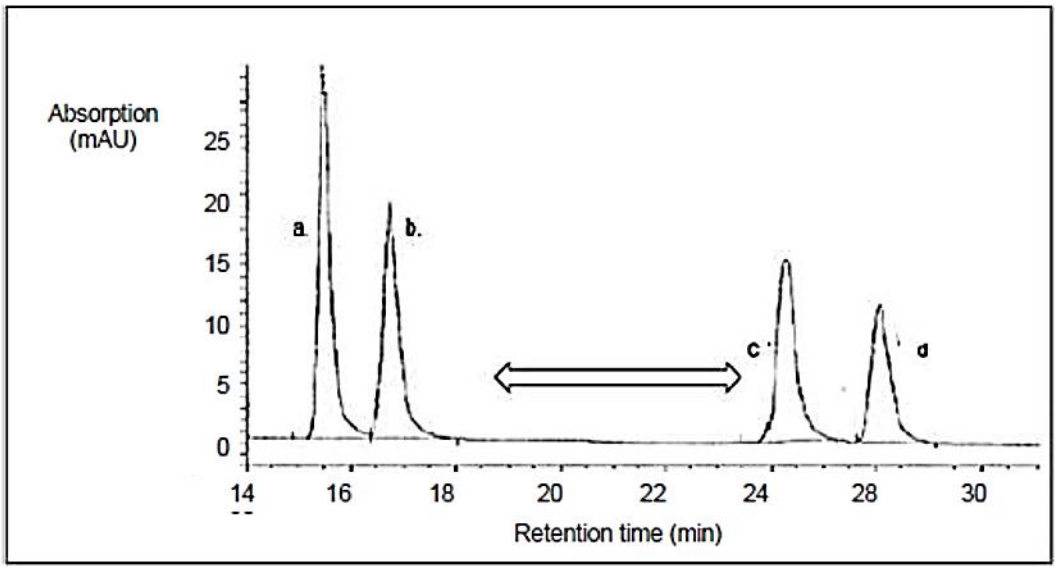


Figure 20. An acceptable chromatographic separation with isocratic HPLC analysis before the application of gradient method.

Note: a: MLP, b: BPD, c: HLP, and d: BRP, at conc. 100 ng/ml. Mobile phase solvent consists of ACN, MeoH, TEMED, and demineralized water. mAU = peak height.

The separation of antipsychotics through the addition of 15% acetonitrile (ACN) to 85% mobile phase C gave a poor result after 20 min chromatographic run. It was observed that the change in the time of admixture (admixture: mixing procedures) partially showed a better resolution of the peaks. The detection of the substances by an addition of 25% ACN to mobile phase C (see figure 21-25 and figure 27-28) was not reliable. An acceptable result was achieved by adding 5% acetonitrile to 95% of mobile phase C at 15.8 min (see figure 26). The table illustrating the gradient mixtures can be found in appendix 3.

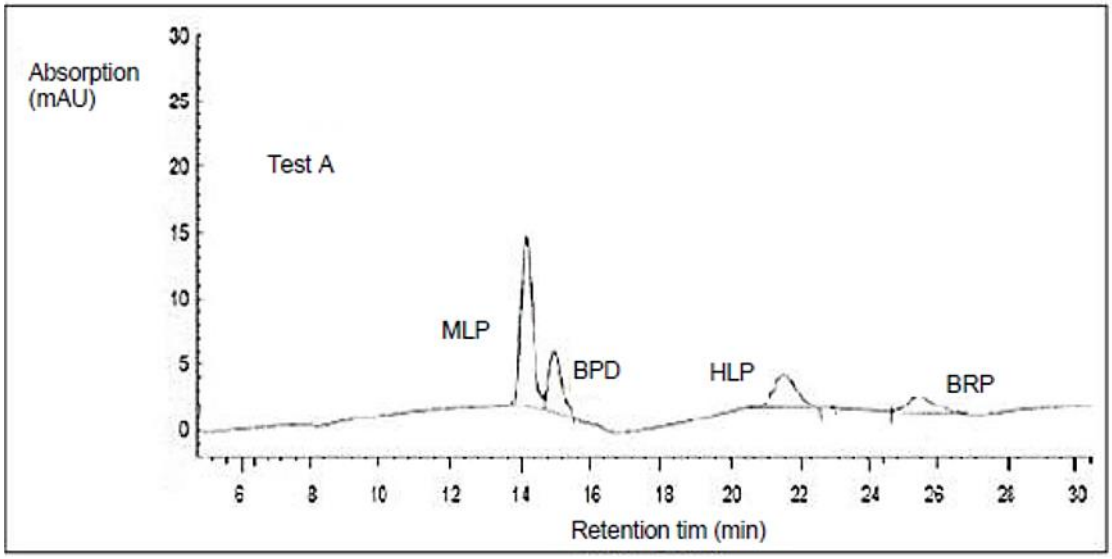


Figure 21. 85% mobile phase C + 15% ACN admixed from 20 min., conc.: 100 ng/ml.

Note: Mobile phase solvent consists of ACN, MeoH, TEMED, and demineralized water. mAU = peak height.

The separation between melperone and benperidol worsened, but the total run time decreased slightly.

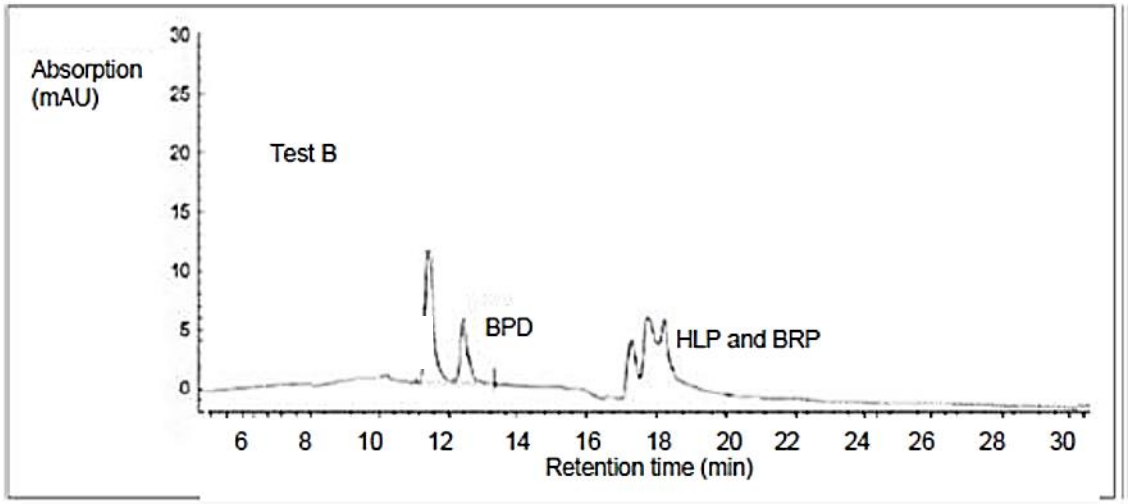


Figure 22. 85% mobile phase C + 15% ACN admixed at 16 min, at conc. 100 ng/ml.

Note: Mobile phase solvent consists of ACN, MeoH, TEMED, and demineralized water. mAU = peak height.

The separation between melperone and benperidol became better, but the separation between haloperidol and bromperidol worsened. The retention time of benperidol was observed to have shortened.

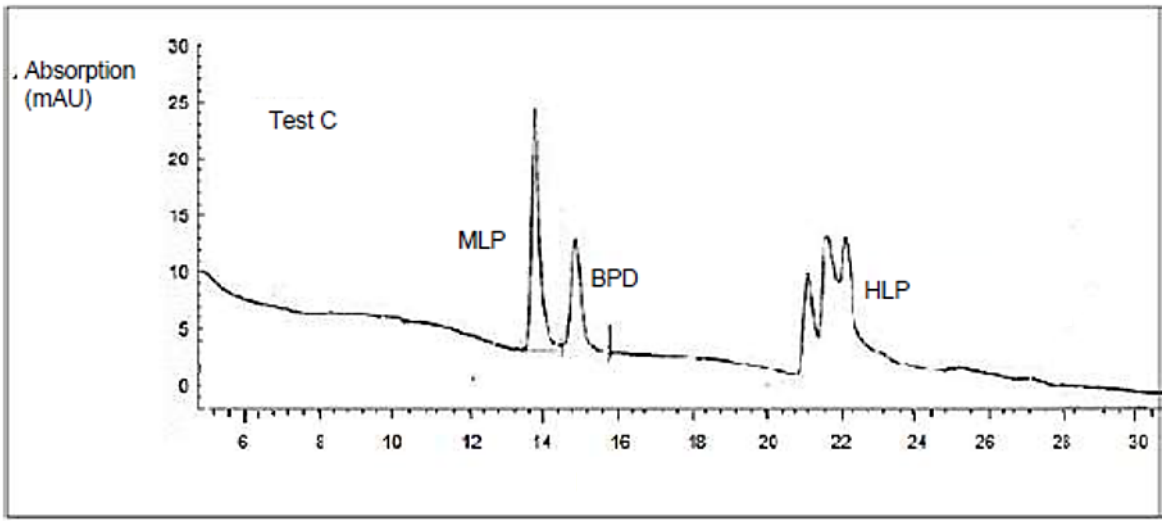


Figure 23. 80% mobile phase C + 20% ACN admixed from 16 min, at conc. 100 ng/ml.

Note: Mobile phase solvent consists of ACN, MeoH, TEMED, and demineralized water. mAU = peak height.

The separation between melperone and benperidol was optimal. The 5% variations between mobile phase C and ACN did not improve the separation of haloperidol and bromperidol.

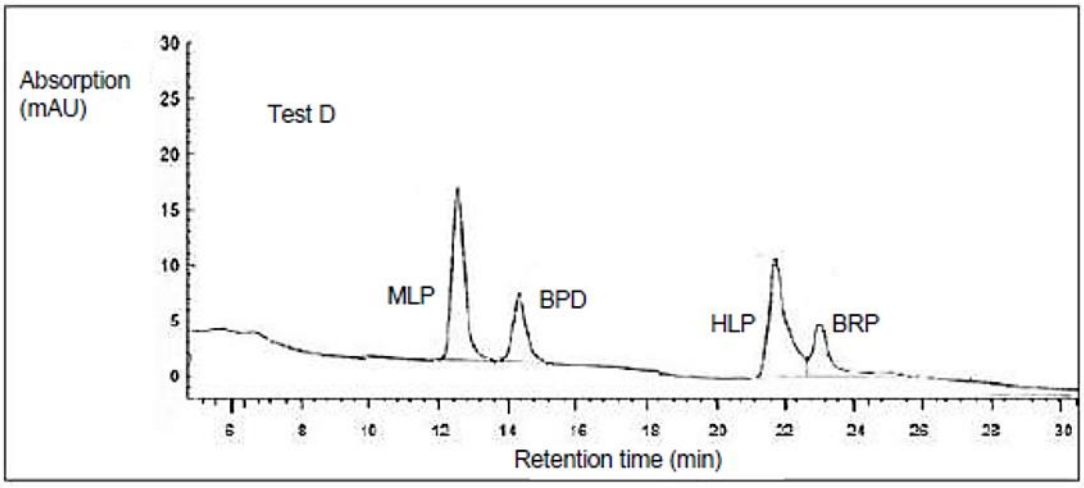


Figure 24. 85% mobile phase C + 15% ACN admixed from 18 min, at conc. 100 ng/ml.

Note: Mobile phase solvent consists of ACN, MeoH, TEMED, and demineralized water. mAU = peak height.

The issue of the haloperidol-bromperidol separation remained.

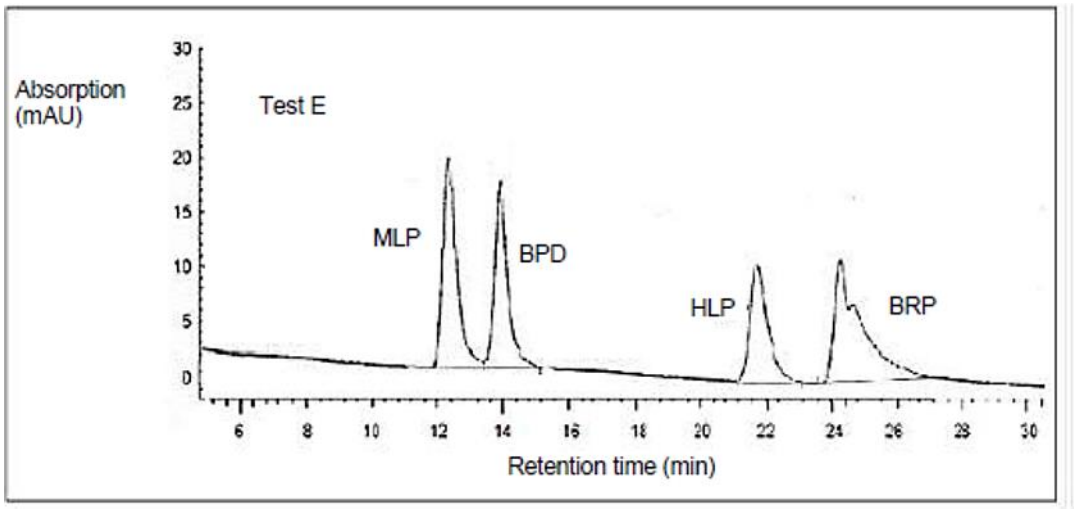


Figure 25. 90% mobile phase C + 10% ACN admixed at 16 min, at conc. 100 ng/ml.

Note: Mobile phase solvent consists of ACN, MeoH, TEMED, and demineralized water. mAU = peak height.

The resolution of the bromperidol peak was not reliable.

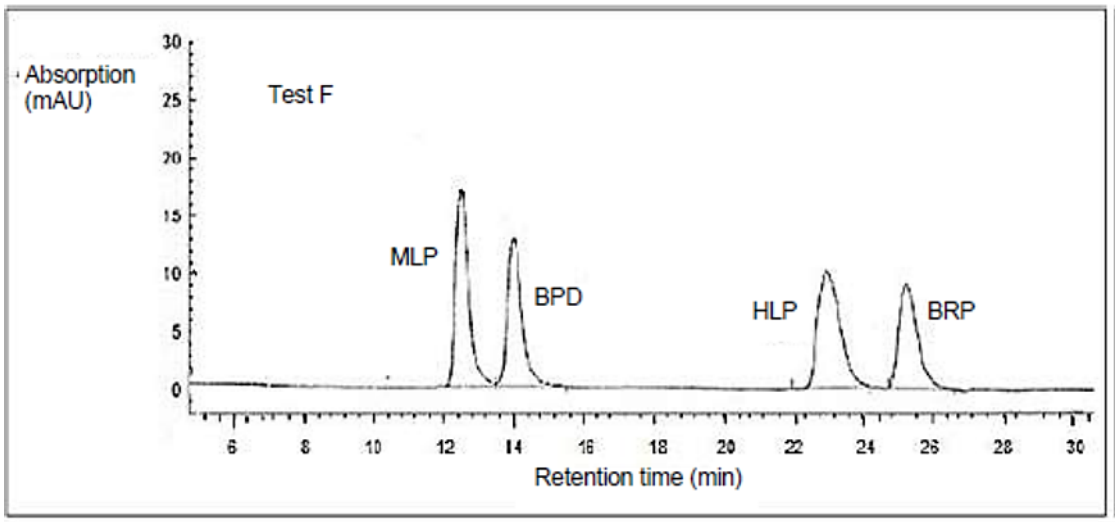


Figure 26. 95% mobile phase C + 5% ACN admixed from 15.8 min, at conc. 100 ng/ml.

Note: Mobile phase solvent consists of ACN, MeoH, TEMED, and demineralized water. mAU = peak height.

An optimal separation of the four substances (MLP, BPD, HLP, BRP) was achieved.

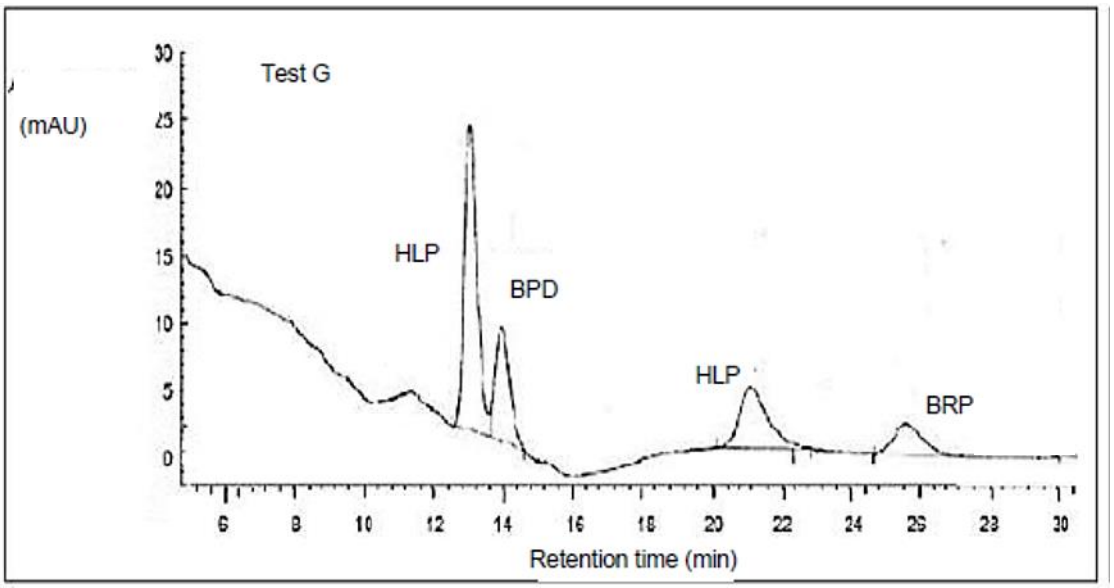


Figure 27. 90% mobile phase C + 10% ACN admixed from 16.2 min at conc. 100 ng/ml.

Note: Mobile phase solvent consists of ACN, MeoH, TEMED, and demineralized water. mAU = peak height.

An irregular base was observed during the measurement.

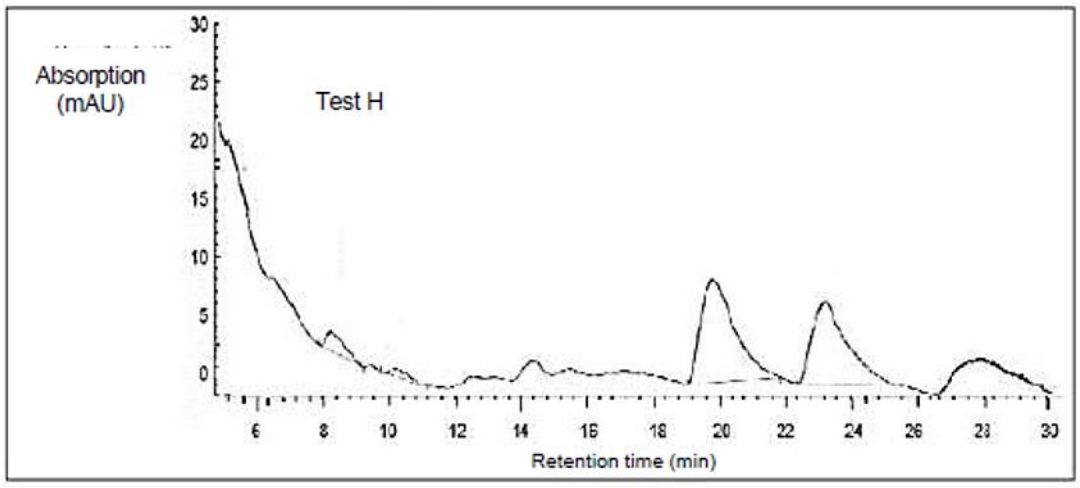


Figure 28. 75% mobile phase C + 25% ACN admixed from 16.2 min, at conc. 100 ng/ml.

Note: Mobile phase solvent consists of ACN, MeoH, TEMED, and demineralized water. mAU = peak height.

No reliable UV-absorption was obtained. UV-absorption: ultra violett absorption.

Comparison of Isocratic and Gradient Separation

The best results from the two developed methods were compared in order to choose the preferred analysis for the method validation. The same analytical column (Luna Phenyl-hexyl 150 x 3.0 mm 3 µm), run time (25 min) and wavelength (245 nm) were used for both the gradient and for the isocratic analysis. The mobile phase for the isocratic separation was constant, while that of gradient speraration was varied. Though the consumption of the analytical solutions was slightly higher by the gradient method (see table 25), the two methods did not show any significant difference in their results.

Table 25. Differences of the isocratic and gradient measurement.

Parameters	Isocratic	Gradient
Column	Luna Phenyl-hexyl 150 x 3.0 mm 3 µm	Luna Phenyl-hexyl 150 x 3.0 mm 3 µm

Run time (min)	25 min	30 min
Separation	Insignificant	Insignificant
Peak height difference	Insignificant	Insignificant
Mobile phase consumption	Less consumption	Higher consumption
Cost	Less expensive	Comparably more expensive

Note: The separation of the substances was carried out with the same parameters for both separation methods as mentioned in the table, in order to enable a valid comparison.

4.1.9 Validation of the Newly Developed Isocratic HPLC-Method

Table 26 shows the parameters for the validation of butyrophenones and phenothiazines. The correction factor of 0.88 for MLP, 0.86 for FLT and 0.85 for ZLT were considered in all the measurements, because the raw substances available were in the form of salt with a molecular weight of 299.8 g/mol for MLP, 503.1 for FLT and 473.9 for ZLT.

Table 26. Parameters for the validation of MLP, BPD, HLP, BRP, FLT and ZLT.

Parameters	Butyrophenones	Phenothiazines
Mobile phase	30 vol% ACN, 13 vol% MeOH, 0.4 vol% TEMED, and 56.6 vol% demineralized water	30 vol% ACN, 13 vol% MeOH, 0.4 vol% TEMED, and 56.6 vol% demineralized water
Mobile phase acidity	pH 5.0	pH 5.0
Extraction column	LiChrosphare ADS-RP-4, 25 µm x 2 mm, 20 µm	LiChrosphare ADS-RP-4, 25 µm x 2 mm, 20 µm
Analytical column	Luna Phenyl-hexyl 150 x 3.0 mm, 3 µm	Nucleodur CN-RP 150 x 4.6 mm, 3 µm
Injection volume	750 µl	1250 µl
Temperature	20 ^{o}C	30 ^{o}C
Flow rate	0.2 ml/min	0.6 ml/min
Wavelength	245 nm	230 nm
Detector	UV/VIS	UV/VIS

Note: ACN: acetonitrile, MeOH: methanol, TEMED: tetramethylethylendiamin, Vol: volume.

4.1.9.1 Calibration and the Calibration Curve

The respective concentrations of the applied antipsychotics were measured seven times successively (see table 27). The result of the mean correlation coefficients which is the measure of linearity was satisfactorily obtained. An example of the calibration curve of zuclopenthixol is shown in appendix 4.

Table 27. Calibration results of the applied antipsychotics.

Substances	Concentration Range (ng/ml)	Correlation Coefficient (r^2)	Linear Equation
Butyrophenones			
Melperone	2-200	0.9958	Y= 0.1729x - 0.3425
Benperidol	2-200	0.9878	Y= 0.1285x + 0.3048
Haloperidol	2-200	0.9856	Y= 0.1045x + 0.1126
Bromperidol	2-200	0.9910	Y= 0.0775x – 0.0651
Phenothiazines			
Flupentixol	5-100	0.9990	Y= 0.0615x – 0.0655
Zuclopenthixol	4-150	0.9958	Y= 0.0591x – 0.0897

Note: The linear equations and correlation coefficients (r^2) of the linearity test were obtained after seven times (n= 7) measurements of each concentration. Y: dependent variable

4.1.9.2 Substance Recovery

The amount of substance lost during the sample preparation and possible interference of serum materials during the measurements were both successfully controlled. A proportional reduction of the amount of substance was observed both in using the peak height as well as the peak area. The evaluated substance recoveries

(see table 28) ranged between 88.8 % and 109.5 %. This result corresponds with the acceptable bias of ± 20% specification of DIN 32645.

Table 28. Mean substance recovery in % ± standard deviation.

Substances	Concentration (ng/ml)	Recovery ± SD (%)
Butyrophenones		
Melperone (factor = 0.88)	22	107.9 ± 2.0
	50.2	104.2 ± 1.0
	101.2	92.4 ± 2.7
Benperidol	2	95.5 ± 1.6
	10	91.3 ± 4.0
	30	95.9 ± 1.1
Haloperidol	2	105.6 ± 2.0
	10	92.1 ± 0.6
	30	88.8± 4.2
Bromperidol	3	103.9 ± 2.6
	15	107.5 ± 1.0
	30	109.5 ± 0.9
Phenothiazines		
Flupentixol (factor = 0.86)	5.2	93.9 ± 3.0
	15.1	108.1 ± 4.0
	30.1	91.2 ± 1.7
Zuclopenthixol (factor = 0.85)	5.1	90 ± 2.6
	51	102.4 ± 1.0
	102	93.3 ± 0.05

Note: The yield of antipsychotics during the test on recovery was carried out six times (n = 6) with three different concentrations for each substance. The factors of melperone, flupentixol, and zuclopenthixol were considered in relation to their different salts. SD: standard deviation.

4.1.9.3 Intraday Precision

Six different chromatographic runs were performed under identical conditions within a day. The measurements evaluated are the substance peak height (mAU). The

variation coefficients obtained were between 0.39% and 13.52%. The required limit for the coefficient of variation (CV) according to DIN 32645 is ± 15%. The obtained results correspond with the given specification (see table 29).

Table 29. The evaluation of intraday measurement.

Substance	Concentration (ng/ml)	Mean (mAU)	SD	CV (%)
Butyrophenones				
Melperone (factor = 0.88)	22	4.7935	0.0742	1.55
	50.2	10.5272	0.0924	0.88
	101.2	20.7535	0.2091	1.01
Benperidol	2	0.2730	0.0110	4.03
	10	1.2612	0.0733	5.81
	30	3.5778	0.0356	0.99
Haloperidol	2	0.1330	0.0072	5.41
	10	0.6407	0.0289	4.51
	30	2.0377	0.0214	1.05
Bromperidol	3	0.1250	0.0169	13.52
	15	0.6158	0.0551	8.95
	30	1.2508	0.0101	0.81
Phenothiazines				
Flupentixol (factor = 0.86)	5.2	0.5133	0.0280	5.45
	15.1	1.4630	0.0080	0.55
	30.1	2.9024	0.0700	2.41
Zuclopenthixol (factor = 0.85)	5.1	0.7590	0.0400	5.27
	51	8.4468	0.0900	1.07
	102.1	18.263	0.0710	0.39

Note: The precision of measurements within a particular day was carried out six times (n=6) for each concentration. SD: standard deviation, CV: coefficient of variation, mAU: peak height.

4.1.9.4 Interday Precision

The measurement was carried out uniformly for six consecutive days. The obtained variation coefficients (CV) were between 0.74 % and 13.12% (see table 30). The values of the coefficient of variation obtained met with the ±15% requirement of DIN 32645.

Table 30. The result of interday measurements.

Substance	Concentration (ng/ml)	Mean (mAU)	SD	CV (%)
Butyrophenones				
Melperone (factor = 0.88)	22	3.2968	0.1074	3.25
	50.2	6.78037	0.2696	3.98
	101.2	13.9556	0.2744	1.97
Benperidol	2	0.1998	0.0129	6.46
	10	0.7727	0.0768	9.94
	30	2.6310	0.0967	4.47
Haloperidol	2	0.1053	0.0080	7.69
	10	0.4438	0.0420	9.42
	30	1.2624	0.0820	6.50
Bromperidol	3	0.1268	0.0108	8.52
	15	0.5331	0.0340	6.38
	30	1.0704	0. 0460	4.30
Phenothiazines				
Flupentixol (factor = 0.86)	5.2	0.1376	0.0012	0.87
	15.1	0.3643	0.0058	1.59
	30.1	0.7470	0.0055	0.74
Zuclopenthixol (factor = 0.85)	5.1	0.3580	0.0300	8.38
	51	5.1011	0.6501	12.74
	102.1	10.1374	1.3302	13.12

Note: The precision of measurements for six days was carried out 4 times (n=4) for each concentra-tion. SD: standard deviation, CV: coefficient of variation, mAU: peak height.

4.1.9.5 The Accuracy of the Method

The evaluation (in bias %) of the deviation of the obtained values proved the accuracy of the method. The values obtained for all the analytes were within the acceptable range of bias at ±15% (see table 31), notwithstanding the possible systematic error.

Table 31. The accuracy of the method for the determination of antipsychotics in human serum.

Substance	Concentration (ng/ml)	Target Value (mAU ± SD)	Measured Value (mAU ± SD)	Bias (%)
Butyrophenones				
Melperone (factor = 0.88)	22	3.4470 ± 0.11	3.7043 ± 0.12	7.46
	50.2	7.1135 ± 0.58	7.4103 ± 0.47	4.17
	101.2	15.8533 ± 0.20	14.6528 ± 0.61	-7.57
Benperidol	2	0.1713 ± 0.02	0.1635 ± 0.03	- 4.55
	10	0.8923 ± 0.16	0.8145 ± 0.01	-8.71
	30	2.6933 ± 0.18	2.5840 ± 0.06	- 4.06
Haloperidol	2	0.1338 ± 0.01	0.1413 ± 0.16	5.61
	10	0.6388 ± 0.03	0.5880 ± 0.05	- 7.95
	30	2.0290 ± 0.02	1.8023 ± 0.05	- 11.17
Bromperidol	3	0.1308 ± 0.01	0.1360 ± 0.00	3.98
	15	0.6340 ± 0.02	0.6818 ± 0.02	7.54
	30	1.2525 ± 0.01	1.3715 ± 0.07	9.50
Phenothiazines				
Flupentixol (factor = 0.86)	5.2	0.1600 ± 0.01	0.1680 ± 0.00	4.87
	15.1	0.4300 ± 0.02	0.4200 ± 0.01	-2.58
	30.1	0.8600 ± 0.02	0.8300 ± 0.03	-3.31
Zuclopenthixol (factor = 0.85)	5.1	0.5641± 0.03	0.6310 ± 0.06	11.88
	51	8.4470 ± 0.09	8.6050 ± 0.01	1.87
	102	18.2630 ± 0.07	17.0810 ± 0.01	- 6.47

Note: The accuracy of the method was carried out 12 times (n=12) for each concentration. SD: stand-ard deviation, mAU: peak height.

4.1.9.6 Lower Detection (LOD) and Quantification Limit (LOQ)

The obtained limit of detection gave the lowest concentration of analytes in the sample material, in which the signal-to-noise ratio is at least 3:1 (S/N=3). The obtained limit of quantification gave the minimum concentration of analyte in the sample that was quantitatively determined with accepted precision and accuracy. The concentrations were detected with a probability of 50% as specified by DIN 32645 and the results obtained correspond to the requirements (see table 32).

Table 32. The result analysis of LOD and LQD.

Substance	Concentration (ng/ml)	Limit of Detection (ng/ml)	Limit of Quantification (ng/ml)
Butyrophenones			
Melperone (factor = 0.88)	5-120 (measured in 13 steps)	7.3	22
Benperidol	1–10 (measured in 6 steps)	0.7	2.0
Haloperidol	1–10 (measured in 6 steps)	0.7	2.0
Bromperidol	3–20 (measured in 6 steps)	1.5	4.5
Phenothiazines			
Flupentixol (factor = 0.86)	1-10 (measured in 6 steps)	1.72	5.2
Zuclopenthixol (factor = 0.85)	2–16 (measured in 8 steps)	1.67	5.01

Note: The limit of detection and limit of quantification were obtained by measuring each concentration four times (n=4). LOD: limit of detection, LQD: limit of quantification.

4.1.9.7 Long-term Stability

The stability of the substances in the sample solution during long-term storage at -20 ^{o}C for 12 months was analyzed with serum and with drug solution . According to DIN

32645, the mean stability of the substances should lie between 90-110% of the mean of the corresponding control samples.

The amount of the freshly prepared test samples served as the 100% yield. The antipsychotic substances in solution were observed to decrease below the given specification after 3 months (see table 33). The stability of the substances in sample solution is therefore proven for 3 months when stored at -20 ^{o}C.

Table 33. Long-term stability of the substances in sample solution.

Substance	Conc. (ng/ml)	Current Test (mAU ± SD)	3 Months Mean % ±SD	6 months Mean % ±SD	12 months Mean % ±SD
Butyrophenones					
Melperone (factor = 0.88)	22	3.60 ± 0.12	93.92 ± 4.4	47.20 ± 0.2	37.00 ± 0.8
	50.2	6.21 ± 0.03	89.62 ± 0.4	51.00 ± 0.6	38.00 ± 1.0
	102.1	12.65± 0.6	90.60 ± 2.8	68.50 ± 1.2	48.00 ± 2.6
Benperidol	2	0.24 ± 0.0	90.00 ± 4.2	60.40 ± 2.1	42.30 ± 3.3
	10	0.65 ± 0.0	92.30 ± 2.5	60.70 ± 3.3	41.90 ± 1.8
	30	1.94 ± 0.2	89.50 ± 1.3	52.10 ± 2.6	36.40 ± 4.2
Haloperidol	2	0.13 ± 0.0	99.00 ± 2.2	45.00 ± 4.0	46.00 ± 3.2
	10	0.69 ± 0.0	91.27 ± 0.8	58.40 ± 2.1	44.80 ± 3.4
	30	2.06 ± 0.0	89.96 ± 5.4	37.00 ± 3.1	56.00 ± 4.2
Bromperidol	3	0.13 ± 0.0	109.90 ± 1.6	66.00 ± 3.3	47.00 ± 2.1
	15	0.63 ± 0.0	91.46 ± 2.2	72.83 ± 2.2	54.50 ± 3.5
	30	1.25 ± 0.0	90.38 ± 1.2	54.00 ± 1.8	65.00 ± 2.7
Phenothiazines					
Flupentixol (factor = 0.86)	5.2	0.52 ± 0.0	91.69 ±3.8	39.00 ± 1.9	32.00 ± 7.6
	15.1	1.52 ± 0.0	90.36 ± 1.9	38.19 ± 3.3	30.79 ± 2.6
	30.1	3.00 ± 0.1	89.89 ± 1.3	37.97 ± 2.7	31.94 ±1.0
Zuclopenthixol (factor = 0.85)	5.1	0.76 ± 0.1	90.78 ± 3.9	66.93 ± 2.1	41.90 ± 3.1
	51	8.60 ± 0.1	90.05 ± 0.2	72.23 ± 1.4	34.93 ± 4.3
	102.1	17.85 ± 0.1	89.74 ± 0.7	72.66 ± 0.8	41.34 ± 0.3

Note: The long-term stability measurements of substance solutions stored at -20 ^{o}C for 12 months were carried out by measuring each concentration four times (n= 4). SD: standard deviation, mAU: peak height.

4.1.9.8 Freeze/ Thaw Stability

The stability of the substances in the sample solution was analyzed under the TDM routine conditions for a given time interval. The solutions were stored in eppendorf caps for 30 minutes at -20^{o}C until frozen. They frozen solutions were thawed within 15 minutes at room temperature under centrifugation, respectively. This process was carried out repeatedly for the sample solutions and four times for each concentration. The results (see table 34) obtained correspond to the specification according to DIN 32645, which is between 90–110% of the mean of the corresponding control value.

Table 34. Freeze/thaw stability test result for antipsychotics.

Substance	Conc. (ng/ml)	Mean Measure-ment (mAU ± SD)	Mean Control Measurement (mAU ± SD)	Mean (%) ± SD
Butyrophenones				
Melperone (factor = 0.88)	22	3.40 ± 0.1	2.28 ± 0.0	149.12 ± 4.4
	50.2	6.72 ± 0.4	5.84 ± 0.1	115.07 ± 6.8
	101.2	13.96 ± 0.4	13.85 ± 0.4	100.79 ± 2.9
Benperidol	2	0.20 ± 0.0	0.14 ± 0.0	142.86 ± 7.1
	10	0.61 ± 0.0	0.62 ± 0.2	98.39 ± 4.8
	30	1.60 ± 0.0	1.89 ± 0.2	84.66 ± 2.1
Haloperidol	2	0.12 ± 0.0	0.13 ± 0.0	92.30 ± 0.0
	10	0.48 ± 0.0	0.64 ± 0.0	75.00 ± 0.0
	30	1.31 ± 0.1	2.03 ± 0.0	64.53 ± 4.9
Bromperiol	3	0.14 ± 0.0	0.13 ± 0.0	107.69 ± 0.0
	15	0.56 ± 0.1	0.63 ± 0.0	88.89 ± 15.9
	30	1.13 ± 0.1	1.25 ± 0.0	90.4 ± 8.0
Phenothiazines				
Flupentixol	5.2	0.54 ± 0.0	0.51 ± 0.0	105.88 ± 0.0

(factor = 0.86)	15.1	1.57 ± 0.0	1.46 ± 0.0	107.53± 0.0
	30.1	3.1 ± 0.0	2.90 ± 0.1	106.90 ± 0.0
Zuclopenthixol (factor = 0.85)	5.1	0.56 ± 0.0	0.62 ± 0.0	90.32 ± 0.0
	51	6.48 ± 0.0	6.56 ± 0.1	98.78 ± 0.0
	102.1	13.94 ± 0.0	13.47 ± 0.0	103.49 ± 0.0

Note: The freeze/thaw stability measurements of substance solutions were carried out by measuring each concentration four times (n=12 for each substance). SD: standard deviation, mAU: peak height.

4.1.9.9 Test of Robustness

The observation made was that the developed method remained unaffected by small and deliberate changes carried out during the measurements. This is proof of the reliability of the method. The pH value of the mobile phase was observed to have an impact on the measurement. There was no substance absorption observed at pH 6.0 (see table 35).

Table 35. Parameters applied to prove the robustness of the developed method.

Parameter	**Standard condition**	**Modifi-cation**	**Impact on the Method**	**Standard condition**	**Modifi-cation**	**Impact on the Method**
Butyrophenones				**Phenothiazines**		
Temperature (^{o}C)	30	40	Noise	30	40	Noise
Total run time (min)	35	25	Wider peak, but still reliable	35	25	Wider peak, but still reliable
pH value	5	4.3	Interference between MLP & BPD	5	5.2	None
		5.2	None		6	No absorption
		6	No absorption			
ACN/ MeOH portion (ml)	130/ 300	100 /330	None	130/ 300	100 /330	None

Acetonitrile (ml)	300	350 (+5%)	Slight peak deformation	300	350 (+5%)	Slight peak deformation
Methanol (ml)	130	80 (-5%)	Slight peak deformation	130	80 (-5%)	Slight peak deformation

Note: The robustness of the method was measured four times (n=4) at a concentration of 100 ng/ml for each substance.

4.1.9.10 Selectivity

The test substances were detected and quantified without the interference of other substances. The examined substances were the substances administered in combination with antipsychotic medications. These substances were developed from the pharmacovigilance data of the AGATE group in the psychiatry. The butyrophenone and the phenothiazine substances were examined at the optimal wavelength of 245 nm and 230 nm, respectively (see appendix 5 and appendix 6).

The results of the validation were successful; the substances meet all the required criteria for the determination of drugs in human serum for the purpose of therapeutic drug monitoring

4.1.9.11 Routine Application of the Validated Method

The validated method was applied for routine measurement of each substance. The blank serum do not contain any substance, the standard sample contains drug substances at known concentrations. The patients' samples were measured in a single procedure together with the blank, and the standard sample (see table 36 and figure 29 - 34).

Table 36. Routine measurement of the substances.

Substance	Conc. (ng/ml)	Peak height (mAU ± SD)	Retention time (min ± SD)	Amount (ng/ml)	Therapeutic reference range (ng/ml)
Melperone	22	4.98 ± 0.19	14.77 ± 0.02	22.09 ± 0.85	30 - 100

	50.2	11.075 ± 0.08	14.65 ± 0.005	49.12 ± 1.1	
	102.1	22.909 ± 0.12	14.64 ± 0.015	102.22 ± 0.54	
Pat. serum		4.187	14.586	18.58	
Benperidol	2	0.156 ± 0.003	16.32 ± 0.03	2.03 ± 0.003	1 - 10
	10	0.741 ± 0.001	16.39 ± 0.005	9.64 ± 0.005	
	30	2.315 ± 0.008	16.33 ± 0.015	30.12 ± 0.011	
Pat. serum		0.171	16.40	2.2	
Haloperidol	2	0.089 ± 0.001	24.13 ± 0.002	2.18 ± 0.01	1 - 10
	10	0.444 ± 0.003	24.22 ± 0.003	10.88 ± 0.6	
	30	1.24 ± 0.002	24.17 ± 0.005	29.79 ± 1.1	
Pat. Serum		0.20	23.90	4.95	
Bromperidol	3	0.1285 ± 0.007	27.25 ± 0.06	3.07 ± 0.16	1 - 10
	15	0.637 ± 0.01	26.75 ± 0.08	15.27 ± 0.19	
	30	1.261 ± 0.012	26.76 ± 0.065	30.64 ± 0.22	
Pat. Serum		0.502	27.05	12.02	
Flupentixol	5.2	0.115 ± 0.02	14.89 ± 0.003	5.24 ± 0.06	1 - 10
	15.1	0.332 ± 0.04	14.94 ± 0.05	15.18 ± 0.04	
	30.1	0.657 ± 0.02	14.96 ± 0.01	30.02 ± 0.02	
Pat. Serum		0.182	14.85	8.28	
Zuclopenthixol	5.1	1.489 ± 0.005	20.75 ± 0.002	5.4 ± 0.005	4 - 50
	51	11.937 ± 0.006	20.70 ± 0.001	51.84 ± 0.003	
	102.1	23.34 ± 0.013	20.69 ± 0.005	102. 09 ± 0.002	
Pat. Serum		8.961	20.65	38.93	

Note.: SD = standard deviation; Amount = concentration obtained after measurement.

Sample Chromatograms of the Substances Measured with the Validated Method

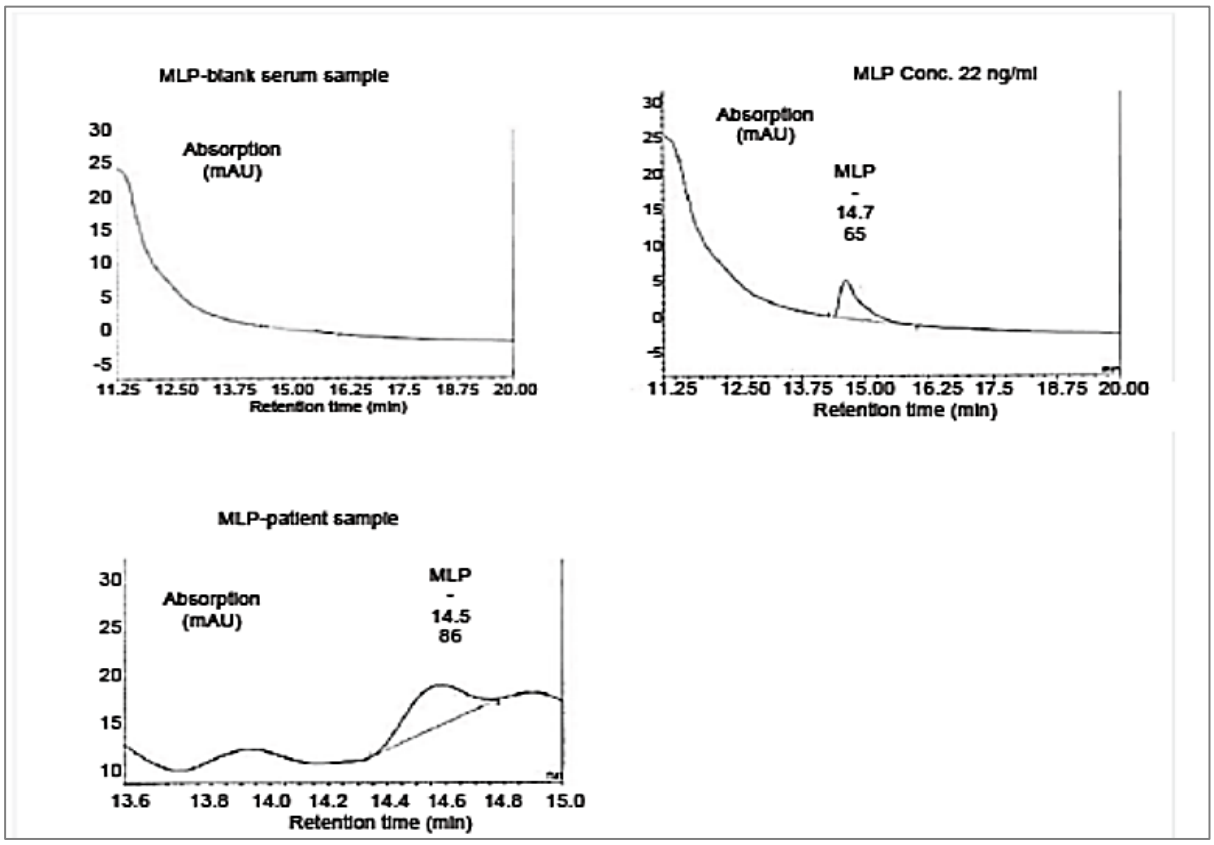


Figure 29. The standard MLP sample used during the measurements.

Note: measured concentration of 22 ng/ml, the blank serum sample (0 ng/ml) and the patient's sample. mAU = peak height.

The blank serum, top left, does not contain MLP. The smallest standard concentration applied is 22 ng/ml, see chromatogram, top right. The chromatogram of the patient's serum with the dose of 25 mg is underneath. The concentration obtained (18.58 ng/ml) is within the therapeutic reference range for MLP (30 - 100 ng/ml). Mobile phase solvent consists of 30 % ACN, 13 % MeOH, 0.4 % TEMED, and 56.6 % demineralized water.

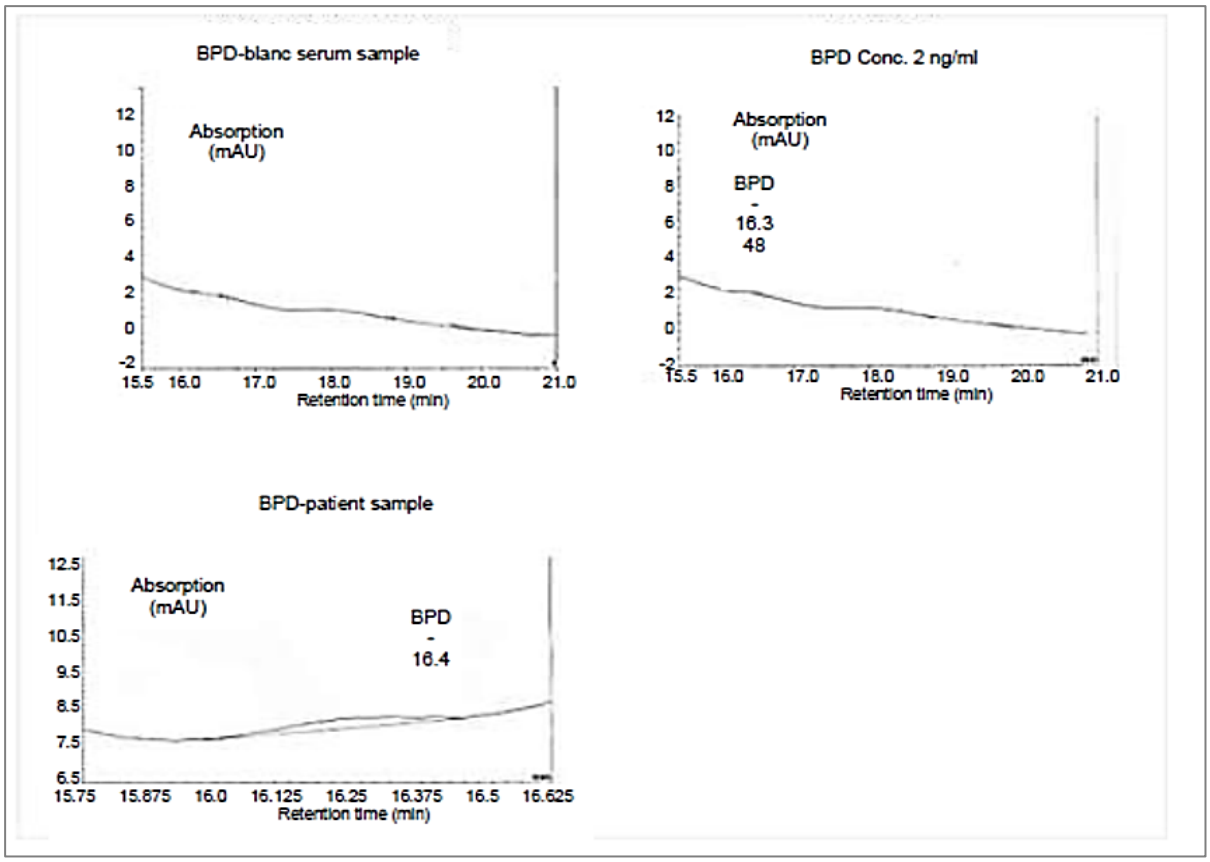


Figure 30. The standard BPD sample used during the measurements.

Note: measured concentration of 2 ng/ml, the blank serum sample (0 ng/ml), and the patient's sample. mAU = peak height.

The blank serum, top left, does not contain BPD. The smallest standard concentration applied is 2 ng/ml, see chromatogram top right. The chromatogram of the patient's serum with the dose of 26 mg is underneath. The concentration obtained (2.20 ng/ml) is within the therapeutic reference range for BPD (1- 10 ng/ml). Mobile phase solvent consists of 30 % ACN, 13 % MeoH, 0.4 % TEMED, and 56.6 % demineralized water.

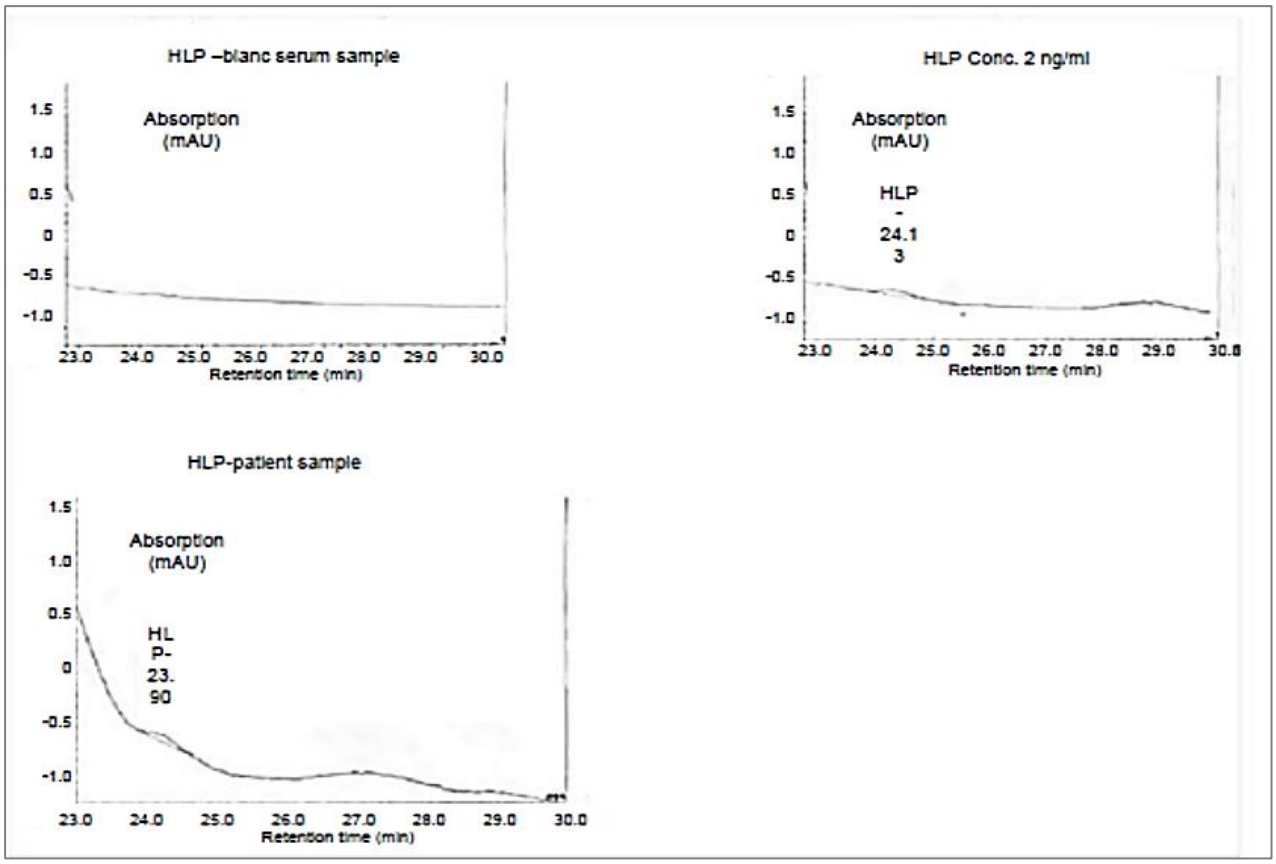


Figure 31. The standard HLP sample used during the measurements.

Note: measured concentration of 2 ng/ml, the blank serum sample (0 ng/ml), and the patient's sample. mAU = peak height.

The blank serum, top left, does not contain HLP. The smallest standard concentration applied is 2 ng/ml, see chromatogram top right. The chromatogram of the patient's serum underneath has no dose information. The concentration (4.95 ng/ml) obtained is within the therapeutic reference range for HLP (1- 10 ng/ml). Mobile phase solvent consists of 30 % ACN, 13 % MeOH, 0.4 % TEMED, and 56.6 % demineralized water.

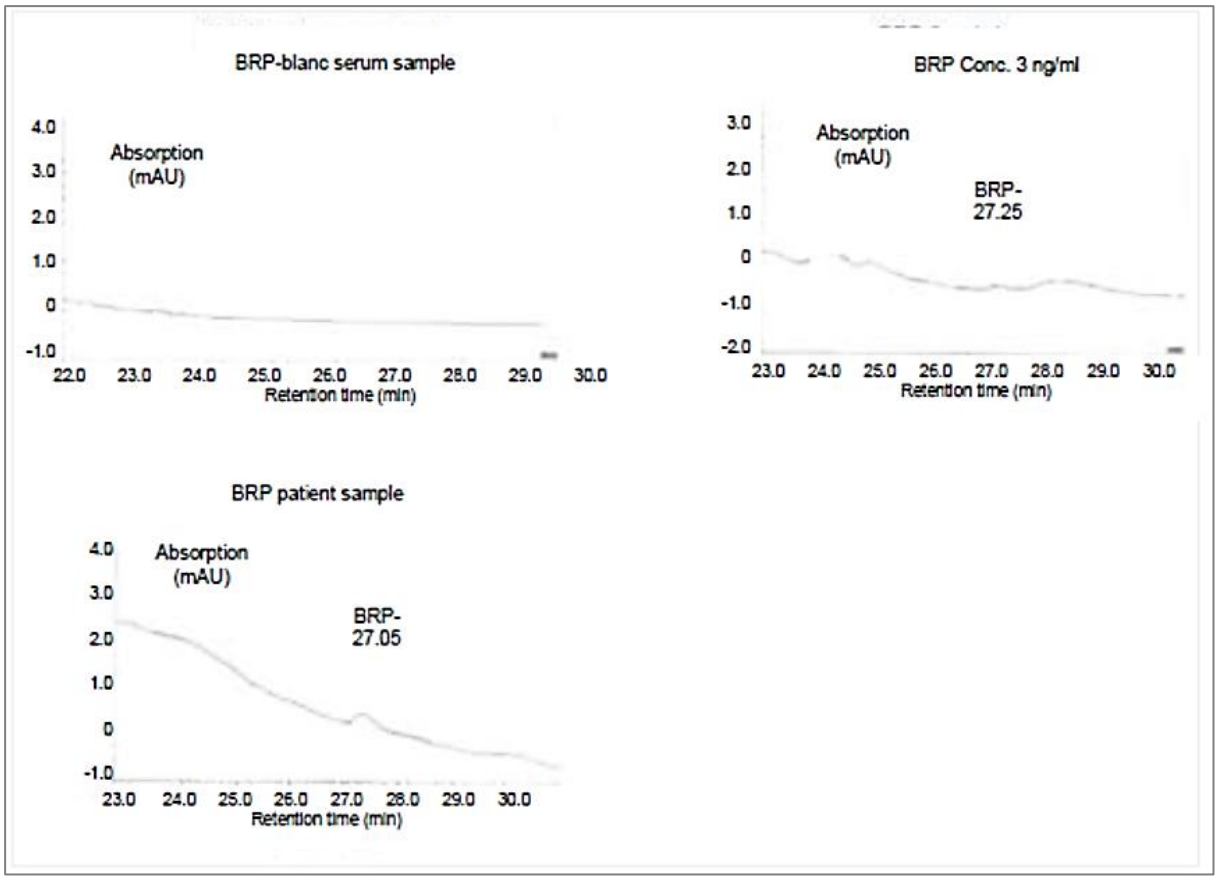


Figure 32. The standard BRP sample used during the measurements.

Note: measured concentration of 3 ng/ml, the blank serum (0 ng/ml) and the patient's sample. mAU = peak height.

The blank serum, top left, does not contain BRP. The smallest standard concentration applied is 3 ng/ml, see chromatogram top right. The chromatogram of the patient's serum has no dose information. The concentration (12.02 ng/ml) obtained is slightly above the therapeutic reference range for BRP (1- 10 ng/ml). Mobile phase solvent consists of 30 % ACN, 13 % MeOH, 0.4 % TEMED, and 56.6 % demineralized water.

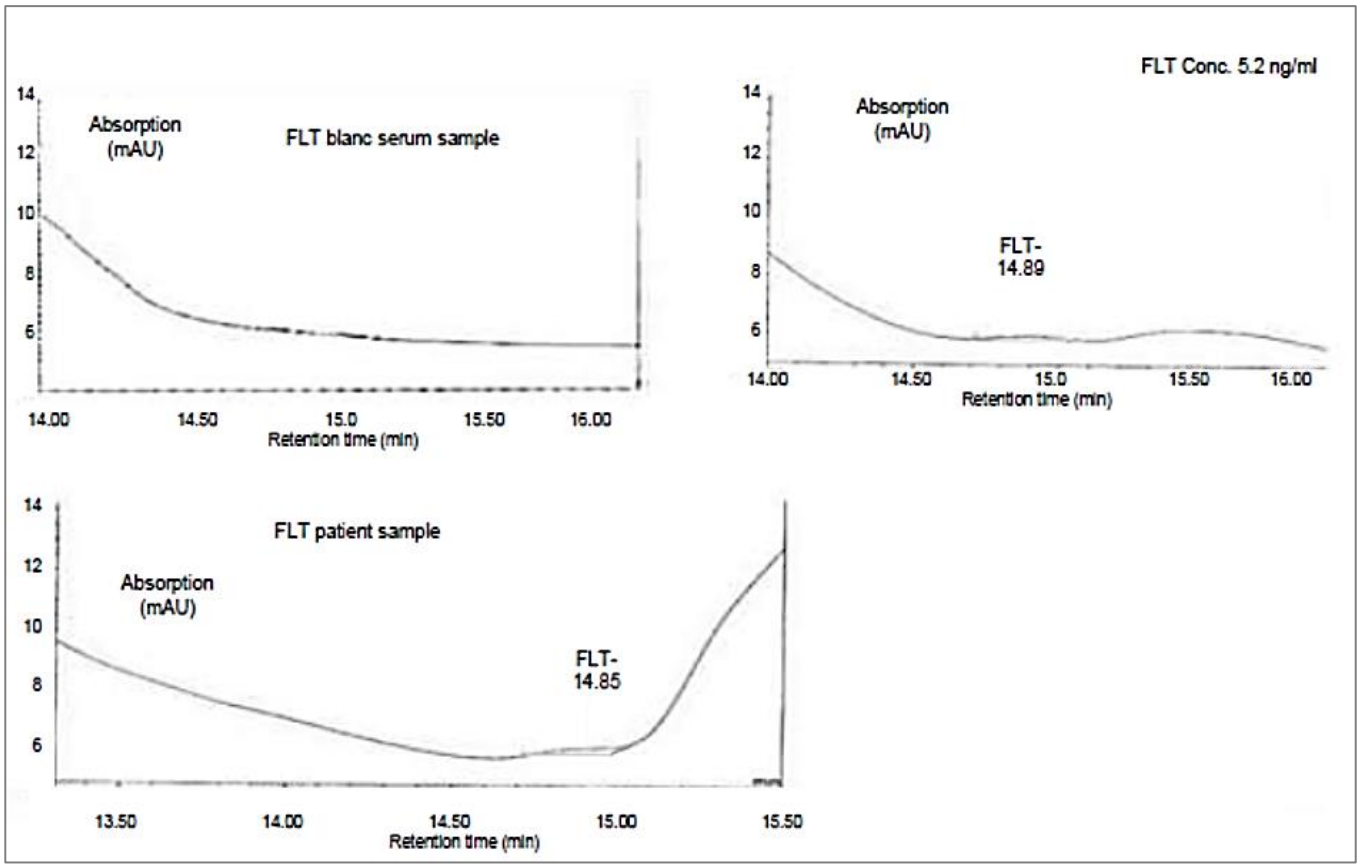


Figure 33. The standard FLT sample used during the measurements.

Note: concentration of 5.2 ng/ml, the blank serum sample (0 ng/ml), and the patient's smaple. mAU = peak height.

The blank serum, top left, does not contain FLT. The smallest standard concentration applied is 5.1 ng/ml, see chromatogram top right. The chromatogram of the patient's serum with the dose of 15 mg is underneath in the diagram. The concentration obtained (8.28 ng/ml) is within the therapeutic reference range for FLT (1- 10 ng/ml). Mobile phase solvent consists of 30 % ACN, 13 % MeOH, 0.4 % TEMED, and 56.6 % demineralized water.

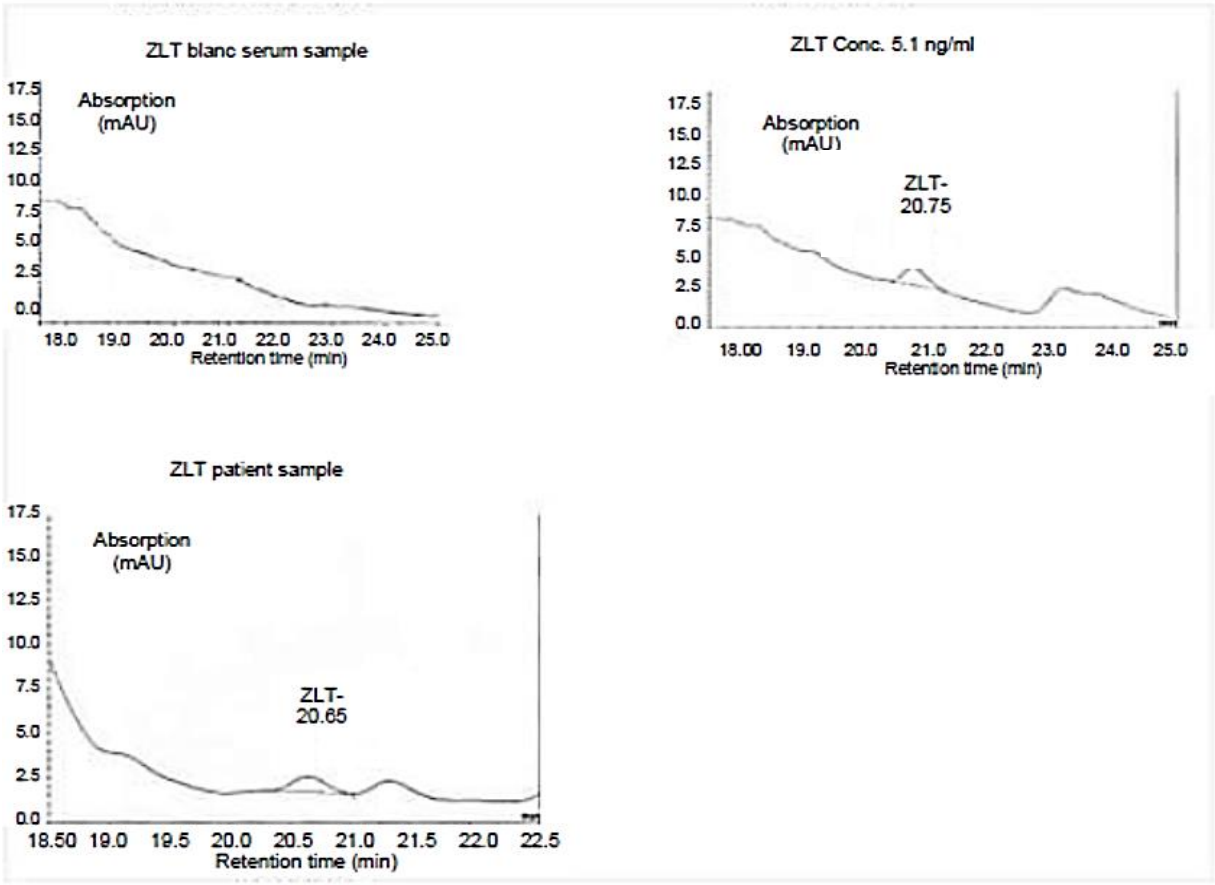


Figure 34. The standard ZLT sample used during the measurements.

Note: concentration of 5.1 ng/ml, the blank serum sample, and the patient's sample. mAU = peak height.

The blank serum, top left, does not contain ZLT. The smallest standard concentration applied is 5.1 ng/ml, see chromatogram top right. The chromatogram of the patient's serum with the dose of 200 mg /14 days (14.29 mg/day) is underneath in the diagram. The concentration obtained (38.93 ng/ml) is within the therapeutic reference range for ZLT (4- 50 ng/ml). Mobile phase solvent consists of 30 % ACN, 13 % MeOH, 0.4 % TEMED, and 56.6 % demineralized water.

4.2 Application of the Developed Method for TDM

The developed method was applied for the therapeutic drug monitoring of the substances administered to patients at different doses. The results of the measured serum samples were either within the expected reference ranges, below or above it. The interpretation of the result depends on individual patients' factors that have impact on the serum concentration such as gender, age, co-medications, clinical status and the history of their sicknesses.

4.2.1 Demographic Data of Study Patients

Some patients' data-information were not given, so that in some cases, the numbers used for the evaluation were less compared to the total number of patients (see table 37). The main diagnoses were determined especially, according to chapter five, mental and behavioural diaorders of the International Statistical Classfication of Diseases and Related Health Problems revison 10, Version for 2010.

Table 37. Summary Data of Study Patients.

Substances	Number of patients (n)	Age (year) (Mean ± SD)	Hight (cm) (Mean ± SD)	Weight (kg) (X ± SD)	BMI (cm /m^2) (X ± SD)	Gender (M, male; F, fe-male)	Main Diagnoses
MLP	7	52.86 ± 11.7	n = 4 168.43	70.57 ± 11.06	26 ± 5.9	M = 5 F = 2	n = 6 F20.0 = 4 F25.1 = 1 F20.5 = 1
BPD	5	48.8 ± 14.1	170.8 ± 7.2	79 ± 18.8	27.2 ± 6.5	M = 1 F = 4	F20.0 = 4 F25.1 = 1

HLP	566	49.75 ±	n = 318 168.6 ± 4.2	n = 461 77.4 ± 12.1	n = 436 22.62 ± 10.3	M = 229 F = 337	n = 467 F20 = 76 F20.0 = 179 F* = 210 G40 = 1 G81.1 = 1
BRP	2	58.6 ± 13.4	NN	n = 1 60.9	26 ± 3.5	F = 2	F20
FLT	41	46.0 ± 10.8	n = 32 171.66 ± 10.8	83.80 ± 19.9	n = 34 29.9 ± 7.9	M = 22 F = 19	n = 32 F20.0 = 27 F25 = 1 F25.2 = 3 F33.2 = 1
ZLT	3	56.7 ± 8.7	n = 2 172.5 ± 0.5	n = 2 85.5 ± 11.5	n = 2 29.0 ± 4.0	M = 2 F = 1	n = 2 F20 = 1 F20.5 =1

Note: F*: These are subdivisions of other main diagnoses stated for HLP. F32=5, F40.2=1, F60.3=1, F03=4, F05.0=1, F06.2=1, F06.8=2, F07.1=1, F10.0=1, F10.1=12, F10.2=2, F10.7=3, F13.2=1, F19.2=7, F20=76, F20.0=179, 20.1=9, F20.2=13, F20.3=2, F20.5=30, F21=2, F22.0=3, F25=3, F25.0=11, F25.1=3, F25.2=16, F25.9=6, F29=16, F31=2, F31.0=1, F31.1=1, F32.2=2, F32.3=1, F33.1=1, F33.2=1, F33.3=5, F41.1=1, F43.1=1, F60=1, F60.2=12, F60.3=3, F60.7=1, F61=1, F70=5, F70.0=2, F71=6, F71.1=4, F72.1=2, F84.1=1, G40=1, G81.1=1. NN = not known.

4.2.2 Doses and Drug Concentrations

The relationship between the administered dose and the serum concentrations obtained, were calculated. Some request forms did not indicate any dose, so that the dose-related concentration could not be calculated. The bioavailability and elimination half-life of the substances were taken from the summary of their product characteristics.

4.2.2.1 Dose- concentration relationship of measured MLP sample and the DRR

The explanation for the calculation of the dose-related reference range with the fraction of concentration and dose is derived from the AGNP Consensus Guidelines for Therapeutic Drug Monitoring in Psychiatry: Update 2011 [Hiemke et al., 2011], which according to the consensus is based on the study of Borgstrom et al. (1982).

The multiplication of the factor 0.14 with the maintenance dose (C/D low x dose) gives the low value of the dose-related reference range (DRR-low) for melperone and the multiplication of the factor 0.28 with the maintenance dose (C/D high) gives the high value of the dose-related reference range (DRR-high) for melperone. One measured serum sample of melperone was within the expected DRR at concentration of 3.5 ng/ml with the administered dose of 25 mg. Three samples were below the DRR and three samples were not calculated, because of the missing dose information (see table 38 and figure 35). The measurements of the samples with missing dose information were requested in other to verify if the patient take the medication. The value of the measured concentration ranges from 4 – 19 ng/ml and the DRR ranges from 0 – 21 ng/ml. All the measured patients' samples contain co-medications.

Table 38 TDM information of MLP in measured patients' serum.

Dose (mg/d)	Conc. (ng/ml)	DRR (ng/ml)	TRR (ng/ml)
30	13	4-8	30-100
25	4	4–7	30-100
NN	13	NN	30-100
NN	4	NN	30-100

25	19	4–7	30-100
75	6	11–21	30-100
NN	15	NN	30-100

Note: NN: not known, DRR: dose-related reference range, Conc: concentration. TRR: therapeutic reference range, Conc: concentration.

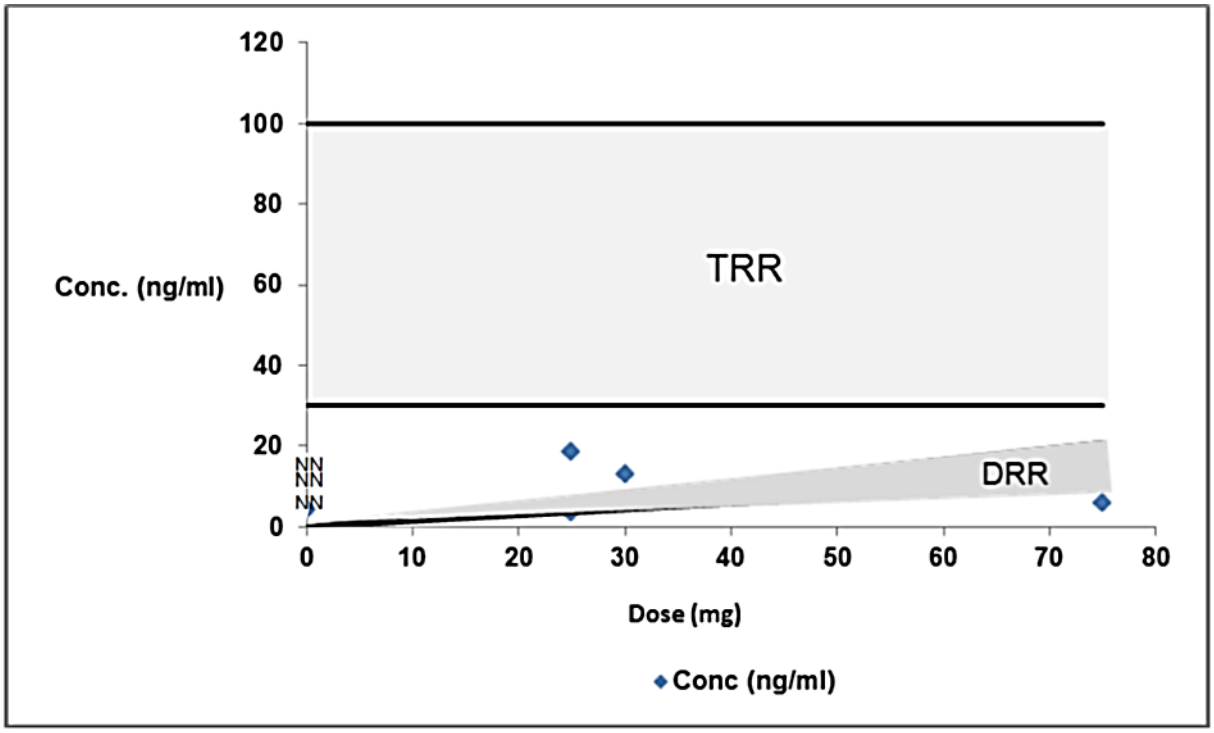


Figure 35. Dose-concentration relationship of seven MLP patients' specimens.

The horizontal area indicates the therapeutic reference range (TRR) and the triangular area the dose-related reference range (DRR).

4.2.2.2 Dose- Concentration Relationship of Measured BPD Sample and the DRR

The explanation for the calculation of the dose-related reference range with the fraction of concentration and dose is derived from the AGNP Consensus Guidelines

for Therapeutic Drug Monitoring in Psychiatry: Update 2011 [Hiemke et al., 2011], which according to the consensus is based on the study of Seiler et al. (1994).

From the given maintenance dose of 40, 26, 32, and 12 mg BPD, a concentration of 7.0, 2.2, 13, and 12 ng/ml were measured, respectively. Two measurements were above the expected therapeutic reference range (see table 39 and figure 36). The value of the measured concentration is higher when compared with that of the corresponding values of DRR. The measured concentration ranges from 2.2 ng/ml to 13 ng/ml and the expected DRR ranges from 1.8 ng/ml to 12.4 ng/ml. All the measured patients' samples contain co-medications.

Table 39 TDM information of BPD in measured patients' serum.

Dose (mg)	Conc. (ng/ml)	DRR (ng/ml)	TRR (ng/ml)
40	7.0	6.0 – 12.4	1-10
26	2.2	3.9 – 8.06	1-10
32	13.0	4.8 – 9.92	1-10
12	12.0	1.8 – 3.72	1-10

Note: DRR: dose-related reference range, TRR: therapeutic reference range, Conc: concentration.

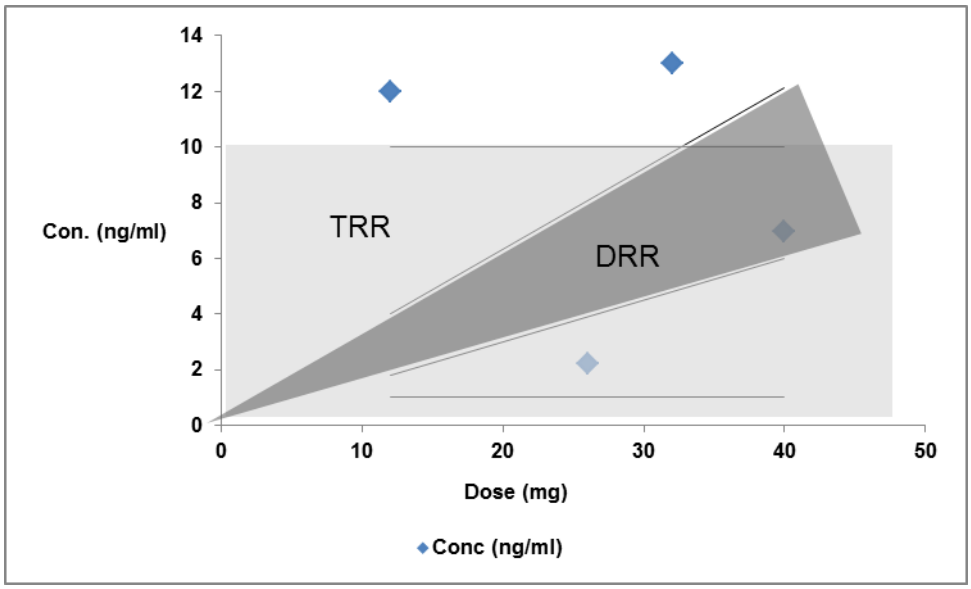


Figure 36. Dose-concentration relationship of four BPD patients' specimens.

The horizontal area indicates the therapeutic reference range (TRR) and the triangular area the DRR.

4.2.2.3 Dose- Concentration Relationship for HLP

The explanation for the calculation (see table 40) of the dose-related reference range with the fraction of concentration and dose is derived from the AGNP Consensus Guidelines for Therapeutic Drug Monitoring in Psychiatry: Update 2011 [Hiemke et al., 2011], which according to the consensus is based on the study of Cheng et al. (1987).

Table 40 Calculation of dose-related reference range for haloperidol.

Factor Multiplication	Result	Value
C/D low x Dose	DRR-low	0.6
C/D high x Dose	DRR-high	1.0

Note. C/ D low and C/ D high are factors obtained from the study of Cheng et al. (1987) for calculating dose-related reference range. DRR = Dose-related reference range.

Thirty-five samples with the maintenance dose of oral haloperidol (see table 41) were measured, respectively. There was no dose information for two samples. Sixteen samples were within the TRR and fourteen samples were above the TRR. No concentration was obtained in three samples (see figure 37). The maximum measured concentration is higher than that of DRR (see figure 38). Six of the measured patients' samples do not contain co-medications (see table 42). The mean measured concentration was 5.67 ng/ml and the median is 6.95 ng/ml. The values are low compared to the data of patients' samples that contain co-medication. Their mean measured concentration was 12.53 ng/ml and the median was 10.00 ng/ml.

Table 41 TDM information of HLP in patients' serum.

Dose (mg)	Conc. (ng/ml)	DRR (ng/ml)	TRR (ng/ml)
NN	0.0	0.0	1 - 10
NN	1.6	0.0	1 - 10
0,5	1.0	0.3 – 0.5	1 - 10
1	1.4	0.6 – 1.0	1 - 10
3	1.4	0.8 - 3.0	1 - 10
5	0.0	3.0 - 5.0	1 - 10
5	3.4	3.0 - 5.0	1 - 10
5	20	3.0 - 5.0	1 - 10
6	1,7	3.6 - 6.0	1 - 10
7,5	8,2	4.5 - 7.5	1 - 10
8	2,9	4.8 - 8.0	1 - 10
8	7	4.8 - 8.0	1 - 10
10	7,3	6.0 - 10.0	1 - 10
10	11	6.0 - 10.0	1 - 10
10	35	6.0 - 10.0	1 - 10
10	5	6.0 - 10.0	1 - 10
10	6,9	6.0 - 10.0	1 - 10
10	49	6.0 - 10.0	1 - 10
10	7,3	6.0 - 10.0	1 - 10

15	12	9.0 - 15.0	1 - 10
15	21	9.0 - 15.0	1 - 10
20	8,8	12.0 - 20.0	1 - 10
20	25	12.0 - 20.0	1 - 10
20	22	12.0 - 20.0	1 - 10
20	18	12.0 - 20.0	1 - 10
20	20,3	12.0 - 20	1 - 10
20	2,9	12.0 - 20.0	1 - 10
20	7,59	12.0 - 20.0	1 - 10
25	13	15.0 - 25.0	1 - 10
30	18	18.0 - 30.0	1 - 10
30	0	18.0 - 30.0	1 - 10
30	21	18.0 – 30.0	1 - 10
30	10	18.0 – 30.0	1 - 10
40	26	24.0 - 40.0	1 - 10
50	1,7	30.0 - 50.0	1 - 10

Note: DRR: dose-related reference range, TRR: therapeutic reference range, Conc: concentration. NN = not known.

Table 42 Data of patients' HLP samples without co-medication.

Dose (mg)	Conc. (ng/ml)	DRR	TRR
7.5	8.2	4.5 – 7.5	1 - 10
8.0	7.0	4.8 -8.0	1 - 10
10.0	6.9	6.0 – 10.0	1 - 10
10.0	7.3	6.0 – 10.0	1 - 10
20.0	2.9	12.0 – 20.0	1 - 10
50.0	1.7	30.0 – 50.0	1 - 10

Note: Six patients's samples were without co-medication. Three concentrations of the measured serum samples were within the dose-related reference range and therapeutic reference range at the maintenance dose of 8.0 mg and 10.0 mg.

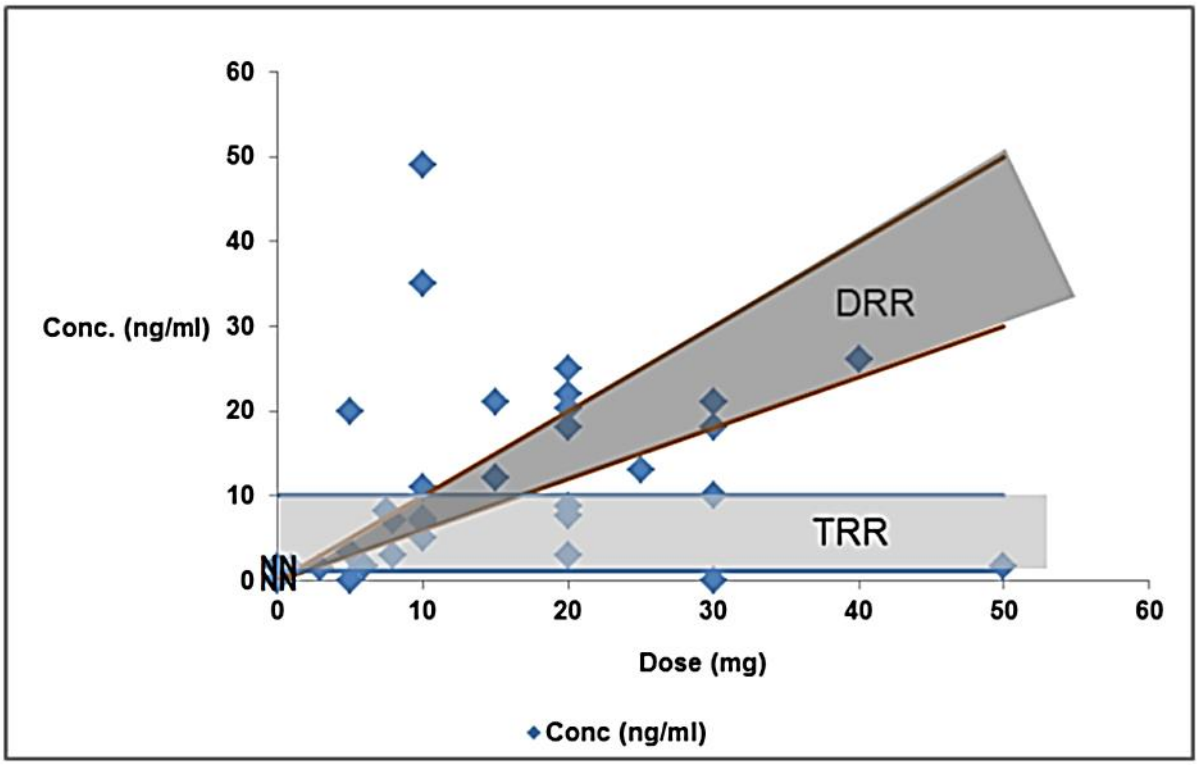


Figure 37. Dose-concentration relationship of thirty-five HLP patients' specimens.

The horizontal area indicates the therapeutic reference range (TRR).The triangular area the dose-related reference range (DRR).

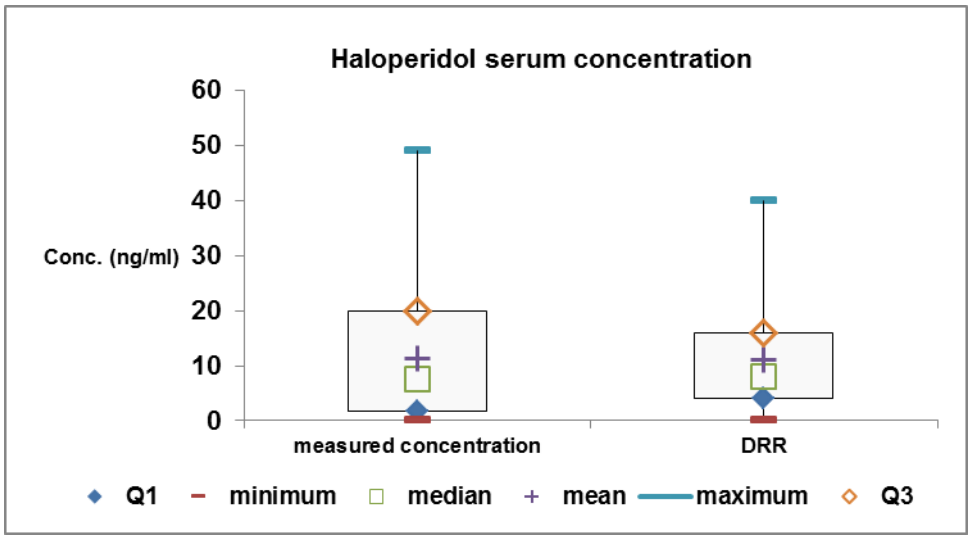


Figure 38. Comparison between the measured concentration and the dose-related reference range (DRR) of haloperidol.

The comparison between the measured concentration and the dose-related reference range (DRR) was evaluated. Q = quartile. For measured concentration, Q1 = 1.7 ng/ml, minimum = 0 ng/ml, median = 7.59 ng/ml, mean = 11.35 ng/ml, maximum = 49 ng/ml, Q3 = 20. For DRR, Q1 = 4.0 ng/ml, minimum = 0ng/ml, median =8.0 ng/ml, mean = 11.6 ng/ml, maximum = 40.0 ng/ml, Q3 = 16 ng/ml. The values of the measured concentrations and the DRR are at close intervals to the mean and the median.

4.2.2.4 Dose- Concentration Relationship of Measured BRP Samples and the DRR

The explanation for the calculation of the dose-related reference range with the fraction of concentration and dose (C/D) is derived from the AGNP Consensus Guidelines for Therapeutic Drug Monitoring in Psychiatry: Update 2011 [Hiemke et al., 2011], which according to the consensus is based on the study of Lee et al. (2006).

Three patients' serum samples containing bromperidol were measured. One of the samples has no dose information (see table 43). The concentrations of 8 ng/ml and 2 ng/ml obtained for two serum samples with doses of 6 mg and 3 mg, respectively, were within the TRR (see figure 39). The DRR was obtained by multiplying each dose with factor 0.09 (C/D low) and 0.19 (C/D high). The measured three samples contain co-medications. The mean measured concentration was 7ng/ml and the median was 8 ng/ml.

Table 43 TDM information of BRP in patients' serum.

Dose	Conc.	DRR	TRR

(mg)	(ng/ml)	(ng/ml)	(ng/ml)
6	8	0.54 – 1.14	1-10
NN	12	0.00	1-10
3	2	0.27 – 0.57	1-10

Note: DRR: dose-related reference range, TRR: therapeutic reference range, Conc: concentration. NN = not known"

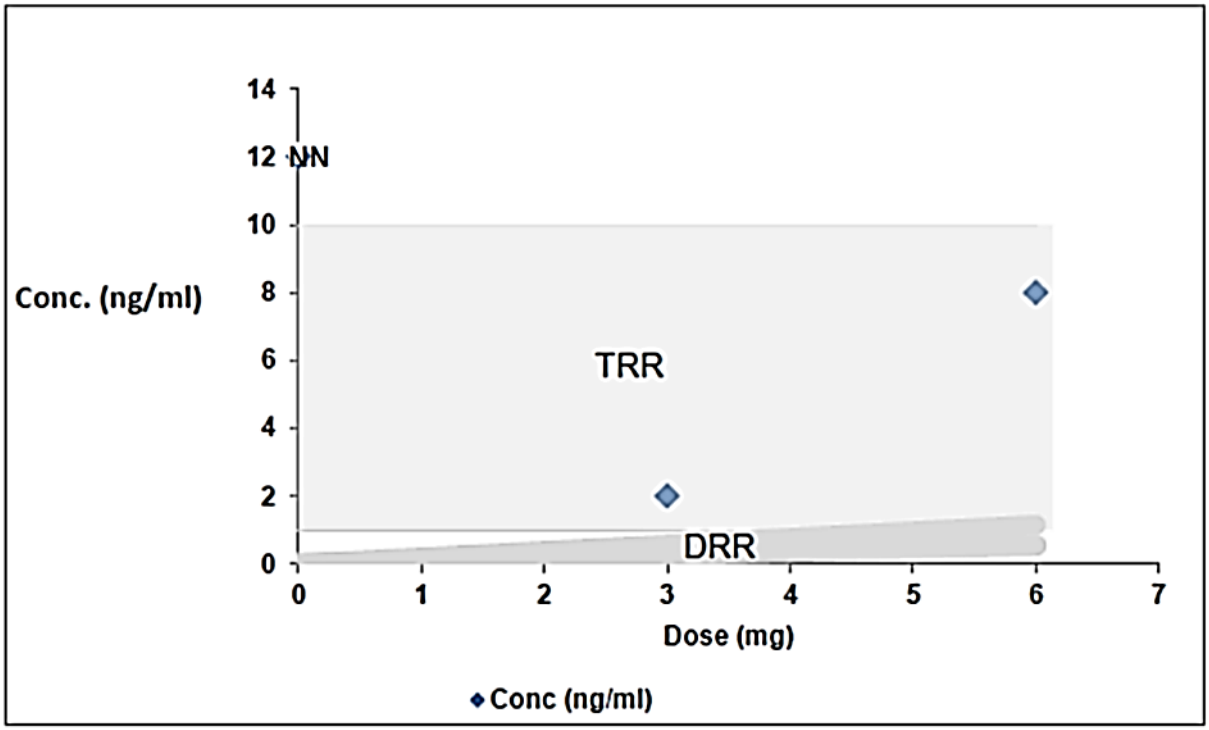


Figure 39. Dose-concentration relationship of three BRP patients' specimens.

The horizontal area indicates the therapeutic reference range (TRR). The triangular area the dose-related reference range (DRR). All the corresponding DRR of the maintenance dose were outside the therapeutic reference range.

4.2.2.5 Dose- concentration relationship of measured FLT samples and the DRR

The explanation for the calculation of the dose-related reference range with the fraction of concentration and dose (C/D) is based on the available pharmacokinetic data

for flupentixol. The values of (C/D low) and C/D high were multiplied with the maintenance dose (see table 44) to obtain the DRR.

Table 44 Calculation of dose-related reference range of flupentixol.

Factor Multiplication	Result	Value
Applied Data		
C/D low x Dose	DRR-low	0.66 (oral); 1.65 (depot)
C/D high x Dose	DRR-high	1.74 (oral); 4.35 (depot)
Consensus Data		
C/D low x Dose	DRR-low	0.78
C/D high x Dose	DRR-high	0.87

Note: C/ D low and C/ D high are factors obtained from the applicable data for calculating dose-related reference range for depot and oral flupentixol. DRR = Dose-related reference range. Applied data = data used in TDM laboratory Regensburg; Consensus data = data derived from AGNP-consensus guideline.

Oral flupentixol dose was administered to 29 patients with schizophrenic and psychotic disorders and 13 patients received depot-flupentixol (administered depot-flupentixol dose were: 100 mg/14d, 60 mg/14d, 65 mg/14d, 50 mg/14d and 17.2 mg/14d).

Forty measured patients' samples contain co-medications. Two samples do not contain co-medication (see table 45). The dose information of two patients out of these forty samples was not given (see table 46) and there was no measurement carried out with one of the patient's FLT serum sample because the sample did not fulfill the laboratory requirements. The sample volume was 0.5 ml instead of recommended 2 ml. The sample collection was carried out after the medication intake instead of the recommended trough concentration by sample collection before the next medication intake. The concentration of 19 samples was within the TRR and 15 samples were above it (see figure 40). The mean measured concentration for samples containing co-medication was 9 ng/ml and the median was 7 ng/ml (see figure 41).

Table 45 Data of patients' FLT samples without co-medication.

Dose (mg)	Conc. (ng/ml)	DRR	TRR
6.0	23	4 – 10	1 - 10
20.0	7	13-35	1 - 10

Note: Monotherapy with oral flupentixol, mean measured concentration was 15.10 ng/ml.

Table 46 TDM information of all measured FLT in patients' serum.

Dose (mg)	**Conc. (ng/ml)**	**DRR (ng/ml)**	**TRR (ng/ml)**
7.14	20	12 - 31	1 – 10
7.14	21	12- 31	1 – 10
10.00	4	7 - 17	1 – 10
7.14	0	12 - 31	1 – 10
20.00	17	13- 35	1 – 10
10.00	24	7 - 17	1 – 10
7.14	3	12 - 31	1 – 10

7.14	9	12- 31	1 – 10
15.00	2	10 - 26	1 – 10
7.14	13	12- 31	1 – 10
5.00	25	3- 9	1 – 10
10.00	5	7 - 17	1 – 10
20.00	0	13- 35	1 – 10
4.29	11	7- 19	1 – 10
7.14	12	12 - 31	1 – 10
NN	7	0	1 – 10
30.00	2	20 - 52	1 – 10
30.00	30	20 - 52	1 – 10
1.00	7	1 - 2	1 – 10
15.00	7	10 - 26	1 – 10
3.57	3	6 - 16	1 – 10
20.00	2	13- 35	1 – 10
4.00	0	3 - 7	1 – 10
10.00	0	7 - 17	1 – 10
1.23	3	2- 5	1 – 10
4.29	7	7- 19	1 – 10
10.00	5	7 - 17	1 – 10
5.00	0	3- 9	1 – 10
6.00	23	4 - 10	1 – 10
15.00	22	10 - 26	1 – 10
20.00	20	13- 350	1 – 10
15.00	0	10 - 26	1 – 10
20.00	7	13- 35	1 – 10
4.00	13	3 - 7	1 – 10
15.00	8	10 - 26	1 – 10
15.00	0	10 - 26	1 – 10
10.00	0	7 - 17	1 – 10
20.00	10	13- 35	1 – 10

NN	18	0	1 – 10
4.64	9	8 - 20	1 – 10
7.14	7	12 - 31	1 – 10
10.00	18	7 - 17	1 – 10

Note: DRR = dose-related reference range, TRR = therapeutic reference range, Conc: concentration. NN = not known.

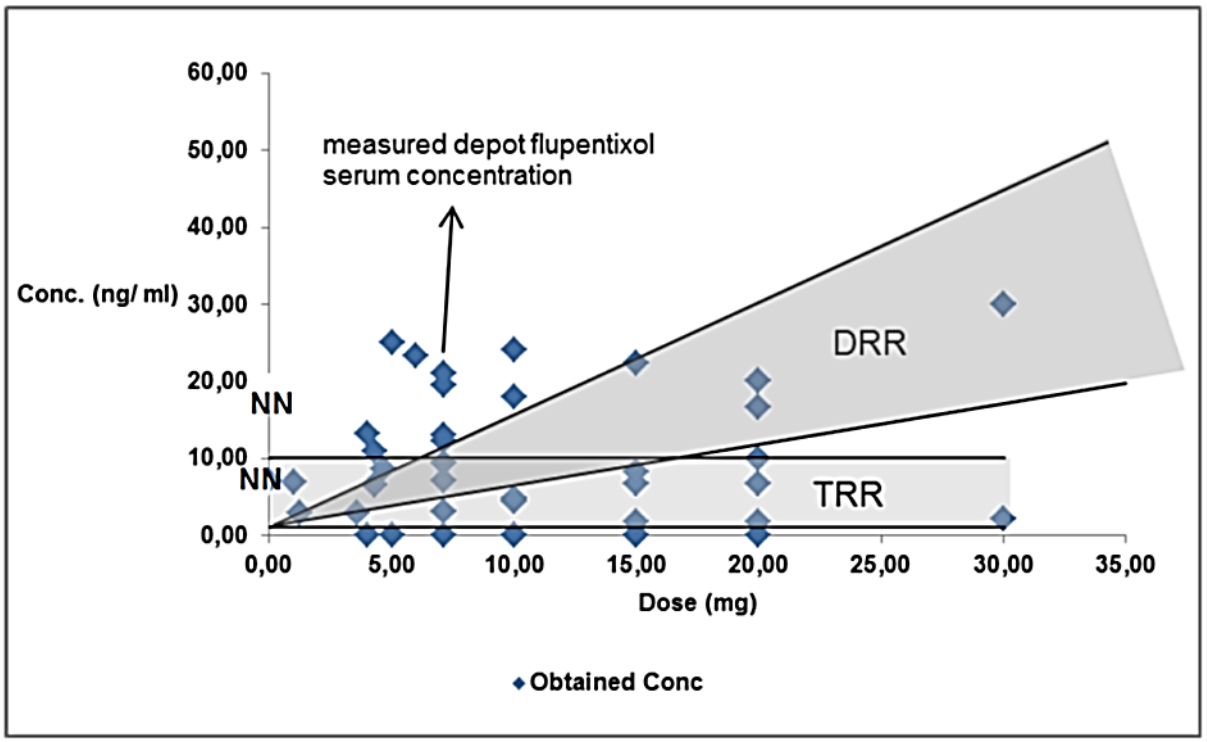


Figure 40. The dose-concentration relationship of fourty-two flupentixol patients' specimens.

The horizontal area indicates the therapeutic reference range (TRR) and the triangular area the dose related reference range (DRR).

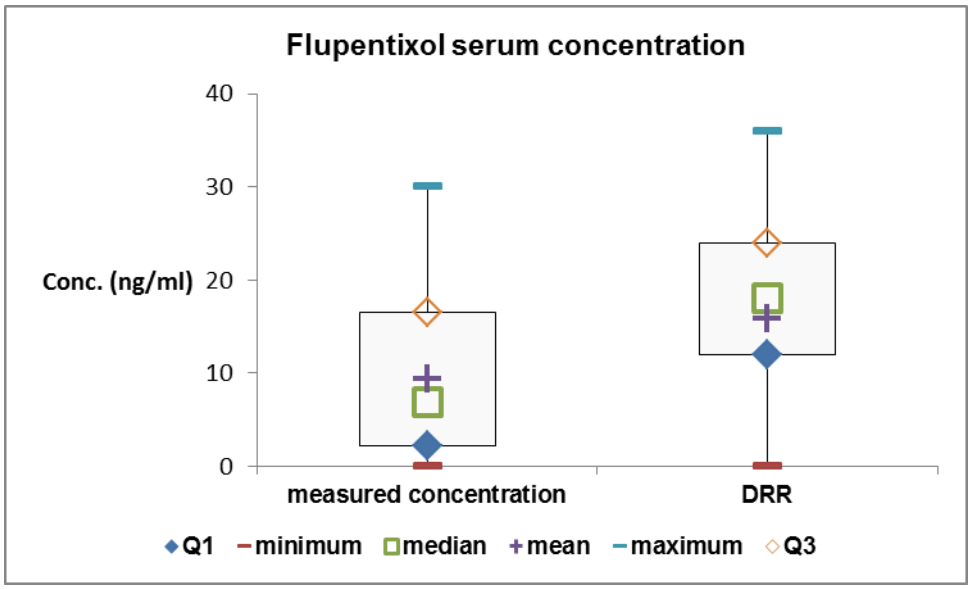


Figure 41. Measured concentration of flupentixol in comparison with the expected DRR.

The comparison between the measured concentration and the dose-related reference range (DRR). Q = quartile. For measured concentration Q1 = 2.2 ng/ml, minimum = 0 ng/ml, mean = 9.35 ng/ml, maximum = 30 ng/ml, Q3 = 16.60 ng/ml, median = 6.95 ng/ml. For DRR Q1 = 12.0 ng/ml, minimum = 0 ng/ml, median = 18 ng/ml, mean = 15.89 ng/ml, maximum = 36.0 ng/ml, Q3 = 24 ng/ml.

Comparison of Consensus DRR-Data with the Applied DRR-Data for FLT

The DRR of the measured concentrations were compared between the consensus data and the applied data. The Flt maintenance doses were multiplied with the consensus data of 0.78 (C/D low) and 0.87 (C/D high), to obtain the corresponding DRR for FLT (see table 47). The calculation with the applied DRR data has been shown in table 46 above.

Table 47 Result of the DRR calculated according to consensus.

Dose (mg)	**Concentration (ng/ml)**		
	Measured conc. (ng/ml)	DRR low	DRR high
7.14	19.5	5.6	6.2
7.14	21.0	5.6	6.2
10.00	4.4	7.8	8.7
7.14	0.0	5.6	6.2
20.00	16.6	15.6	17.4
10.00	24.0	7.8	8.7
7.14	3.0	5.6	6.2
7.14	9. 5	5.6	6.2
15.00	1.7	11.7	13.1
7.14	13.0	5.6	6.2
5.00	25.0	3.9	4.4
10.00	4.8	7.8	8.7
20.00	0.0	15.6	17.4
4.29	10.9	3.4	3.7
7.14	12.3	5.6	6.2
NN	7.0	0.0	0.0
30.00	2.2	23.4	26.1
30.00	30.0	19.8	52.2
1.00	6.9	0.78	0.87
15.00	6.7	1.7	13.1

3.57	2.9	2.8	3.1
20.00	1.8	15.6	17.4
4.00	0.0	3.1	3.5
10.00	0.0	7.8	8.7
1.23	2.8	1.0	1.1
4.29	6.5	3.4	3.7
10.00	4.5	7.8	8.7
5.00	0.0	3.9	4.4
6.00	23.4	4.7	5.2
15.00	22.3	11.7	13.1
20.00	20.0	15.6	17.4
15.00	0.0	11.7	13.1
20.00	6.8	15.6	17.4
4.00	13.3	3.1	3.5
15.00	8.3	11.7	13.1
15.00	0.0	11.7	13.1
10.00	0.0	7.8	8.7
20.00	10.0	15.6	17.4
NN	18.0	0.0	0.0
4.64	8.6	3.6	4
7.14	7.0	6.0	6.2
10.00	18.0	7.8	8.7

Note: The DRR was calculated according to values in the TDM consensus. DRR = dose-related reference range. NN = not known.

The consensus DRR data is narrow and closer to the therapeutic reference range while the applied DRR data is wider and above the therapeutic reference range. The low DRR value of the consensus data is comparably higher than that of the applied data (see figure 42). More than half of the measured concentrations were above the consensus DRR range.

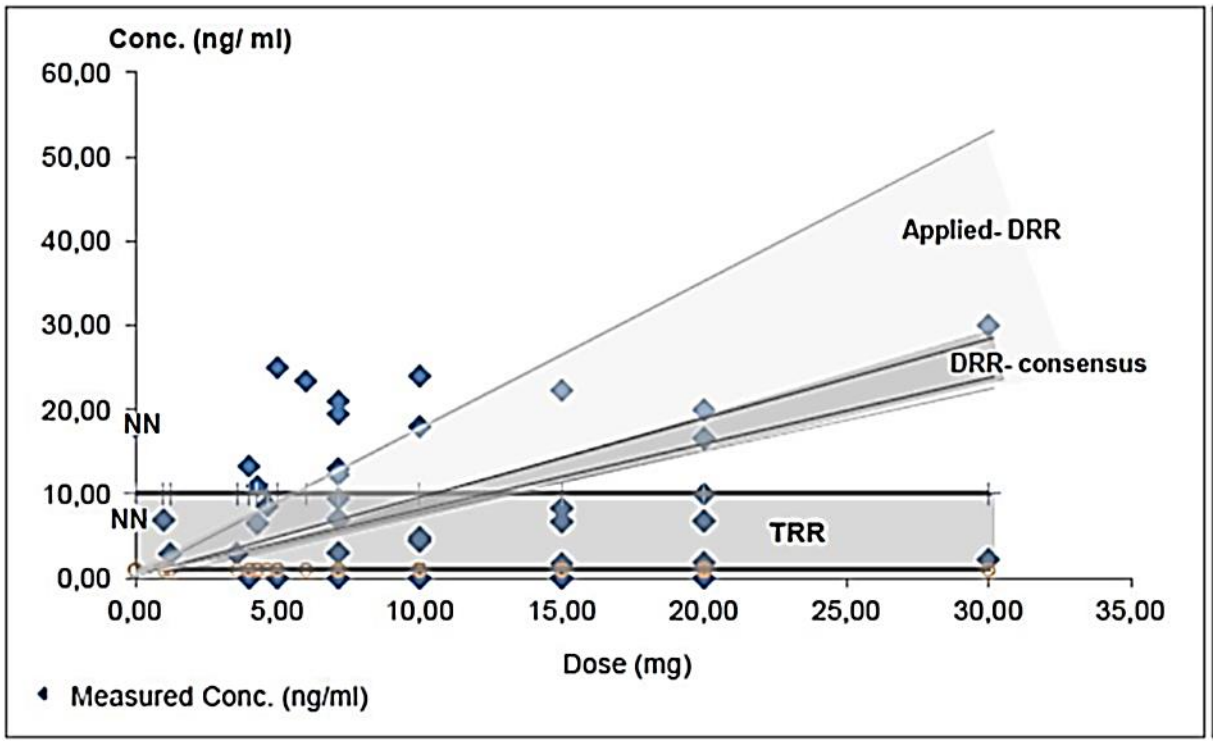


Figure 42. Consensus FLT DRR data in comparison with the applied FLT DRR data.

4.2.2.6 Dose- concentration relationship of measured ZLT samples and the DRR

The explanation for the calculation of the dose-related reference range with the fraction of concentration and dose is derived from the AGNP consensus for therapeutic drug monitoring in the psychiatry: update 2011, which according to the consensus is based on the study of Jerling et al. (1996). The DRR was obtained by multiplying each dose with factor 0.13 (C/D low) and 0.35 (C/D high).

Three ZLT serum samples comprised of 2 oral ZLT and 1 depot ZLT. Oral ZLT dose administered were 12 and 20 mg/day, depot ZLT administered was 200 mg/14 days (see table 48). Two out of the three measured samples contain co-medications. One

of the samples was administered as monotherapy with a depot application. All the measured concentrations are within the TRR but above the DRR (see figure 43).

Table 48 TDM information of ZLT in measured patients' serum.

Dose (mg)	Conc. (ng/ml)	DRR (ng/ml)	TRR (ng/ml)
200/14 day (14.29/day)	39	2 – 5	4 - 50
20	27	3 – 7	4 - 50
12	9	2 - 4	4 – 50

Note: DRR = dose-related reference range, TRR = therapeutic reference range, Conc: concentration.

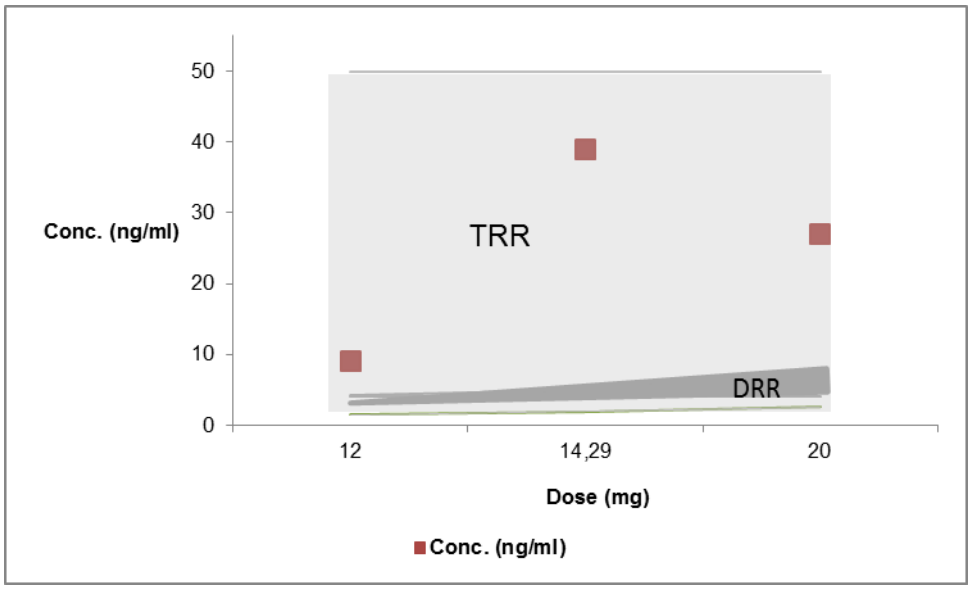


Figure 43. The The Dose-Concentration-Relationship of three zuclopenthixol patients' specimens.

The horizontal area indicates the therapeutic reference range (TRR) and the triangular area the dose related reference range (DRR).

4.2.3 Results of Antipsychotic TDM Data Evaluation

The routine application of the developed and validated method was successful. The recorded data in konbest and other databases applicabale to this work were reveiwed and compared with the study substances under the application of different parameters. The results of the data evaluations were successfully applied in the clinic pharmacological interpretations of the laboratory values of individual patients and in the pharmacist advice to the attending clinicians at the clinic ward. For the study substances, the serum concentrations were measured with the validated method. A reliable interpretation of the values were satisfactorily carried out and then submitted to the attending clinicians. Possible drug interactions between the recorded co-medications in konbest and the administered substances were also reviewed during the TDM clinic-pharmacological interpretation. The evaluation cost of haloperidol was carried out with the help of ABDA database. The recorded pharmacovigilance data of melperone and benperidol in the psychiatry was carried out with AGATE database. The comparison of C/D values of AGNP consensus data with the measured concentration of the study susbstances were satisfactorily carried out through the data extraction of the AGNP Consensus Guideline: update 2011.

4.2.3.1 The Konbest Data of Melperone

Seven patient' specimens (= 100%) were measured. The maintenance dose of melperone was not given in 42.86% of the delivered patients' sample. 57.86% of the samples indicated the maintenance dose, from which the dose-related reference range were analyzed and evaluated. The Konbest data showed a maintenance dose between 25 to 75 mg. The measured concentrations calculated from the maintenance dose were between 4 and 19 ng/ml; the recorded dose-related

reference ranges were between 4 and 21 ng/ml. Melperone was administered as reserve medication in all the study patients. The patients received other medications alongside with melperone. The co-medications in two of the measured samples lead to an increase of the concentration. The co-medications are; risperidon, clozapin, and propranolol.

4.2.3.2 The Konbest Data of Benperidol

Serum samples of different patients containing BPD and other co-medications from different hospitals were measured in the TDM laboratory using the described validated method. The samples were prepared in one single operation with three standard serum solutions (2, 10 and 30 ng/ml). The results of the measured patients' samples are on table 39. According to the patients' information recorded in the konbest, all the four patients were seriously sick before the administration of benperidol. Three patients got better, but one patient got worse at a serum concentration of 11.80 ng/ml, the maintenance dose of 12 mg and with the corresponding dose- related-reference range of 1.8– 3.72 ng/ml. Recorded co-medications were biperidene, bisoprolol, clozapine lorazepam, pirenzepine, valproic acid and zuclopenthixol.

4.2.3.3 The Konbest Data of Haloperidol

The total of 566 (100%) patients' haloperidol data was recorded in the Konbest program. 59.54% of the patients were male and 40.46% were female. The average age of the patients treated with haloperidol was 49.75 ± 5.6 years. 66 recorded undesired effects were observed, but could neither be traced to haloperidol serum concentration nor to the co-medications alone. 16 patients experienced undesired

effects though their serum concentrations were below the therapeutic reference range. Inter-individualities of the obtained patients' serum concentrations were observed. 28 patients got better at haloperidol serum cencentration above the therapeutic reference range. At concentration below the therapeutic reference range, 17 patients got better. 75 patients whose haloperidol serum concentrations were within the expected therapeutic reference range got better. Other patients health statues were not recorded.

Frequency of TDM HLP Request

The frequency of HLP-request has increased continuously since the development of konbest, and the tendency of further increase is accordingly anticipated from the data (see figure 44). The frequency of HLP request in 2008 slightly, but insignificantly declined when compared with that of 2007. The lowest frequency was registered in 2006 and the highest in 2011.

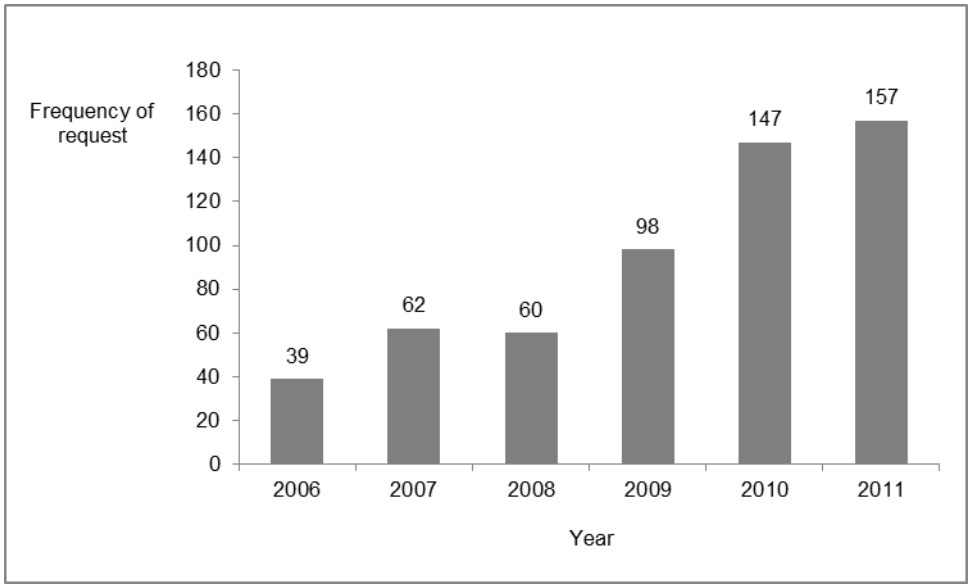


Figure 44. The annual request of haloperidol.

Administration of Haloperidol in Combination with other Medications

The number of co-medications evaluated as active substances which was administered to the patients during haloperidol therapy was 206 (see table 49). The total frequency of administration was 2077 (100%). The most frequently co-administered medications to haloperidol were biperidene (7.17%), lorazepam (6.45%), olanzapine (6.07%), valproic acid (5.58%), pantoprazole (4.53%), clozapine (4.24%). Sixty-nine co-medications (active substances) have the same metabolic pathway with haloperidol (CYP 1A2, CYP 2D6, and CYP 3A4) as substrates. Fifty-one are CYP enzyme inhibitors and fifteen CYP enzyme inducers. Some active substances act as inhibitors, inducers as well as substracts (see table 49).

CYP –enzyme inhibitors cause an elevation of drug serum concentration in a toxic level which for example lead to undesired drug effect. CYP-enzyme inducers cause the reduction of drug serum level which among others lead to lack of or reduced effect. It was observed that co-medications were administered because of comorbidities and for the treatment of undesired effects.

Table 49. The recorded HLP co-medications according to Konbest data.

Co-medication (active substance)	Frequency of administration	Percentage frequency of administration	CYP inhibitor	CYP in-ducer	Substrates
Acarbose	3	0.14	-	-	-
Acetylcystein	4	0.19	-	-	-
Acetylsalicylsäure	34	1.64	-	-	-
Agomelatin	2	0.10	-	-	1A2
Algeldrat	1	0.05	-	-	-
Allopurinol	20	0.96	-	-	-
Alprazolam	1	0.05	-	-	3A4
Ambroxol	1	0.05	-	-	-
Amisulprid	17	0.82	-	-	-
Amitriptylin	23	1.11	1A2, 2D6	-	3A4
Amlodipin	9	0.43	2D6, 3A4	-	-

Aripiprazol	27	1.37	-	-	2D6, 3A4
Ascorbinsäure	1	0.05	-	-	-
Atenolol	1	0.05	-	-	-
Atorvastatin	2	0.10	3A4	-	-
Atropa belladona	2	0.10	-	-	-
B-acetyldigoxin	3	0.14	-	-	-
Baclofen	1	0.05	-	-	-
Beclometason	2	0.10	-	3A4	-
Benperidol	4	0.19	-	-	1A2, 2D6, 3A4
Bezafibrat	3	0.14	-	-	3A4
bifidobacterium longum	1	0.05	-	-	-
Biperiden	149	7.17	2D6	-	-
Bisacodyl	3	0.14	-	-	-
Bisoprolol	31	1.49	-	-	-
Bornaprin	1	0.05	-	-	(x)15?
Brimonidin	2	0.10	-	-	-
Bupropion	2	0.10	2D6	-	1A2, 3A4
Buspiron	1	0.05	-	-	2D6, 3A4
Butylscopolamin	1	0.05	-	-	(x)12?
Calcitriol	2	0.10	-	3A4	-
Calcium	2	0.10	-	-	-
Candesartan	2	0.10	-	-	-
captopril	2	0.10	-	-	2D6
Carbamazepin	21	1.01	-	1A2, 3A4	2D6
Carbimazol	2	0.10	-	-	-
Cefazolin	1	0.05	-	-	-
Ceftriaxon	1	0.05	-	-	-
Chlorprothixen	36	1.73	2D6	-	1A2,3A4 (x)23,21
Ciclosporin A	1	0.05	3A4	-	-
Ciprofloxacin	1	0.05	1A2, 3A4	-	-
Citalopram	33	1.59	1A2, 2D6	-	3A4
Clindamycin	2	0.10	-	-	-
Clobazam	1	0.05	-	-	3A4
Clomethiazol	1	0.05	-	-	2D6, 34
Clomipramin	2	0.10	2D6	-	1A2, 3A4
Clonazepam	3	0.14	-	-	3A4
Clonidin	2	0.10	-	-	3A4
Clotrimazol	1	0.05	3A4	-	-
Clozapin	88	4.24	-	-	1A2, 2D6, 3A4
Colecalciferol	2	0.10	2D6	-	-
Crataegutt	1	0.05	-	-	-

Diazepam	30	1.4	3A4	-	1A2
Diclofenac	5	0.24	-	-	1A2, 2D6, 3A4
Digitoxin	4	0.19	-	-	3A4
Dikaliumclorazepat	1	0.05	-	-	3A4
Donepezil	4	0.19	-	-	2D6, 3A4
Dorzolamid	2	0.10	-	-	3A4
Doxazosin	1	0.05	-	-	-
Doxepin	4	0.19	-	-	1A2, 2D6, 3A4
Duloxetin	4	0.19	2D6	-	1A2
Efeublätter	1	0.05	-	-	-
Eisen II	8	0.39	-	-	-
Enalapril	9	0.43	-	-	3A4
Enoxaparin	9	0.43	-	-	-
Escitalopram	1	0.05	2D6	-	3A4
Esomeprazol	10	0.48	-	-	3A4
Estradiol	1	0.05	1A2	3A4	2D6
Estriol	3	0.14	-	-	-
Ethosuximid	1	0.05	-	-	3A4
Eucalyptusöl	1	0.05	-	-	-
Fenofibrat	1	0.05	-	-	3A4
Fenoterolhydrobromid	1	0.05	-	-	-
Fludrocortisonacetat	1	0.05	-	-	-
Flunitrazepam	1	0.05	-	-	3A4
Fluoxetin	5	0.24	1A2, 2D6, 3A4	-	-
Flupentixol	12	0.58	-	-	2D6
Fluphenazin	1	0.05	1A2, 2D6	-	-
Flupredniden	1	0.05	-	-	-
Fluticason	2	0.10	-	3A4	-
Fluvoxamin	2	0.10	1A2, 2D6, 3A4	-	-
Formoterol	1	0.05	-	-	2D6
Furosemid	25	1.20	-	-	-
Ginkgo-biloba	2	0.10	-	-	-
Glibenglamid	2	0.10	-	-	3A4
Glimepirid	6	0.29	-	-	-
Gramicidin	1	0.05	-	-	-
Hydrchlorothiazid	13	0.63	-	-	-
Ibuprofen	2	0.10	-	-	-
Insulin	15	0.72	-	1A2	-
Ipratropiumbromid	1	0.05	-	-	-
Isosorbiddinitrat	10	0.48	-	-	3A4
Kalium	8	0.39	-	-	-

Kaliumiodid	2	0.10	-	-	-
Lactobacillus gasseri	1	0.05	-	-	-
Lactulose	11	0.53	-	-	-
Lamotrigin	17	0.82	-	-	-
Levetiracetam	3	0.14	-	-	-
Levomepromazin	39	1.88	2D6	-	1A2
Levonorgestrel	1	0.05	-	-	3A4
Levothyroxin	43	2.07	-	-	-
Lisinopril	2	0.10	-	-	-
Lithium	50	2.41	-	-	-
Lorazepam	134	6.45	-	-	-
Losartan	2	0.10	-	-	3A4
Macrogol	15	0.72	-	-	-
Magnesium	10	0.48	-	-	-
Maprotilin	1	0.05	-	-	2D6
Medroxyprogesteron	3	0.14	-	3A4	-
Melperon	36	1.73	2D6	-	-
Metamizol	13	0.63	-	3A4	-
Metformin	31	1.49	-	-	-
Metoclopramid	1	0.05	2D6	-	1A2
Metoprolol	25	1.20	2D6	-	3A4
Metronidazol	1	0.05	3A4	-	-
Miconazol	1	0.05	3A4	-	-
Mirtazapin	14	0.67	1A2, 3A4	-	2D6
molsidomin	5	0.24	-	-	-
Multivitamin	1	0.05	-	-	-
Mycophenolatmofetil	1	0.05	-	-	-
Natriumchlorid	5	0.24	-	-	-
Natriumdihydrogen-phosphate	1	0.05	-	-	-
Natriummonohydro-genphosphat	1	0.05	-	-	-
Natriumpicosulfat	3	0.14	-	-	-
Neomycin	1	0.05	-	-	-
Nitroxolin	1	0.05	-	-	-
Nortriptylin	1	0.05	2D6	-	1A2, 3A4
Olanzapin	126	6.07	1A2, 2D6, 3A4	-	-
Omeprazol	4	0.19	2D6, 3A4	1A2, 3A4	-
Opipramol	2	0.10	-	-	2D6
Oxazepam	9	0.43	-	-	2D6
Oxcarbazepin	2	0.10	-	3A4	-
Oxybutynin	1	0.05	2D6, 3A6	-	-
Oxycodon	1	0.05	2D6, 3A4	-	-

Paliperidon	3	0.14	-	-	2D6
Pankretin	1	0.05	-	-	-
Pantoprazol	94	4.53	-	1A2, 3A4	-
Paracetamol	10	0.48	-	-	1A2, 2D6, 3A4
Paroxetin	1	0.05	1A2, 2D6, 3A4	-	-
Perazin	10	0.48	-	-	2D6, 3A4
Perphenazin	1	0.05	1A2, 2D6	-	3A4
Phenytoin	1	0.05	-	1A2, 3A4	-
Pioglitazon	1	0.05	-	3A4	-
Pipamperon	39	1.88	-	-	-
Pirenzepin	28	1.35	-	-	-
Piretanid	2	0.10	-	-	-
Polymyxin B	1	0.05	-	-	-
Polyvinyllkohol	1	0.05	-	-	-
Prednisolon	2	0.10	3A4	3A4	-
Pregabalin	15	0.72	-	-	-
Promethazin	14	0.67	2D6, 3A4	-	-
Propiverin	1	0.05	-	-	-
Propranolol	3	0.14	1A2, 2D6	-	3A4
Prothipendyl	4	0.19	-	-	-
Quetiapin	80	3.85	-	-	2D6
Ramipril	32	1.54	-	-	-
Ranitidin	1	0.05	1A2, 2D6	-	-
Reboxetin	5	0.24	-	-	3A4
Repaglinid	3	0.14	-	-	3A4
Risperidon	41	1.97	2D6, 3A4	-	-
Rivastigmin	1	0.05	-	-	-
Saccharomyces cerevi-siae	1	0.05	-	-	-
Salbutamol	1	0.05	-	-	3A4
Salmeterol	2	0.10	-	-	3A4
Sertralin	2	0.10	1A2, 2D6, 3A4	-	-
Simeticon	3	0.14	-	-	-
Simvastatin	13	0.63	2D6	-	3A4
Solifenacin	1	0.05	-	-	-
Spironolacton	1	0.05	-	-	-
Sulfamethoxazol	1	0.05	-	-	3A4
Sulprid	1	0.05	-	-	-
Tacrolimus	1	0.05	3A4	-	-
Tamsulosin	10	0.48	-	-	2D6, 3A4
Temazepam	23	1.11	-	-	2D6, 3A4
Terazosin	1	0.05	-	-	-

Tetryzolin	1	0.05	-	-	-
Theophyllin	2	0.10	1A2	-	2D6, 3A4
Thiamin	1	0.05	-	-	-
Thioridazin	1	0.05	1A2, 2D6	-	3A4
Tiaprid	1	0.05	-	-	-
Ticlopidin	4	0.19	1A2, 2D6, 3A4	-	-
Tilidin	1	0.05	-	-	-
Timolol	5	0.24	2D6	-	-
Tiotropiumbromid	3	0.14	-	-	-
Tolperisonhydrochlorid	1	0.05	-	-	-
Topiramat	4	0.19	-	3A4	-
Torasemid	7	0.34	-	-	-
Tramadol	1	0.05	-	-	2D6, 3A4
Travoprost	1	0.05	-	-	-
Trazodon	2	0.10	2D6, 3A4	-	-
Triamteren	1	0.05	-	-	-
Trihexylphenidylhydro-chlorid	2	0.10	-	-	-
Trimethoprim	1	0.05	-	-	3A4
Trimipramin	7	0.34	-	-	2D6, 3A4
Trospiumchlorid	3	0.14	-	-	-
Valproinsäure	116	5.58	2D6, 3A4	-	-
Venlafaxin	12	0.58	2D6, 3A4	-	-
Verapamil	3	0.14	1A2, 2D6, 3A4	-	-
Xipamid	1	0.05	-	-	-
Zaleplon	1	0.05	-	-	3A4
Zink	1	0.05	-	-	-
Ziprasidon	5	0.24	2D6, 3A4	-	1A2
Zolpidem	4	0.19	-	-	1A2, 2D6, 3A4
Zopiclon	22	1.06	-	-	3A4
Zotepin	2	0.10	-	-	1A2, 3A4
Zuclopenthixol	13	0.63	-	-	2D6
Total	**2077**	**100**			

Note: These co-medications were recorded in the patients' TDM-request forms and were transferred to Konbest upon receipt. Substances with the percentage frequency above 4% were regarded as most frequently administered co-medications, when compared to other substances. CYP 1A2, 2D6, 3A4 inhibitors, inducers and substrates were derived from CYP-table compiled by Ekkehard Haen and Goepfert Christine on 28.10.2010 and TDM-consensus, update 2011.

Total number of patients that received other medications alongside with haloperidol was 535 patients. 323 of them were male and 212 were female (see appendix 7). The number of administered co-medications per patient ranges from 1 to 15. One female patient received the highest number of co-medications (15). Most patients received the combination of 1 - 3 medications. 114 patients received the combination of 3 medications, 94 patients received 2 and 90 patients received 1 (see figure 45).

The active substances contained in the 15 co-medications for this female patient were oxcarbazepine, ciprofloxacine, clindamycine, cholecalciferole, levothyroxine, torasemid, potassium, lorazepam, agomelatine, metamizole, Oxycodon, Insulinglargin, human insulin, enoxaparin, salmeterol, and fluticasonpropionate. The measurement of her haloperidol serum concentration gave a concentration of 1.1 ng/ml at a haloperidol maintenance dose (oral) of 50 mg. The concentration was within the expected therapeutic reference range (1-10 ng/ml) but below the dose-related reference range (30 – 50 ng/ml). The administered co-medications contain CYP-inhibitors as well as CYP-inducers (see table 49). The patient's health condition improved under these medications.

The 14 co-medications recorded for the male patient were amisulprid, biperidene, sodium picosulfate, amlodipine, clonidin, bisoprolol, ramipril, bisacodyl, magnesium, zopiclon, metamizol, fludpredniden, macrogol, and lorazepam. The measured haloperidol serum concentration of 37.3 ng/ml at a maintenance dose (oral) of 40 mg was above the theraupeutic reference range, but within the dose-related reference range (24- 40 ng/ml). The patient smoked during the therapy. There was no information about the health condition of the patient.

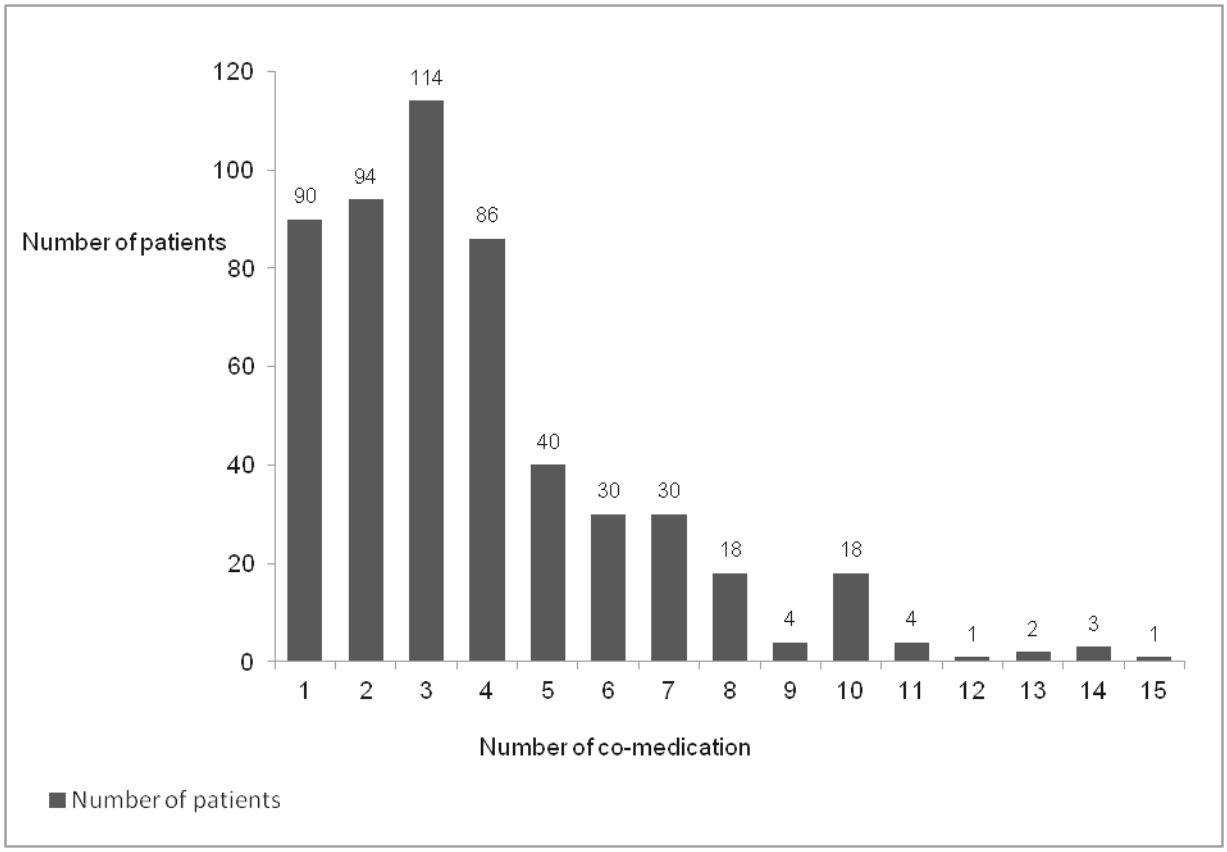


Figure 45. Number of co-medictions administered to each patient during haloperidol therapy.

Note: The number of co-medication recorded for each patient was evaluated according to the name of the administered medications (generic name) and not according to the active substances. In some cases, the same co-medications were administered to more than one patient.

Overview of haloperidol TDM Results with the Nine-Fold Table

Five hundred and sixty-three Konbest data records for the therapeutic drug monitoring of haloperidol were evaluated. The serum samples of the patients submitted to TDM laboratory were measured and analysed for the concentration of haloperidol in serum. The obtained results were classified in too low, expected and too high based on the given specification for the therapeutic reference range (1- 10 ng/ml) and dose-related reference range (C/D = 0.6 – 1.0). The expected DRR of each dose was calculated with the C/D factor haloperiol given in the TDM consensus.

104 patients were below the therapeutic (TRR) and dose-related reference ranges (DRR), 79 patients were above them while 380 patients were within the expected ranges (see table 50). A satisfactory overview of the state of the recorded serum concentrations was obtained. The result of the overview shows that the applied TDM was effective in the control of haloperidol serum concentration. More than 50 % of TDM-controlled serum concentration was within the expected therapeutic and dose-related reference ranges. Samples with concentrations below or above the expected ranges were further interpreted based on the patients' health condition, patients' compliance, the administered co-medications, time and duration of drug administration, and the occurrence of undesired drug effects.

For example, in cases of too low TRR where the patient is compliant, no improvement in his health condition and no deterioration either, no influence of co-medication to haloperidol, and do not have any undesired drug effect, then an increase of medication dose and further monitoring was advised (see figure 46).

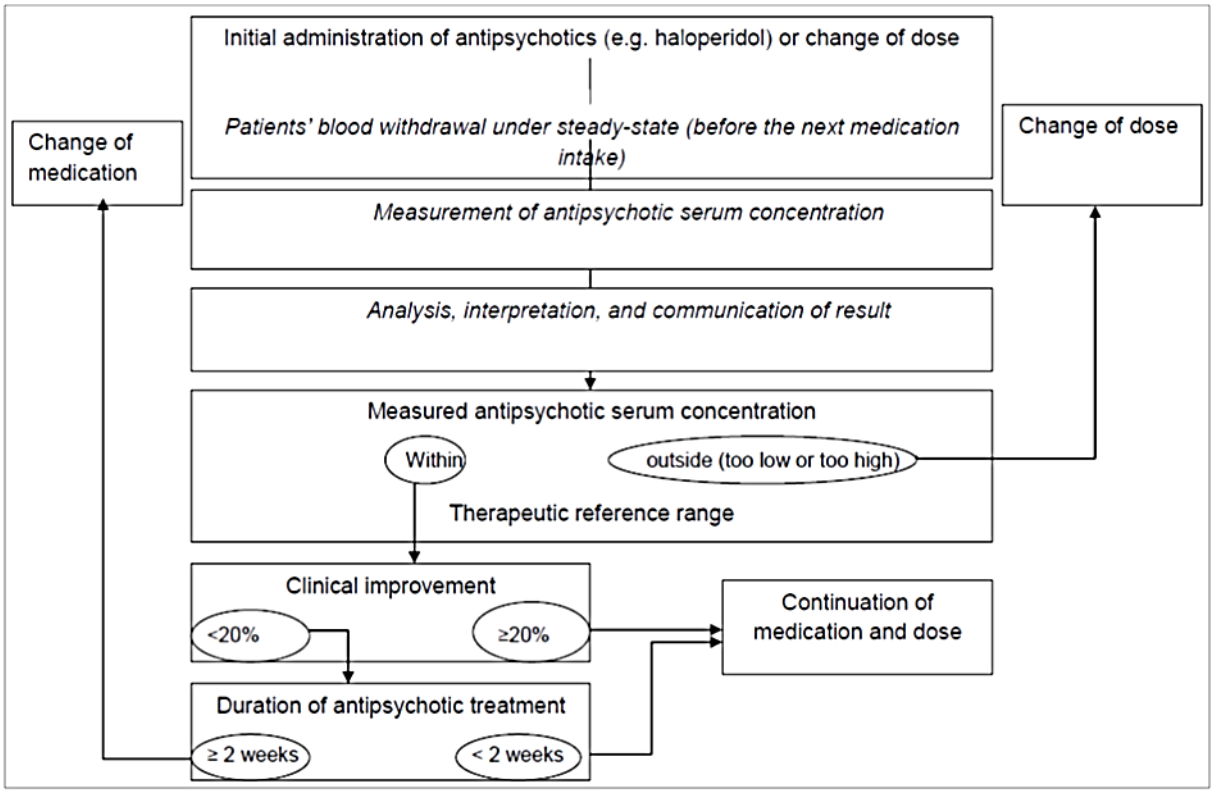


Figure 46. TDM-Recommendation scheme for antipsychotic therapy from [Hiemke et al., 2011] and adjusted by the author.

Note: This scheme is based on the value obtained from the measured antipsychotic serum concentration such as haloperidol. Steady-state concentration is reached after five elimination half-lives ($t_{1/2}$) of the drug. The $t_{1/2}$ of haloperidol is 12-38 hours according to the summary of product characteristics Haldol® 2012.

In cases of too high TRR with the occurrence of undesired drug effect, deterioration of patients' health condition, and with co-medications that inhibit CYP 2D6, a change of co-medication and further monitoring was advised and if need be, the attending clinician was advised to change the medication, especially, if a constant dose of the medication has been administered and compliantly taken for more than two weeks (see figure 46).

Table 50. The nine-fold table of Konbest data collection for haloperidol-TDM.

	Concentration in relation to the therapeutic reference range			
		Too low	**As expected**	**Too high**
Concentration in relation to the dose-related ref-erence range	Too low	104 patients		
	As expected		380 patients	
	Too high			79 Patients

Note: The concentrations of the patients' sample that were measured in TDM laboratory Regensburg were grouped in relation to the dose-related reference range (DRR) and then the number of patient for each group was countered.

4.2.3.4 The Konbest Data of FLT and ZLT

The total number of patients studied during this work was 45. Fourty-two patients' samples contained FLT and 3 samples contained ZLT. The recorded co-medications, evaluated as active substances was 62 (see table 51).The total frequency of administration was 157 (100%). The most administered co-medications (active substances) were lorazepam (12.10%), biperidene (7.64%), bisoprolol, (5.10%), olanzapine (5.10%), pantoprazole (5.10%), quetiapin (4.46%). Nine co-medications have the same metabolic pathway with flupentixol and zuclopenthixol as substrates of CYP 2D6. Fifteen co-medications were CYP 2D6 inhibitors. 14 patients who received flupentixol got better during the therapy, the health condition of 9 patients deterioriated and the health condition of 11 patients did not change. The health status of other patients was not recorded.

Table 51. Recorded co-medications and their active substances.

Active Substance	**Frequency of administration**	**Percentage frequency of administration**	**CYP 2D6 Inhibitor**	**CYP 2D6 Inducer**	**CYP 2D6 substrate**

Active Substance	Frequency of administration	Percentage frequency of administration	CYP 2D6 Inhibitor	CYP 2D6 Inducer	CYP 2D6 substrate
Allopurinole	1	0.64	-	-	-
Amlodipine	2	1.27	x	-	-
Aripiprazole	1	0.64	-	-	x
Benperidol	2	1.27	-	-	(x)
Betamethason	1	0.64	-	-	-
Biperidene	12	7.64	x	-	-
Bisoprolol	8	5.10	-	-	-
Budesonide	1	0.64	-	-	
Carbamazepine	3	1.91	-	-	x
Cefuroxime	1	0.64	-	-	-
Chlorprothixene	2	1.27	x		
Citalopram	1	0.64	x	-	-
Clomipramine	2	1.27	x		
Clozapine	4	2.55	-	-	x
Cyanocobalamine	1	0.64	-	-	-
Cyproterone Acetate	1	0.64	-	-	-
Desmopressin	1	0.64	-	-	-
Diazepam	4	2.55	-	-	-
Pipamperone	2	1.27	-	-	-
Drospirenone	1	0.64			
Enoxaparin	2	127	-	-	-
Estraldiol	1	0.64	-	-	x
Formoterol	1	0.64	-	-	x
Furosemide	1	0.64	-	-	-
Hydrochlorothiazide	2	1.27	-	-	-
Hydroxycarbamide	1	0.64	-	-	-
Lamotrigine	2	1.27	-	-	-
Levofloxacin	1	0.64	-	-	-
Levomepromazine	3	1.91	X	-	-
Levothyroxin	4	2.55	-	-	-
Lithium	1	0.64	-	-	-
Lorazepam	19	12.10	-	-	-
Magnesium	1	0.64	-	-	-
Melperone	2	1.27	X	-	-

Active Substance	Frequency of administration	Percentage frequency of administration	CYP 2D6 Inhibitor	CYP 2D6 Inducer	CYP 2D6 substrate
Metformine	1	0.64			
Olanzapine	8	5.10	x	-	-
Oxazepam	1	0.64			x
Pantoprazole	8	5.10	-	-	-
Phenprocoumon	1	0.64	-	-	-
Piperacillin	1	0.64	-	-	-
Pirenzepine	1	0.64	-	-	-
Potassium	1	0.64	-	-	-
Prednisolon	1	0.64	-	-	-
Pregabaline	1	0.64	-	-	-
Prothipendyl	3	1.91	-	-	-
Pyridoxine	1	0.64	-	-	-
Quetiapine	7	4.46	-	-	x
Ramipril	2	1.27	-	-	-
Risperidon	3	1.91	x	-	-
Salbutamol	1	0.64	-	-	-
Salicylic Acid	1	0.64	-	-	-
Simvastatine	1	0.64	X	-	-
Tazobactam	1	0.64	-	-	-
Thiamine	1	0.64	-	-	-
Tiotropium Bromide	2	1.27	-	-	-
Triamterene	1	0.64	-	-	-
Trihexyphenidyl	1	0.64	-	-	-
Valproic Acid	11	3.82	x	-	-
	5	3.18	x	-	-
Venlafxine	1	0.64	x	-	-
Ziprasidon	3	1.91	x	-	-
Zolpiderm	1	0.64	-	-	x
Total	**157**	**100,07**			

Note: The co-medications were recorded during the TDM of flupentixol and zuclopenthixol. CYP 2D6 inhibitors and substrates were derived from CYP-table compiled by Ekkehard Haen and Goepfert Christine on 28.10.2010 and TDM consensus update 2011. The percentage frequency of drug administration was evaluated based on the active substances alone. CYP 2D6 is recorded as flupentixol and zuclopenthixol metabolizing enzyme. X = drugs that act as inhibitor or substrate of CY 2D6. (X) = discrepancy in the literatures.

The patients received different number of co-medications, respectively, during the therapy with flupentixol and/or zuclopenthixol. The highest number of co-medications administered per patient was 15 and the least number was 1 (see figure 47).

The 15 co-medications administered to the male patient were amlodipine, biperidene, budesonide, citalopram, formoterol, furosemid, metoprolol, quetiapine, potassium, ramipril, pantoprazole, pregabaline, salbutamol, tiotropium bromide, and oxazepam. The co-medications comprise of CYP 2D6 inhibitors as well as substrates (see table 53). No flupentixol serum concentration was measured at a maintenance dose of 20 mg per day. The patient smoked 10 cigarettes in a day and there was no improvement in his health condition.

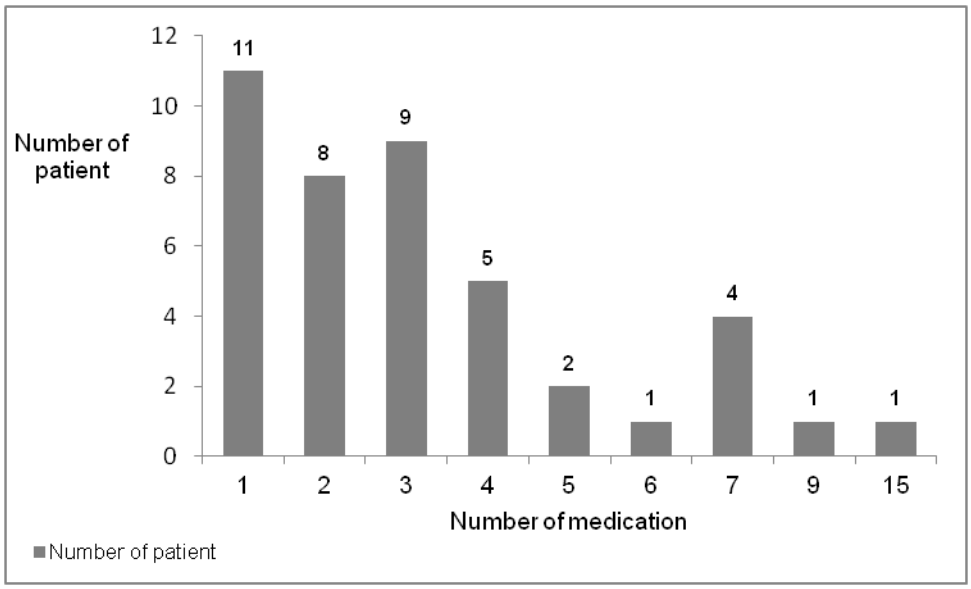


Figure 47. Number of co-medication administered to each patient during flupentixol and/or zuclopenthixol therapy.

Note: The number of co-medication recorded for each patient was evaluated according to the name of the administered medications (invented name) and not according to the active substances. In some cases, the same co-medications were administered to more than one patient.

4.2.3.5 ABDA Haloperidol Data Evaluation

The price list of haloperidol medication stated in ABDA database was applied for the evaluation of the cost of the documented haloperidol therapy. The haloperidol request applied during the therapy was documented in Konbest program of the TDM laboratory, at the university of Regensburg between 2006 and 2011.

The total haloperidol request of 562 gave a total cost of 184.892,58 €. The request and the cost reduced in 2008 and showed a significant leap between 2009 and 2010 (see figure 48). The cost obtained by the year 2011 was reduced due to the general reduction of the medication cost in the German Health Care System. The least cost at 7.13% of the total cost was registered by the year 2006 and the highest cost at 27.32% of the total cost was documented by 2010.

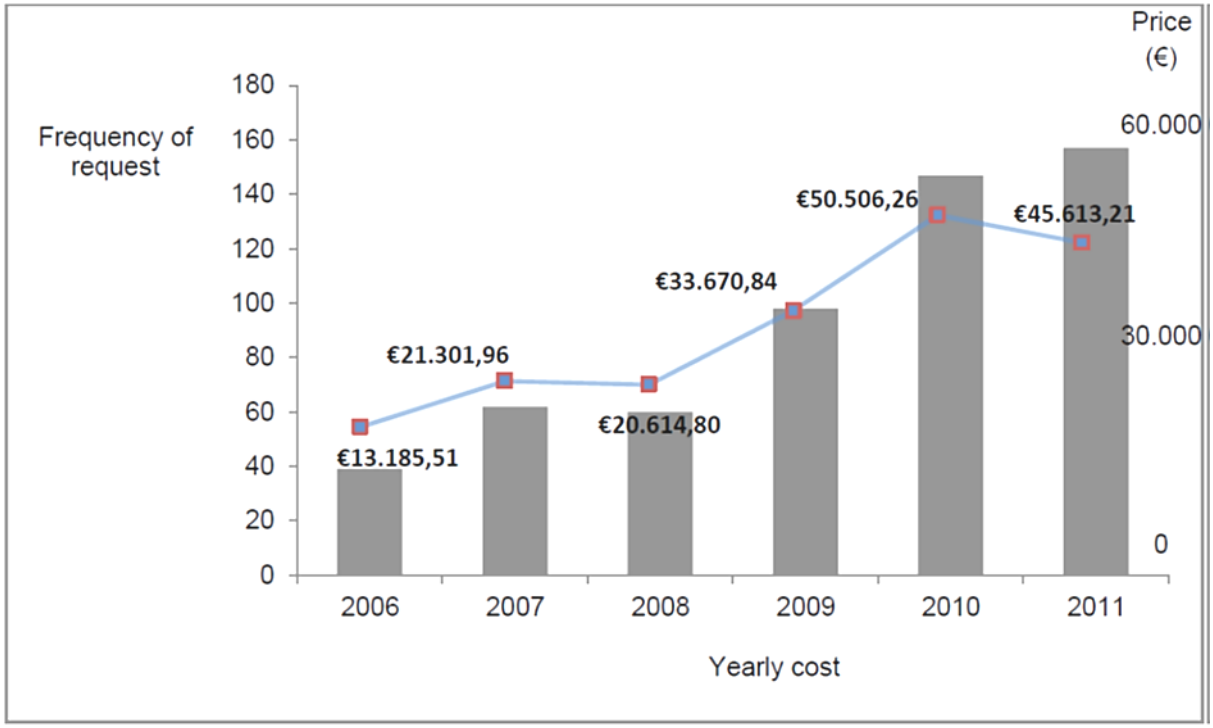


Figure 48. The cost evaluation of haloperidol yearly request in euro (€).

Note: The pillars represent the yearly request of haloperidol TDM.

4.2.3.6 AGATE Melperone-Benperidol Data Evaluation

On the two index days in the years between 1995 and 2010, the AGATE identified 9,540 (100%) frequency administration of MLP and/ or BPD for 9,483 patients (100%).

8,055 (84.43%) MLP was frequently administered to 7,999 (84.35%) patients and 1,485 (15.57%) BPD to 1,484 patients (15.65%). 82 (0.86%) patients received the combination of MLP and BPD. Less than one-sixth of the total patients was admistered only BPD (see figure 49).

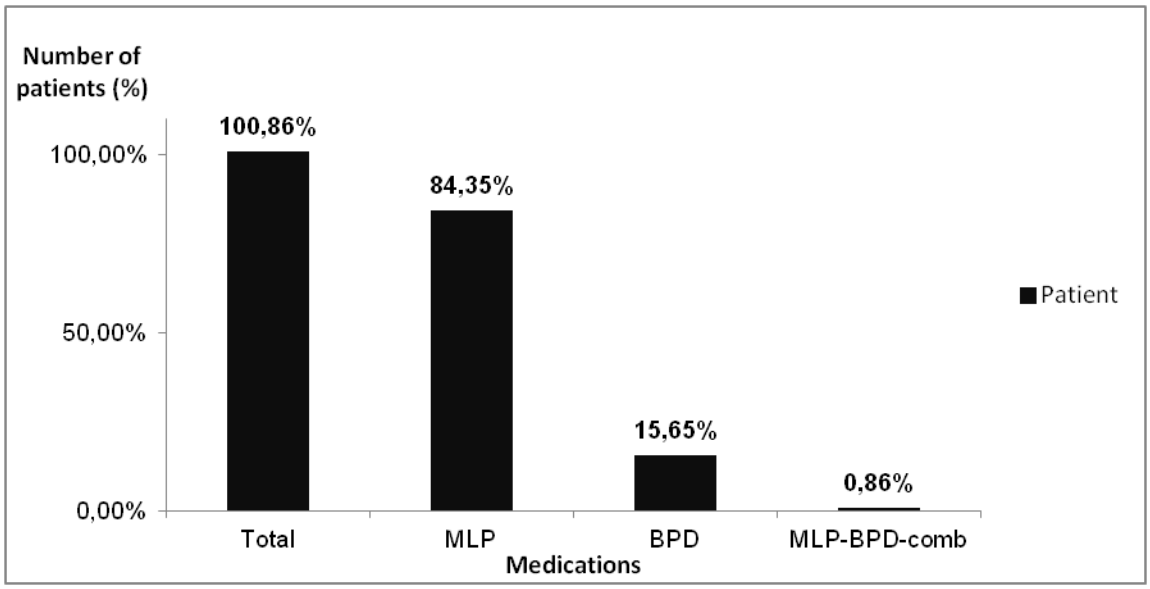


Figure 49. Data comparison of single and combined administration of melperone (MLP) and benperidol (BPD).

From the other co-administered medications, the most administered group of psychotropic medications (see table 52) were antipsychotics (20.9%) and anticonvulsants (18.4%).The diagnoses for the administration of melperone and/ or benperidol are listed in appendix 12. According to TDM consensus update, 2011, melperone and benperidol are under level 3 of TDM recommendation. Under level 3, TDM is recommended to be applied for special indications or problem solving

[Hiemke et al., 2011]. The observation of the data evaluation shows that the rate of TDM request for MLP and BPD is low. Two-hundred and ten case reports were recorded. Only 81 of these cases were requested for labor TDM of MLP and/or BPD.

Table 52. Administration of combined medications to MLP and/or BPD.

Abbreviation	Medication Groups	N prescriptions	%Polymed
AD	Antidepressants	17	4.0
AEP	Anticonvulsants	78	18.4
APM	Anti-Parkinson drugs	44	10.4
HYP	Hypnotics	9	2.1
NL	Antipsychotics	89	20.9
BZD	Benzodiazepine	52	12.2
AND	Others	136	32.0
Total	7	425	100.0

Note: Groups of the applied polymedications (Polymed), N: total number of medication prescribed in a group.

4.2.4 Influence of Smoking on HLP and FLT serum Concentration

Polycyclic aromatic hydrocarbons in tobacco induce cytochrome P450 enzymes [Lucas c and Martin J, 2013] which can lead to decreased drug concentrations. The evaluation of the effect of smoking on serum concentrations of HLP and FLT were compared in smokers and non-smokers. All the study patients who smoked during the therapy with flupentixol also received other co-medications.

4.2.4.1 Influence of Smoking on Haloperidol Serum Concentration

The number of patients treated with haloperidol was 366 (100 %). 197 (64.21%) were smokers, and 35.79% of them were female. The mean serum concentration for smokers was 9 ± 10ng/ml, at a mean dose of 17 ± 15mg per day. The mean serum concentration for non-smokers was 10 ± 12 ng/ml, at a mean dose of 15 ± 17 mg per

day (see appendix 8). Dose related concentrations were different between smokers and non-smokers (see figure 50 and 51). In two outliers, co-medication was, melperone at a dose of 100 mg for one sample which might explain the outlier value, since melperone is an inhibitor [Hiemke et al., 2011] and haloperidol a substrate of CYP2D6 [Kudos and Ishizaki, 1999]. For the other outlier, no co-medication was recorded.

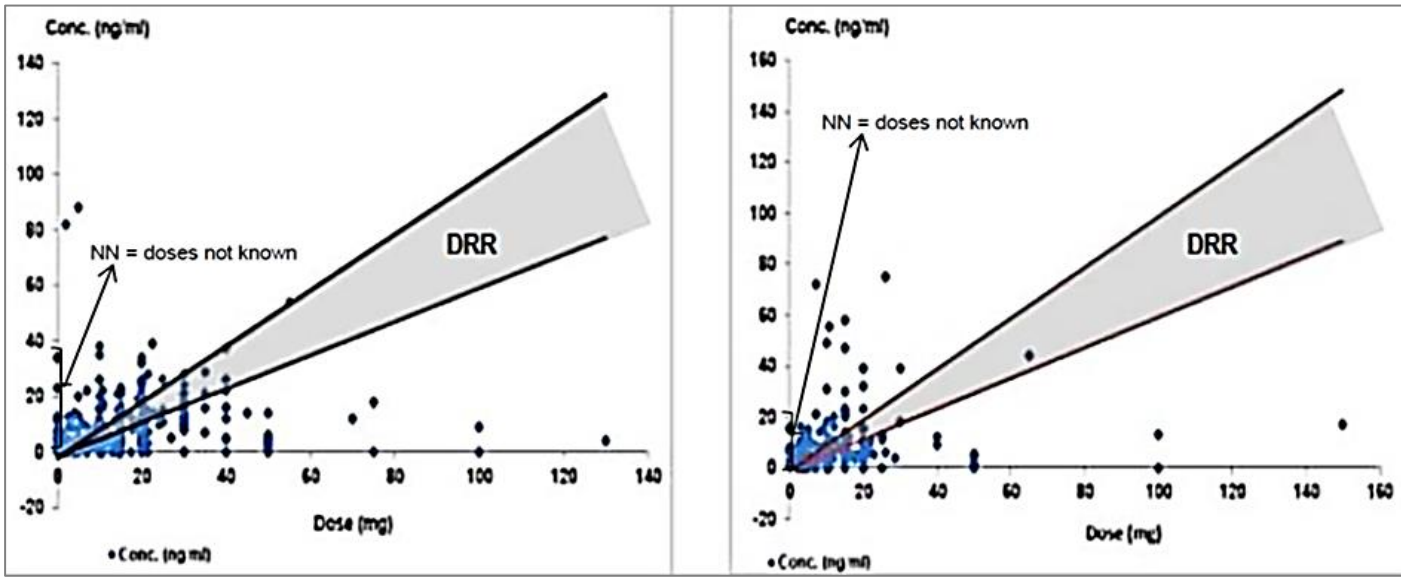


Figure 50. C/D received from the measured samples of haloperidol for smokers (left panel) and non-smokers (right panel).

Note: DRR = dose-related reference range.The measured concentrations both by smokers and non-smokers were clustered between 0 and 20 ng/ml. The slope of the graph for both patients' groups is similar. DRR for samples without dose information was not applicable, as it can be observed in the graph. C = concentration, D = administered dose.

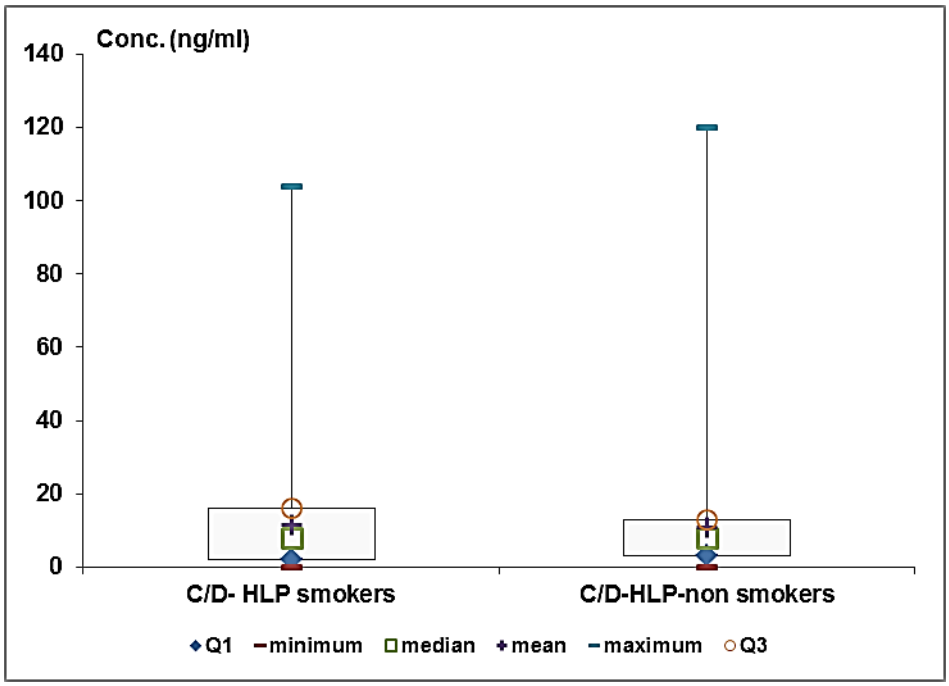


Figure 51. Comparison of C/D-low and C/D high for smokers and non-smokers during therapy with HLP.

Note: Comparison with patients who smoked during the administration of HLP with that of patients who didn't smoke during the therapy. Q = quartile. For C/D HLP-smokers; Q1 = 2.0 ng/ml, minimum = 0 ng/ml, median = 8 ng/ml, mean = 11.57 ng/ml, maximum = 104 ng/ml, Q3 = 16.0 ng/ml. For C/D HLP- non-smokers; Q1 = 3.2ng/ml, minimum = 0 ng/ml, median = 8 ng/ml, mean = 10.75 ng/ml, maximum = 120.0 ng/ml, Q3 = 12.8 ng/ml. C/D-low and C/D are factors for calculation of dose-related reference ranges.

Haloperidol Serum samples with and without Co-medication by Smokers

Four patients who smoked during haloperidol therapy did not receive co-medications. Patients under sole administration of haloperidol received an average dose of 22 ± 19 mg and reached an average serum concentration of 5 ± 3 ng/ml. Their average C/D was 18 ± 16 ng/ml. Patients who received haloperidol alongside with other medications reached an average serum concentration of 9 ± 10ng/ml at an

average dose of 17 ± 15mg. Their average C/D was 12 ± 13ng/ml. Patients who were treated with haloperidol monotherapy received higher dose compared with the patients who received other medications alongside with haloperidol. During monotherapy, smokers reached a lower level of haloperidol serum concentration. Patients' smokers under polytherapy, though received lower haloperidol doses, reached higher level of haloperidol serum concentration (see figure 52). The average serum concentration reached in both cases was below the expected average dose-related reference range.

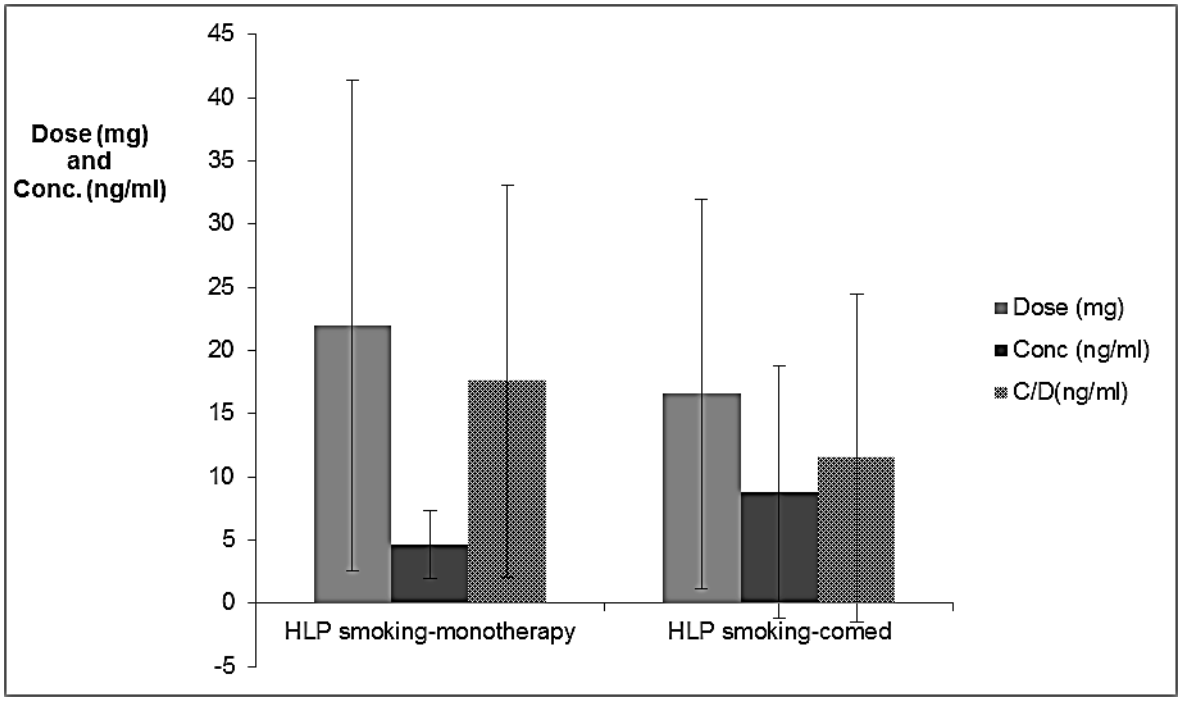


Figure 52. comparison of haloperidol serum concentration in smokers with and without co-medications.

Note: For patients who received only HLP: Dose = 22 ± 19.4 mg, Conc = 4,63 ± 2.7 ng/ml, C/D = 17.6 ± 15.5 ng/ml. For patients who received HLP alongside with other medications: Dose = 16.56 ± 15.4 mg, Conc = 8.8 ± 10.0 ng/ml, C/D = 11.5 ± 13.0 ng/ml. Comed = co-medication

4.2.4.2 Influence of Smoking on Flupentixol Serum Concentration

Thirty-three patients smoked under the therapy with flupentixol and ten patients did not smoke. The mean serum concentration found in smokers was 9.3 ± 8.2 ng/ml, at a mean dose of 10.0 ± 6.7 mg per day. The mean serum concentration obtained by non-smokers was 9.4 ± 9.3 ng/ml, at a mean dose of 11.9 ± 8.8 mg per day (see appendix 9). The results of the evaluation for the impact of smoking on the serum concentration of these patients show no significant different when compared with the serum concentration of the patients who did not smoke (see figure 53). The serum concentration of the depot administration of FLT is not linear to the corresponding DRR. The distribution of the obtained values both by smokers and non-smokers are similar. The mean DRR value for smokers is 16.2 ng/ml while that of non-smokers is 15.1 ng/ ml (see figure 54). The number of patients' smokers who were administered FLT were more when compared to that of nonsmokers. The serum concentration reached by smokers is lower than the concentration reached by nonsmokers.

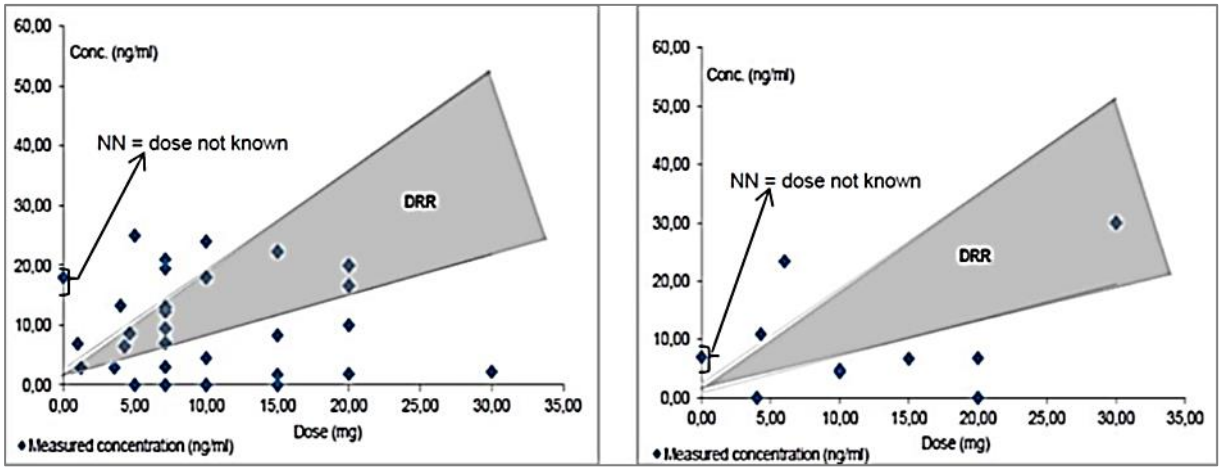


Figure 53. C/D received from the measured samples of flupentixol for smokers (left panel) and non-smokers (right panel).

The measured concentrations both by smokers and non-smokers were clustered between 0 and 20 ng/ml. The slope of the graph for both patients' groups is similar. The C/D obtained for samples containing depot flupentixol are not linear to the administered dose.

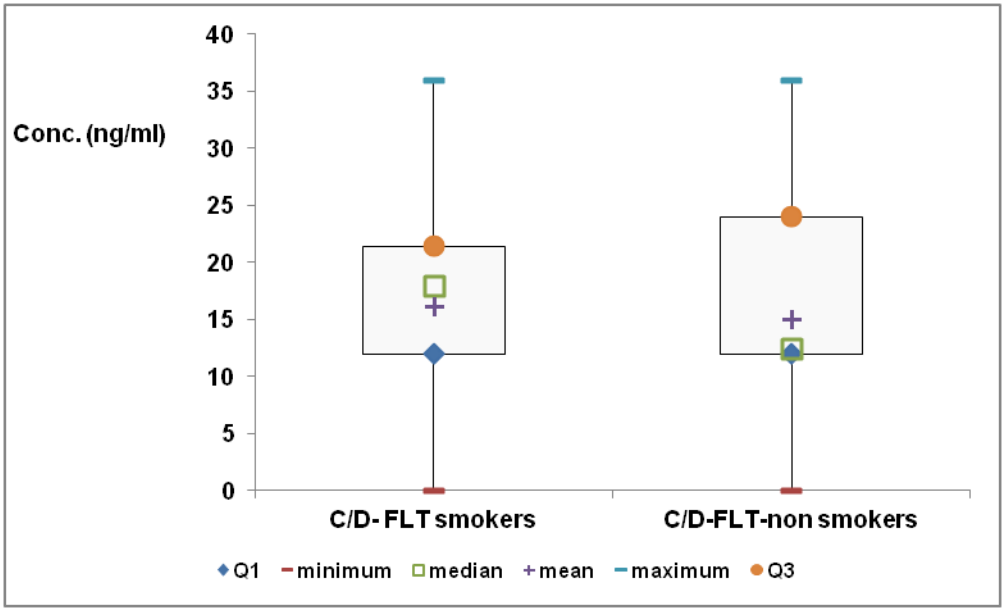


Figure 54. Comparison of C/D-low and C/D high for smokers during therapy with FLT.

Note: Comparison with patients who smoked during the administration of FLT with that of patients who didn't smoke during the therapy. C/D-low and C/D high are factors for calculation of dose-related reference range.

Q = quartile. For C/D FLT-smokers; Q1 = 12.0 ng/ml, minimum = 0 ng/ml, median = 18 ng/ml, mean = 16.19 ng/ml, maximum = 36 ng/ml, Q3 = 21.43 ng/ml. For C/D FLT- non-smokers; Q1 = 12.0 ng/ml, minimum = 0 ng/ml, median = 12.44 ng/ml, mean = 15.09 ng/ml, maximum = 36.0 ng/ml, Q3 = 24.0ng/ml.

4.3 Application of TDM at the Clinic Ward

The TDM at the clinic ward was not restricted to antipsychotic medications alone. Medications of different pharmacological groups reported by the attending clinicians and patients were treated by the pharmacist. There are different reasons observed for requesting the therapeutic drug monitoring of antipsychotic drugs. Clarification of the request is based on the result of the measured patients' sample and the patients' information on the request form, which were recorded in the konbest program. Cases pertaining melperone (MLP), benperidol (BPD), haloperidol (HLP), bromperidol (BRP), flupentixol (FLT) and zuclopenthixol (ZLT) were discussed.

4.3.1 Konbest Case Report of the Applied Antipsychotics

The record of the measured patients' serum samples including their sickness and medication histories are covered with the help of konbest. The metabolic pathways of the administered medications are arranged in such a way as to give one an overview of any interaction between the metabolizing enzyme of the applied medication and other co medications. Enzyme inducers lead to a reduced serum concentration level of the substrate medication, resulting to a reduced efficacy of the administered medication. Enzyme inhibitors lead to an increased concentration of the medication in the serum, resulting to undesired drug effects.

4.3.1.1 Reported Headache under Medication with Melperone

An elderly female patient, aged 85 years, received as reserve medication, melperone 75 mg per day as sedative, for more than one week. The patient reported of continues headache anytime she takes melperone. Her diagnosis was disorganised schizophrenia according to ICD F20.1. She received clozapine 350 mg per day for

the treatment of schizophrenia. Other co-medications were metformine 850 mg, three times per day, januvia 100 mg (active substances = metformine and sitagliptine) and delix plus 5 mg (active substances = ramipril and hydrochlorothiazide). Information to her health situation under these medications was not reported. The TDM of melperone gave a serum concentration of 6 ng/ml, which is below the therapeutic reference range of 30 – 100 ng/ml. Because of the persistent headache at low concentration of MLP, the patient was advised to cease the intake of melperone. Alternative sleeping bills from plant basis, which will not alter the patients' medications, was suggested by the pharmacist. The mentioned co-medications do not have common metabolic pathways with melperone; they were not adjusted. According to the patient, the intensity of the headache reduced after the intake of melperone was stopped, but started again after two days even without the intake of melperone. Melperone was therefore ruled out as the reason for the headache with the help of TDM, which was also confirmed with the measured serum concentration below the therapeutic reference range. There was no follow-up or more information to this as the patient was transferred to another clinic before the next visit.

4.3.1.2 Case Report of Hypersalivation with Benperidol and Zuclopenthixol

Serum sample of a 66 years old female patient, diagnosed of schizophrenia, was sent for measurement, because of undesired drug effect- hypersalivation, which started after an intake of zuclopentixol and benperidol. She received 12 mg ciatyl-z (active substance = zuclopenthixol) and 26 mg glianimon (active substance = benperidol). Her other co-medications were clozapine, orfiril ret (valproic acid), akineton (biperiden), tavor (lorazepam), bisoprolol, and gastrozepin (pirenzepine). The measured serum concentration was 2 ng/ml for benperidol and 9 ng/ml for

zuclopenthixol. Both measured concentrations are within the therapeutic reference range (1-10 ng/ml for benperidol and 4 – 50 ng/ml for zuclopenthixol) clozapine, valproic acid, and zuclopenthixol have a common metabolic pathways at CYP 2D6 (see table 53). Since the patient was getting better under this condition, and the reported side effect is tolerable, it was advised that the medication scheme should be maintained, and the patient should be monitored, in case of an increase in the reported undesired drug effect.

Table 53 Interaction table for cytochrom P450 relating to the concerned medication.

substances	CYP 1A	CYP 2A6	CYP 2B6	CYP 2C8	CYP 2C9	CYP 2C19	CYP 2D6	CYP 2E1	CYP 3a4/5/6	Ugt 1a4
Benperidol	n.a									
Bisoprolol									X subst	
Clozapine	X subst	X subst				X subst	X subst		X subst	
Lorazepam										X subst
Pirenzepine	n.a									
Valproic acid			X subst	X inh	X inh	X inh	X inh	X subst	X inh	
zuclopenthixol							X subst			

Note: n.a = not available, subst = substrate, inh= inhibition, ind = induction

4.3.1.3 Reported Case of Non- Compliance with Haloperidol

A female patient (age unknown), has been on haldol (dose unknown) for the last five years. She has paranoid schizophrenia and Haldol helped her with her hallucination. Because of weight gain, she rejected the further intake of haldol. The measurement of her serum haloperidol concentration was not performed, because she has already stopped the intake of haldol before the report . She was switched to quetiapine at a dose of 600 mg per day. The TDM of her serum sample gave a serum quetiapine

concentration of 192 ng/ml. Her health condition remained stable and the weight gain was reduced. No co-medication was mentioned.

4.3.1.4 Reported Case of Bleeding during Bromperidol Therapy

A 48 years old male patient reported of bleeding after the intake of Impromen. He was administered Impromen (active substance = bromperidol) for the treatment of psychotic disorder at a dose of 6 mg per day. He stopped the intake of bromperidol on his own; two days before his admission in the hospital. As co-medication during the therapy with Impromen, he received risperidon. According to him, he will prefer not to have a similar experience. The TDM of his serum sample showed a rest concentration of bromperidol at 8 ng/ml. The monotherapy with risperidon dose of 0.5 mg per day was continued and targeted to 1 mg per day. He was compliant to the instructions of his risperidon medication intake and to the therapy.

4.3.1.5 Reported Hyponatremia during Therapy with Flupentixol

A 56 years old schizophrenic male patient was recorded to have suffered a server side effect of hyponatremia when flupentixol was co-administered with quetiapine. Unfortunately the dose of flupentixol and quetiapine that lead to the named side effect was not reported. The flupentixol therapy was discontinued, and the patient got better under the continuous mono therapeutic administration of quetiapine dose at 150 mg. The measured quetiapine serum concentration during TDM was 74 ng/ml, which is below the therapeutic reference range of 100 -500 ng/ml according to consensus. There was no clinic pharmacological report to this effect, because some relevant information such as the administered dose, period of the therapy before the onset of the reported side effect, and the co-medications were not mentioned.

Other reported undesired effects were tremor, tardive dyskinesia and dry mouth. Tremor and tardive dyskinesia reduced after the withdrawal of flupentixol, but dry mouth persisted. According to the information the pharmacist received on enquiry, the dry mouth symptomatic was locally treated by advising the patient to slip water regularly and/or to chew sugar-free gums or candies to stimulate the flow of saliva.

4.3.2 TDM Processing at the Clinic Ward

TDM was applied for some medications for the clarification of some requests at the clinic ward. The samples of the patients' serum were first sent to TDM laboratory and measured, if need be. The clinic-pharmacological interpretation of the result was prepared with the given information in the request form. Discussions on compliance, change and/ or addition of medication, continuation of the therapy with a particular medication, dose increase or reduction were made based on the outcome of the serum concentration measurement and the health condition of the patients.

4.3.2.1 Indications for the requested TDM

The attending clinicians requested for TDM of their patients' serum samples because of various reasons (see table 54) but for one goal, which is the optimization of the therapy of their individual patients. TDM requests because of drug interactions and dose adjustment were made at the admission of a new patient, if the attending clinicians see the need to reorganize the patient's medication scheme. Compliance-check request was made when the patient is not responding to treatment and when the clinical statue deteriorates instead of getting better. TDM request because of lack of response to therapy was also made, to find out if the applicable dose yields the

expected therapeutic serum concentration. The samples were accompanied with a completed request forms, for each sample, respectively.

Table 54 Reasons for the request of TDM at the clinic ward.
Compliance
Drug interactions
Lack of response to a therapy
Change in the clinical status of the patients
Dose adjustment

4.3.2.2 Time Frame of the Analysis

The entire TDM requests from the clinic ward were treated in accordance with the measurements standards and criteria at the TDM laboratory in Regensburg. Samples were grouped and measured at different days and times in a week, with the corresponding methods. The laboratory measurements last between 15 mins to 60 mins depending on the substance.

Results and interpretation of the measured serum concentrations were available within 1 – 2 days. Late samples were measured with another applicable and validated method, in order to supply the result within the given time frame. Results and interpretation of measured samples that arrived later, after all the measurements have been concluded, were available within the next five working days. Measurements and interpretation of the study substances melperone, benperidol, haloperidol, bromperidol, flupentixol and zuclopenthixol were carried out daily and lasted 40 mins including the clinic-pharmacological interpretation, whenever they were requested. Prompt alerting of the attending clinicians in cases where the measured drug concentration is highly above the expected dose-related reference range and therapeutic reference range were applied. This alert was done by making

a direct call to the clinic ward to the attending clinician. The requested medications during the study are listed in table 55.

Table 55 Requested medications applied in TDM.

Abilify
Clomipramine
clozapine
Cymbalta
Ergenyl
Fluanxol
Haldol
Imipramine
Lamotrigine
Mirtazapin
Nortrilen
Olanzapine
Perazine2
Quetiapine
Risperidon
Seroquel
Tavor
Valproate
Venlafaxine

4.3.2.3 Clinic-Pharmacological Reports and Communication of Result

Time serum samples were taken, time the last dose was taken, duration of the dosing regimen, indication for the therapeutic drug monitoring, co-medications were considered. The patients' data and health status were as well taken into consideration during the clinic-pharmacological interpretation of the results. The interaction table for the applied medication and co-medications generated by konbest was included in the interpretation sheet. The unit of the measured concentration was given in ng/ml. The dose-related reference range and the therapeutic reference

range of the concerned medication were presented, so that the attending clinicians easily had an overview of the measured concentration in comparison with the expected concentration. The result and the report were communicated to the clinicians within 1-2 days after measurement. Prompt alerting via telephone was for example, applied once in this work, when a flupentixol measurement gave the concentration of 400 ng/ml. The serum sample was collected after the medication intake, so that the peak concentration instead of the steady-state concentration was measured. The concerned flupentixol sample was therefore nullified.

4.3.3 Pharmacist's Support at the Clinic Ward

The pharmacist visited the clinic ward after the laboratory measurements. The serum concentration of melperone, benperidol, haloperidol, bromperidol, flupentixol and zuclopenthixol was reliably measured using the validated method. Some samples for these measurements were made available from the patients at clinic ward 1B and from the employees of the clinic and polyclinic for psychiatry and psychotherapy, University of Regensburg. The clinical pharmacological interpretations of the obtained values were successfully done by the pharmacist and further clarified through the clinic ward visitations. As mentioned already, the medications treated at the clinic ward were not only antipsychotics. Other medication groups which are not applicable in TDM were also treated.

4.3.3.1 Clinic Ward Visitation

The pharmacist's clinic ward visitations, conducted with four attending clinicians, one pharmacist, four nurses and psychologists, two social workers, and three interns were successful. During field work, the number of intern students varied and one

attending clinician transferred to another ward. On two occasions, visiting clinicians were present during the discussion. Three attending clinician participants followed the activities of the pharmacist at the ward to the end of three years research study. Specific requests about drug information and their relationship with adverse drug reactions and the laboratory measurements were discussed only with the attending clinicians. Many reports and requests regarding adverse drug reactions and the reasons for non-compliance were obtained in the triple team discussion (patient-clinician-pharmacist) and in the discussion with the attending clinicians.

4.3.3.2 Therapeutic Measures at the Clinic Ward

The groups of drugs applied during the therapy were mostly antidepressants, antipsychotics, mode stabilizers, anxiolytics, and sedative-hypnotics. Drugs from each group were either administered alone (mono-therapy) or in combination with another drug (co-medication) of the same group or of another group to achieve a better therapeutic result. Accepted standardized diagnostic criteria were used in accordance with the International Statistical Classification of Diseases and Related Health Problems (ICD). The drug administration was carried out by the attending clinicians applying the guidelines of the German Association for Psychiatry and Psychotherapy (DGPPN).

The station offers an integrative therapeutic approach to support drug therapy during hospitalization. With the aid of well-documented therapeutic procedures such as psycho-educational groups, social skills training, cognitive training and occupational therapy, the patients were disposed to contribute to a successful therapy during their

hospitalization. These non-drug therapies were carried out under consideration of their medication intake and state of health.

4.3.3.3 Clinical Requests during Clinic Ward Visitation

There were two types of requests made during the ward visitations. Questions about adverse drug reactions and specific drug information were asked by the clinicians as well as the patients. There were cases in which requests about adverse drug reactions were made before beginning a drug therapy for clarification purposes. Information on drugs was also provided in cases where the encountered undesired drug effects had not been reported before. In such cases, different studies from reliable sources of journal were provided to help the clinicians in their daily clinical decisions by supplying them with the expected information on the drug. Recommendations to implement therapeutic drug monitoring for the determination of drug serum concentration were given by the pharmacist in reports concerning undesired drug effects, dosing, compliance problems, and a lack of effect.

By higher serum concentrations, the dose was titrated until an optimal dose for each patient was achieved just as it was in the case of lower serum concentration. The therapeutic strategy was changed in cases where no therapeutic success was achieved within two to four weeks. ADR (adverse drug reaction) reports and reports concerning undesired drug effects given during the visit, were documented in the report forms (see appendix 12) of the working group for pharmacovigilance in psychiatry (AMÜP).

4.3.3.4 Groups of Clinic Therapeutic Request

Seventy-nine requests were made during ward visitation. These requests were grouped into two subsets, namely requests about drug information (29 requests) and reports of adverse drug reactions or undesired drug effects (50 requests). Requests concerning information on the drug which were based on an adverse drug reaction or had connection to such a reaction were grouped under adverse drug reaction (see figure 55).

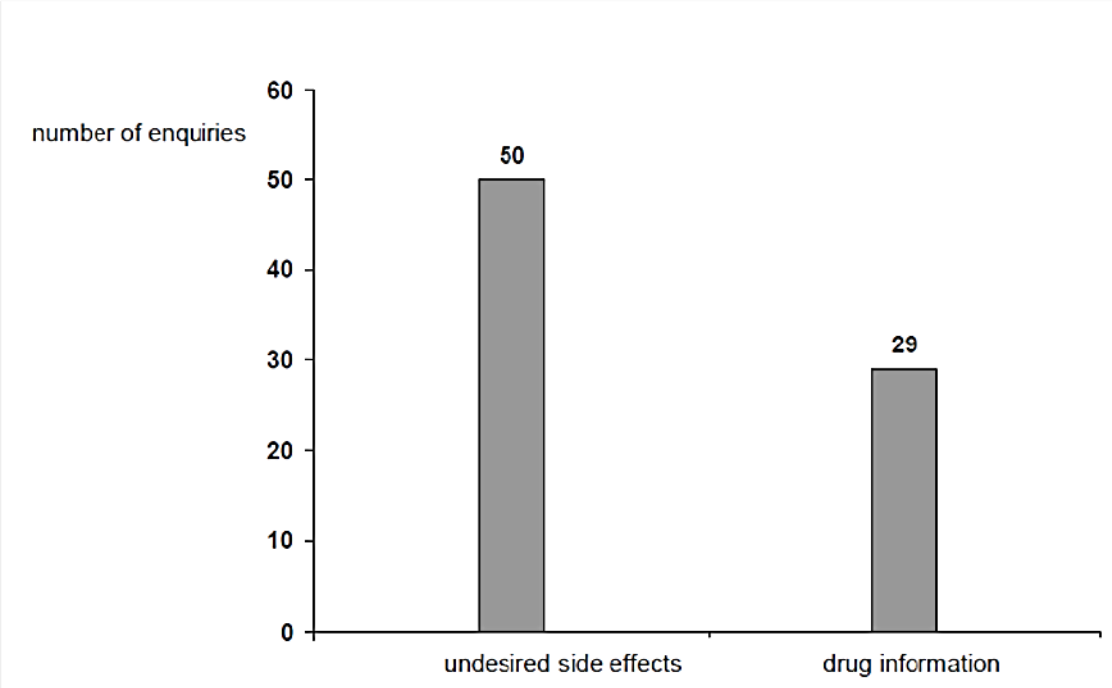


Figure 55. The group of requests treated during the clinic ward visitation.

Information on drug and processing period

The processing period of the information required varied from one day to 30 days depending on the type of information that was sought after. Information on drug that required intensive research and was not urgently needed for the therapy, took more time to procure than types. 48.28% of the received drug information was processed

within one day, 34.48% was processed within 7 days, and 17.24% within 28 days (see figure 56). Several intensive researches regarding unexplained undesired drug effects were carried out with the Red-List®-Drug Directory for Germany [Rote Liste® Arzneimittelverzeichnis, 2012] and other recognized sources of scientific literatures.

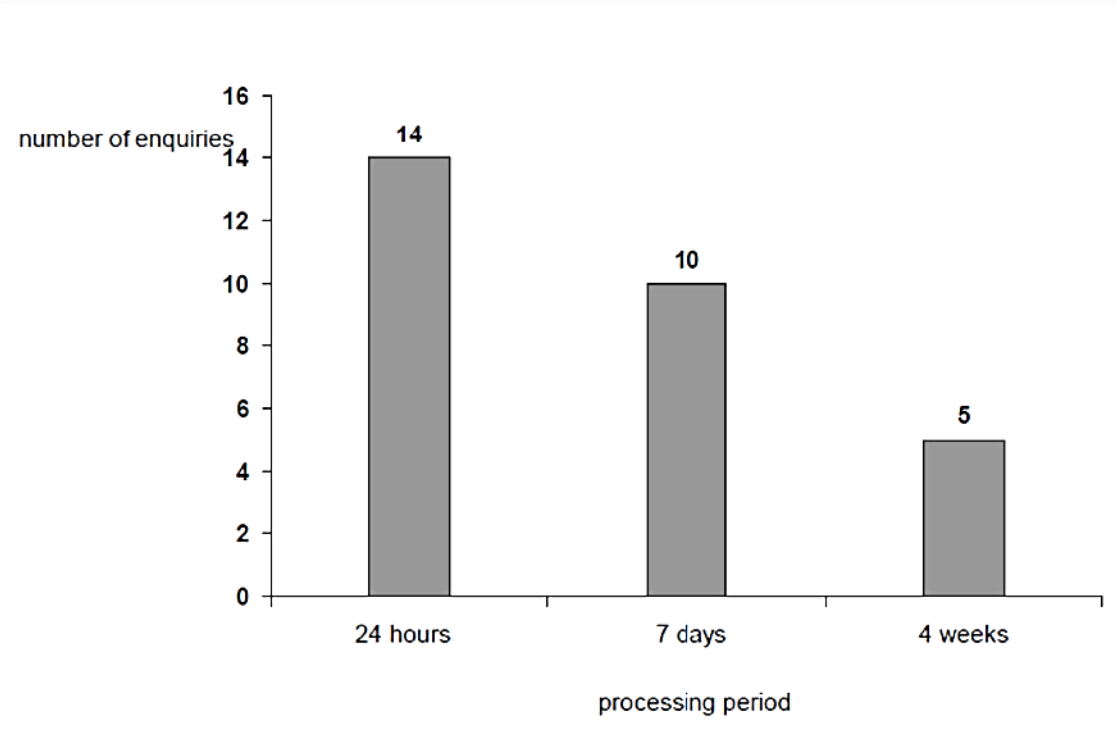


Figure 56. The average period of time applied for clinic therapeutic answers and advices.

Certain required information was observed to appear in more than one group depending on what they entailed, and some did not directly involve any medication but rather appeared between one illness and the other. Therefore another evaluation was carried out based on drugs in relation to other drugs (18 requests), drugs in relation to illness (14 requests), and illness in relation to other illnesses (2 requests). See table 56 for type of drug information and their corresponding request.

Table 56. Number of requests concerning drugs and illnesses.

Classification of the Received Drug Enquiries	Number of Drug Information	Drug Information (%)
Drug-Drug	18	52.94
Drug- Illness	14	41.18
Illness- Illness	2	5.88
Total	34	100

The illnesses associated with the requested drug information were dissociative symptoms, nightmare, bipolar disorder, attention deficit hyperactivity disorder (ADHD), borderline personality disorder, fatigue, flash back symptoms, post traumatic stress disorder (PTSD), serotonin syndrome, diabetes, delirium syndrome, psychotic state, QTC-time increase, glaucoma, increased blood flow, and severe depressive episodes. The obtained information regarding drugs in relation to other drugs was further differentiated into 13 groups following the similarity of the required information and reports. Table 57 shows the related enquiry for each type of drug information.

Table 57. Differentiation of requests on drug information.

Type of drug information	Enquiry
Pharmacokinetic properties of drugs	• The elimination half-life of fluphenazine retard • The elimination half-life of topiramate
Combination of drugs	• Possibility of increased seizure under the following co-medications: bupropion/nortriptyline and bupropion/citalopram • Influence of co-medications (methylphenidate ret / citalopram/ quetiapine) on the anticontraceptive

	femigoa (ethinylestradiol/levonogestrel) • Combination of sertraline and warfarin and its effect • The effect of the following co-medications amitriptyline-clozapine-lithium-quetiapine
Off-label use of drugs	• General off-label use of antidepressants • Ineffectiveness of lorazepam at higher doses • Effect of methylphenidate on ADHS patients • Off-label application of methylphenidate and moclobemide • The risk of dosing citalopram above 40 mg
Self medication	• The contents of Morinda Citrifolia and grape; the relationship between grapes and morinda citrifolia
Contents of foreign food items used by certain patients during the therapy	• The contents of morinda citrifolia and grapes
Differences within medication groups	• Triptans, serotonine and antidepressants. • What differentiated Nortriptyline, fluoxetine, and doxepine
Development of sickness through medication intake	• Elontrile (bupropion) and glaucoma • Mirtazapine influence on diabetes • The possible development of diabetes through lamotrigine • QTC-time increase by citalopram and other SSRIs
Triggering of an illness through a pre-existing illness	• ADHD and borderline syndrome
Explanation of sicknesses that can terminologically be misused	• Differences between delirium syndrome and psychotic state
Clarification of non-documented contraindications experienced by individual patients	• Agomelatine and cardiac contraindication • Agomelatine and trigger effect by bipolar disorder • Absence of withdrawal symptoms by sudden discontinuation of lorazepam therapy
Drug formulations and their application methods	• Application possibilities of different risperidon formulations (Risperidon oral, risperidon Consta and xeplion)
Comparison of European drug	• QTC-time increase by citalopram according to FDA

information with other countries	
Journal researches	• Possible application of resolor by patients with bipolar disorder • Combined application of jatrosom and lithium • The effect of mirtazapine on the brain • The use of topiramate for the treatment of PTSD • The treatment of flash-back symptoms with prazosin, by patients with PTSD • The use of naltrexone to treat nightmares • The influence of naltrexone after a long-term therapy by patients with dissociative symptoms

Note: Different requests reported as regard to drug information were grouped according to similarity of the reports.

Treated Medications for request concerning drug Information

Information concerning 33 (100%) drugs and groups of drug were treated during the clinic ward visitation. As shown in table 58, 42.42% of the total drugs were repeatedly mentioned in different requests concerning drug information. 57.58% of the requests resulted from the therapeutic drug monitoring of patients' drug in serum and dose adjustment. The most repeatedly requested drugs were citalopram (12.12%), bupropion (9.09%), methylphenidate (9.09%), nortriptyline (6.06%), lithium (6.06%), agomelatine (6.06%), flupentixol (6.06%), lorazepam (6.06%), mirtazapine (6.06%), quetiapine (6.06%), risperidon (6.06%), topiramate (6.06%), and phenprocoumon (6.06%).

Table 58. The active substances of the reported medications.

Drug	Active substance
Agomelatine	Agomelatine
Amitriptyline	Amitriptyline
Natural grapes	Natural grapes

Bupropion	Bupropion
Citalopram	Citalopram
Clozapine	Clozapine
Doxepine	Doxepine
Fluanxol	Flupentixol
Femigoa	Levonorgestrel + Ethinylestradiol
Fluoxetine	Fluoxetine
Grapes	Vitamines
Jatrosom	Tranylcypromine
Lamotigine	Lamotrigine
Lithium	Lithium
Lorazepam	Lorazepam
Lyogen ret	Flupehnazine
Methylphenidate	Methylphenidate
Mirtazapine	Mirtazapine
Moclobemide	Moclobemide
Morinda citrifolia (Noni)	Phytoestrogene + Oligosaccharides + Polysaccharides + Flavonide+ Iridoide + Catechin + Epicatechin + Betasitosterol + Alkaloide.
Naltrexon	Naltrexon
Nortrilen	Nortrilen
Prazosin	Prazosin
Quetiapine	Quetiapine

Resolor	Prucaloprid
Risperidon	Risperidon
Seroquel	see Quetiapin
Sertraline	Sertraline
Topiramate	Topiramate
Marcumar	Phenprocoumon
Antidepressant	Drug groups
Triptanes	Drug groups
SSRIs	Drug groups

Note: List of active substances of the drugs; the last three lines show the enquiry on groups of medication.

Enquiries about Adverse Drug Reactions

Fifty (100%) adverse drug reactions (ADRs) were reported during the clinic ward visitation (see figure 57). 72% of the reported ADRs are documented in the record sheet (see appendix 13) of Pharmacovigilance in the Psychiatry (AMÜP: Arzneimittel Überwachung in der Psychiatrie) at the university of Regensburg. 28% of adverse drug reactions are not included in the data collection sheet, because some important information needed to evaluate the data such as age and exact medication history of the patients were not reported before their discharge. All the reports concerning adverse drug reactions were treated immediately and the clinic therapeutic advices were given in less than seven days to help the clinicians in their therapeutic decisions.

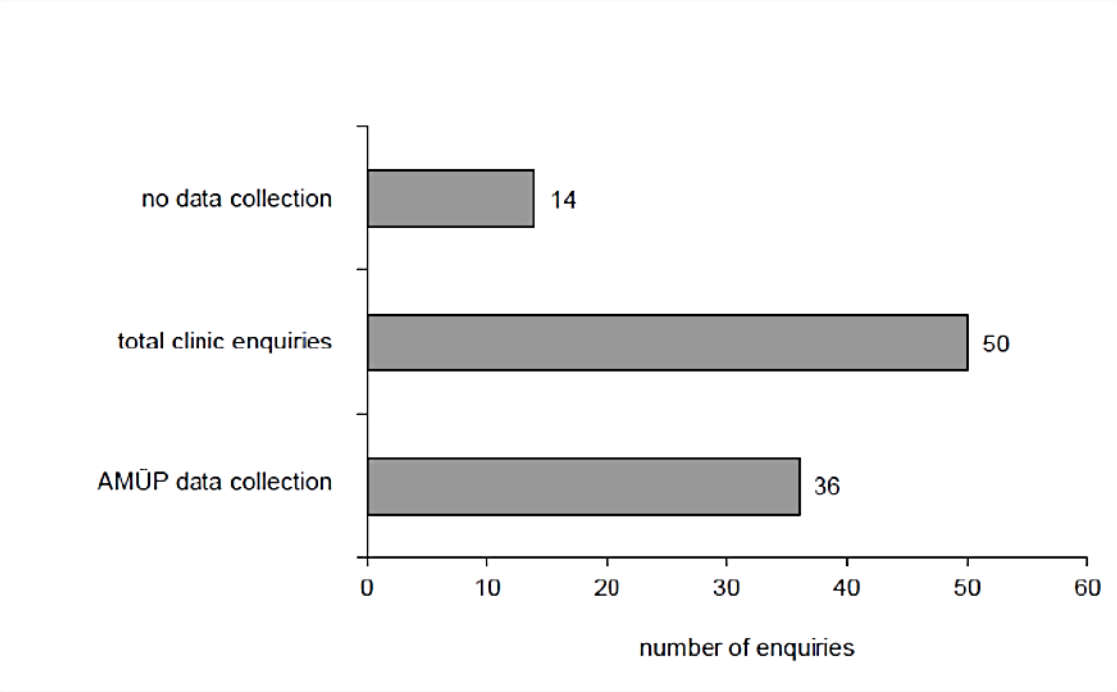


Figure 57. Number of collected data records of undesired drug effects.

The reported adverse drug reactions were grouped in psychotic reactions, neuronal symptoms, gastrointestinal tract reactions, skin reactions, lack of drug efficacy, hormonal dysfunction, clotting problems, edema, liver problems, urinary tract problems, cardiac problems, heart and blood circulatory system, immune system, drug interactions, vitamins, and water regulation of the body (see table 59).

Table 59. List of drugs and the reported adverse drug reaction.

Adverse Drug Reaction	Related Drug
Psychotic reactions	Ciprofloxacine, Infliximab, Cortisone, Metronidazole
Delirious symptoms	Nortriptyline, Lorazepam, Carbegolin, Seroquel, Abilify
Missing hangover effect	Remergil
Lack of drug efficacy	St. Johns word
Parkinson symptoms	Fluphenazine

Weight gain	Seroquel, Risperidon, Abilify
Constipation and sudden diarrhea	Valdoxan, Bisoprolol, Agiocur, Vitamins B_{12}
Restlessness	Quetiapine
Dry mouth	Risperidon, Quetiapine, Olanzapine
Decrease in neutrophilia	Valproate, Perazine
Pressure in the heart	Quetiapine, Risperidon, Lyrica
Addiction	Tilidin, Tramadol
Encephalopathy	Valproate, Perazine
Dry skin	Clomipramine
State of confusion	Lyrica
Urine retention	Imipramine, Clomipramine
Hyper-salivation	Olanzapine
Vocal-tics	Nortrilen
Spasms	Valproic acid
Heavy legs	Gabapentin
Itching	Pregabalin
Erectile dysfunction	Fluphenazine, Mirtazapine
Massive alopecia	Venlafaxine
Change of heart clapper	Amphetamine
Tremor	Lyrica
Vomit	Seroquel
Sexual dysfunction	Cymbalta

Hyponatremia	Fluanxol, quetiapine, seroquel
Nightmares	Pregabalin
Reslex	Quetiapine
Suicide attempt	Risperdal
Increase of eosinophil	Mirtazapine
Akathisia	Risperdal, Quetiapine
Increased bladder pressure	Cymbalta
Fatigue	Diazepam
Steadily increase of transaminase	Seroquel, Venlafaxine
Seizure	Ergenyl
Hypotension	Bisoprolol, clozapine, venlafaxine
Edema	Jatrosom, Seroquel, Risperdal, L- thyroxine
Haggle feeling	Sertraline
Diffuse hair loss	Valproic acid
Platelet function and bleeding under medication intake	Valproic acid
Scabies	Lamotrigine
Nausea	Venlafaxine
QTC-time extension	Zeldox, Quetiapine
Sudden drop in blood pressure	Prazosin
Tiredness	Tavor
Vitamin B12 deficiency	Topiramate
Elevation of the Leucocytes	Diazepam

Abnormal sleep	Nortrilen

Note: The table shows the adverse and the undesired drug reactions treated during the advice on drug information. For each ADR, the relevant drug is listed.

Frequency of Medication Reports Concerning Adverse Drug Reactions

Forty-one (100%) different medications were involved in the reported adverse drug reactions. The most reported active substances were quetiapine (31.71%), risperidon (17.07%), valproic acid (14.63%), pregabalin (12.20%), venlafaxine (9.76%), Nortriptyline (7.32%), aripiprazole (4.88%), bisoprolol (4.88%), clomipramine (4.88%), diazepam (4.88%), duloxetine (4.88%), fluphenazine (4.88%), lorazepam (4.88%), mirtazapine (4.88%), olanzapine (4.88%), and piperazine (4.88%).

4.3.3.5 Clinic Ward Questionnaires

After the analysis of the reports received during the clinic ward visitation, a general evaluation regarding the impact of the pharmacist at the clinic ward activities were summarized in the follow-up questionnaires.

Evaluation of the Clinicians' Questionnaire

There were four factors considered during the evaluation of the returned questionnaires (see appendix 10) namely the non-drug therapeutic forms, therapeutic decision undertaken through the pharmaceutical advice, the competency of the advisory pharmacist for the therapeutic decision of the clinicians, and general comments (see table 60). The therapeutic decisions were for example the decisions taken to reduce or increase the medication dose and/or to choose an alternative medication to improve individual therapy.

Table 60. Factors used for the clinician's questionnaire.

Factors	Question Numbers
The non-drug therapeutic forms	3-5
The action taken on the occurrence of adverse drug reactions	4-10
The competency of the advisory pharmacist for the therapeutic decision of the clinicians.	11-15
General comments	-

Note: Non-drug therapeutic forms are therapies such as ergotherapy, and psychological advices and practices.

The age of the attending clinicians in ward 1B as at the time of the field work ranges from 34 to 64 years. 33.3% of the questioned clinicians treated both out-patient and inpatients, 66.7% treated only inpatients. All the attending clinicians have treated more than 20 patients within the period of this study. Their main diagnostic groups according to the ICD-10 (International Statistical Classification of Diseases) are listed in table 61. The secondary diagnoses were given to be F10 (mental and behavioural disorders due to use of alcohol), F12 (mental and behavioural disorders due to use of cannabinoids) and F13 (mental and behavioural disorders due to use of sedatives and hypnotics).

Table 61. The diagnosis according to ICD-10.

ICD-10 Classification	Diagnoses
F06	Organic hallusinosis
F20.0	Paranoid schizophrenia
F20.1	Hebephrenic schizophrenia

F31.1	Bipolar affective disorder, current episode manic without psychotic symptoms
F31.3	Bipolar affective disorder, current episode mild or moderate depression
F32	Depressive episode
F33	Recurrent depressive disorder
F33.2	Recurrent depressive disorder, current episode severe without psychotic symptoms
F40	Phobic anxiety disorder
F41	Other anxiety disorders
F41.1	Generalized anxiety disorder
F43	Reaction to severe stress, and adjustment disorders
F44	Dissociative (conversion) disorders
F60	Specific personality disorders
F61	Mixed and other personality disorders

Note: ICD-10 diagnostic groups [World Health Organisation, 1993] of the treated sickness as recorded by the attending clinicians.

The result obtained by the evaluation of clinician questionnaire gave information about the medications and group of medications applied by the attending clinicians for the treatment of the diagnoses mentioned in table 61. The medications included, among others, antidepressants, antipsychotics and mood stabilizers. Non-drug therapeutic forms that accompanied the drug therapy were mostly cognitive training, cognitive psychotherapy, psycho-educative groups, occupational therapy, and psychotherapy sessions with an orientation towards behavioral therapy. The

attending clinicians (n=4) assessed their patients with regard to drug therapy. 5% of their patients were assessed to be very satisfied, 70% satisfied, 15% slightly satisfied and 10% dissatisfied. Other answers given by the clinicians to the questions on the questionnaire are reported on table 62. The clinicians recommended the continuation of the pharmaceutical advices through drug researches and drug information, because they contributed to their therapeutic decisions and helped in the optimization of an individual therapy.

Table 62. The evaluation of the result of the clinician questionnaire (n = 4).

Questions	**Yes (%)**	**No (%)**	**Not Really (%)**	**No Idea (%)**
Did adverse drug reactions occur?	4 (100)	-	-	-
Did adverse effect occur only after the administration of certain drugs?	2 (50)	1 (25)	1 (25)	-
Are there alternative explanations (apart from medication) for the occurrence of adverse drug reactions?	3 (75)	1 (25)	-	-
Were the adverse drug reactions confirmed by documented guidance?	3 (75)	1 (25)	1 (25)	-
Did the advice of the pharmacist help in therapeutic decisions?	4 (100)	-	-	-
Are the answers and information supplied as regards to medication questions complied with your expectations?	4 (100)	-	-	-

Did the supplied answers to your questions about adverse effect met your expectation?	4 (100)	-	-	-
Did the presence of a pharmacist contributed to a successful therapy?	4 (100)	-	-	-
	Good	Not Good	Bad	No Idea
How would you rate the work of the pharmacist in your station?	4 (100)	-	-	-

Note: The table contains the summary of the fifteen questions in the clinician questionnaire. The last question has another form of answer which does not coincide with the previous questions in the table.

Evaluation of the Patients' Questionnaire

The patients' questionnaire (see appendix 11) was carried out on a voluntary and anonymous base. The four sections of this questionnaire were gender, age, sickness progression and the involvement of patients in the treatment decisions as well as in the ongoing management of future treatment. Twenty-five patients were questioned. Any question point that was not answered was grouped under "no idea" (see table 63). 60% of the patients that attended to the questionnaire were female and 40% of them were male. Their ages were between 32 and 51 years. 80% of the patients were inpatients and 20% of them were outpatients. The ICD-10 diagnoses of the patients were from F06 to F61 (mental and behavioural disorders). All the patients were medically advised from their attending clinicians. The therapeutic goals and expectations were clarified before and during the therapy. Through the collaboration of the clinic ward psychologists and nurses, the therapeutic goals were successfully strived after during the patients' admission.

Table 63. The results of the patients' questionnaire (n = 25).

Questions	Yes (%)	No (%)	Not Really (%)	No Idea (%)
Were you informed of the name of the consultant, nurse or therapist?	20 (80)	-	5 (20)	-
Were you given all the privacy needed during your treatment?	18 (72)	7 (28)	-	-
Were the procedures and next steps in your treatment explained in a way you could understand?	20 (80)	4 (16)	-	1 (4)
Did you feel involved in deciding about your treatment plan?	23 (92)	-	-	2 (8)
Was the effectiveness of your medication explained to you?	19 (76)	3 (12)	-	3 (12)
Were you informed of the possible occurrence of adverse side effect of your medication?	15 (60)	-	2 (8)	8 (32)
Have you already experienced adverse side effect?	22 (88)	3 (12)	-	-
If you were to continue your medication or treatment at home, were you given a clear explanation and written instructions of what to do?	24 (96)	-	-	1 (4)
Did you feel you were given all the time and attention needed?	20 (80)	-	1 (4)	4 (16)

Note: This table contains the summary of the thirteen questions in the patient questionnaire.

The patients' satisfaction with the attending clinicians, the pharmacist, and the clinic staff regarding the explanations about their therapy and their drug-intake show a

good indication of treatment adherence and thus a high chance of successful treatment. The attending clinicians were supported in their timely therapeutic decisions through the help of the pharmacist in drug-related issues. They were also of the opinion that pharmaceutical knowledge is an inevitable help for clinicians to calculate dose adjustments or to analyze interactions with other drugs. All the answers showed that the presence of the pharmacist in the clinic ward was appreciated and will always be needed

4.3.4 Survey of Literature on the Importance of a Pharmacist in the Clinic Ward

Different reports were studied regarding the activities of pharmacists at different clinics, the ideas derived from the literature survey was successfully reviewed and modified for a reliable field work at the clinic ward 1B.

Marino et al. reported in 2010 on the differences in pharmacy interventions at a psychiatric hospital. The 2,220 interventions made by pharmacist during their study were based on the documentation of medication errors, undesired effects and the reduction of undesired effects following pharmaceutical advices. Their results show the positive contribution of pharmacists on clinical therapeutic interventions for the optimization of individual patient therapy [Marino et al., 2010].

The development of a standard form for the collection of data such as drug and drug related problems, type of interventions, and the estimation of their significance during pharmacy intervention was reported by Alderman in 1997. According to his report, medical staffs accepted that 91.7% interventions with antidepressants top rated as

the cause of the intervention. The recommendation for drug therapy and patient monitoring brought about an optimization of treatment of outcomes [Alderman, 1997].

Van Wijk (2005) reported some discrepancies regarding the intervention outcomes of community pharmacists in the medline research carried out in 2005. Pharmaceutical advices, drug monitoring, and training served as effective tools for the pharmacy intervention in some studies and were also reported as ineffective by other studies. The problem was attributed to lack of well-designed and well-conducted studies on the effectivenss of interventions by pharmacist, which according to this report need to be improved [Van Wijk, 2005].

A systematic review of clinical pharmacist and inpatients medical care carried out by Kaboli et al. (2006) showed that the addition of clinical pharmacist services in the care of inpatients generally resulted in improved care, with no evidence of harm. Interacting with the health care team on patient rounds, interviewing patients, reconciling medications and providing patient discharge counseling and follow-up all resulted in improved outcomes after the revision of 36 studies consisting of 10 evaluating pharmacists' participation on rounds, 11 medication reconciliation studies, and 15 drug-specific pharmacist services [Kaboli et al., 2006].

During this work, the ward clinicians acknowledged the work of the pharmacist in the ward and acted on her suggestions for interventions regarding drug-related problems. Drugs reviewed by the pharmacist and follow-up discussions with attending clinicians and patients resulted in a reduction in suboptimal prescriptions among hospitalized patients in psychiatric ward 1B. This was proven from the TDMs carried out for different patients in that ward and out-patients as well.

5 Discussion

This study aimed at developing a new analytical procedure for the accurate and specific determination of antipsychotics with low therapeutic concentrations and ranges such as melperone (MLP), benperidol (BPD), haloperidol (HLP), bromperidol (BRP), flupentixol (FLT) and zuclopenthixol (ZLT), in human serum. In this context, the application of simple, reliable, less expensive HPLC equipment and parameters (HPLC) and an ultra violet (UV) detector is demonstrated to be effective in providing high sensitivity and selectivity. The developed method provides the possibility of measuring these substances simultaneously and individually at low cost especially for routine applications at small scale TDM laboratories, where these substances could not be tested reliably until now.

The HPLC/UV, a less expensive analytical equipment was employed in two separation methods dedicated respectively to the qualitative and quantitative serum determination of MLP, BPD, HLP, BRP, FLT, and ZLT. The procedures were designed by first testing the dissolution character of each substance in the mobile phase solvents, columns were tested and short sized columns with high resolution were preferably chosen, because this will keep the total chromatographic run time short.

Several experiments were carried out to determine a pH value applicable for detecting the smallest concentration of all the substances, so as to ensure that all the substances remain stable in the solution during the simultaneous determination and throughout the chromatographic process. The applied UV detector has a special feature (diode array detector) of measuring the substances with variable wavelengths, so that wavelength that gave the expected peak character at small

concentration of each substance was chosen. Medium wavelengths between 230 nm and 245 nm were preferred and chosen, to reduce the risk of interference with other substances including serum materials, which can easily be detected at lower and higher wavelengths [Gunasekaran und Uthra, 2008]. With this design, this study succeeded to establish a method to determine lower concentrations of antipsychotics. This method is less expensive and easy-to-use, especially at small-scale TDM laboratories.

The isocratic separation method allowed a simultaneous serum determination of MLP, BPD, HLP, BRP, FLT, and ZLT with a time gap between MLP/BPD and HLP/BRP. The multistep gradient method was employed to reduce the gap between the peaks, thereby reducing the total run time. The same solvent that was applied for the isocratic separation (ACN/ MeOH/ TEMED/ H2O) was also applied for the gradient separation, but at different volumes and at different times. The determination with the gradient separation method was successful, but however the expectations were not met. The gap between the peaks (MLP/BPD and HLP/BRP) were not reduced, the total run time was longer and more volume of solvents were used for the measurement, when compared with isocratic separation method. The gradient method was therefore not continued for the validation.

The developed HPLC/UV analytical method with isocratic separation was validated by following different international validation guidelines acceptable for the validation in human serum such as ICH validation guideline, the guidelines of GTFCH, FDA and the guidance for industry on biomedical method validation. The results of validation were all within the acceptance criteria and proved that this method is reliable for the serum quantification of the applied substances. The sensitivity, selectivity and the

precision of the validated analytical procedure enable measurements of low serum concentrations of the substances without additional purification steps, which proved less-time consuming. The method was successfully applied to the quantification of MLP, BPD, HLP, BRP, FLT, and ZLT in human serum.

The method can be used in all TDM laboratories, especially small scale laboratories, because it is among others, precise, robust and cheap. Preliminary experiments suggest that it can also be applied in the serum measurements of other substance groups apart from antipsychotics. Only the set parameters and conditions used during the validation of this method should be applied for a reliable result for the measurement of melperone, benperidol, haloperidol, bromperidol, flupentixol and zuclopenthixol in human serum. No special experts are needed to operate this method. Users of this method in small-scaled TDM laboratories can easily operate it once they know the principle of HPLC/UV detector system and by following the validated set parameters and conditions.

It has to be emphasized that this method is applied for the first time at TDM laboratory in Regensburg for the determination of the study substances. Similar analytical procedures have been discussed by different researchers. Seiler et al. (1994) and Furlant et al. (1987) succeeded respectively, with the application of HPLC/EC method to determine benperidol in human serum. Furlant et al. (1987) successfully reached a low detection limit of 0.2 ng/ml with 1 ml volume. Comparing to Furlant et al. (1987), this study reached a higher limit of detection at 0.7 ng/ml, but this limit is acceptable because it is below the expected therapeutic reference range of 1- 10 ng/ml, so that serum concentrations within the expected TRR will reliably be reached with this method. Moreover, the developed method will be applied not only

for the determination of benperidol but also the determination of five other substances simultaneously or individually in human serum. With a much lower detection limit of 0.1 ng/ml, Hiroshi et al. (2000) succeeded in the simultaneous serum determination of five butyrophenones, but with the use of an expensive method. The HPLC/MS, requires special experts for its application. This solution is not easily accessible for small-scale TDM laboratories.

The HPLC/UV method described in this thesis is practicable to both small and large-scale TDM laboratories. Zhou et al. (2004) in their HPLC/MS method though succeeded to simultaneously determine four antipsychotics. They had to alkalinize and extract the preparations twice before they could measure [Zhou et al. 2004]. These preparation steps are not required in this method, which is an advantage for a faster measurement and communication of the result. Both volatile and non volatile substances can be measured with the elaborated method of this study unlike the GC/MS method described by Zhu et al. (1998), where they succeeded after an intensive preparation of the samples, to use the obtained results for the pharmacokinetics of clozapine.

Tanaka et al. (2007) used a HPLC/UV method just like in this study to successfully and simultaneously determine twelve phenothiazines in human serum. However, with the application of a pre-step method and the fourfold higher detection limit of 3.2 – 5.5 ng/ml, their method is of less advantage in the serum determination of antipsychotics with low and narrow therapeutic ranges.

Kirchherr et al. (2006) and Jain et al. (2011) both described analytical methods used for determination of haloperidol in human serum. Kirchherr successfully developed a HPLC/MS method for a simultaneous determination of forty-eight antipsychotics both

for butyrophenone and phenothiazine groups. The expected results were reached and the limit of detection for the substances showed good covering of therapeutic and subtherapeutic ranges [Kirchherr et al., 2006]. Though the number of substances that can simultaneously be measured with the developed method in this thesis is much lower, this method has unlike the method of Kirchherr et al. (2006) the advantage that it can be applied in all TDM laboratories, because of its simple and also reliable application. The HPLC/MS method and procedures applied by Kirchherr et al. (2006) are not only expensive, the samples also undergo an intensive preparation before they could be measured, which is not easily practicable in daily routine measurements in some TDM laboratories.

Jain et al. (2011) described the successful application of HPLC/UV to determine haloperidol, which was effectively applied for routine estimation of haloperidol in psychiatric patients [Jain et al., 2011]. The disadvantage of their method is that it needs a pre-step extraction procedure before the measurement. Another disadvantage is the high limit of detection for haloperidol obtained at 1.1 ng/ml, which is above the set therapeutic reference range for haloperidol according to AGNP Consensus Guideline, Update 2011.

The void observed by previous researches as explained above were all covered in the new method developed during this study. More than one substance can be measured individually and simultaneously. No pre-step and intensive preparations are needed. Both volatile and non-volatile substances are applicable. Cheap analytical equipment that does not require specialist for its operation can be applied. The disadvantage observed in this method is however, the slight higher detection limit obtained for flupentixol at 1.7 ng/ml, which is above the expected therapeutic

reference range of 1 - 10 ng/ml. Therefore, future work may be necessary here to obtain a lower detection limit applicable for flupentixol.

The results of the validation corroborate the observation, described also by other researchers, that with HPLC/UV analytical method, substances can reliably be determined in human serum. The applied variable wavelength UV detector (the diode array detector) allows for the best wavelength to be selected for the analysis [Titier et al., 2003]. A good separation demands an optimal resolution of the individual samples, especially when the routine application is to be used to analyze a number of patient samples [Snyder et al., 2012].

The isocratic separation of the substances was successfully achieved with the column Luna Phenyl-hexyl 150 x 3.0 mm, 3 µm for the applied butyrophenones and Nucleodur CN 150 x 4.6 mm, 3µm for applied phenothiazines. Both columns were selected for this method development because they gave satisfactory resolution in the mobile phase, consisting of acetonitrile/methanol/ TEMED/ water/ Orthophosphoric acid solvent system and they are compared with other columns less expensive.

According to studies, some proteins in serum-analyte have the ability to interfere with or attenuate the measurement signals of the applied substances [Hiroshi et al., 2000]. The test on interference carried out with 108 substances shows no interference with MLP, BPD, HLP, BRP, FLT, and ZLT. Additionally, standard samples were measured alongside with the unknown samples, which helped to control any unexpected interference. The Amount (concentration) obtained from the measurements of the standard solutions of melperone, benperidol, haloperidol, bromperidol, flupentixol, and zuclopenthixol correspond to the expected results. The

absorption increases with an increase in the concentration. A reduction in the molecular absorption unit during the long term stability test could be observed, though linear to the given concentrations. It can be related to redox-reactions that must have taken place during the preservation of the substances in the methanol solution. According to the reports of many journals such as Einosuke et al., (2007), the mentioned substances were stable for three months when preserved in -20 °C [Einosuke et al., 2007].

The developed method was successfully applied in the TDM routine measurements. Patients' samples were received together with the TDM request form. Results of the samples requested by different hospitals were evaluated with Konbest program [Wirkstoffkonzentrationsbestimmung, 2012]. The information recorded in the request form was considered for the clinic-pharmacological reports of the TDM result of the concerned substance. Some discrepancies already mentioned in some journals were observed in the completed form, such as dosing information, duration of the therapy, and serum samples at steady state concentration. According to the work of Vuille et al., 1991 and Zernig et al., (2004), such inadequate request lead to misinterpretation of results and to wrong dose adjustments [Vuille et al.,1991; Zernig et al, 2004]. Zernig et al., 2007 also mentioned the non-conformance with steady-state conditions and errors on the application form as other typical errors [Zernig et al., 2007]. Therefore, just as Hiemke, (2008) and Conca et. al, (2011) state, the current use of TDM in the mental health care needs to be improved [Hiemke, 2008; Conca et. al, 2011].

During this study, in cases of discrepancies on the TDM request form, the attending clinicians were contacted for a clarification of the issue before the clinic-

pharmacological report was written. The disadvantage is that it extended the time needed to communicate the result and moreover, only indicated errors were clarified. Errors regarding wrong information in dosing and dosage form were difficult to indicate, especially when the measured concentration was not significantly above or below the expected DRR and TRR.

The plotted dose-concentration diagrams of the substances were carried out with few patients' samples with the exemption of haloperidol and flupentixol. It is therefore advisable that more samples should be tested in future to confirm the results obtained in this study.

Studies regarding the metabolic pathway(s) of benperidol have also not yet been clarified, so that the interpretation of the measured concentrations above the expected DRR could not be given in this study. Sun und Scott (2011) reported that 4-aminopiperidens, example benperidol are metabolized by CYP3A4. The catalytic mechanism is however not yet clarified. The result of their experiments suggests that there are interactions between 4-aminopiperiden substrates and CYP3A4 active site [Sun and Scott, 2011]. It has to be mentioned that the samples above the DRR and TRR were co-administered with other medications which could have an impact on the metabolism of benperidol.

The consensus data (DRR) comparison with the obtained concentration of the measured samples were carried out to evaluate the behavior of the obtained concentrations with the expected DRR, by sole administration and by co-administration with other substances. The C/D values applied for the evaluation of DRR for flupentxiol in this study was derived from the applicable value in TDM laboratory Regensburg. These values differ from that of consensus. In the consensus

data according to AGNP guideline, Update 2011, [Hiemke et al., 2011] the given range of (0.78 – 0.87 ng/ml/mg) in consensus is not differentiated between the oral and intravenous (depot) application. Therefore two different evaluations were carried out in this study; firstly the comparison between the measured concentrations and the values obtained with DRR data from TDM laboratory, secondly the comparison of the measured concentration with the values obtained with the given consensus DRR data. The C/D calculation of consensus with regard to flupentixol should be re-evaluated. The different behavior of the depot and oral application in serum should be considered, because of non-lineal relationship observed in the presented dose-concentration diagram of flupentixol with the applied TDM-data (data from TDM Laboratory Regensburg).

Jorgensen (1980) and Jann et al., (1985) reported about the observed differences in the pharmacokinetics of depot and oral antipsychotics. Jorgensen evaluated the pharmacokinetic studies of flupentixol for oral and depot application form. He observed that the intravenous flupentixol followed a multi-compartment model, which data he however did not succeed to fix to a particular compartment. Slow absorption was observed after oral administration while the serum concentration after intramuscular injection of flupentixol decanoate clearly related a depot effect. He mentioned the probability that the approximate half-life obtained during the study was not the elimination half-life but the release of drug from the oil depot [Jorgensen, 1980]. This finding is supported by Jann et al., (1985) in their study on the kinetic properties, the relationship of plasma concentrations to clinical effects, and conversion from oral to injectable therapy. They reported the observation in the absorption rate constant of depot antipsychotics, which is slower than the elimination

rate constant and therefore the depot antipsychotics exhibit “flip-flop” kinetics regarding the time to steady-state and the concentration at steady state [Jann et al., 1985]. The observation made in this study regarding the dose-concentration diagram of the administered flupentixol, did not however distinguish the application of flupentixol in intravenous and intramuscular form. As mentioned already, the correct administered dosage form should be recorded in the TDM request form and the C/D value in the AGNP Consensus Guideline for flupentixol should consider the differences in serum concentrations of flupentixol using different application forms.

There are other factors that can influence the concentration of drugs in human serum. There are discrepancies in literature findings concerning the influence of smoking on antipsychotics such as the haloperidol and flupentixol and the sickness schizophrenia.

Shimoda et. al, (1999), reported the impact of smoking on plasma haloperidol concentrations of patients who smoked ten cigarettes per day. They satisfactorily observed that at low maintenance doses /kg body weight, smokers showed significantly lower concentrations than nonsmokers. They did not observe any significant difference in doses more than 0.2 mg/kg body weight [Shimoda et. al., 1999]. They however, did not pursue the impact of therapeutic monitoring of the haloperidol serum concentration of smokers. The data used for the evaluation during this study, the study of the influence of smoking on haloperidol and flupentixol serum concentration, did not specifically monitor the number of cigarette smoked by patients during therapy with haloperidol and flupentixol, because this information was not given for all the smokers, and also the information regarding the body weight and body mass index (BMI) of some patients was not recorded. This study however,

confirms as a major finding, that individual haloperidol and flupentixol serum concentrations of smokers and nonsmokers were well controlled through TDM, so as to minimize concentrations at toxic plasma levels and undesired drug effect. Therefore, no significant influence between smokers and nonsmokers as shown in the comparison of the DRR for smokers and non-smokers was observed for flupentixol. The slight difference in the high concentration of smokers is referred to the influence of the administered co-medication and individual variations. The two outlier observed in dose-concentration diagram of haloperidol by smokers were caused by the prescribed co-medications which have the same metabolic pathways like haloperidol. No co-medication was given for one of the samples with outlier serum concentration, and there was no evidence of drug interactions or unsual metabolism status of the patient. The information about the health status of the patient and the undesiered effect (UDE) were not given. An individual variation should therefore not be excluded, under the conditon that the TDM request form is correctly completed and that the serum sample was collecetd at steady-state as advised.

Lucas and Martin, (2013), reported a significant increase of the activities of Cytochrome P450 (CYP) 1A2 in patients who smoked more than 20 cigarettes a day, compared with nonsmokers. They attribute this to the pharmacokinetic interactions of cigarette smoking, which induces CYP1A2. Alongside with clozapine, haloperidol is mentioned in their study as one of the substrates of CYP1A that can be induced through smoking [Lucas and Martin, 2013]. They could however not confirm how the number of daily smoked cigarettes and the inter-individuality of the patients affect CYP induction.

Jiang et al., (2013), researched the mechanism associated with heavy smoking by male schizophrenic patients and the suggested hypothesis about the alleviation of schizophrenic symptoms and reductions of undesired effect. They successfully concluded their analysis with the insight that smoking is associated with improved negative symptoms which could account for heavier smoking among schizophrenic patients [Jiang et al., 2013]. The study presented in this thesis has shown that through TDM and the correct data records in konbest, serum concentration of individual patients under the consideration of their inter-individualities can be controlled.

During the therapeutic drug monitoring of haloperidol and flupentixol, an adjustment of the maintenance dose was repeatedly carried out by the attending clinicians to reach the therapeutic serum concentration for individual patients. Since smokers reached lower haloperidol and flupentixol serum concentration than non-smokers, respectively, they were prescribed higher dose by the attending clinicians, in order to obtain serum concentrations at their individual therapeutic level. Because of the desired and controlled individual dose adjustment during TDM, patients' therapy were monitored effectively. The collected konbest data was enough to support the fact that smoking reduces the haloperidol, but not the flupentixol serum concentration.

The data evaluation on the influence of smoking on haloperidol and flupentixol carried out in this work is focused on the serum concentration obtained by smokers, irrespective of the number of cigarettes smoked, and the difference when compared with nonsmokers. This is to improve an effective individual therapy through dose adjustment and reduction of undesired drug effect. However, for better

understanding, control and optimization of antipsychotic therapy by schizophrenic patients, a compound controlled TDM study with possibly schizophrenic patients from different ethnicity should be carried out. It should comprise the type and the number of cigarettes smoked, selected antipsychotics and co-medications, measurement of serum concentrations before and after dose adjustments and the impact of smoking on the schizophrenic ailment of these patients before and after the study. The knowledge that will be obtained in this research will throw more light on the relationship between serum antipsychotic concentration, smoking, schizophrenic symptoms and sickness progression, among individual patients. It will also enhance an effective individual therapy. A compound controlled TDM study mentioned here, is a study in TDM context and guidelines consisting of the aformentioned factors.

The outstanding aspect of this work is the close communication with the attending clinicians in terms of clarification of TDM and pharmaceutical advice at the clinic ward 1B, not only for antipsychotics but also for other medication groups. One of the reasons observed during this study for the request of TDM by the attending clinicians is the prediction of the compliance of their patients. This coincides with the studies of Byerly et al., (2007) and Sajatovic et al., (2010). They state that clinicians cannot reliably predict their patients' compliance due to lots of individual differences and the avoidance of intolerable antipsychotic undesired effects [Byerly et al.,2007; Sajatovic et al., 2010]. Patients' samples that gave, for example, the concentration of 0 ng/ml in this study was suspected of compliance problem when the maintenance dose was continuously given for at least one week and especially, when the concentration of other co-medications gave reasonable values. In cases of oral medications, the attending clinicians were advised to discuss with their patients and switch to

intravenous application or to change the medication depending on their clinical decision.

The limit of clinic-pharmacological interpretations lies on the absence of the clinical impression of the unseen individual patient. This limitation was handled through the clinic ward visits, where the measured concentrations were discussed with the attending clinicians and patients. The mentioned case reports were not handled directly at the general ward discussion, but during the triple discussion between the patients, the attending clinicians, and the pharmacist, during the discussion between the pharmacist and the patients alone and during the discussion between the attending clinician and the patients alone. Data of the patients were collecetd later from the attending clinicans. Some data could not be supplied by the attending clinicians, especially in cases where out-patients were involved.

Some authors have already studied the effectiveness of the presence of a pharmacist at the clinic ward. Fertleman et al. (2005), reported the improvement in the accuracy of drug history documentation, reduction in prescribing costs, and a decrease in the potential risk to patients through the assistance of a pharmacist at the clinic ward [Fertleman et al., 2005].

The clinic ward visits carried out during this study corresponds with the findings of Fertleman et al. (2005), that clinically significant drug-related problems can effectively be identified and solved by pharmacists. This can be achieved through therapeutic drug monitoring of the suspected substances and pharmaceutical advices based on the current state of research on the administration and guidelines of the concerned medication.

Marino et al. reported in 2010 the differences in pharmacy interventions at a psychiatric hospital. The 2,220 interventions made by pharmacist during their study were based on the documentation of medication errors, undesired effects and the reduction of undesired effects following pharmaceutical advices. Their results show the positive contribution of pharmacists on clinical therapeutic interventions for the optimization of individual patient therapy [Marino et al., 2010]. Their analysis is however not focused on the control of the serum concentrations of the suspected drugs through TDM, for a timely prevention and reduction of undesired effect and also lack of effect. Though with limited number of patients, these issues were effectively considered and treated in this study as shown in the case report examples.

Through the evaluation of the clinic ward questionnaires, the work of the pharmacist at the clinic ward was assessed as positive both by the patients and the attending clinicians. Alderman (1997) developed a standard form for the collection of data such as drug and drug related problems, type of interventions, and the estimation of their significance during pharmacy intervention. Her study suggests an optimization of treatment outcomes through recommendations for drug therapy [Alderman, 1997]. The form created in this work is to assess how patients understand their therapy and the value of their drugs, and to find out how compliant they are with respect to the prescribed drug. The evaluation form for the attending clinicians was developed with a focus on the use and application of the recommendations and advice on the therapy of individual patient and the impact of the pharmacist activities during the ward visits. As confirmed by the attending clinicians and participating patients, the

advice of the pharmacist on different drugs during clinic admissions of the patients and on discharge resulted in better outcomes.

The reported undesired drug effects obtained during clinic visits at the clinic ward 1B were documented in AGATE pharmacovigilance form. This is of value in further research of other drugs. Antipsychotic dosing guided by TDM has proven to be very effective and less expensive [Ostad-Haji et al., 2013]. This work has confirmed the application of therapeutic drug monitoring (TDM) in the realization of effective cost therapy from laboratory measurement of serum concentrations to the routine application of TDM and its clinical advantages for the health enhancement of individual patients.

6 Summary

This study aimed at the development of a new analytical procedure for the qualitative and quantitative determination of antipsychotics with low therapeutic reference ranges such as melperone (MLP), benperidol (BPD), haloperidol (HLP), bromperidol (BRP), flupentixol (FLT) and zuclopenthixol (ZLT) in human serum, both individually as well as simultaneously. This objective was successfully achieved. The procedure uses on-line solid phase sample cleanup coupled to high-performance liquid chromatographic (HPLC) separation with ultra violet (UV) detection. The procedure is inexpensive and affordable for TDM routine applications at small scale TDM laboratories, where these substances could not be tested reliably until now.

The development and validation of the method was carried out with the analytical columns Luna phenyl-hexyl 3 µm, 150 x 3.0 mm column and Nucleodur CN-RP 3 µm, 150 x 4.6 mm column for butyrophenones and phenothiazines, respectively. The mobile phase was composed of 30 vol% ACN, 13 vol% MeOH, 0.4 vol% TEMED and 56.6 vol% demineralized water. The wavelength for the detection was set at 245 nm and at 230 nm for butyrophenones and phenothiazines, respectively. The detection limits were satisfactorily obtained at 7.3 ng/ml for MLP, 0.7 ng/ml for BPD and HLP, 1.6 ng/ml for BRP, 1.7 ng/ml for FLT and 1.7 ng/ml for ZLT.

The developed method was successfully applied for TDM routine measurements. It has sufficient sensitivity to detect steady-state concentration in patients. The main diagnoses of the study patients were determined according to chapter five (mental and behavioral disorders) of the International Statistical Classification of Diseases and Related Health Problems. Dose-concentration diagrams of the substances and the boxplots were plotted to compare the obtained serum concentrations with dose-

related reference ranges (DRR) and therapeutic reference ranges (TRR) of the respective substances. The clinic-pharmacological interpretation of the measured concentrations relied on the information given in TDM request form, namely, the co-medications, development of undesired drug effects, duration of the therapy, dosage regimen, time the sample was taken, and indication for monitoring.

The understanding of antipsychotic TDM was improved during this study through the evaluation of old and new medication data. This included the Konbest data base for the measured antipsychotics, ABDA data for HLP cost evaluation, and AGATE data for the concomitant applications of MLP and BPD. Consensus C/D data were compared, concentrations measured in serum of patients under monotherapy and polytherapy. Differences between oral and depot form of flupentixol were observed. This study also shows that the serum concentrations of HLP and FLT for smokers and nonsmokers can be controlled through TDM.

Case reports of the study substances were successfully evaluated by the pharmacist in the context of the clinic ward visitation. The results of the clinic ward questionnaires proved that through the application of the less expensive TDM methods in the clinic ward, attending clinicians were able to effectively monitor the serum drug concentrations of individual patients, make correct therapeutic decisions in time, and were therefore able to begin the therapy without unwanted delay.

The validated isocratic HPLC separation method was the appropriate method for the serum determination of the study substances and through the simple developed HPLC procedures.

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Appendix

List of appendices

Appendix 1. Column Selection by Measurement with Buffer Mobile Phase

a) Nucleodur CN-RP 150 x 4.6 100-3

Mobile phase constitution	ACN/MeoH/Buffer (ml) (300/ 100/ 600)
PH value	5.0
Temperature (°C)	30
Injection volume (µl)	100.0
Concentration (ng/ml)	100
Test Substance	flupentixol

b) Thermo Betasil C6 250 x 4.6 mm 5 µm

Mobile phase constitution	ACN/MeoH/Buffer (ml) (300 / 100 / 600)
PH-value	4.3
Temperature (°C)	25
Injection volume (µl)	100
Concentration (ng/ml)	2000, 400 and 200
Test substance	bromperidol

c) Luna Phenyl- hexyl 150 x 3.0 mm 3 µm (and 5 µm)

Mobile phase constituents	ACN/MeOH/Buffer (ml) (300ml/100ml/600)
PH-value	4.0
Temperatur (oC)	30
Injection volume (µl)	100
Concentration (ng/ml)	10
Columns	Luna Phenyl-hexyl 3 µm 150 x 3.0 mm Luna Phenyl- hexyl 5 µm 150 x 3.0 mm

d) Hypersil ODS 250 x 4.6 mm 5 µm

Mobile phase constituents	ACN/MeOH/Buffer (300ml/100ml/600ml)
PH-value	4.3
Temperatur (°C)	30
Injection volume (µl)	100
Concentration (ng/ml)	100
Test substance	bromperidol

e) SphereClone ODS (2) 150 x 4.6 mm 5 µm

Mobile phase constituents	ACN/MeOH/Buffer (ml) (300/ 100/ 600)
PH-value	4.3
Temperatur (°C)	30
Injection volume (µl)	100
Concentration (ng/ml)	100 und 1000
Test substance	bromperidol

Note: Parameters applied during the selection of different analytical columns with buffer mobile phase for the HPLC method development.

Appendix 2. Column Selection with TEMED Mobile Phase

a) PerfectSil 120 ODS-L 250 x 4.6 mm 5 µm

Mobile phase constitution	ACN/ MeOH/ TEMED/ H2O (ml) (330/ 100/4 /566)
PH-value	6.5
Temperatur (°C)	30
Injection volume (µl)	100
Concentration (ng/ml)	100 and 200
Test substance	bromperidol

b) Gemini-Nx C18 5 µm 150 x 4.6 mm and 250 x 4.6 mm with Bromperidol Sample

Mobile phase constitution	ACN/MeOH/H2O/TEMED (ml) (330/ 100/4 / 566)
PH-value	4.3
Temperatur (°C)	30
Injection volume (µl)	100
Concentration (ng/ml)	200 , 2 and 5
Test substance	bromperidol

c) Gemini C6 5 µm 150 x 4.6 mm

Mobile phase constitution	ACN/ MeOH/ TEMED/ H2O (300 ml/100 ml/566 ml/4 ml)
PH-value	4.3
Temperature	30°C
Injection volume	100 µl
Concentration	2000 ng/ml and 10 ng/ml
Test substance	bromperidol

d) Nucleodur CN-RP 150 x 4.6 mm 100-3

Mobile phase constitution	ACN/ MeOH/ TEMED/ H2O (ml) (300 /130 /4 / 566)
PH-value	5.0
Temperatur (°C)	30
Injection volume (µl)	1250

Concentration (ng/ml)	100
Test substance	Flupentixol and zuclopenthixol

e) Luna Phenyl- hexyl 5 µm 150 x 3.0 mm

Luna Phenyl-hexyl 3 µm 150 x 3.0 mm

Mobile phase constitution	ACN/ MeOH/ TEMED/ H2O (ml) (300 /130 /4 /566)
PH-value	5.0
Temperatur (°C)	20
Injection volume (µl)	750
Concentration (ng/ml)	100
Test substances	Melperone, benperidol, haloperidol, bromperidol

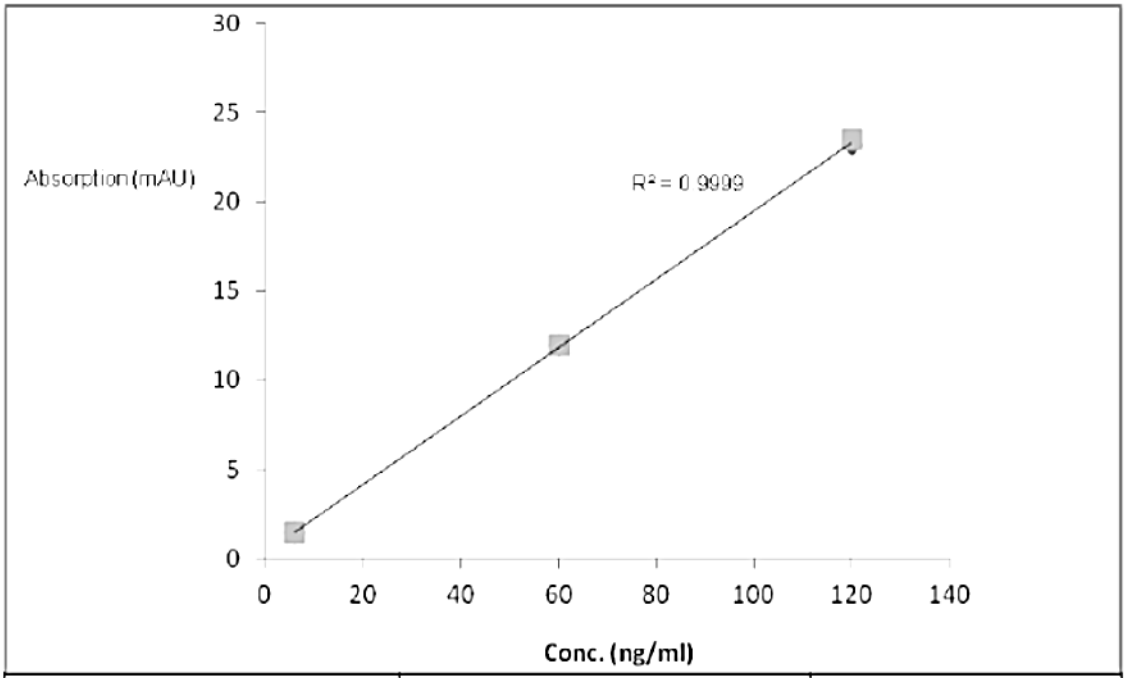
Note: The addition of TEMED in the mobile phase for the selection of analytical column the serum determination of MLP, BPD, HLP, BRP, FLT, and ZLT.

Appendix 3. Application of Gradients HPLC Method Development

Mobile phase C (%)	Solvent	ml	%	ml	%	ml	%	ml	%
		ACN		MeOH		TEMED		H2O	
95	A	285	28.5	123.5	12.35	3.8	0.38	537.7	53.77
	B	50	5	-	-	-	-	-	-
	Total	335	33.5	123.5	12.35	3.8	0.38	537.7	53.77
90	A	270	27	117	11.7	3.6	0.36	509.4	50.94
	B	100	10	-	-	-	-	-	-
	Total	370	37	117	11.7	3.6	0.36	509.4	50.94
85	A	255	25.5	110.5	11.05	3.4	0.34	481.1	48.11
	B	150	15	-	-	-	-	-	-
	Total	405	40.5	110.5	11.05	3.4	0.34	481.1	48.11
80	A	240	24	104	10,4	3,2	0,32	452,8	45,28
	B	200	20	-	-	-	-	-	-
	Total	440	44	104	10.4	3.2	0.32	452.8	45.28
75	A	225	22.5	97.5	9.75	3.0	0.3	424.5	42.45
	B	250	25	-	-	-	-	-	-
	Total	475	47.5	97.5	9.75	3.0	3.2	424.5	42.45

Note: Illustration of the percentage volume of solvent applied during the gradient HPLC method development. The mobile phase C and acetonitrile of HPLC grade were admixed with the help of multistep gradient method. Mobile phase C comprises of 300 ml ACN, 130 ml MeOH, 4 ml TEMED, and 566 ml H2O. Solvent A shows the volume and percentage portion of the constituents of mobile phase C at each chromatographic run, while solvent B on the table shows the volume and the percentage of the HPLC-grade acetonitrile added to the mobile phase C.

Appendix 4. Example of a Calibration Curve of Zuclopenthixol



Conc (ng/ml)	Mean (mAU)	SD
6	1.489	0.03
60	11.937	0.01
120	23.492	0.02

Note: The calibration curve of zuclopenthixol carried out during the TDM application of the developed method (n = 7). The linearity of the curve shows the precision and reliability of the method, for the qualitative and quantitative analysis of drugs in patients' serum. With the curve of linearity, the concentration of an unknown substance can be calculated.

Appendix 5. The Simultaneous Test on Interference for Butyrophenones

Medication	Number of Administration	Percentage (%) Administration	Retention Time (min)
Benperidol	48	0.13	12.04
Bromperidol	19	0.05	21.52
Haloperidol	826	2.29	19.12
Melperone	177	0.49	10.98
9-OH-Risperidone (met)	(2444)	6.79	13.8
Acetylsalicylic acid	1229	3.41	nd
Alprazolam	1	0.00	28.60
Amantadine HCl (salt)	8	0.02	nd
Amisulpride	586	1.63	nd
Amitriptylinoxide x $2H_2O$ (met)	185	0.51	nd
Amlodipine	3	0.01	21.19
Aripiprazole	1090	3.03	nd
Ascorbic acid	5	0.01	nd
Biperiden HCl (salt)	164	0.46	nd
Bisoprolol	267	0.74	nd
Bromazepam	4	0.01	17.83
Bupropion	1	0.00	9.86
Buspirone HCl (salt)	21	0.06	11.62
Caffein	-	-	nd
Carbamazepine	465	1.29	19.88
Chlordiazepoxide	1	0.00	24.74
Chlorprothien HCl (salt)	160	0.44	nd
Cisaprid	-	-	19.55
Citalopram	1161	3.22	16.20
Citalopram- HBr (salt)	(1161)	3.22	21.22
Clobazam	6	0.02	nd
Clomipramine	325	0.90	nd
Clozapine	4080	11.33	15.31
Clozapine-N-Oxide (met)	(4080)	11.33	16.10
Demethylvenlafaxine (met)	(2312)	6.42	nd
Desipramine HCl (salt)	3	0.01	27.41
Desmethylcitalopram (met)	(1161)	11.33	15.14
Dextran	-	-	nd
Diclofenac-Na (salt)	12	0.03	nd
Dihydroaripiprazole (met)	(1090)	3.03	nd
Dihydrocodeine hydrogentartrate (met)	1	0.00	nd
Dimethylbiguanide HCl (salt)	111	0.31	nd

Doxepine HCl(salt)	733	2.04	17.01
Duloxetine HCl (salt)	616	1.71	24.93
Enalapril maleate(salt)	343	0.95	nd
Estradiol	2	0.01	nd
Flupentixol 2HCl(salt)	44	0.12	nd
Fluperlapine	-	-	15.62
Fluphenazine decanoate (salt)	10	0.03	nd
Fluvoxamine maleate(salt)	1	0.00	19.92
Furosemide	648	1.8	nd
Gabapentin	57	0.16	nd
Galantamine	15	0.04	19.86
Glimepiride	154	0.43	nd
Hydrochlorothiazide	181	0.5	nd
Imipramine HCl	205	0.57	24,24
Lamotrigine	2646	7.35	nd
Levodopa	247	0.69	nd
Levomepromazine	140	0.39	34.8
Lorazepam	688	1.91	24.87
Maprotillin HCl (salt)	30	0.08	31.35
Melperone	177	0.49	10.98
Memantine HCl (salt)	30	0.08	nd
Methylrisperidone (met)	(2444)	6.79	19.10
Metoprolol	376	1.04	nd
Mianserin HCl (salt)	3	0.01	20.47
Mirtazapine	764	2.12	nd
Monohydrocarbamazepine	-	-	nd
Nateglinide	1	0.00	nd
N-Dealkylated quetiapine (met)	(2936)	8.15	19.15
N-Desmethylclozapine (met)	(4080)	11.33	13.82
N-Desmethylolanzapine (met)	(3673)	10.2	nd
Nitrazepam	1	0.00	26.46
Norclomipramine HCl (met)	325	0.9	nd
Nordiazepam(met)	-	-	33.96
Nordoxepine HCl (met)	(733)	2.04	20.84
Nortriptyline HCl (met)	241	0.67	24.77
O- Desmethylvenlafaxine (met)	(2312)	6.42	nd
Olanzapine	3673	10.20	nd
Omeprazole	584	1.62	17.33
Oxazepam	4	0.01	24.27

Oxcarbazepine	181	0.5	15.99
Pantoprazole-Na (salt)	27	0.07	19.86
Paroxetine HCl (salt)	9	0.02	27.66
Perazinbishydrogenmalonate	48	0.13	34.60
Phenytoin	5	0.01	20.73
Pipamperone	820	2.28	14.70
Pregabalin	1	0.00	nd
Pirenzepin HCl	27	0.07	nd
Primidone	22	0.06	nd
Promethazine HCl (salt)	119	0.33	24.63
Propranolole HCl (salt)	33	0.09	16.72
Quetiapine (base)	2936	8.15	21.03
Quetiapine fumarate (salt)	(2936)	8.15	20.89
Ramipril	395	1.1	nd
Reboxetine	44	0.12	nd
Risperidone	2444	6.79	15.82
Rivastigmine	21	0.06	nd
Sertraline	28	0.08	18.78
Sultiame	-	-	nd
Sumatriptan	1	0.00	nd
Testosterone	3	0.01	32.21
Theobromine	-	-	nd
Theophylline	126	0.35	nd
Topiramate	5	0.01	nd
Triamcinolone acetonide	-	-	24.07
Triamterene	2	0.01	nd
Triazolam	-	-	27.64
Trimipramine maleate	179	0.5	nd
Valproic acid	5	0.01	nd
Venlafaxine HCl (salt)	2312	6.42	nd
Ziprasidone	291	0.81	21.08
Zopiclone	174	0.48	nd
Zuclopentixol 2HCl (salt)	36	0.1	nd
Total	36003	100.00	

Note: Interference test with Butyrophenones and the combined medications used during the therapy with butyrophenones was carried out with Luna Phenyl-hexyl column 3µm at an optimal wave length of 245 nm. The raw substances used for the test were obtained from the TDM laboratory at the University of Regensburg. In brackets are the values of the drug products (met). The substances beyond the detection limit set for butyrophenone method were not detected (nd).

Appendix 6. Test on Interference for Flupentixol and Zuclopenthixol

Medication	Number of Administration	Percentage (%) Administration	Retention Time (min)
Flupentixol	31	0.10	15.38
Zuclopenthixol	5	0.12	14.25
9-OH-Risperidone (met)	(2444)	7.6	nd
Acetylsalicylic acid	292	0.91	nd
Alprazolam	8	0.02	12.58
Amantadine	3	0.01	nd
Amisulpride	586	1.82	9.15
Amitriptyline-oxide (met)	185	0.58	13.4
Amlodipine	28	0.09	11.10
Aripiprazole	1090	3.39	13.69
Ascorbic acid	11	0.03	nd
Benperidol	25	0.08	10.89
Biperiden	1988	6.18	12.08
Bisoprolol	108	0.34	9.41
Bromazepam	4	0.01	nd
Bromperidol	2	0.01	12.76
Bupropion	6	0.02	10.10
Buspiron	2	0.01	10.27
Caffeine	1	0.00	nd
Carbamazepine	333	1.03	10.6
Chlordiazepoxide	-	-	11.48
Chlorprothixene	511	1.59	14.14
Cisapride	5	0.02	12.93
Citalopram (base)	1161	3.61	11.19
Citalopram HBr (salt)	(1161)	3.61	11.98
Clobazam	1	0.00	12.58
Clomipramine	325	1.01	14.37
Clozapine	4080	12.68	10.95
Clozapine-N-oxide (met)	(4080)	12.68	11.91
Desipramine HBr (salt)	3	0.01	12.88
Desmethylcitalopram (met)	(1161)	3.61	10.94
Desmethylvenlafaxine (met)	(2312)	7.19	nd
Dextrene	1	0.00	nd
Diclofenac	41	0.13	13.6
Dihydroaripiprazole (met)	(1090)	3.39	13.19
Dihydrogencodeinhydrogentartrate	-	-	nd
Doxepine	733	2.28	11.51

Duloxetine	616	1.91	12.86
Enalapril	37	0.11	nd
Estradiol	10	0.03	11.79
Fluerlapine	-	-	11.14
Fluoxetin	25	0.08	n.d
Fluphenazine	4	0.01	nd
Fluvoxamine	8	0.02	11.3
Furosemide	49	0.15	nd
Gabapentin	6	0.02	nd
Galantamine	33	0.10	nd
Glimepride	12	0.04	15.4
Haloperidol	826	2.57	12.39
Hydrochlorothiazide	162	0.50	nd
Imipramine	205	0.64	12.41
Lamotrigine	2646	8.22	nd
Levodopa	7	0.02	nd
Levopromazine	220	0.68	12.94
Lorazepam	1478	4.59	11.72
Maprotilin	30	0.09	12.6
Melperone	144	0.45	10.47
Memantine HCl	30	0.09	nd
Methylrisperidone$^{(met)}$	(2444)	7.6	11.88
Metformine	49	0.15	nd
Metoprolol	174	0.54	nd
Mianserine	20	0.06	12.08
Mirtazapine	764	2.37	9.7
Monohydrocarbamazepine$^{(met)}$	(333)	1.03	nd
N-Alkylated quetiapine $^{(met)}$	(2936)	9.12	10.5
Nateglinide	-	-	nd
N-Desmethylclozapine $^{(met)}$	(4080)	12.68	10.56
N-Desmethylolanzapine $^{(met)}$	(3673)	11.41	nd
Nitrazepam	-	-	11.88
Norclomipramine	325	1.01	13.92
Nordiazepam	-	-	11.9
Nordoxepine$^{(met)}$	(733)	2.28	11.27
Nortriptyline	241	0.75	12.39
Olanzapine	3673	11.42	9,5
Omeprazole	12	0.04	12.48
Oxazepam	4	0.01	11.26

Oxcarbazepine	181	0.56	10.07
Pantoprazole	219	0.68	10.85
Paroxetine	9	0.03	12.5
Perazine bihydrogenmaleate	167	0.52	12.68
Phenytoin	9	0.03	10.75
Pipamperone	161	0.50	10.12
Prasidone	-	-	11.41
Pregabalin	19	0.06	nd
Pirenzepine hcl$^{(salt)}$	32	0.1	nd
Primidone	2	0.01	nd
Promethazine	186	0.58	12.57
Propranolol	51	0.16	11.28
Quetiapine $^{(base)}$	2936	9.12	10.93
Quetiapine fumarate $^{(salt)}$	(2936)	9.12	11.7
Ramipril	116	0.36	nd
Reboxetine	24	0.07	10.80
Risperidone	2444	7.6	10.68
Rivastigmine	21	0.07	nd
Sertraline	28	0.09	14.4
Sultram	-	-	nd
Sumatriptane	-	-	nd
Testosterone	3	0.01	11.87
Theobromine	-	-	nd
Theophylline	19	0.06	nd
Topiramate	6	0.02	nd
Triamacinolonacetonide	-	-	11.01
Triamterene	43	0.13	nd
Triazolam	-	-	12.48
Trimipramine maleate	179	0.56	13.88
Venlafaxine	2312	7.19	nd
Zopiclone	164	0.51	13.93
Total	32178	100.00	

Note: The medications for the interference test were the combined medications during the therapy with flupentixol and/ or zuclopenthixol. The test was carried out with Nucleodur CN-RP 150 x 4.6 mm, 3µm at an optimal wave length of 230 nm. The raw substances used for the test were obtained from the TDM laboratory at the University of Regensburg. In brackets are the values of the drug products (met). The substances beyond the detection limit set for butyrophenone method were not detected (nd).

Apendix 7: Number of administered co-medication per patient

Number of patients	**Number of co-medication per patient**
90	1
94	2
114	3
86	4
40	5
30	6
30	7
18	8
4	9
18	10
4	11
1	12
2	13
3	14
1	15
535	**120**

Note: Some haloperidol co-medications are repeatedly recorded for more than one patient. Invented name of the medications were calculated just as administered for each patient.

Appendix 8. Haloperidol Serum Concentration Data for Smokers and Nonsmokers

Haloperidol Data for Smokers

Dose (mg)	Conc. (ng/ml)	C/D-low x De (DRR-L)	C/D-high x De (DRR-H)
0	0	0	0
0	0	0	0
0	0	0	0
0	1,3	0	0
0	1,6	0	0
0	2,1	0	0
0	2,4	0	0
0	4,1	0	0
0	4,5	0	0
0	5,4	0	0
0	7	0	0
0	8	0	0
0	11	0	0
0	12	0	0
0	12,6	0	0
0	23	0	0
0	34	0	0
0	34	0	0
0,5	1	0,3	0,5
0,7	3,9	0,42	0,7
1	0	0,6	1
1	0	0,6	1
1	1	0,6	1
1	1,4	0,6	1
1	4	0,6	1
1	6,5	0,6	1
2	0	1,2	2
2	0	1,2	2
2	0	1,2	2
2	0,5	1,2	2
2	1,3	1,2	2
2	2	1,2	2
2	3,1	1,2	2
2	4	1,2	2
2	4,8	1,2	2
2	82	1,2	2
2,5	0	1,5	2,5
2,5	1	1,5	2,5
2,5	8,8	1,5	2,5
2,5	12,5	1,5	2,5
3	0	1,8	3
3	0	1,8	3
3	1,4	1,8	3
3	3	1,8	3
3	4	1,8	3
3,5	8,9	2,1	3,5
4	0	2,4	4

Haloperidol Data for Smokers

4	0	2,4	4
4	0	2,4	4
4	0	2,4	4
4	3	2,4	4
4	4	2,4	4
4	13	2,4	4
4	14	2,4	4
5	0	3	5
5	0	3	5
5	0	3	5
5	0	3	5
5	1	3	5
5	2,4	3	5
5	2,8	3	5
5	2,8	3	5
5	3,4	3	5
5	3,8	3	5
5	4	3	5
5	4	3	5
5	4,2	3	5
5	4,5	3	5
5	5,1	3	5
5	5,7	3	5
5	6	3	5
5	8,5	3	5
5	13	3	5
5	20	3	5
5	88	3	5
6	1,7	3,6	6
6	4,7	3,6	6
6	10	3,6	6
7	0	4,2	7
7	0	4,2	7
7	0	4,2	7
7	0	4,2	7
7	1,5	4,2	7
7	2,1	4,2	7
7	4,2	4,2	7
7	9	4,2	7
7	22	4,2	7
7,14	3	0,45	0,86
7,14	3,3	1	1,71
7,14	6	0,45	0,86
7,5	8,2	4,5	7,5
8	1,9	4,8	8
8	2	4,8	8
8	2	4,8	8
8	2,3	4,8	8

Haloperidol Data for Smokers

8	2,9	4,8	8
8	4	0,75	1,29
8	5,9	4,8	8
8	7	4,8	8
9	3,7	5,4	9
9	4,6	5,4	9
9	9	5,4	9
10	0	6	10
10	0	6	10
10	0	6	10
10	0	6	10
10	0	6	10
10	0,8	6	10
10	1,4	6	10
10	2	6	10
10	2,1	6	10
10	2,8	6	10
10	2,9	6	10
10	3,1	6	10
10	3,1	6	10
10	3,2	6	10
10	3,2	6	10
10	3,5	6	10
10	3,6	6	10
10	4	6	10
10	4,5	6	10
10	4,6	6	10
10	5	6	10
10	5	6	10
10	5	6	10
10	5,1	6	10
10	6	6	10
10	6	6	10
10	6	6	10
10	6	6	10
10	6,3	6	10
10	6,3	6	10
10	6,9	6	10
10	7	6	10
10	7	6	10
10	7,6	6	10
10	8	6	10
10	10	6	10
10	10	6	10
10	11	6	10
10	11	6	10
10	11	6	10
10	11	6	10

Haloperidol Data for Smokers

10	12	6	10
10	12	6	10
10	15	6	10
10	16	6	10
10	20	6	10
10	26	6	10
10	35	6	10
10	38	6	10
10,71	0	0,75	1,29
10,71	0	0,75	1,29
10,71	0	0,75	1,29
10,71	2	0,75	1,29
10,71	3	0,75	1,29
10,71	4	0,75	1,29
10,71	4	0,75	1,29
10,71	4,2	0,75	1,29
10,71	5	0,75	1,29
10,71	6,5	0,75	1,29
10,71	8	0,75	1,29
10,71	10	0,75	1,29
10,71	16	0,75	1,29
10,71	17	0,75	1,29
10,71	18	0,75	1,29
10,71	22	0,75	1,29
10,71	22	0,75	1,29
10,71	22	0,75	1,29
12	6	7,2	12
12	7,5	7,2	12
12,5	3	7,5	12,5
13	0	7,8	13
14	5	8,4	14
14	10	8,4	14
14	12	8,4	14
14,29	0	1	1,71
14,29	0	1	1,71
14,29	0	1	1,71
14,29	1,2	1	1,71
14,29	1,6	1	1,71
14,29	1,6	1	1,71
14,29	1,8	1	1,71
14,29	2	1	1,71
14,29	3	1	1,71
14,29	3,2	1	1,71
14,29	3,4	1	1,71
14,29	3,4	1	1,71
14,29	3,6	1	1,71
14,29	3,6	1	1,71
14,29	4	1	1,71

Haloperidol Data for Smokers

14,29	4	1	1,71
14,29	4,3	1	1,71
14,29	4,4	1	1,71
14,29	5	1	1,71
14,29	5,5	1	1,71
14,29	5,6	1	1,71
14,29	10	1	1,71
14,29	12	1	1,71
14,29	12	1	1,71
14,29	15	1	1,71
14,29	21	1	1,71
15	0	9	15
15	0	9	15
15	0	9	15
15	0	9	15
15	0	9	15
15	1,9	9	15
15	1,9	9	15
15	3	9	15
15	3	9	15
15	3,3	9	15
15	4	9	15
15	4,3	9	15
15	4,5	9	15
15	5	9	15
15	5,4	9	15
15	5,8	9	15
15	6,6	9	15
15	7	9	15
15	7	9	15
15	7,5	9	15
15	8	9	15
15	8	9	15
15	8	9	15
15	9	9	15
15	9	9	15
15	10	9	15
15	12	9	15
15	12	9	15
15	13	9	15
15	14,64	9	15
15	15	9	15
15	15	9	15
15	16,5	9	15
15	21	9	15
15	21	9	15
15	23	9	15
17	13	10,2	17

Haloperidol Data for Smokers

17,5	0	10,5	17,5
17,86	8	1,25	2,14
17,86	9,5	1,25	2,14
17,86	12	1,25	2,14
19	19	11,4	19
20	0	12	20
20	0	12	20
20	1,7	12	20
20	2	12	20
20	2,4	12	20
20	2,9	12	20
20	3,5	12	20
20	4	12	20
20	4,1	12	20
20	5	12	20
20	5	12	20
20	5,4	12	20
20	6	12	20
20	6	12	20
20	6,2	12	20
20	7	12	20
20	7	12	20
20	7,5	12	20
20	7,5	12	20
20	7,6	12	20
20	8,4	12	20
20	8,9	12	20
20	10	12	20
20	10	12	20
20	11	12	20
20	11	12	20
20	12	12	20
20	12	12	20
20	14	12	20
20	17	12	20
20	18	12	20
20	19	12	20
20	20	12	20
20	20,3	12	20
20	22	12	20
20	24	12	20
20	25	12	20
20	25	12	20
20	26	12	20
20	32	12	20
20	32	12	20
20	34	12	20
21,43	0	1,5	2,57

Haloperidol Data for Smokers

21,43	3	1,5	2,57
21,43	7	1,5	2,57
21,43	7,9	1,5	2,57
21,43	12	1,5	2,57
21,43	13	1,5	2,57
21,43	13	1,5	2,57
21,43	14	1,5	2,57
21,43	15	1,5	2,57
21,43	28	1,5	2,57
22,5	39	13,5	22,5
23	14	13,8	23
24	10	14,4	24
25	11	15	25
25	11	15	25
25	15	15	25
25	26	15	25
27	5	16,2	27
30	0	18	30
30	0	18	30
30	7,9	18	30
30	8	18	30
30	9,2	18	30
30	9,3	18	30
30	9,7	15	30
30	10	18	30
30	10	18	30
30	11	18	30
30	11	18	30
30	12	18	30
30	12	18	30
30	13	18	30
30	13	18	30
30	13	18	30
30	14	18	30
30	15	18	30
30	15	18	30
30	15	18	30
30	17	18	30
30	18	18	30
30	18,7	0,75	1,29
30	21	18	30
30	21	18	30
30	24,4	18	30
30	28	18	30
35	7	21	35
35	16	21	35
35	16	21	35
35	21	21	35

Haloperidol Data for Smokers

35	29	21	35
40	0	24	40
40	4,5	24	40
40	5	24	40
40	12	24	40
40	16	24	40
40	16	24	40
40	22	24	40
40	26	24	40
40	37,3	24	40
45	14	27	45
50	0	30	50
50	0	30	50
50	1,1	30	50
50	1,7	30	50
50	2,7	30	50
50	3	30	50
50	3,1	30	50
50	4	30	50
50	4,4	30	50
50	5	30	50
50	5	30	50
50	6,1	30	50
50	14	30	50
55	54	33	55
70	12	42	70
75	0	45	75
75	18	45	75
100	0	60	100
100	9	60	100
130	4	78	130

Haloperidol Data for Nonsmokers

Dose (mg)	Conc. (ng/ml)	C/D-low x De (DRR-L)	C/D-high x De (DRR-H)
0	0,6	0	0
0	1	0	0
0	3	0	0
0	6	0	0
0	6,2	0	0
0	7	0	0
0	8	0	0
0	15	0	0
0	16	0	0
1	0,9	0,6	1
1	1,9	0,6	1
1	3,5	0,6	1
1,5	6,3	0,9	1,5
1,79	8	0,88	1,5
2	0	1,2	2
2	1	1,2	2
2	2,8	1,2	2
2	6	1,2	2
3	0	1,8	3
3	2	1,8	3
3	3	1,8	3
3	11	1,8	3
3	16	1,8	3
3,5	3,6	2,1	3,5
4	0	2,4	4
4	0	2,4	4
4	0	2,4	4
4	0	2,4	4
4	1,2	2,4	4
4	3	2,4	4
4	4	2,4	4
4	7,6	2,4	4
4	10	2,4	4
4	12	2,4	4
5	0	3	5
5	2	3	5
5	2,6	3	5
5	3	3	5
5	4,2	3	5
5	4,9	3	5
5	7	3	5
5	8,1	3	5
5	13	3	5
5	15	3	5
6	0,9	3,6	6
6	2	3,6	6
6	2,2	3,6	6

Haloperidol Data for Nonsmokers

6	2,5	3,6	6
6	10	3,6	6
7	0	4,2	7
7	0	4,2	7
7	1,7	4,2	7
7	3,1	4,2	7
7,14	3	0,5	0,86
7,14	3	0,5	0,86
7,14	5	0,5	0,86
7,14	9	0,5	0,86
7,14	21	0,5	0,86
7,14	21	0,5	0,86
7,14	72	0,5	0,86
7,5	5,5	4,5	7,5
8	3,7	4,8	8
8	5	4,8	8
8	5	4,8	8
8	6	4,8	8
8	14	4,8	8
9	3,6	5,4	9
9	4,9	5,4	9
9	9	5,4	9
9	15	5,4	9
10	0	2,4	10
10	0	6	10
10	0,4	6	10
10	1,3	6	10
10	1,9	6	10
10	3	6	10
10	3	6	10
10	3	6	10
10	3	6	10
10	3,1	6	10
10	3,6	6	10
10	4,1	6	10
10	4,1	6	10
10	4,2	6	10
10	4,6	6	10
10	5	6	10
10	5	6	10
10	5,6	6	10
10	6,5	6	10
10	7,3	6	10
10	8	6	10
10	8,4	6	10
10	8,8	6	10
10	9,8	6	10
10	10	6	10

Haloperidol Data for Nonsmokers

10	13,3	6	10
10	18	6	10
10	31	6	10
10	49	6	10
10,71	0	0,75	1,29
10,71	3	0,75	1,29
10,71	4	0,75	1,29
10,71	5	0,75	1,29
10,71	11	0,75	1,29
10,71	11	0,75	1,29
10,71	55,4	0,75	1,29
12	3,7	7,2	12
12	11	7,2	12
12	16	7,2	12
12	20	7,2	12
12,5	7	7,5	12,5
12,5	9,2	7,5	12,5
13	9	7,8	13
14,29	6	1	1,71
15	0	9	15
15	2,1	9	15
15	2,7	9	15
15	3,9	9	15
15	3,9	9	15
15	4	9	15
15	4,5	9	15
15	5	9	15
15	6,6	9	15
15	6,7	9	15
15	7	9	15
15	8	9	15
15	8,2	9	15
15	9	9	15
15	10	9	15
15	10	9	15
15	12	9	15
15	14	9	15
15	14	9	15
15	14,1	9	15
15	20	9	15
15	21	9	15
15	22	9	15
15	23	9	15
15	23	9	15
15	30	9	15
15	47	9	15
15	58	9	15
16	6,9	9,6	16

Haloperidol Data for Nonsmokers

16	12	9,6	16
17,5	3,5	10,5	17,5
17,86	3,4	1,25	2,14
18	4,9	10,8	18
20	0	12	20
20	0	12	20
20	0	12	20
20	2,5	12	20
20	2,6	12	20
20	5	12	20
20	5,1	12	20
20	5,6	12	20
20	6,4	12	20
20	6,4	12	20
20	7	12	20
20	7	12	20
20	8,8	12	20
20	9,4	12	20
20	10,4	12	20
20	11	12	20
20	11	12	20
20	15	12	20
20	15	12	20
20	15	12	20
20	15,8	12	20
20	23	12	20
20	32	12	20
20	39	12	20
20	39	12	20
21,43	5,9	1,5	2,57
21,43	9	1,5	2,57
22	11	13,2	22
22,86	5,3	1,6	2,74
25	0	15	25
25	11	15	25
25	12	15	25
25	13	15	25
26	6	15,6	26
26	75	15,6	26
28,51	3,8	2	3,43
30	18	18	30
30	39	18	30
40	9	24	40
40	12	24	40
50	0	30	50
50	1,1	30	50
50	1,6	30	50
50	4	30	50

50	5,5	30	50
65	44	39	65
100	0	60	100
100	13	60	100
150	17	90	150

Note: DRR = dose-related reference range. The DRR is obtained by multiplying the factors C/Dlow and C/Dhigh with the administered dose. Conc. is the serum concentration obtained after measuring the patients' haloperidol samples.

Appendix 9. Flupentixol Serum Concentration Data for Smokers and Nonsmokers

Flupentixol Data for Smokers

Dose (mg)	Conc. (ng/ml)	DRR low	DRR high	TRR low	TRR high
0,00	18,00	0,00	0	1	10
1,00	6,90	11,79	1,74	1	10
1,23	2,87	2,03	5,35	1	10
3,57	2,90	5,89	15,53	1	10
4,00	13,28	2,64	6,96	1	10
4,29	6,50	7,07	18,66	1	10
4,64	8,60	7,66	20,18	1	10
5,00	0,00	3,30	8,7	1	10
5,00	25,00	3,30	8,7	1	10
7,14	0,00	11,79	31,07	1	10
7,14	3,00	11,79	31,07	1	10
7,14	7,00	11,79	31,07	1	10
7,14	9,45	11,79	31,07	1	10
7,14	12,30	11,79	31,07	1	10
7,14	13,00	11,79	31,07	1	10
7,14	19,50	11,79	31,07	1	10
7,14	21,00	11,79	31,07	1	10
10,00	0,00	6,60	17,4	1	10
10,00	0,00	6,60	17,4	1	10
10,00	4,50	6,60	17,4	1	10
10,00	18,00	6,60	17,4	1	10
10,00	24,00	6,60	17,4	1	10
15,00	0,00	9,90	26,1	1	10
15,00	0,00	9,90	26,1	1	10
15,00	1,70	9,90	26,1	1	10
15,00	8,30	9,90	26,1	1	10
15,00	22,30	9,90	26,1	1	10
20,00	1,83	13,20	34,8	1	10
20,00	9,99	13,20	34,8	1	10
20,00	16,60	13,20	34,8	1	10
20,00	19,98	13,20	34,8	1	10
30,00	2,20	19,80	52,2	1	10

Flupentixol Data for Nonsmokers

Dose (mg)	Conc:(ng/ml)	DRR low	DRR high	TRR low	TRR high
0,00	7,00	0,00	0	1 - 10	1 - 10
4,00	0,00	2,64	6,96	1 - 10	1 - 10
4,29	10,90	7,07	18,66	1 - 10	1 - 10
6,00	23,40	3,96	10,44	1 - 10	1 - 10
10,00	4,40	6,60	17,4	1 - 10	1 - 10
10,00	4,80	6,60	17,4	1 - 10	1 - 10
15,00	6,70	9,90	26,1	1 - 10	1 - 10
20,00	0,00	13,20	34,8	1 - 10	1 - 10
20,00	6,80	13,20	34,8	1 - 10	1 - 10
30,00	30,00	19,80	52,2	1 - 10	1 - 10

Note: DRR = dose-related reference range, TRR = therapeutic reference range. The DRR is obtained by multiplying the factors C/D_{low} and C/D_{high} with the administered dose. Conc. is the serum concentration obtained after measuring the patients' flupentixol samples.

Appendix 10. Questionnaires for the Attending Clinicians

Auswertungsbogen zur Pharmazeutischen Beratung der Ärzte durch die Apotheker

(Erstellt von Mary Remigius Onuoha zur Auswertung der Stationsvisite: 2009 - 2012)

Abschnitt 1. Zur Person

□ weiblich
□ männlich

Station:

Alter:

Genaues Alter: TTMMJJ

Abschnitt 2. Verwendung der Beratung in der Therapie und Patientenbetreuung

1) Welche Patientengruppe haben Sie betreut?

□ Stationär
□ Ambulant
□ Beide
□ Keine Ahnung

Kommentare: ----------

2) Wie viele Patienten haben Sie Ihrer Einschätzung nach auf dieser Station betreut?

□ Weniger als 10
□ 10 - 20
□ Mehr als 20
□ Keine Ahnung

Kommentare: ----------

Auswertungsbogen zur Pharmazeutischen Beratung der Ärzte durch die Apotheker

(Erstellt von Mary Remigius Onuoha zur Auswertung der Stationsvisite: 2009 - 2012)

3) Patientengruppe welcher Diagnose haben Sie im Allgemein betreut?

□ ICD - 10:
□ Andere:
□ Nebendiagnose:
□ Keine Ahnung

Kommentare: ----------

4) Welche Arzneimittelgruppe haben Sie bei der Therapie öfters verwendet?

□ Antidepressiva
□ Antipsychotika
□ Stimmungsstabilisatoren
□ Alle

5) Welche andere Therapieformen wurden zusätzlich zur medikamentösen Therapie angewendet?

□ Psychoedukative Gruppe (Info- Gruppe)
□ Soziales Kompetenz-Training
□ Kognitives Training
□ Beschäftigungstherapie

Andere: ----------

6) Wie können Sie die Zufriedenheit Ihrer Patienten bezüglich der medikamentösen Therapie einschätzen?

□ Sehr zufrieden
□ Zufrieden
□ Eher zufrieden
□ Unzufrieden

Auswertungsbogen zur Pharmazeutischen Beratung der Ärzte durch die Apotheker

(Erstellt von Mary Remigius Onuoha zur Auswertung der Stationsvisite: 2009 - 2012)

7) Sind unerwünschte Arzneimittelwirkungen (UAW) aufgetreten?

□ Ja
□ Nein
□ Nicht wirklich
□ Keine Ahnung

8) Sind die UAW erst nach der Verabreichung bestimmter Medikamente aufgetreten?

□ Ja
□ Nein
□ Es ist keine unerwünschte Arzneimittelwirkung aufgetreten
□ Keine Ahnung

9) Gibt es alternative Erklärungen (abgesehen von den Medikamenten) für das Auftreten unerwünschter Arzneimittelwirkungen?

□ Ja
□ Nein
□ Es ist keine unerwünschte Arzneimittelwirkung aufgetreten
□ Keine Ahnung

Erklärung: ----------

10) Wurden die unerwünschten Arzneimittelwirkungen durch belegte Anhaltspunkte bestätigt?

□ Ja
□ Nein
□ Es ist keine unerwünschte Arzneimittelwirkung aufgetreten
□ Keine Ahnung

Auswertungsbogen zur Pharmazeutischen Beratung der Ärzte durch die Apotheker

(Erstellt von Mary Remigius Onuoha zur Auswertung der Stationsvisite: 2009 - 2012)

11) Hat Ihnen die Beratung durch den Apotheker bei therapeutischen Entscheidungen geholfen?

□ Ja
□ Nein
□ Ich brauchte keine Beratung
□ Keine Ahnung

12) Haben die Antworten und gelieferten Informationen im Bezug auf Ihre Fragen zu Medikation Ihren Erwartungen entsprochen?

□ Ja
□ Nein
□ Ich brauchte keine Beratung
□ Keine Ahnung

13) Haben die Antworten und gelieferten Informationen im Bezug auf Ihre Fragen zu Nebenwirkungen Ihren Erwartungen entsprochen?

□ Ja
□ Nein
□ Ich brauchte weder Antwort noch Information
□ Keine Ahnung

14) Hat die Anwesenheit des Apothekers zu einer erfolgreichen Therapie beigetragen?

□ Ja
□ Nein
□ Nicht wirklich
□ Keine Ahnung

Auswertungsbogen zur Pharmazeutischen Beratung der Ärzte durch die Apotheker

(Erstellt von Mary Remigius Onuoha zur Auswertung der Stationsvisite: 2009 - 2012)

15) Wie beurteilen Sie die Arbeit des Apothekers auf Ihrer Station?

- □ Sehr zufrieden
- □ Zufrieden
- □ Nicht zufrieden
- □ Keine Ahnung

Ihre allgemeinen Kommentare können Sie hier eintragen

Ort, Datum

Unterschrift

Note: Questionnair prepared for the attending clinicians for the evaluation of the activities of the Pharrmacist at the clinic ward 1b during the research study.

Appendix 11. Patients' Questionnaire for the Evaluation of Pharmacist's Activities at the Clinic Ward

Auswertungsbogen zur Optimierung der individualisierten Arzneimitteltherapie der Patienten durch die pharmazeutische Beratung

(Erstellt von Mary Remiglus Onuoha zur Auswertung der Stationsvisite: 2009 - 2012)

Abschnitt 1. Geschlecht des Patienten

□ weiblich
□ männlich

Abschnitt 2. Alter der Patienten **Genaues Alter: TTMMJJ**

Abschnitt 3. Krankheitsverlauf

A) Diagnose

- ICD - 10:
- Andere:

B) Krankheitsdauer von ---------- bis ----------

C) Stationär □ Ambulant □

Abschnitt 4. Die Einbeziehung der Patienten in die Behandlungsentscheidungen sowie in die kontinuierliche Führung der künftigen Therapie.

1) Wurden Ihnen die Namen des Beraters mitgeteilt?

Auswertungsbogen zur Optimierung der individualisierten Arzneimitteltherapie der Patienten durch die pharmazeutische Beratung

(Erstellt von Mary Remigius Onuoha zur Auswertung der Stationsvisite: 2009 - 2012)

□ Ja
□ Nein
□ Nicht wirklich
□ Keine Ahnung

2) Von wem wurden Sie beraten?

□ Arzt
□ Psychologe
□ Pflgepersonal
□ Ich wurde nicht beraten

3) Wurde alle notwendige Privatsphäre während Ihrer Behandlung respektiert?

□ Ja, vollständig
□ Ja, zu einem gewissen Grad
□ Nein
□ Keine Ahnung

4) Wurden das Verfahren und die nächsten Schritte in der Behandlung erklärt, so dass, Sie diese verstehen konnten?

□ Ja, vollständig
□ Ja, zu einem gewissen Grad
□ Nein
□ Ich brauchte keine Erklärung

5) Von wem wurden Sie bezüglich des Therapieziels und der Erwartungen beraten?

□ Arzt
□ Psychologe
□ Pflegepersonal
□ Ich brauche keine Erklärung

Auswertungsbogen zur Optimierung der individualisierten Arzneimitteltherapie der Patienten durch die pharmazeutische Beratung

(Erstellt von Mary Remigius Onuoha zur Auswertung der Stationsvisite: 2009 - 2012)

6) Hatten Sie das Gefühl bei der Entscheidung über Ihre Behandlungspläne einbezogen zu werden?

□ Ja vollständig
□ Ja zu einem gewissen Grad
□ Nein
□ Nicht anwendbar

7) Wurden Ihnen die Arzneimittelwirkung Ihrer Medikamente erklärt?

□ Ja vollständig
□ Ja zu einem gewissen Grad
□ Nein
□ Keine Ahnung

8) Wurden Ihnen das Auftreten unerwünschter Arzneimittelwirkungen Ihrer Medikamente erklärt?

□ Ja vollständig
□ Ja zu einem gewissen Grad
□ Nein
□ Keine Ahnung

9) Haben Sie das Gefühl, dass Ihnen die Zeit und Aufmerksamkeit, die Sie benötigten, wurden gegeben?

□ Ja vollständig
□ Ja zu einem gewissen Grad
□ Nein
□ Keine Ahnung

10) Sind Unerwünschte Arzneimittelwirkungen bereits aufgetreten?

□ Ja, nur einmal

Auswertungsbogen zur Optimierung der individualisierten Arzneimitteltherapie der Patienten durch die pharmazeutische Beratung

(Erstellt von Mary Remigius Onuoha zur Auswertung der Stationsvisite: 2009 - 2012)

□ Ja, mehrmals
□ Nein
□ Keine Ahnung

11) Wenn Sie Ihr Medikament zu Hause einnehmen oder eine Therapie fortführen müssen: haben Sie klare Erklärungen und Anweisungen bekommen, was zu tun ist?

□ Ja vollständig
□ Ja zu einem gewissen Grad
□ Nein
□ Ich brauchte keine Erklärung

Ort, Datum Unterschrift

Note: Questionnaire prepared for the patients for the evaluation of the activities of the Pharrmacist at the clinic ward 1b during the research study.

Appendix 12. Diagnoses for which MLP and/or BPD was Administered

Group ICD-9 + ICD-10	Diagnoses	Diagnoses (number)	Diagnoses (%)
349 + F0	Organic disorders including dementia, delirium and diseases of the nervous system	2,349	24.2
304 + 305 + F1	Disorders due to psychoactive substances, addictive disorders	680	7.0
290-294 + 295 + F2	Schizophrenia, delusional disorders and psychotic conditions	3,686	38.0
296.3 + 296.1 + F3	Affective disorders, depression and mania	2,073	21.4
300 + F4	Neuroses, stress and somatoform disorders	382	3.9
F5	Behavioral problems with physical disorders	35	0.4
F6	Personality and behavioral disorders	269	2.8
F7	Intellectual deficit	152	1.6
F8	Developemental disabilities	26	0.3
F9	Mental disorders in childhood and adolescence	38	0.4
	Total	9.690	100.00

Note: N diagnoses= total number of diagnosis in a group. The diagnoses were grouped according to the grouping of the International Classification of diseases 2009 and 2010.

Appendix 13. AMÜP Form for the Record of ADR-Reports in the Psychiatry

Arzneimittelüberwachung in der Psychiatrie (AMÜP)
Erfassungsbogen für UAW

Stammdaten
Fallkonferenzdatum : Fall-Nr. :

Geburtsdatum : Geschlecht (m/w) : Initialen (N/V) :

Aufnahmedatum: Entlassdatum: Erstaufnahme ☐ ja ☐ nein Jahr d. Ersterkrankung :

Fallart : ☐ stationär ☐ ambulant ☐ Aufnahme-UAW
☐ Sonderfall (schwere/ungewöhnliche UAW ohne Absetzen des beschuldigten Medikaments)

bereits gemeldet : ☐ nein ☐ ja, und zwar an ☐ AkdÄ ☐ BfArM
☐ Firma

Diagnosen (ICD-10)
psychiatr. Hauptdiagnose: Nebendiagnose(n):

somat. Diagnose(n) : Klartext.

Aktuelle UAW
☐Suizid/Suizidversuch ☐Medikamentenmissbrauch/-abhängigkeit

☐andere UAW, Art:

Beschreibung der UAW, angeschuldigtes Medikament, Begründung des Wahrscheinlichkeitsgrades:

Dauer der UAW von: bis: Weitere UAW zeitgleich : ☐ ja ☐ nein

Beurteilung nach GCP : (=Good Clinical Practice)
☐ lebensbedrohlich
☐ bleibender Schaden
☐ Verlegung
☐ Tod
☐ Aufenthaltsverlängerung
☐ stationäre Aufnahme
☐ Schwangerschaft

mögliche Risikofaktoren : ☐ nein
☐ ja, weil Vorschädigung d. Organs
☐ ja, weil hohe Einstiegsdosis
☐ ja, weil schnelle Dosissteigerung

Beschreibung der Risikofaktoren :

Alternativerklärung: (nicht medikamentös) ☐ nein ☐ ja, eher wahrscheinlich ☐ ja, weniger wahrscheinlich

Angaben zur Alternativerklärung:

Maßnahmen nach UAW : ☐ keine
☐ Reduktion
☐ Absetzen
☐ Verlegung
☐ Weiterbehandlung
☐ medikamentöse Gegenmaßnahmen
☐ nichtmedikamentöse Gegenmaßnahmen
☐ Konsil

nähere Beschreibung zu den Maßnahmen :

Verlauf der UAW :
☐ abgeklungen ☐ im Abklingen ☐ unverändert ☐ bleibender Schaden ☐ Exitus ☐ Verlauf unbekannt

nähere Angaben zum Verlauf :

Frühere Exposition

☐ *n*ein ☐ *j*a, gleiche UAW ☐ *j*a, aber keine solche UAW ☐ *j*a, Verlauf unbekannt

nähere Angaben bei früherer Exposition (Dosis, Datum, Verlauf, auch ggf. bei ähnlichen Medikamenten):

Reexposition nach UAW

☐ *n*ein ☐ *j*a, gleiche UAW ☐ *j*a, aber keine solche UAW ☐ *j*a, Verlauf unbekannt

☐ *W*eitergabe ohne Absetzen

nähere Angaben bei Reexposition (Dosis, Datum, Verlauf) :

Fall-Nr.:

Medikation vor UAW - bitte genaue Dosierungen und zeitlichen Verlauf
- bei längerer Einnahme unbedingt Angabe der letzten Dosisveränderungen i. d. Wochen vor UAW-Begir
- auch Nicht-Psychopharmaka aufführen

UAW-Beginn:

Medikamente (Handelsname)	Tagesdosis	von bis	UAW-Dosis (mg)	W.Grad
	-			

W.Grad (= Wahrscheinlichkeit des Zusammenhanges mit der beobachteten UAW)

0 = kein Zusammenhang
1 = möglich
2 = wahrscheinlich
3 = sicher
4 = unwahrscheinlich/ nicht beurteilbar

5 = Interaktion-nicht durch Blutspiegeldaten belegt
51 = Interaktion möglich
52 = Interaktion wahrscheinlich
53 = Interaktion sicher
54 = Interaktion nicht beurteilbar
6 = Interaktion- durch Blutspiegeldaten belegt

Relevante Laborbefunde und Zusatzdiagnostik

☐ keine

Datum:									
Leukozyten (/nl)									
Neutrophile (%)									
Eosinophile (%)									
Thrombozyten(/nl)									
GOT (U/l)									
GPT (U/l)									
γ-GT (U/l)									
GLDH(U/l)									
AP (U/l)									
CK (U/l)									
CRP (mg/dl)									
Na (mmol/l)									
K (mmol/l)									
Kreatinin (mg/dl)									
Lithium									
Valproat									
TCA									
Carbamazepin									
RR (mm/Hg)									
Puls (/min)									
BMI (kg/m²)									
Temperatur (°C)									

Relevante EKG-Befunde:

Andere Diagnostik :

Falls Suizid(versuch) oder Medikamentenmissbrauch/-abhängigkeit: bitte nächste Seite beachten!

Suizidversuch
(nur auszufüllen bei Suizid/ Suizidversuch!)

Ergänzung soziale Anamnese: ☐ ledig, ☐ verheiratet, ☐ geschieden, ☐ verwitwet

Beruf:

Finanzielle Situation

Suizidanamnese:
bisher Suizidalität ☐ nein ☐ ja, und zwar ☐ latent ☐ akut, ☐ manifest

Anzahl der bisherigen Suizidversuche
Suizidversuche während stationärer Aufenthalte

bisherige Suizidmethoden:

Suizid/-versuch
Zeitpunkt: Ort:

Art der Suizidhandlung: ☐ parasuizidal ☐ ernsthaft

Nähere Beschreibung, Anlass und Begründung des Suizidversuchs:

Folgen: ☐ allgemeine Maßnahmen ☐ Verlegung ☐ sonstige Folgen
Beschreibung.

Medikamentenmissbrauch / -abhängigkeit
(nur auszufüllen bei Medikamentenmissbrauch / -abhängigkeit!)

☐ Med.missbrauch ☐ Med.abhängigkeit

Suchtanamnese
Substanzmissbrauch ☐ ja ☐ nein
zus. Missbrauch and. Substanzen ☐ ja ☐ nein

Symptomatik
Entzugssymptomatik ☐ ja ☐ nein
Dosissteigerung ☐ ja ☐ nein
Craving ☐ ja ☐ nein
andere Ursache ☐ ja ☐ nein

Beschreibung:

Spezifische Maßnahmen nach Erkennen des Medikamentenmissbrauchs:

Entzugsbehandlung ☐ ja ☐ nein

Verlauf der UAW:

Note: The AMÜP-form for recording the reported undesired drug effects during the clinic ward visits.

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