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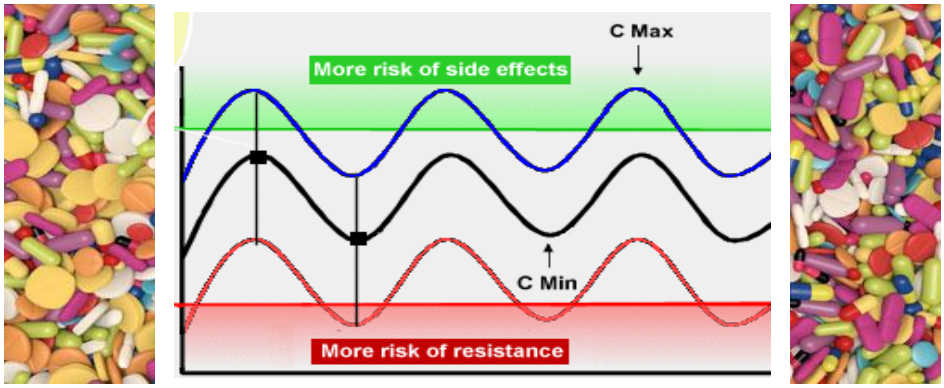
THERAPEUTIC DRUG MONITORING AND DETECTION OF INTOXICATIONS FOR PSYCHOTROPIC DRUGS USED IN RWANDA



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Abstract

The use of psychotropic medications in Rwanda is not limited to treatment of usual mental illnesses only, but these drugs are used also in management of some sequels of various atrocities experienced by Rwandan population especially the 1994 Genocide against Tutsi. The optimisation of treatment with psychotropic medications requires therapeutic drug monitoring as they are associated with a great interindividual variability in treatment susceptibility. In Rwanda, no therapeutic drug monitoring of psychotropic drugs is applied, which results into difficult treatment optimisation and exposition of patients under treatment to a high risk of toxicity or medication ineffectiveness.

The aim of this work was to assess the clinical implication of blood concentration levels of psychotropic drugs in Rwandan patients in order to initiate Therapeutic Drug Monitoring (TDM) in Rwandan clinical practices.

The first step consisted in validation of an analytical technique that can be applied in therapeutic drug monitoring of psychotropic drugs commonly prescribed in Rwanda. An HPLC/DAD method was validated according to FDA (Food and Drug Administration) criteria and to the total error approach for the determination in serum of 27 psychotropic drugs: alprazolam, amitriptyline, bromazepam, carbamazepine, chlorpromazine, citalopram, clomipramine, clonazepam, diazepam, droperidol, fluoxetine, flupentixol, haloperidol, imipramine, levomepromazine, lorazepam, midazolam, nordiazepam, olanzapine, phenobarbital, phenytoin, pipamperone, risperidone, sulpiride, thiopental, zolpidem and zuclopenthixol.

The method was then applied in the determination of blood concentration levels of psychotropic drugs in Rwandan patients, with the aim of identifying problems associated with the lack of therapeutic drug monitoring. Serum samples from Rwandan patients were analysed

in the laboratory of Clinical, Forensic, Environmental and Industrial Toxicology of the University Teaching Hospital of Liège. Analytical results showed that Rwandan patients under psychotropic treatment are exposed to both the risk of drug ineffectiveness (47%) and the risk of toxicity (8%) with only 46% of results within therapeutic reference range. Based on these results, the need to carry out therapeutic drug monitoring for optimisation of psychotropic treatment in Rwandan patients was obvious.

To carry out therapeutic drug monitoring activities in Rwanda, an analytical method suitable for such activities was required. It is in this regard, that a transfer in Rwanda of the analytical method previously validated in Belgium was envisaged. A method based on high performance liquid chromatography with diode array detection for the determination of psychotropic drugs in serum, suitable for both therapeutic drug monitoring and detection of intoxications, was transferred from the Laboratory of Clinical, Forensic, Environmental and Industrial Toxicology/University Teaching Hospital-Liège to the Laboratory of Analysis of Foodstuffs, Drugs, Water and Toxics/University of Rwanda.

This work indeed constitutes a paramount contribution to the initiation of TDM activities for psychotropic drugs in Rwanda. Patients under treatment in Rwandan hospitals, starting with those treated in Butare University Teaching Hospital will soon start to benefit from these activities as the first and immediate implementation phase.

Résumé

L'utilisation des médicaments psychotropes au Rwanda ne se limite pas au traitement des maladies mentales habituelles, mais ces médicaments sont également utilisés dans la prise en charge des séquelles des atrocités qu'a vécues la population Rwandaise, plus spécialement le génocide contre les Tutsi de 1994. Le suivi thérapeutique pharmacologique est nécessaire à l'optimisation du traitement avec les médicaments psychotropes, sachant que ces derniers sont associés à une grande variabilité interindividuelle. Le manque du suivi thérapeutique pharmacologique pour ces médicaments au Rwanda rend difficile l'optimisation du traitement et expose les patients à un haut risque de toxicité et d'inefficacité du traitement.

L'objectif de ce travail était d'évaluer l'implication clinique des concentrations plasmatiques des médicaments psychotropes chez les patients Rwandais dans le but d'initier le suivi thérapeutique pharmacologique dans les pratiques cliniques Rwandaises.

La première étape consistait à valider une technique analytique pouvant être appliquée dans le suivi thérapeutique pharmacologique des médicaments psychotropes communément prescrits au Rwanda. Une méthode HPLC/DAD a été validée selon les critères de la FDA (Food and Drug Administration) et selon le principe d'erreur totale pour le dosage dans le sérum des 27 médicaments psychotropes: alprazolam, amitriptyline, bromazépam, carbamazépine, chlorpromazine, citalopram, clomipramine, clonazépam, diazépam, dropéridol, fluoxétine, flupentixol, halopéridol, imipramine, lévomépromazine, lorazépam, midazolam, nordiazépam, olanzapine, phénobarbital, phénytoïne, pipampérone, rispéridone, sulpiride, thiopental, zolpidem et zuclopenthixol.

En suite, la méthode validée a été utilisée pour déterminer la concentration des médicaments psychotropes dans le sang des patients Rwandais, à fin d'identifier les problèmes liés au manque du suivi thérapeutique pharmacologique. Les échantillons de sérum des patients

Rwandais ont été analysés au sein du Laboratoire de Toxicologie Clinique, Médico-légale, de l'Environnement et en Entreprise du Centre Hospitalier Universitaire (CHU) de Liège. D'après les résultats d'analyse, les patients Rwandais sous traitement psychotrope sont exposés aussi bien au risque d'inefficacité du traitement (47%) qu'au risque de toxicité (8%), avec seulement 46% des résultats dans l'intervalle thérapeutique. Ces résultats démontrent clairement la nécessité de mener les activités de suivi thérapeutique pharmacologique dans le but d'optimiser le traitement psychotrope au Rwanda.

Pour réaliser les activités de suivi thérapeutique pharmacologique au Rwanda, une technique analytique appropriée était requise. C'est dans ce cadre qu'un transfert au Rwanda de la technique analytique précédemment validée en Belgique a été envisagé. Une méthode basée sur la chromatographie liquide haute performance couplée à un détecteur à barrette de diodes (HPLC/DAD) pour le dosage des médicaments psychotropes dans le sérum, appropriée aussi bien pour le suivi thérapeutique pharmacologique que pour le dépistage des intoxications, a été transférée du Laboratoire de Toxicologie Clinique, Médico-légale, de l'Environnement et en Entreprise/CHU-Liège au Laboratoire d'Analyse des Denrées Alimentaires, Médicaments, Eau et Toxiques/Université du Rwanda.

Ce travail apporte donc une importante contribution à l'initiation au Rwanda des activités de suivi thérapeutique pharmacologique pour les médicaments psychotropes. Les patients sous traitement dans les hôpitaux Rwandais, en commençant par ceux traités au sein du CHU-Butare, vont bientôt commencer à bénéficier de ces activités.

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PREAMBLE AND WORK PLAN

Therapeutic drug monitoring (TDM) refers to the measurement of serum concentration of a drug in a single or multiple time points in a biological matrix after a conventional drug dose, with the purpose of achieving maximum efficacy and minimum adverse reactions of drug by individualizing the dosage. TDM can also be defined as individualization of drug dosage by maintaining plasma or blood drug concentrations within a targeted therapeutic range (1, 2). The International Association for Therapeutic Drug Monitoring and Clinical Toxicology considers TDM as the measurement made in the laboratory of a parameter that, with appropriate interpretation, will directly influence prescribing procedures. In other words, in addition to the measurement of the concentration of a drug in a biological matrix, TDM involves the proper interpretation of the result using pharmacokinetic parameters, drawing appropriate conclusion regarding the drug concentration and dose adjustment (3, 4). In general, the measurement of serum concentration levels concerns prescribed xenobiotics but sometimes it may also concern endogenous compounds prescribed as a replacement therapy in patients physiologically or pathologically deficient in those compounds (4).

Therapeutic drug monitoring was introduced in clinical practice in the early 1970s and since then it has been used to individualize medication therapy with the goal of optimizing pharmacological responses while avoiding adverse effects of various medications (5-11). In fact, pharmacological effects can be better predicted with serum concentrations than with dose for drugs monitored on routine basis in clinical laboratories. In addition, TDM is also used in monitoring of patient's medication compliance and identification of potential drug interactions (1, 4).

The use of TDM is not recommended for all drugs and the following are categories of drugs requiring therapeutic drug monitoring:

- drugs with narrow therapeutic range i.e. the dose of a drug that produces the desired

therapeutic concentration is near the dose that may produce toxic serum concentration;

- drugs without clearly defined clinical parameter that allows dose adjustments;
- drugs for which the relationship between dose and clinical outcome is unpredictable;
- drugs with toxicity that may lead to hospitalization, irreversible organ damage or death;
- drugs with a proven correlation between serum concentration and efficacy as well as toxicity (4, 12).

Psychotropic drugs are among drugs for which the relevance of TDM has been demonstrated. The main reason of using TDM for optimisation of psychotropic medications is a considerable interindividual variability in the pharmacokinetic properties of these drugs (13). Various factors including genetic peculiarities, concurrent disease, age, concomitant medications, etc., affect patients' ability to absorb, distribute, metabolise and eliminate psychotropic drugs, which results in a great variation in blood concentration levels for a drug administered at the same dose to different patients (14-18).

For a large number of psychotropic medications, there is evidence that incidence of undesirable effects is often dose-related and the same correlation has been observed for therapeutic effects and plasma levels in most cases (14, 19-22). For these drugs, a dose adjustment based on serum concentration levels, may be more useful rather than routine assessment of a patient. For example, the adjustment of phenytoin dose in patients based on their serum concentrations rather than seizure frequencies results not only in a decrease of morbidity but it also prevents unnecessary drug toxicity (4). In addition to clinical benefits of TDM, patients may also benefit economically from it, as it has the potential to improve the cost-effectiveness of psychopharmacotherapy when used appropriately (1, 2, 13).

The importance of TDM use for dose adjustment of psychotropic drugs has obviously been demonstrated; however to fully benefit from it, TDM should be adequately integrated into the

treatment process. The use of TDM in psychiatry remains suboptimal (23, 24). As it has been demonstrated by various studies, inappropriate use of TDM is common (1, 13, 25). When used inappropriately, TDM will not only be a waste of laboratory resources but its results may also mislead clinical decision making (25). For tricyclic antidepressants for example, between 25 and 40% of the requests for TDM in psychiatric university hospital settings were found to be inappropriate and TDM results were responsible for about 20% of inappropriate therapeutic adjustments (13, 24).

A large number of guidelines for TDM of psychotropic medications have been published since the last decade (1, 13, 26-34). The AGNP consensus guidelines for therapeutic drug monitoring in psychiatry are among well known guidelines (1, 13). These guidelines issued by the TDM group of the "Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie" (AGNP), were published for the first time in 2004 and an updated version was published in 2011. With respect to these guidelines, depending on the medication, TDM may be strongly recommended, recommended, useful, probably useful or not recommended (1, 13).

The measurement of plasma levels to titrate drug dose after initial prescription or after dose change is rational for drugs with well defined therapeutic reference ranges or with narrow therapeutic indexes. For these drugs even when there is no specific problem, there is enough evidence that patients under treatment will benefit from TDM. This is the case for lithium, where TDM is even compulsory for safety reasons, but also for tricyclic antidepressants, several antipsychotics or anticonvulsants (1, 13, 35).

When it comes to a suspicion of medication non-compliance or lack of clinical improvement under recommended doses, the use of TDM is valid for all psychotropic medications commonly used in clinical practice. A high risk of medication non-compliance is a major

problem of long-term treatment (36-38). In psychiatric patients, the prevalence of medication non-compliance varies between 10 and 69% (37-40). Various studies have shown that it is not possible to reliably predict patients' adherence using classical methods including pill counting, examining case-note recordings, interviewing patients or noting the attending physicians' clinical judgement about adherence (41-46). Therefore, measuring drug plasma level remains advantageous as it is an objective method showing the prescribing physician if the drug is in the body at a concentration needed to produce expected clinical response.

Like any other diagnostic test, the request of TDM should only be made in case of evidence that the result will help to improve treatment or to solve a well defined problem. The validity of TDM indications in psychiatry should be examined on an individual basis and with an individual evaluation for each case. The following are typical TDM indications for psychotropic medications according to AGNP guidelines:

- dose optimisation after initial prescription or after dose change;
- drugs for which TDM is mandatory for safety reasons (e. g., lithium);
- suspected complete or partial non-adherence (non-compliance) to medication;
- lack of clinical improvement under recommended doses;
- adverse effects and clinical improvement under recommended doses;
- combination treatment with a drug known for its potential or suspected drug interaction;
- pharmacovigilance programs;
- relapse prevention under maintenance treatment;
- recurrence under adequate doses;
- presence of a genetic particularity concerning drug metabolism (genetic deficiency, gene multiplication);
- pregnant or breast feeding patients;
- children and adolescent patients;

- elderly patients;
- individuals with intellectual disabilities;
- patients with pharmacokinetically relevant comorbidities (hepatic or renal insufficiency, cardiovascular disease);
- forensic cases;
- problems occurring after switching from an original preparation to a generic form and vice versa (1, 13).

Therapeutic drug monitoring of psychotropic medications has now interred routine activities of clinical laboratories in many developed countries (13, 20-22). However, this is far from being the case in developing countries including Rwanda. Actually, in addition to usual use of psychotropic medications, in Rwanda these drugs are used also in management of some sequels of various atrocities experienced by Rwanda population especially the 1994 Genocide against Tutsi. Psychotropic medications are used without TDM in Rwanda and this makes difficult the treatment optimisation . Patients are therefore exposed to a high risk of drug ineffectiveness but also severe side effects or toxicity of these drugs. This also increases the risk of medication discontinuation and medication non-compliance.

According to data from the Ministry of Health-Mental Health Department, in July 2015 cases of mental problems that could involve the use of psychotropic drugs in Rwanda were 64,038 and epilepsy was the most frequent problem representing 81% of the total cases. Females were representing 51% of the total cases with the age ranges of 0-19 years and 20-39 years representing respectively 35 and 42% of the total cases. Between June 2014 and July 2015, 10,909 new cases of mental problems were registered. Information about the quantities of psychotropic drugs used in Rwanda was only available for controlled drugs. For example, data from the Pharmacy Task Force/Ministry of Health showed that about 20,000 grams of diazepam and 450,000 grams of phenobarbital are imported each year (47).

So far in Rwanda, there is no structure to control blood concentration levels of psychotropic drugs. The objective of this study is to contribute to the initiation in Rwanda of activities of therapeutic drug monitoring and detection of intoxications for psychotropic drugs commonly used in Rwanda.

The first chapter of this work gives a reminder of pharmacokinetic, pharmacodynamic and pharmacogenetic properties of psychotropic drugs. Clinical use and problems associated with the use of these drugs as well as their therapeutic drug monitoring are also described in this chapter.

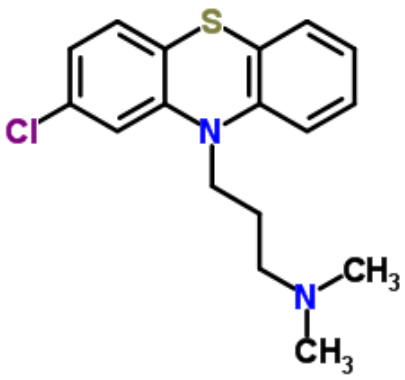
The second chapter describes the validation process of the analytical method developed for the determination in serum of psychotropic drugs. An analytical method based on High Performance Liquid Chromatography (HPLC), was validated in the University Teaching Hospital of Liège (Belgium) for 27 psychotropic drugs commonly prescribed in Rwanda: alprazolam, amitriptyline, bromazepam, carbamazepine, chlorpromazine, citalopram, clomipramine, clonazepam, diazepam, droperidol, fluoxetine, flupentixol, haloperidol, imipramine, levomepromazine, lorazepam, midazolam, nordiazepam, olanzapine, phenobarbital, phenytoin, pipamperone, risperidone, sulpiride, thiopental, zolpidem and zuclopenthixol.

In the third chapter, after being validated, the analytical method was applied to determine serum concentration levels of psychotropic drugs in Rwandan patients in order to identify problems associated with the lack of TDM of these drugs in Rwanda. Serum samples were collected from Rwandan patients under psychotropic treatment and samples from 128 patients were analysed. Analytical results were interpreted based on therapeutic reference ranges of various drugs.

Finally, the forth chapter describes the transfer of the validated analytical method from the Laboratory of Clinical, Forensic, Environmental and Industrial Toxicology of the University Teaching Hospital-Liège (Belgium) to the Laboratory of Analysis of Foodstuffs, Drugs, Water and Toxics of the University of Rwanda, for its application in therapeutic drug monitoring activities. Among various approaches used in analytical method transfer, revalidation of the method in the receiving laboratory was adopted.

CHAPTER I. INTRODUCTION

The term "psychotropic drugs" was introduced for the first time in 1957 by Ralph Gerard, an American neurophysiologist. This term was used to designate all drugs that can affect mental activity and human behavior. The first six psychotropic drugs to be introduced in clinical practice consisted of two antipsychotics (chlorpromazine and reserpine), two antidepressants (iproniazid and imipramine), an anxiolytic (meprobamate) and a mood stabilizer (lithium carbonate) (48). Following the introduction and the success of chlorpromazine (fig. 1) into clinical practice in 1950s, many other psychoactive medications were developed and now around 130 psychotropic drugs are available (13).



Molecular Formula **$C_{17}H_{19}ClN_2S$**

Average mass **318.864 Da**

Figure 1. Chemical structure of Chlorpromazine (49)

I.1. Pharmacokinetics of psychotropic drugs

Drug's pharmacokinetics refers to what the body does to the drug and it determines the concentration of the latter in the body. Pharmacokinetics involves four phases:

- Absorption of the drug from the site of administration
- Distribution through the body
- Biotransformation or metabolism leading to more polar metabolites
- Elimination from the body

The dosing rate and the clearance of the drug are two important parameters which determine the concentration of the drug (50).

I.1.1. Absorption

A pharmacokinetic parameter used to assess the absorption of a drug is the bioavailability referring to the portion of a drug absorbed from the site administration. For psychotropic drugs, the principal route of administration is oral and the absorption generally occurs in the small bowel. The drugs pass then through the portal circulation and enter the liver. Drug metabolism by cytochrome P450 enzymes can occur in both the bowel wall and the liver before reaching the systemic circulation (first-pass effect). Being lipophilic in most cases, psychotropic medications readily enter the central nervous system after passing the blood-brain barrier. Moreover, due to their high lipophility these drugs have in common other features including rapid and complete absorption, rapid and extensive tissue compartment distribution, high first-pass effect and large volume of distribution (51, 52).

The more polar a compound, the slower the absorption from gastrointestinal tract and the slower the penetration into the brain, the target organ for psychotropic drugs. For example, oxazepam, the most polar benzodiazepine, is slowly absorbed into both the systemic circulation and the brain. This pharmacokinetic profile is not desired in hypnotic-sedative where a rapid penetration in both compartments is normally needed and lorazepam is a good example (50, 53). However, fast absorption is not always desirable as adverse effects may be a function of the maximum concentration (C_{max}). In some cases, the division of the dose into smaller amount with more frequent administrations can indeed help to avoid severe side effects. In this case, the peak concentration becomes lower and the trough concentration higher, but the average plasma concentration over the dosing interval and the amount absorbed usually remains the same (50).

Various routes of administration are used for psychotropic drugs. The route of absorption can affect the rate of absorption and the ratio of parent compound to its metabolites as well. Compared to oral route, the time corresponding to the maximum concentration (T_{max}) usually reduces and the C_{max} is higher with intramuscular administration due to more rapid absorption. Nevertheless, exceptions can be observed with some psychotropic medications. For example the bioavailability of chlordiazepoxide and diazepam decreases when administered intramuscularly versus oral administration. This is actually due to the fact that these drugs are unstable and tend to crystallize in tissue at a pH of 7.4 (50, 53).

When administered orally, drugs are typically absorbed in the small bowel, enter the portal circulation, and then pass through the liver. Before reaching the systemic circulation, a portion of drugs can be metabolized by CYP isoenzymes in both the bowel and the liver; this is the first-pass metabolism also known as first-pass effect. The latter can be broadly affected by various conditions including diseases (e.g. cirrhosis, portacaval shunting, persistent hepatitis and congestive heart failure) and some drugs like alcohol and ketoconazole, with influence on the peak concentrations and the ratio parent compound: metabolites (54).

When a drug undergoes a first-pass metabolism, metabolites are excreted into the bile and then the small bowel. The lipid-soluble ones are reabsorbed and eventually enter the systemic circulation. These metabolites may have similar or totally different pharmacological profile from their parent drugs. For example, around 70 metabolites have been identified in blood and tissue after an extensive hepatic biotransformation of chlorpromazine. Though weaker than the parent compound, some of these metabolites are dopamine receptor-blockers and it is not easy to separate the effects of chlorpromazine from those of its metabolites. To avoid the first-pass effect drugs are administered parenterally. Drugs like olanzapine, ziprazidone, aripiprazole and risperidone are more potent with intramuscular administration (50, 54).

I.1.2. Distribution

Once in systemic circulation, drugs distribute to organs according to their fat and protein content. The accumulation rate is a function of the vascularity of an organ. Drugs with high lipid-solubility accumulate to same extent in adipose tissue and the brain; however the rate of accumulation is much faster in the latter (55).

Being quite lipophilic with large volumes of distribution, psychotropic drugs reach tissue concentrations usually 10 to 100 times greater than their plasma concentrations. In fact, the initial concentration drop is rather a function of the rate of uptake into other bodily compartments than elimination process. This is particularly important for intravenous administration of the first dose of a psychotropic. For most of psychotropic drugs, the acute effects of a single dose are terminated by redistribution. The acute sedative effects of intravenously administered lorazepam would be a typical example. This drug rapidly enters the brain from the blood with a greater amount of the dose in the brain compared to peripheral adipose tissue. As the drug redistributes into the plasma and then into other peripheral compartments, brain concentration subsequently falls which put an end to the acute psychoactive effects of lorazepam (54).

Most of psychotropic drugs are highly protein bound and the bound amount often account for more than 90% of the total plasma concentration (56). Knowing that the drug effect is determined by the free-drug fraction, any change in the ratio of bound to free drug can change the magnitude of effect. A functional decrease in amount of circulating protein can be the result of various conditions including malnutrition, wasting, aging and concomitant drugs competing for protein binding sites (50, 56).

Increasing the amount of the free fraction can increase toxicity, but most assays used in routine Therapeutic Drug Monitoring (TDM) do not distinguish between free and bound-drug and thus will not detect such changes. Nevertheless, the usefulness of TDM using plasma

concentration to ensure the adequacy of dose is based on the fact that it relays on steady state concentration. Under steady state conditions, there is a proportional relationship between the plasma compartments and the tissue. Thus, even though psychotropic drug effects are not exerted in plasma, their plasma concentration and tissue levels are in equilibrium (56).

I.1.3. Biotransformation

Most psychotropic drugs undergo phase-I metabolism leading to the formation of more polar metabolites excreted in urine. This metabolism includes oxidative (e.g., hydroxylation, dealkylation, oxidation to N-oxides, S-oxidation to sulfoxides or sulfones), reductive (e.g., carbonyl reduction to secondary alcohols) or hydrolytic reactions. These reactions are predominantly catalysed by cytochrome P450 (CYP) enzymes comprising more than 200 isoenzymes. For psychotropic drugs the most important isoenzymes are CYP1A2, CYP2B6, CYP2D6, CYP2C9, CYP2C19 and CYP3A4/5. The table 1 shows CYP enzymes involved in metabolism of various psychotropic drugs (57-59).

The role of phase-I reactions is generally to introduce a polar functional group making possible a phase-II conjugation reaction with highly polar molecules such as glucuronic or sulphuric acids. For psychotropic medications with functional groups in the parent compound, the essential pathway of metabolism may be represented by glucuronidation of an N-H group (e.g., olanzapine) or a hydroxyl group (e.g., oxazepam or lorazepam). Tertiary amine groups can be conjugated with the formation of quaternary ammonium glucuronides as well. In fact phase II enzymes are not specific regarding substrates and much overlap is observed between the isozymes when affinity for substrates is considered (60). Other enzymatic systems that may also be involved include ketoaldehyde oxidases which reduce ziprasidone to its dihydro-derivative or naltrexone to naltrexol (61-63).

The metabolism of drugs mainly occurs in the liver and to a minor degree in extrahepatic tissues such as the intestinal mucosa or the brain (64-66). Variation in activities of drug-metabolizing enzymes results into inter- and intra-individual differences in plasma concentrations of psychotropic drugs. The enzyme activity can be modified by renal and hepatic diseases but it may also decrease with age (67). Although findings are inconsistent with no clear clinical relevance, gender differences have been reported for psychotropic drugs (68-71).

Table 1. CYP enzymes involved in the metabolism of some psychotropic medications (13)

Substrates	**Main metabolizing enzymes**	**Other metabolizing enzymes**
Alprazolam	CYP3A4/5	-
Amitriptyline	CYP2C19, CYP2D6	CYP1A2, CYP2C9, CYP3A4/5
Brotizolam	CYP3A4	-
Carbamazepine	CYP3A4/5	CYP1A2, CYP2B6, CYPC8
Chlorpromazine	CYP1A2, CYP2D6	-
Citalopram	CYP2C19	CYP2D6, CYP3A4
Clomipramine+ norclomipramine	CYP2C19, CYP2D6	CYP1A2, CYP3A4
Clozapine	CYP1A2, CYP2C19	CYP3A4
Desipramine	CYP2D6	-
Diazepam, nordiazepam, oxazepam & temazepam	CYP2C19	CYP2B6, CYP3A4
Doxepin & nordoxepin	CYP2C9, CYP2C19, CYP2D6	-
Duloxetine	CYP1A2	CYP2D6
Estalopram	CYP2C19	CYP2D6, CYP3A4
Fluoxetine + norfluoxetine	CYP2C9, CYP2C19, CYP2D6	CYP2B6
Flupentixol	CYP2D6	-
Haloperidol	CYP3A4	CYP2D6
Imipramine	CYP1A2, CYP2C19, CYP2D6	CYP3A4
Levomepromazine	-	CYP2C19, CYP2D6
Midazolam	CYP3A4	-
Mirtazapine	-	CYP1A2, CYP2B6, CYP2D6, CYP3A4
Nortriptyline	CYP2D6	-
Olanzapine	CYP1A2	CYP2D6
Paroxetine	CYP2D6	CYP1A2, CYP3A4
Quetiapine	CYP3A4	CYP2D6
Risperidone	CYP2D6	CYP3A4
Trimipramine+ nortrimipramine	CYP2C19, CYP2D6	CYP2C9
Venlafaxine	CYP2D6	CYP2C19, CYP3A4
Zolpidem	CYP3A4	CYP1A2, CYP2D6
Zopiclone	CYP3A4	CYP2C8
Zuclopenthixol	CYP2D6	-

I.1.4. Elimination

Most psychotropic drugs are eliminated from the body via the kidneys. Most compounds are eliminated via urine after conversion into polar metabolites more water-soluble and less lipid-soluble than parent compounds. It is obvious that renal failure will delay drug clearance resulting in the accumulation and higher concentrations of polar metabolites. Patients may thus accumulate compounds with less effectiveness, more toxicity or both compared to parent compounds and depending on the pharmacological profile of metabolites. The same results can also be the consequence of dehydration as it decreases the glomerular filtration rate (54, 55).

Elimination half-lives of psychotropic drugs generally vary between 12 and 36 hours. However, some of these drugs have relatively short elimination half-lives (about 2-10 hours) and this is the case for venlafaxine, nefazodone, trazodone, tranylcypromine, moclobemide, quetiapine, rivastigmine and ziprasidone, whereas others like aripiprazole and fluoxetine have long elimination half-lives (72 hours for aripiprazole and 3-15 days for fluoxetine, taking into account its active metabolite nor-fluoxetine) (72).

I.2. Pharmacodynamics of psychotropic drugs medications

Pharmacodynamics refers to what a drug does once it gets in interactions with its receptor at adequate concentrations. With regard to drug effect, two critical factors are to be considered: the affinity for drug's attachment to a target and its intrinsic activity (full or partial agonist or antagonist at a receptor). Psychotropic medications affect specific biochemical processes and their activity involves in most cases enzymes, receptors or ion channels (50).

The first psychotropic drugs of the modern era including lithium, first-generation antipsychotics, tricyclic and monoamine oxidase inhibitor antidepressants, were discovered by chance and they have a wide range of central biochemical effects. As matter of fact, they

usually affect simultaneously more than one neurotransmitter system with multiple repercussions (50). Modifications made to chemical structures of the early psychotropics helped to reduce such undesired qualities by developing a new generation of drugs with enhanced selectivity. For example, bupropion, venlafaxine, nefazodone, mirtazapine and duloxetine are serotonin reuptake inhibitors developed to eliminate many of the adverse effects of earlier-generation antidepressants (73). Actually, the first era of psychopharmacotherapy where drugs were discovered serendipitously has given the way to the second one with refinement of drugs based on known biochemical effects. The compounds of the next era will be synthesized based on specific interactions at newly discovered components of the neuron (50).

Mechanisms of action of psychotropic drugs are different but they all have in common the target of specific molecular sites that have great effects on neurotransmission. There are only few sites of action (fig. 2) for over 100 essential psychotropic drugs utilized in clinical practice. Actually, about 30% of psychotropic medications target one of the transporters for a neurotransmitter, other 30% target receptors coupled to G proteins, while enzymes are targeted by around 10%. The remaining part of these drugs (around 30%) targets various types of ion channels. Therefore, to understand the mechanisms of actions of psychotropic agents, one needs just to master how these molecular sites regulate neurotransmission (74).

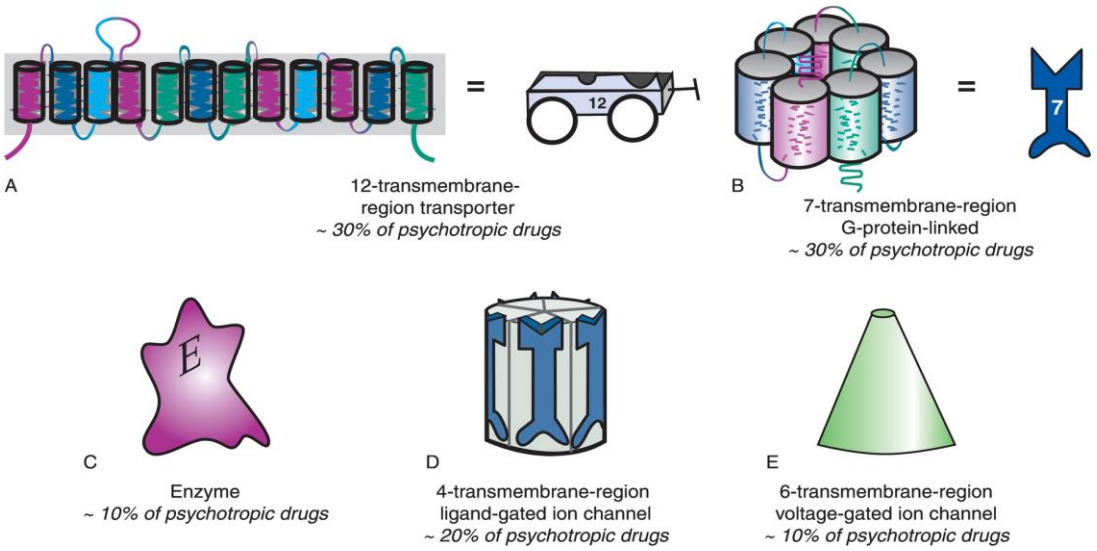


Figure 2. The molecular targets of psychotropic drugs. Approximately one-third of psychotropic drugs target one of the twelve-transmembrane-region transporters for a neurotransmitter (A), while another third target seven-transmembrane-region receptors coupled to G proteins (B). The sites of action for the remaining third of psychotropic drugs include enzymes (C), four-transmembrane-region ligand-gated ion channels (D), and six-transmembrane-region voltage-sensitive ion channels (E) (74).

I.3. Clinical use of major classes of psychotropic drugs

I.3.1. Antipsychotics

I.3.1.1. Clinical use

Antipsychotics also known as neuroleptics are used in treatment of psychosis in such disparate disorders as schizophrenia, schizophreniform disorder, schizoaffective disorder, delusional disorder and brief psychotic disorder. These drugs are also indicated in cases of psychosis secondary to nonpsychiatric medical condition and depression or mania with mood-congruent or mood-noncongruent psychotic symptoms (4).

Based upon their ability to cause neurological adverse effects, antipsychotic medications are made of first-generation antipsychotics (FGAs) and second-generation antipsychotics (SGAs). Actually, compared to conventional antipsychotics (FGAs), atypical antipsychotics (SGAs) have the clinical profile of an equal positive symptoms (antipsychotic actions), but low

extrapyramidal symptoms and less hyperprolactinemia (74). All antipsychotics marketed before clozapine are referred to as FGAs. The SGAs also known as atypical antipsychotics are the most commonly prescribed antipsychotic agents in most countries including the United States and Canada. The table 2 presents both FGAs and SGAs still in use in clinical practice (50, 74).

Table 2. Conventional and atypical antipsychotics used in Belgium vs. Rwanda

Conventional antipsychotics			
Belgium		**Rwanda**	
Generic name	**Brand name**	**Generic name**	**Brand name**
Amisulpride	Solian	Chlorpromazine	Largactil
Bromperidol	Impromen	Droperidol	Orap
Clotiapine	Etumine	Flupentixol	Fluanxol
Droperidol	Dihydrobenzperidol	Levomepromazine	Nozinan
Flupentixol	Fluanxol	Haloperidol	Haldol
Fluspirilene	Imap	Pimozide	Orap
Haloperidol	Haldol	Pipamperone	Dipiperon
Levomepromazine	Nozinan	Sulpride	Dogmatil
Pipamperone	Dipiperon	Zuclopenthixol	Clopixol
Pimozide	Orap		
Prothipendyl	Dominal		
Sulpride	Dogmatil		
Tiapride	Tiapridal		
Zuclopenthixol	Clopixol		
Atypical antipsychotics			
Aripiprazole	Abilify	Olanzapine	Zyprexa
Asenapine	Sycrest	Risperidone	Risperidal
Clozapine	Leponex		
Olanzapine	Zyprexa		
Paliperidone	Invega		
Quetiapine	Seroquel		
Risperidone	Risperidal		
Sertindole	Serdolect		

I.3.1.2. Mechanism of action

Talking about the mechanism of action of antipsychotics, one should distinguish conventional antipsychotics from atypical antipsychotics. Since the 1970s, the key pharmacologic property of all neuroleptics with antipsychotic effects was recognised to be their ability to block dopamine D_2 receptors. It has been proven that this action is not only responsible for the antipsychotic efficacy, but also for the most of undesirable effects of conventional antipsychotic medications. Specifically, therapeutic effects of these drugs is the result of the blockade of D_2 receptors in the mesolembic dopamine pathway (74). This results into the reduction of the hyperactivity in this pathway postulated to cause psychosis positive symptoms. The blockade of enough number of D_2 receptors in the mesolimbic dopamine pathway to quell positive symptoms causes a simultaneous blockade of the same number of D_2 receptors throughout the brain and this is the cause of undesirable effects of conventional antipsychotic drugs. Extrapyramidal side effects of conventional antipsychotics seem to be the result of the use of these drugs at doses producing striatal D_2 receptor blockade that exceeds 80%. At doses that do not produce this level of receptor occupancy, these drugs can be used therapeutically without producing these side effects (74-76).

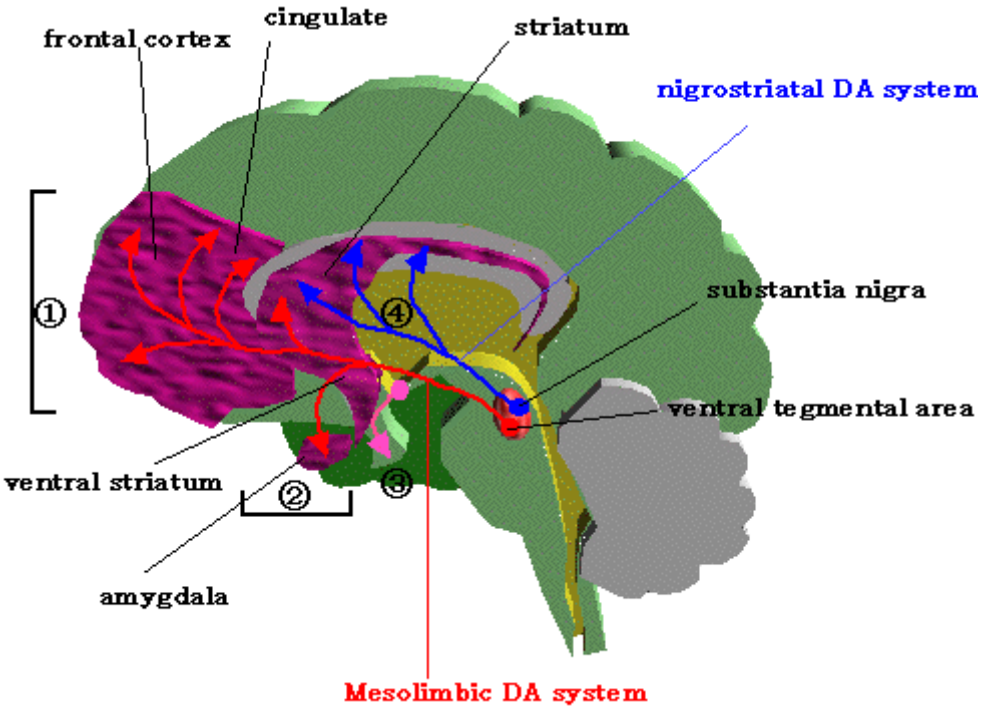


Figure 3. Actions and side effects of antipsychotic drugs on dopaminergic neurons (77). Antipsychotic and sedative actions are the results of drugs' works on mesocortical DA system (1) and mesolimbic DA system (2). The hyperprolactinemia is caused by DA blockade in the hypothalamus and hypophysis system (3), while extrapyramidal effects are caused by the inhibition of D2 receptor of the nigrostriatal system (4).

On the other side, the mechanism of action of atypical antipsychotics involves another neurotransmitter in addition to dopamine and this is serotonin. These drugs are defined as serotonin-dopamine antagonists, because their dopamine D_2 receptor antagonism is accompanied by a simultaneous serotonin $5HT_{2A}$ receptor antagonism. Other pharmacologic actions that can hypothetically mediate the clinical profile of atypical antipsychotics (low extrapyramidal symptoms and less hyperprolactinemia) include partial agonist actions at both $5HT_{1A}$ and D_2 receptors (74, 75, 78).

I.3.1.3. Undesirable effects and overdose

The efficacy of any medication must be balanced against its undesirable effects. For antipsychotic medications, undesirable effects can vary from those with mildly discomfort to those that can seriously affect people's health. Reported adverse effects of antipsychotics are so many and this may suggest that most of them are experienced by patients to a significant level. Actually, some of these effects such as dry mouth or tremor will be experienced by almost all patients treated with these drugs, but they are usually transitory and disappear with time, medication reduction, or discontinuation. Most of these effects are not serious or irreversible and in general the typical complications with antipsychotics are not worse than those associated with other medications (50). Undesirable effects of antipsychotics are usually the result of the blockade of various receptors by these drugs and the table 3 presents the main side effects due to this action.

Table 3. Receptor blockade and antipsychotic side effects (50, 78)

Receptor type	**Side effects**
Dopamine (D_2)	Extrapyramidal symptoms (EPS), prolactin elevation
Muscarine (M_1)	Cognitive deficits, dry mouth, constipation, increased heart rate, urinary retention, blurred vision
Histamine (H_1)	Sedation, weight gain, dizziness
α_1	Hypotension
Serotonin (5-HT_{2A})	Anti-EPS
Serotonin (5-HT_{2C})	Satiety blockade

Antipsychotic overdose can be potentially serious as it can be associated with various cardiac complications. Nevertheless, the overdose of these drugs is often associated with low morbidity and mortality (79). Overdosing antipsychotics results in a gamut of manifestations affecting multiple organ systems. Cardiovascular system and the CNS are involved in the most serious toxicity. In overdose, both conventional and atypical antipsychotics can produce

a pronounced sedation as a result of CNS histamine H_1 receptor blockade; this is particularly the case for clozapine and quetiapine (80, 81). Tachycardia, mild hypotension and prolongation of the QT interval are commonly observed cardiovascular effects of antipsychotic overdose (82). Other symptoms that may be seen in acute overdose of antipsychotic drugs include nausea and vomiting, miosis or mydriasis, confusion, hallucinations, agitation, electrolyte imbalance, drowsiness progressing to coma and respiratory depression or apnoea and extrapyramidal symptoms (79, 83).

I.3.2. Antidepressants

I.3.2.1. Clinical use

As indicated by their name, antidepressants are class of psychotropic drugs used in treatment of various types of depression. The latter constitutes a heterogeneous group of mood, neurovegetative and cognitive disorders. Mood symptoms include depressed, low or irritable mood and diminished interest or pleasure. Neurovegetative symptoms include weight and appetite change (increased or decreased), dyssomnia (insomnia, hyposomnia and hypersomnia), psychomotor retardation or agitation and fatigue or energy loss. Cognitive symptoms are indecisiveness, diminished attention or concentration, worthlessness/guilt feelings, hopelessness feelings and suicidality. The disorder may occur at any age, however the average age at onset is the late twenties (50, 84, 85). The depression incidence increases dramatically from adolescence to early adulthood. According to the World Health Organization, by 2020 and worldwide, this disorder will be the second leading cause of disability (86).

Selective serotonin reuptake inhibitors (SSRIs) form the most commonly prescribed class of antidepressants. These drugs are largely prescribed to the extent that their prescription rate

could be estimated to six prescriptions per second in the US alone and their use worldwide is dramatically increasing (74).

I.3.2.2. Mechanism of action

Antidepressant mode of action is classically the blockade of one or more of the transporters for serotonin, norepinephrine, and/or dopamine. Classic antidepressants include monoamine oxidase inhibitors (MAOIs) and tricyclic antidepressants (50, 74).

The MAOIs were the first clinically effective antidepressants to be discovered. Their discovery occurred by accident when an anti-tuberculosis drug, iproniazid, was found to have antidepressant effects in some patients with coexistence of tuberculosis and depression. The antidepressant effects of iproniazid were found to be the results its inhibition of the MAO enzyme. The latter exists in two subtypes, A and B. The MAO-A metabolizes preferentially the monoamines most closely linked to depression while the MAO-B intervenes preferentially in the metabolization of trace amines like phenylethylamine. In addition to their antidepressant effects, MAOIs are also highly effective for certain anxiety disorders including panic disorders and social phobia (74).

On the other side, tricyclic antidepressants (TCAs), named so because of the presence of three rings in their chemical structure, were discovered to block the reuptake pumps for either both norepinephrine and serotonin or for norepinephrine alone. These drugs were actually synthesized about the same time as other three-ringed molecules such as chlorpromazine which were shown to have antipsychotic effects. When they were tested for schizophrenia they were discovered to be rather antidepressants (40). This has been the case for imipramine, a derivate of Chlorpromazine that was originally synthesized as possible antipsychotic. Some TCAs (e.g., clomipramine) have equal or greater potency for serotonin inhibition whereas others (e.g., desipramine) are more selective for norepinephrine inhibition (50, 74). However,

for most tricyclic antidepressants the reuptake is blocked to some extent for both serotonin and norepinephrine. In addition, some tricyclic antidepressants have antagonist actions on $5HT_{2A}$ and $5HT_{2C}$ that could contribute to their therapeutic profile. These drugs have also in common the blockade of muscarinic cholinergic receptors, H_1-histamine receptors, α_1-adrenergic receptors and voltage-sensitive sodium channels, which explains their various undesirable effects (74).

The new generation of antidepressants is mainly made of selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs). If the antidepressant effect of serotonin inhibition has been demonstrated, this is not the case for the norepinephrine inhibition because most selective norepinephrine reuptake inhibitors (Selective NRIs) have failed in clinical trials and the question whether this mechanism alone is able to produce antidepressant effect of a clinical relevance remains (50, 74).

I.3.2.3. Undesirable effects and overdose

The choice of an antidepressant is often based on its adverse effect profile as a large number of these drugs provide comparable therapeutic effects. Compared to TCAs and MAOIs, modern antidepressants present relatively improved safety and tolerability. This is actually due to the fact that the latter affect selectively the sites of action that appear to mediate antidepressant efficacy (e.g., serotonin uptake pump) which is not the case for the former affecting even unnecessary sites of actions (e.g., Ach receptor, fast sodium channels). Potentially serious undesirable effects commonly reported for antidepressants include sedation, anticholinergic effects, orthostatic hypotension and cardiac effects (50). The adverse effect profiles of SSRIs are almost opposite to those of TCAs. For the former, adverse effects principally result from excessive serotonin agonism, whereas for the latter these effects result from their blockade of multiple neuroreceptors (histamine, muscarinic, Ach and α_1-

adrenergic), the uptake pumps for serotonin and norepinephrine and at quite high concentrations, fast sodium channels (cardiotoxicity of TCAs) (50, 74).

Antidepressant overdose cases are frequent in psychiatric patients as depression is the most frequent psychiatric disorder in people dying by suicide (86). In overdose, tricyclics are more toxic than newer antidepressants and they are frequently identified in self poising along with paracetamol, benzodiazepines and alcohol (88, 89). Compared to other tricyclics, amitriptyline and dosulepin (dothiepin) have shown a relatively greater toxicity, the latter being the commonest tricyclic involved in fatal overdose. The table 4 shows effects of tricyclic overdose on various body systems (90).

Table 4. Clinical features of tricyclic antidepressant overdose

CNS	**Peripheral autonomic system**	**Cardiovascular system**
Drowsiness	Dry mouth	Sinus tachycardia
Coma	Blurred vision	Prolonged PR/QRS/QT
Convulsions	Mydriasis	ST/T wave changes
Pyramidal signs	Urinary retention	Heart block
Rigidity	Absent bowl sounds	Vasodilatation
Delirium	Pyrexia	Hypotension
Respiratory depression	Myochronic twitching	Cardiogenic chock
Ophtalmologia		Ventricular fibrillation
		Asystole

I.3.3. Antianxiety and Sedative-Hypnotic drugs

I.3.3.1. Clinical use

Bromides and barbiturates were the first drugs of this group to be synthesized and used to reduce anxiety, tension and agitation in the nineteenth century. However, these drugs have in common treatment-limiting and potentially life-threatening disadvantages including rapid development of tolerance to their therapeutic effects, high risk of dependence, significant

withdrawal effects, serious undesirable effects and lethality in overdose. Later on, meprobamate was introduced expecting an improvement over barbiturates, but unfortunately this was not the case, because this drug showed the same disadvantages (50).

Since their introduction in 1960s till now, benzodiazepines remain the most widely prescribed of anxiolytics and sedative-hypnotics. Their effectiveness for a large number of disorders, safety in overdose alone as well as in combination with most drugs and mildness in terms of undesirable effects are among other advantages of benzodiazepines (74).

I.3.3.2. Mechanism of action

The barbiturates and benzodiazepines bind to sites on $GABA_A$ receptors which are different from the GABA (γ-aminobutyric acid) recognition site but linked to the latter (fig. 4). The action of GABA is usually to balance the effects of the primary excitatory neurotransmitter, glutamate. In addition, the recognition sites for GABA receptors were found to be coupled to chloride channels. The binding of GABA to its receptors result in opening of these channels followed by the flow of chloride ions into the neuron, which make it more resistant to excitation. $GABA_A$, $GABA_B$ and $GABA_C$ are three pharmacologically and physiologically different classes of receptors involved in actions of GABA (50, 74, 91).

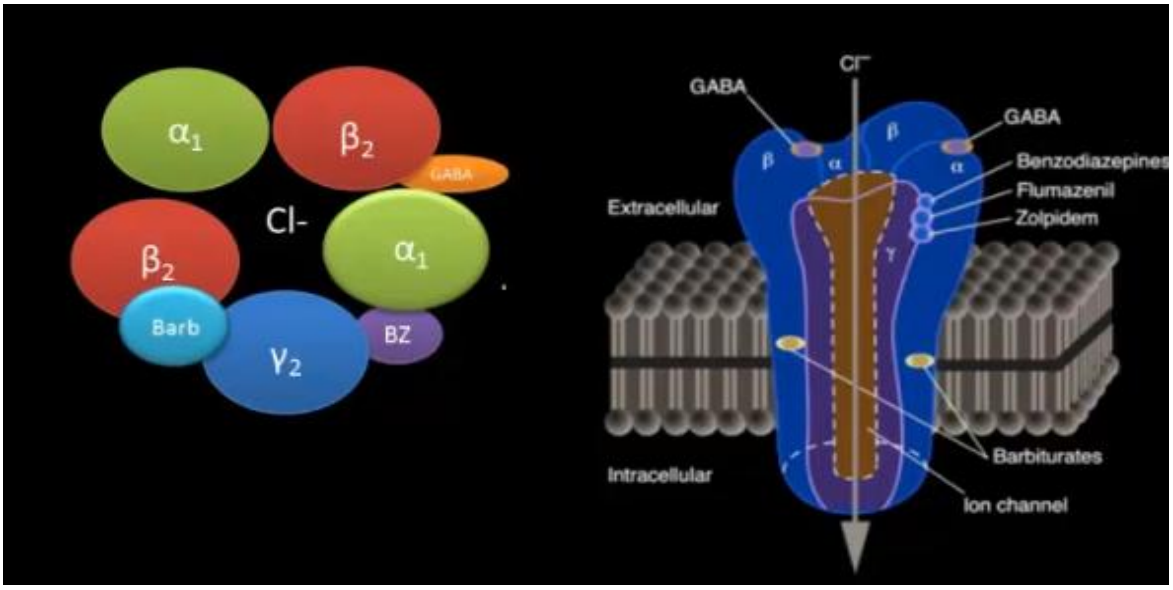


Figure 4. Mechanism of action of barbiturates and benzodiazepines (92)

On one side, barbiturates seem to interact with sites directly related to the chloride channel resulting in prolongation of the duration of its opening by around four to five-fold (38). On the other side, benzodiazepines potentiate inhibitory action of GABA by enhancing the affinity of its recognition site and thus increase the frequency of channel openings. In fact, benzodiazepines act as indirect $GABA_A$ agonists facilitating GABA-mediated neurotransmission (93, 94).

I.3.3.3. Undesirable effects and overdose

Drowsiness, dizziness, lightheadedness, headache, sedation and unsteadiness are the most common side effects of barbiturates. Other side effects include depression, confusion and unusual excitement. These drugs can also cause the bleeding sores on the lips, chest pain, fever, muscle or join pain and skin problems (rash, hives,...), swollen eyelids, face, or lips and wheezing. Barbiturate overdose can result in difficulty thinking, slow speech, slow and unsteady walking, lack of coordination, shallow breathing, drowsiness and in serious cases respiratory depression coma and death (50, 74).

The most well-known initial complication for benzodiazepines is sedation. This effect usually diminishes with the emergence anxiolytic action of benzodiazepines, in about one week. Other less frequent side effects of these drugs include agitation, ataxia, confusion, excitement, gastrointestinal distress, transient hypotension and vertigo. In general, acute treatment with benzodiazepines is associated with fewer undesirable effects compared to other antipsychotics (38). Acute benzodiazepine overdose alone can lead to death in extremely rare cases. Even when massive doses are ingested, a relatively rapid recovery appears without serious complications. Nevertheless, the combination of benzodiazepines with other central nervous system (CNS) depressants (alcohol, barbiturates, narcotics or TCAs) may cause severe CNS and respiratory depression or hypotension. In fact the severity of symptoms is determined

more by the type and quantity of other drugs associated to benzodiazepines than by the plasma level of the latter (50, 74).

I.4. Pharmacogenetics of psychotropic medications

Pharmacogenetics is an important variable determining the biological state of a given patient, which in turn can affect the susceptibility to treatment (e.g., efficacy, safety, and/or tolerability). In clinical practice, the recognition of the importance of pharmacogenetic factors in the pharmacokinetics and pharmacodynamics of psychotropic medications is increasing (95-98). Genetic variability is observed among drug-metabolising enzymes, especially CYP isoenzymes (58).

A deviation in the alleles affecting at least 1 % of the population is considered as genetic polymorphism. The expression enzyme (phenotype) is determined by the number of active alleles in a gene. Individual genetic disposition basically determines the drug-metabolising enzyme activity. People lacking functional alleles are poor metabolisers (PM), while those with an active and an inactive allele (or an allele with reduced activity) or having 2 alleles with reduced activity are intermediate metabolisers (IM). In extensive metabolisers (EM) there is a presence of 2 active alleles, while for ultra-rapid metabolisers (UM) there is an amplification of functional alleles (99).

On one hand, an increase in plasma concentrations with unexpected adverse reactions and toxicity may occur in PM, while on the other hand, subtherapeutic plasma concentrations resulting in non-response may occur in UM (100). Genetic polymorphism is also exhibited by other enzyme systems with unclear clinical relevance in pharmacopsychiatry such as UDP-glucuronosyltransferases (101).

Methods for genotyping of CYP are becoming more and more available and guidelines for their use in clinical practice have been published (98, 102). Nevertheless, the functional significance of many genotypes is not clearly defined. Regardless of the fact that they show a wide interindividual variability in their activity, a genetic polymorphism is not clearly demonstrated for some enzymes. Even though it may be advantageous to use phenotyping methods in some cases, their results may be influenced by environmental factors such as smoking or comedications. Actually genotyping remains advantageous due to the fact that environmental factors have no influence on its results, in other words, its result has a lifetime value (103-105).

The relevance of the drug efflux transporter P-glycoprotein (P-gp) in the intestinal mucosa and blood-brain barrier has also been indicated for the pharmacokinetic variability of psychotropic drugs (106). The P-gp encoded the multidrug resistance gene (MDR1, ABCB1), belongs to the family of ATP-cassette binding (ABC) transporter protein. This protein displays a genetic polymorphism that may have the same considerable clinical relevance as has been demonstrated for drug-metabolizing enzymes (107-110). A genotype dependent association of drug response was demonstrated for antidepressants; drugs known to be substrates of P-gp (111, 112). It also has been shown that plasma concentrations and clinical effectiveness of quetiapine depend on the P-gp genotype of patients (23). Even though more researches are needed to evaluate the clinical relevance of the genetic polymorphisms of drug transporters, some reports suggest the influence of the genetic polymorphism of P-gp on the occurrence of desired or undesired clinical effects of psychotropic medications (113, 114).

I.5. Therapeutic drug monitoring of Psychotropic medications

As reminder, therapeutic drug monitoring (TDM) is the measurement of drug levels that, with appropriate clinical pharmacological interpretation, will directly affect prescribing

procedures. Monitoring plasma concentrations is less necessary if a drug produces immediate pharmacological effects. In contrast, when a relatively long period (e.g., weeks) is needed for an administered drug to produce its effect, monitoring plasma levels is necessary and this is often the case in psychopharmacotherapy. Doses can be more rapidly adjusted to achieve proper levels, if the plasma concentrations needed for clinical response are known (50).

When among patients there are large differences in drug metabolism, monitoring plasma levels is also useful. More precise guidelines to individualize dose adjustment can be provided by the knowledge of the optimal therapeutic range. Therapeutic drug monitoring is based on the assumption that there is a relationship between plasma levels and clinical response. To understand this relationship there are three basic assumptions:

- there is an optimal concentration at which maximal pharmacological response will occur;
- there is a relationship between the drug concentration in plasma and at the site of action;
- the drug reaching the receptor site is affected by pharmacogenetic and environmental factors in different individuals (50).

I.5.1. Drug dose and its blood concentration

In most cases where TDM is used to optimize the dose, drug administration is realised in a series of repeated doses to reach a steady state concentration within a given therapeutic reference range. This state is attained when the rate of medication input (absorption) equals the rate of medication output (elimination) and normally this happens approximately after 4 to 5 elimination half-lives. One week of maintenance dosing is required to reach such a steady-state for more than 90% of all psychotropic medications. If the dosing interval (τ), the clearance (Cl) and the bioavailability (F) for the drug in a particular patient are known, one

can determine the dose required to reach a steady-state concentration of a drug in plasma. For psychotropic drugs, such data are available from studies where drug concentrations were measured in plasma of healthy volunteers or patients treated with fixed doses (13, 115). The population involved in such clinical trials is usually made up of adult people (18-65 years old) without relevant comorbidity, comedication, and genetic abnormalities in drug metabolism. However in clinical practice this is not always the case (13).

The basis of TDM is the assumption that there is a relationship between plasma concentrations of a drug and its clinical effects (therapeutic and adverse effects). It is also based on the assumption that there is a plasma concentration range associated with maximal effectiveness and maximal safety of a drug, the so-called "therapeutic window"(13). This range also known as "therapeutic reference range" defines a range of drug concentrations specifying a low limit below which therapeutic response of a drug is relatively unlikely to be obtained and an upper limit above which there is a decrease of tolerability or it is unlikely possible to enhance therapeutic improvement. However, one should be aware that the therapeutic reference range is a population based range which may not necessarily be applicable to all patients. Optimal therapeutic response of some individuals may be obtained with a medication concentration different from the therapeutic reference range. The latter remains an orienting range and the best way would be to identify individual therapeutic concentration of the patient (1, 13, 20).

I.5.2. Sample collection, storage and shipment

To carry out TDM plasma or serum samples are generally used. In psychiatry the analysis of whole blood for TDM has been abandoned (13). Studies demonstrating clearly differences in the drug concentrations using either plasma or serum are still lacking. In accordance to few available comparisons, values obtained from two matrices can be used interchangeably (116).

Most psychotropic drugs are highly bound to plasma proteins and concentrations commonly reported in TDM refer the total fraction of the drug (13).

Therapeutic drug monitoring relies on trough steady-state drug plasma concentrations, with few exceptions. Therefore, one should wait at least 4 drug elimination half-lives after the start of treatment or a change in dosage to collect blood, and the collection must be done during the terminal ß-elimination phase. Apart from fluoxetine and aripiprazole with longer elimination half-lives or quetiapine, trazodone and venlafaxine with elimination half-lives of around 6 h, elimination half-lives of most psychotropic drugs vary between 12 and 36 h. In clinical practice, one week after stable daily dosing and immediately before ingestion of the morning dose, is the appropriate time for sample collection for most psychotropic drugs. In case of treatment with depot preparations, sampling should be realised immediately before the next injection (6). In case of unexpected side effects, it is not necessary to measure trough levels. TDM may be carried out at any time after drug ingestion but for interpretation, the dosing schedule should be reported (1, 13).

If collected samples have to be stored and sent frozen, the preparation of serum or plasma before freezing is a requirement as to make this preparation on frozen blood is impossible. Apart from few exceptions, the storage of serum or plasma samples can be realised at 4°C for at least 24 h, and for most psychotropic drugs, samples can be shipped without freezing. A special care is needed for light/or oxygen sensitive drugs (117).

I.5.3. Sample analysis

To conduct TDM successfully, sensitive and selective analytical methods for quantitative analysis of drugs are essential. The methods must be validated in order to guarantee the reliability of analytical results. Preferable methods for psychotropic drug analysis are generally chromatographic techniques including gas chromatography (GC), high-performance

liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC), in combination with suitable detection techniques (118). These techniques are accurate and precise enough and can be used to analyse a larger number of psychotropic drugs. Their major disadvantage is a limited sample throughput due to the need of sample preparation before chromatographic separation. To overcome this challenge some laboratories have introduced HPLC with column switching allowing direct injection of sample into the HPLC system and such techniques are available for a number of psychotropic drugs (119-124). Another method that can be applied for a large number of psychotropic medications and their metabolites is the liquid chromatography coupled with mass spectroscopy (LC-MS) and especially the tandem MS (LC-MS/MS). This method is more sensitive and selective but its use can be limited by its high cost and the lack of suitable calibration standards (125, 126).

Laboratory analysis does not concern only drugs but also their active metabolites. Even when metabolites do not contribute to the clinical effect of drugs, their determination remains useful in monitoring medication compliance, determination of patient capacity to metabolize drugs, or interpretation of drug-drug interactions. Internal and external quality control procedures are required for quality assurance and reliability of analytical results. Control samples are required for each series of samples to be analysed. For analytical results to be reported, quality control results must be within expected range, otherwise the reason needs to be clarified and documented (13).

I.5.4. Result communication and interpretation

Laboratory results should be reported with therapeutic reference ranges for both psychotropic drugs and their actives metabolites. Concentrations are expressed in mass units (e.g. ng/mL, µg/L) or molar (nmol/L, µmol/L) units. To relate the concentration to dose easily, the use mass units is recommended. In case of concentrations below the limit of quantification

(LOQ), this limit should be indicated. Results should be reported within clinically reasonable time for a decision making (13).

To ensure the full clinical benefit of TDM, adequate interpretation of a drug concentration measurement as well as adequate use of the information are essential. Result interpretation should not be limited to the consideration of whether the plasma concentration of the drug is within the therapeutic reference range. The consistence of plasma concentration with the drug dose has also to be considered. A plasma concentration outside the therapeutic reference range may simply be the result of a low or high dose that was taken. Moreover, the level of evidence underlying the therapeutic reference range of the particular drug should also be considered. One should also consider if the daily dose of the drug was taken as single or multiple dose (13).

The interpretation of plasma concentration requires a clinical presentation in mind. The most frequent advice remains the recommendation on dose change. Other information such as genetic polymorphisms or risks of pharmacokinetic interactions could also be helpful for the decision making (1, 13).

CHAPTER II. DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD

The first step of this work was the identification of psychotropic drugs commonly used in Rwanda, in order to develop an analytical method that could be used in their determination in blood for therapeutic drug monitoring purpose or in case of intoxications. Following the survey carried out in various institutions involved in management of psychotropic drugs in Rwanda, 27 drugs were selected: alprazolam, amitriptyline, bromazepam, carbamazepine, chlorpromazine, citalopram, clomipramine, clonazepam, diazepam, droperidol, fluoxetine, flupentixol, haloperidol, imipramine, levomepromazine, lorazepam, midazolam, nordiazepam, olanzapine, phenobarbital, phenytoin, pipamperone, risperidone, sulpiride, thiopental, zolpidem and zuclopenthixol.

II.1 Identification and quantification of psychotropic drugs in blood

An analytical method based on High Performance Liquid Chromatography (HPLC) coupled to a Diode array Detector (DAD) was developed for the determination in serum of selected drugs. This method allows a simultaneous determination of several psychotropic drugs in case of polymedication.

The high performance liquid chromatography operates with a liquid mobile phase made of aqueous buffer and organic solvent used either in isocratic mode or in gradient mode. The stationary phase consist of a column containing small particles (3.5 µm diameter) tightly packed. The application of a high pressure is compulsory for the passage of mobile phase through the column (127).

The liquid chromatography is certainly the most polyvalent method nowadays. The principle of this technique is based on equilibrium of concentrations of compounds to separate between a stationary phase (column) and a mobile phase moving on the contact of the former. The solutes are distributed into different phases according to their affinity for the mobile and stationary phases, and they are trained by the mobile phase at different speeds resulting into

their separation. Passing through the column, analytes are unequally retained by the stationary phase. This difference in retention explains the reason why compounds get out of the column ones after others and thus separated. Once out of the column, a detector coupled to an integrator (computer) allows to obtain a graph known as chromatogram representing the detector response as function of time, where each peak corresponds to the exit of a compound (127).

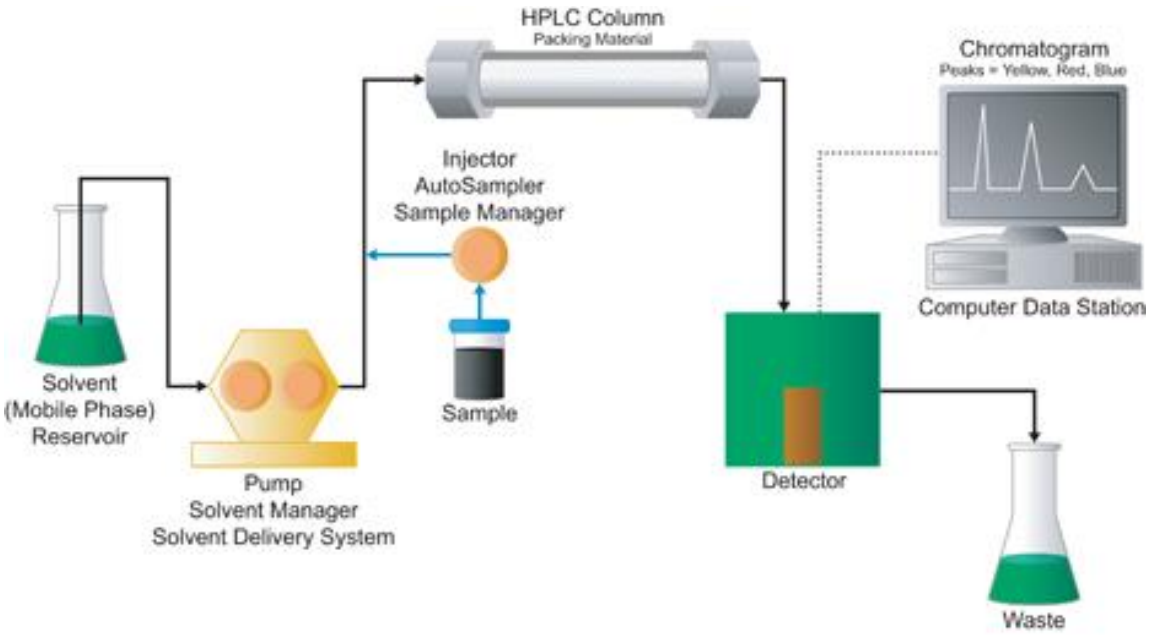


Figure 5. High-Performance Liquid Chromatography System (127)

Specifically for this work, the chromatographic separation was performed on a Symmetry® C8 analytical column (4.6mm×250mm, 5µm, Waters). The mobile phase consisted of acetonitrile and sodium dihydrogenophosphate buffer used in gradient elution mode (see table 5).

Table 5. Mobile phase gradient

Time (min)	Flow (mL/min)	% A	% B
0	1.0	13.0	87.0
9.0	1.0	35.0	65.0
28.0	1.5	80.0	20.0
30.0	1.5	80.0	20.0
31.0	1.5	13.0	87.0
32.0	1.0	13.0	87.0
45.0	1.0	13.0	87.0

A-Acetonitrile; **B**-Sodium dihydrogenophosphate buffer, pH 3.8

Differently from detectors with monochromatic variable wave lengths which follow eluted compounds on a single wave length (the wave length of the maximum absorbance of the compound of interest), the diode array detector (DAD) scans a large range of wave lengths in a couple of milliseconds generating continuously a UV-visible spectrum. The DAD consists of a row of diodes, each indicating the mean absorbance on a narrow wave length interval, one minute generally. The diode array detector delivers tridimensional information, namely the determination of absorbance at each wave length as function of time. The reading of generated chromatograms allows to identify eluted compounds by comparison to reference spectra of libraries, and quantify them giving at the same time information about the eluate purity (128).

II.2 Sample preparation

To extract various psychotropic drugs from serum, liquid-liquid extraction using a mix of organic solvents was used. This mix was made up of diethyl ether, dichloromethane, hexane and n-amyl alcohol in proportions of 50, 30, 20 and 0.5 (V/V). To enhance the ionic strength of the sample and facilitate the passage of analytes into organic phase, sodium carbonate (Na_2CO_3) was used. Five milliliters and five hundred microliters were used respectively for the organic

mix and sodium carbonate to extract 1 mL of serum sample after addition of 100 µL of internal standard. Details on sample preparation process are provided in the publication 1.

II.3 Analytical validation

The analytical validation was realized with respect to the general guidelines for validation of analytical methods and according to the principle of total error measure (129-132). Validation parameters assessed are presented in the publication 1. To calculate various validation parameters e-noval® (Arlenda) software was used.

Parameters assessed during the analytical validation process

The **response function** of an analytical procedure stands for the relationship existing, within a specified range, between the response (signal) and the concentration of analyte in the sample. This parameter was assessed using 6 calibration standards prepared by dilution a methanolic solution containing various compounds with a drug-free serum. These standards were prepared in duplicates on three consecutive days. The linear model was used for all compounds.

The **selectivity** of an analytical method is the ability of the method to discriminate between the analytes and interfering compounds. It refers to the extent to which the method can determine the particular analyte (s) in a complex mixture without interference from other components of the mixture. To assess this parameter retention times and UV-visible spectra were used. UV-visible spectra between 200 and 400 nm of all analytes are presented in appendix 1.

The **linearity** of a method refers to the relationship between introduced concentration and the concentration back-calculated from the calibration curve. This criterion shows the ability of the method to obtain results directly proportional to concentrations of analyte in samples

within a specified range. Results for the linearity of this method for various analytes are presented in appendix 2.

The **trueness** of an analytical method refers to the closeness of agreement between conventionally accepted value or reference value and the average value obtained from a large series of tested results. The trueness is expressed in terms of bias, relative bias or recovery and gives information on systematic error. Results for the assessment of trueness of this method are found in the publication 1.

The **precision** of an analytical method refers to the closeness of agreement between series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. The precision provides information on random error. It is expressed in terms of standard deviation, relative standard deviation or coefficient of variation. Both repeatability and intermediate precision were assed and results are found in the publication 1.

The **measurement uncertainty** is a parameter that characterizes the dispersion of the values that could be attributed to the measurand. The expended measurement uncertainty is presented in the appendix 3.

The **accuracy** of an analytical method expresses the closeness of agreement between the test result and the value accepted either as the reference or conventional true value. Actually, this closeness of agreement results from the total error related to test result, i.e. random and systematic errors. The accuracy expresses therefore the sum of precision and trueness of an analytical procedure. It is estimated from the accuracy profile obtained by joining between them, lower boundaries on one side and on the other side high boundaries of the tolerance interval, boundaries determined for each concentration level. The method is considered as valid for the determination interval where the accuracy profile is within prefixed acceptance limits. Accuracy profiles of various compounds are presented in the appendix 4.

The trueness, the precision, the measurement uncertainty and the accuracy were determined based on 3 validation standards prepared in triplicates on 3 days, by spiking the drug-free serum with a methanolic solution containing various compounds.

The **limit of detection** of an analytical procedure was defined as the lowest amount of analyte in a sample that can be detected. The **low** and **upper limits of quantification** were respectively defined as the lowest and the highest quantities of analyte in the sample that can accurately be quantitatively determined. To determine the LOD and LLOQ the signal-to-noise (S/N) ratio approach was used. The S/N ratios of 3:1 and 10:1 were considered respectively for the LOD and LLOQ.

The **stability** of various compounds was assessed on a single level of concentration for a storage temperature of -20°C. The recovery rates as function of storage time for various compounds are presented in the table 6. After 8 months of storage at -20°C, at least 70% of the initial concentrations were found for all compounds.

Details on validation process and results obtained for various validation parameters assessed were subject of the publication 1.

Table 6. Percentage of initial concentration remaining over time

Compounds	**Initial Conc. (ng/mL)**	**% of initial concentration**	
		After 1 month	After 8 months
Alprazolam	106	102	87
Amitriptyline	210	106	96
Bromazepam	509	107	101
Carbamazepine	9567	105	106
Chlorpromazine	223	81	83
Citalopram	250	103	97
Clomipramine	381	92	90
Clonazepam	124	86	82
Diazepam	1104	83	79
Droperidol	179	97	93
Flupentixol	98	108	108
Fluoxetine	577	93	84
Haloperidol	57	93	77
Imipramine	191	98	93
Levomepromazine	114	89	81
Lorazepam	211	83	88
Midazolam	541	81	82
Nordiazepam	1012	107	104
Olanzapine	81	102	100
Phenobarbital	50	87	92
Phenytoin	19600	106	103
Pipamperone	1123	79	80
Risperidone	101	101	94
Sulpiride	1488	86	70
Thiopental	3625	84	70
Zolpidem	593	106	103
Zuclopenthixol	103	99	97

PUBLICATION 1

Validation of an analytical method for the determination in serum of psychotropic drugs by High-Performance Liquid Chromatography with Diode Array Detection.

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Validation of an analytical method for the determination in serum of psychotropic drugs by High-Performance Liquid Chromatography with Diode Array Detection

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Abstract

Background: Inter-individual variability in clinical response to psychotropic drugs remains problematic in management of mental illnesses. The patients differ in their ability to absorb, distribute, metabolize and eliminate drugs due to genetic peculiarities, concurrent disease, age, or concomitant medication **Methods:** A simple and sensitive high performance liquid chromatography method with ultraviolet detection was validated for the determination of 27 psychotropic drugs in serum. The analysis by High-Performance Liquid Chromatography (HPLC) was performed on serum spiked with analyte(s), and prazepam was used as an internal standard. To get validation parameters, analytical results were processed based upon the total error concept using Enoval software. **Results:** The validated method was linear over the tested dosing intervals with a coefficient of determination of at least 0.99 for all molecules. The relative standard deviation (%-RSD) and bias were less than 15 % for all validation standards and the recovery varied between 92.7 % and 112.9 %. The accuracy of the method was demonstrated over the used dosing intervals. **Conclusion:** The method is suitable for both therapeutic drug monitoring and confirmation of drug poisoning, except for haloperidol, flupentixol and zuclopenthixol where it is only applicable for the confirmation of intoxication.

Key words: Psychotropic drugs, serum, validation, HPLC-DAD

Introduction

The inter-individual variability in clinical response to psychotropic drugs remains problematic in the management of mental illnesses (Malhotra, Murphy & Kennedy, 2004; Vecchione et al., 2012). The eventual toxicity of these drugs, especially barbiturates, antipsychotics and antidepressants can worsen the patient status and may be due to a poorly adapted dosing. Furthermore, due to the widespread use of these drugs, cases of deliberate and accidental poisoning with these drugs have become a major medical problem (Sanchez, Martinez & Almarza, 2005; Smink et al. 2004).

During the last 60 years, around 130 drugs have been developed in psychiatry. Despite enormous medical and economic benefits of the drugs, for many patients therapeutic outcomes are still far from satisfactory (Addington, 2009; Adli, Baethge, Heinz, Langlitz & Bauer, 2005; Jeffrey at al., 2005; Trivedi et al., 2007). Instead of continuing to focus on the development of new drugs, as it has been the case for more than 5 decades, there is growing evidence suggesting that substantial benefit to patients may be brought by improving the way the available medications are administered (Bates & Gawande, 2003; Hiemke et al. 2011). In psychotropic therapy, it has been demonstrated that incidence of undesirable effects is often dose-related and for some psychotropic drugs, the same correlation has been observed for therapeutic effects and plasma levels (Raggi, 2002; Bengtsson, 2004) To tailor the dosage of the prescribed medication(s) to the individual characteristics of a patient, therapeutic drug monitoring (TDM) is a valuable tool. Patients differ in their ability to absorb, distribute, metabolize and eliminate drugs due to genetic peculiarities, concurrent disease, age, or concomitant medication. At the very same dose a more than 20-fold inter-individual variation in the medication's steady state concentration in the body can be observed (Raggi, 2002; Brosen, 1996; Hiemke, 2008a; Hiemke, 2008b, Klotz, 2009). The use of TDM helps to determine the dose of individual patients in order to obtain an optimal drug concentration. Moreover, TDM has also a potential to improve psychopharmacotherapy cost-effectiveness (Preskorn & Fast, 1991; Touw, Neef, Thomson & Vinks., 2005). To adjust dose for a considerable number of psychotropic drugs, the determination of their plasma levels has become a clinical routine. For a large number of these drugs including tricyclic antidepressants, antipsychotic drugs and conventional mood stabilizing drugs clear evidence of benefits of TDM has been exhibited (Baumann et al.,

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2004; Müller et al., 2004). To disclose abnormal levels in patients with atypical metabolic rates or in forensic practice measurements of serum concentrations of psychotropic drugs and their metabolites may also be useful (Cutroneo, Beljean, Tan Luu & Siouffi, 2006; Sheng, Lei, Ju, Song & Zhang, 2010).

So far, several techniques based on liquid chromatography (Bugamelli et al., 2002), spectrophotometry, immunoassay (Zhang, Heineman & Halsall, 1999), electrochemistry (Wilhelm, Battista & Obendorf, 2000), gas chromatography and electrophoresis (Wang, Fan, Zhang & Cao, 2006) have been proposed for the determination of psychotropic drugs in biological fluids. Since most of these drugs are thermally labile and water soluble, reversed-phase liquid chromatography has interesting features in routine TDM (Cutroneo et al., 2006; Sheng et al., 2010). This technique can thus be used to carry out TDM of psychotropic drugs in Rwanda and therefore optimize treatment with these drugs.

Psychotropic drugs are used in Rwanda to treat usual mental illnesses but also to manage some of the psychological problems directly related to the genocide against Tutsi.. So far in Rwanda, to the best of our knowledge, no control of plasma concentration levels is done to optimize the treatment with these drugs and reduce the risk of toxicity to patients.

This prompted us to undertake a study aiming to develop and validate an HPLC-DAD technique that can be used to determine in serum psychotropic drugs most commonly used in Rwanda. To identify the concerned drugs, a survey on of psychotropic drug use in Rwanda has been conducted in various hospitals and institutions involved in management of psychotropic drugs in Rwanda. Visited sites were: Butare University Teaching Hospital (CHUB), Kigali University teaching Hospital (CHUK), King Faisal Hospital (KFH), Rwanda Military Hospital, Ndera Neuropsychiatric Hospital (HNPN), the Pharmacy Task Force in the Ministry of Health (PTF-MoH), the Psychosocial Consultation Service-CHUK (SCPS-CHUK), and Rwanda Biomedical Center-Medical Procurement (RBC-MP).

Based upon the results of this survey, the following drugs have been selected for the present study: alprazolam, amitriptyline, bromazepam, carbamazepine, chlorpromazine, citalopram, clomipramine, clonazepam, diazepam, droperidol, fluoxetine, flupentixol, haloperidol, imipramine, levomepromazine, lorazepam, midazolam, nordiazepam, olanzapine, phenobarbital, phenytoin, pipamperone, risperidone, sulpiride, thiopental, zolpidem and zuclopenthixol.

For an analytical method to be used in routine activities, analytical validation process is compulsory. This process aims to appreciate the performance of the method and evaluate it by experimentation if the method meets the expected requirements. Response function, linearity, limits of quantification and detection, selectivity, trueness, precision and accuracy are validation parameters commonly tested during the validation process (Rozet et al., 2007; Hubert et al., 2007a).

Methods

Chemicals and reagents

Compounds used as reference standards were purchased from various suppliers. Alprazolam and lorazepam have been obtained from Pfizer (Brussels, Belgium); amitriptyline, carbamazepine, clomipramine, and imipramine from LGC GmbH (Luckenwalde, Germany); bromazepam, clonazepam, and midazolam from Roche (Brussels, Belgium); citalopram, flupentixol, and zuclopenthixol from Lundbeck (Brussels, Belgium); chlorpromazine, diazepam, fluoxetine, nordiazepam, olanzapine, phenobarbital and zolpidem from Cerilliant (Texas, USA); droperidol from Prostrakan (Saint Claude, France); haloperidol, pipamperone, and risperidone from Jassen-Cilag (Antwerp, Belgium); levomepromazine and sulpiride from Sanofi-Aventis (Diegen, Belgium); phenytoin from Kela Pharma (Sint Niklaas, Belgium) and thiopental from Inresa (Freiburg, Germany). Prazepam was purchased from Certa (Braine-l'Alleud, Belgium). Sodium carbonate and sodium dihydrogenophosphate were respectively purchased from Merck (Darmastadt, Germany) and J.T. Baker (Deventer, the Netherlands). Acetonitrile was purchased from Lab Scan (Dublin, Ireland); n-Amyl alcohol and dichloromethane from J.T. Baker (Deventer, the Netherlands); n-hexane and methanol from Lab Scan (Sowinskeigo, Poland)and diethyl ether and acetonitrile supra gradient from Biosolve (Valkenswaard, the Netherlands). All organic solvents were certified for HPLC use. Blank human serum was obtained from CHU Liege blood bank.

Chromatographic conditions

The used chromatographic system consisted of a Waters Alliance 2695 Separations Module (Zellik, Belgium), equipped with a quaternary, low-pressure mixing pump, a degassing line and a thermostated autosampler and coupled to a 2996 photodiode array detector. The HPLC instrument was piloted by Empower® software (Waters) which was used also for data processing (area integration, calculation and plotting of chromatograms). Baselines were visually inspected with manual adjustment whenever necessary. A Symmetry® C8 analytical column (4.6mm×250mm) packed with 5µm diameter particles (Waters), together with a guard column (20mm×4.6 mm)

packed with identical material were used for separation performed at 30°C. An injection volume of 40 µL, a carousel temperature of 25°C and a run time of 45 min were fixed. The mobile phase consisted of acetonitrile (A) and sodium dihydrogenophosphate buffer (B) used in gradient elution mode: the run started with 13% (A) which was increased to 35% in 9 min and 80% in 28 min. This proportion was maintained for 2 min before decreasing and turning back to starting conditions held till the end of the run. The flow varied between 1and 1.5 mL/min. UV–visible spectra were recorded in the range 200–400 nm.

Solutions

Standard stock solutions were obtained either immediately from suppliers or prepared by dissolving various compounds in methanol. The same solvent was also used whenever dilution was necessary. Stock solutions were refrigerated between 2 and 8°C. Calibration and validation standard samples were prepared by spiking blank serum with an adequate amount of standard stock solutions. Sodium carbonate solution used in extraction was prepared by dissolving 21.2 g of Na_2CO_3 into 200 mL of bidistilled water. Phosphate buffer solution was obtained by dissolving 6.0 g of sodium dihydrogenophosphate into 1000 mL of bidistilled water and the pH was adjusted to 3.8 using phosphoric acid. The filtration prior to use was compulsory for the buffer solution.

Sample preparation

One milliliter of serum sample was needed for the analysis and 100 µL of prazepam 10 mg/L (internal standard) were added to the sample prior to extraction. To make the extraction more efficient, 500 µL of sodium carbonate were used to increase the sample ionic strength and thus decrease the water solubility of our analytes (organic compounds) and facilitate their transfer to the organic phase. The extraction was performed using 5 mL of a mix of organic solvents: diethyl ether/dichloromethane/ hexane/n-amyl alcohol (50/30/20/0.5: V/V/V/V). After shaking during 10 min and centrifuging during 10 min at 2000 rounds/min, 3.5 mL of the supernatant were picked up and evaporated to dryness under the nitrogen at 40°C maximum. Seventy microliters of a mix of acetonitrile and bidistilled water were used in a 50/50 ratio for recovery. After a 5 min centrifugation into Eppendorf tube, the supernatant was put into a vial for HPLC analysis.

Method of validation

Validation parameters assessed

1. Response function

The response function of an analytical procedure stands for the relationship existing, within a specified range, between the response (signal) and the concentration (quantity) of analyte in the sample (Rozet et al., 2007; Hubert et al., 2007a).

2. Selectivity

The selectivity of an analytical method refers to the extent to which the method can determine the particular analyte (s) in a complex mixture without interference from other components of the mixture. In other words this parameter refers to the ability of the method to discriminate between the analytes and interfering compounds (Rozet et al., 2007; Hubert et al., 2007a).

3. Linearity

The linearity of an analytical procedure refers to the relationship between introduced quantity (concentration) and the concentration back-calculated from the calibration curve. This criterion shows the ability of the method within a specified range, to obtain results directly proportional to concentrations of analyte in samples (Rozet et al., 2007; Hubert et al., 2007a).

4. Trueness

The trueness stands for the closeness of agreement between conventionally accepted value or reference value and the average value obtained from a large series of tested results. The trueness usually expressed in terms of bias, relative bias or recovery gives information on systematic error (Rozet et al., 2007).

5. Precision

According to various regulatory documents, the precision of an analytical procedure is defined as closeness of agreement between series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. This validation parameter provides information on random errors. Standard deviation, relative standard deviation (RSD %) or coefficient of variation (CV) are used to express the precision (Hubert et al., 2007a).

6. Accuracy

The accuracy of an analytical method refers to the closeness of agreement between the test result and the value accepted either as the reference value or conventional true value. Actually, this closeness of agreement results from the total error related to test result, i.e. random and systematic errors. Therefore, the accuracy expresses the sum of precision and trueness of an analytical procedure

(Rozet et al., 2007; Kratzsch, Peters, Kraemer, Weber & Maurer, 2002).

7. Limits of detection and quantification

The limit of detection of an analytical procedure is the lowest amount of analyte in a sample that can be detected. Low and upper limits of quantification (LLOQ and ULOQ) are respectively the lowest quantity and the highest quantity of analyte in the sample that can accurately be quantitatively determined. Among other approaches used to determine the LOD and LLOQ there is the signal-to-noise (S/N) ratio approach. According to the International Conference on the Harmonization, the S/N ratios of 3:1 and 10:1 are considered respectively for the LOD and LLOQ (Rozet et al., 2007).

Validation process

Validation process was carried out according to the general guidelines for validation of analytical methods (Rozet et al., 2007; Hubert et al., 2007a).

Calibration standard samples were prepared in duplicates for three consecutive days at six levels of concentration within a range covering molecule therapeutic windows to evaluate the response function relationship of the method. Calibration curves were obtained by plotting ratios of analyte peak area over internal standard peak area versus the analyte concentrations in spiked samples.

In line with the above mentioned guidelines, three levels of concentration covering therapeutic windows (Table 1) for various molecules have been prepared in triplicates on three consecutive days to demonstrate the linearity, precision, trueness, measurement uncertainty and accuracy of the method. Results were processed according to the total error concept with the Enoval V3.0 software (Arlenda, 2011).

The limits of detection (LOD) and quantification (LOQ) were defined as the lowest concentrations of analyte in a sample that can be detected and quantified. The LOD and LOQ were determined on the basis of signal-to-noise ratios (S/N) of 3:1 and 10:1 respectively (Rozet et al., 2007).

Table 1. Concentration ranges used in preparation of calibration and validation standards

Drugs	Reference values (ng/mL)	Calibration Standards (ng/mL)	Validation standards (ng/mL)
Alprazolam	10 - 50	10 – 400	40 - 300
Amitriptyline	50 - 200	20 – 800	60 - 600
Bromazepam	80 - 170	50 – 2000	150 - 1250
Carbamazepine	6000 - 12000	1000 - 50000	2500 – 50000
Chlorpromazine	30 - 300	20 – 1000	80 – 800
Citalopram	20 - 200	20 – 1000	50 – 1000
Clomipramine	100 - 250	50 – 1200	120 – 1000
Clonazepam	20 - 80	10 – 500	40 – 400
Diazepam	125 - 1500	100 - 4000	400 – 3000
Droperidol	5 - 50	25 – 600	60 – 500
Fluoxetine	100 - 450	50 – 2000	150 – 1500
Flupentixol	1 - 15	10 - 500	25 – 500
Haloperidol	5 - 17	5 – 200	15 – 150
Imipramine	45 - 250	20 – 800	60 – 600
Levomepromazine	15 - 60	10 – 500	40 – 400
Lorazepam	20 -250	20 – 1000	80 – 800
Midazolam	80 - 250	50 – 2000	200 – 1500
Nordiazepam	200 - 1800	100 – 4000	300 – 2500
Olanzapine	20 - 80	10 – 400	30 – 300
Phenobarbital	15000 - 40000	5000 - 200000	15000 - 150000
Phenytoin	10000 - 20000	2000 - 80000	6000 – 50000
Pipamperone	100 - 400	100 – 4000	400 – 3000
Risperidone	20 - 60	10 – 500	40 – 400
Sulpiride	200 - 1000	100 – 5000	400 – 4000
Thiopental	1000 - 5000	500 - 20000	1500 – 12500
Zolpidem	80 - 300	50 – 2000	150 – 1250
Zuclopenthixol	4 - 50	10 – 500	25 – 500

Reference values stand for therapeutic windows of various molecules, calibration standards are points (prepared concentration levels) of calibration curves, while validation standards stand for concentration levels used to determine various validation parameters.

Results

Response function

Calibration standards prepared in duplicates at six levels of concentration (Table 1) on three consecutive days were used to assess this criterion. A linear response function was obtained and generated calibration curves had at least 0.99 as coefficient of determination (R^2). These curves were used to determine analyte concentrations in validation standards.

Selectivity

Retention times and UV spectra (Fig. 2) were parameters used to assess the selectivity of detection of the method. As exhibited by the chromatograms (Fig. 1), the method allows simultaneous separation of several molecules and peaks with good resolution were obtained. However, a simulations separation of molecules with relatively same retention times was not easy to get and this was the case for carbamazepine, imipramine and levomepromazine; alprazolam and lorazepam; chlorpromazine, fluoxetine and zuclopenthixol; clonazepam and flupentixol.

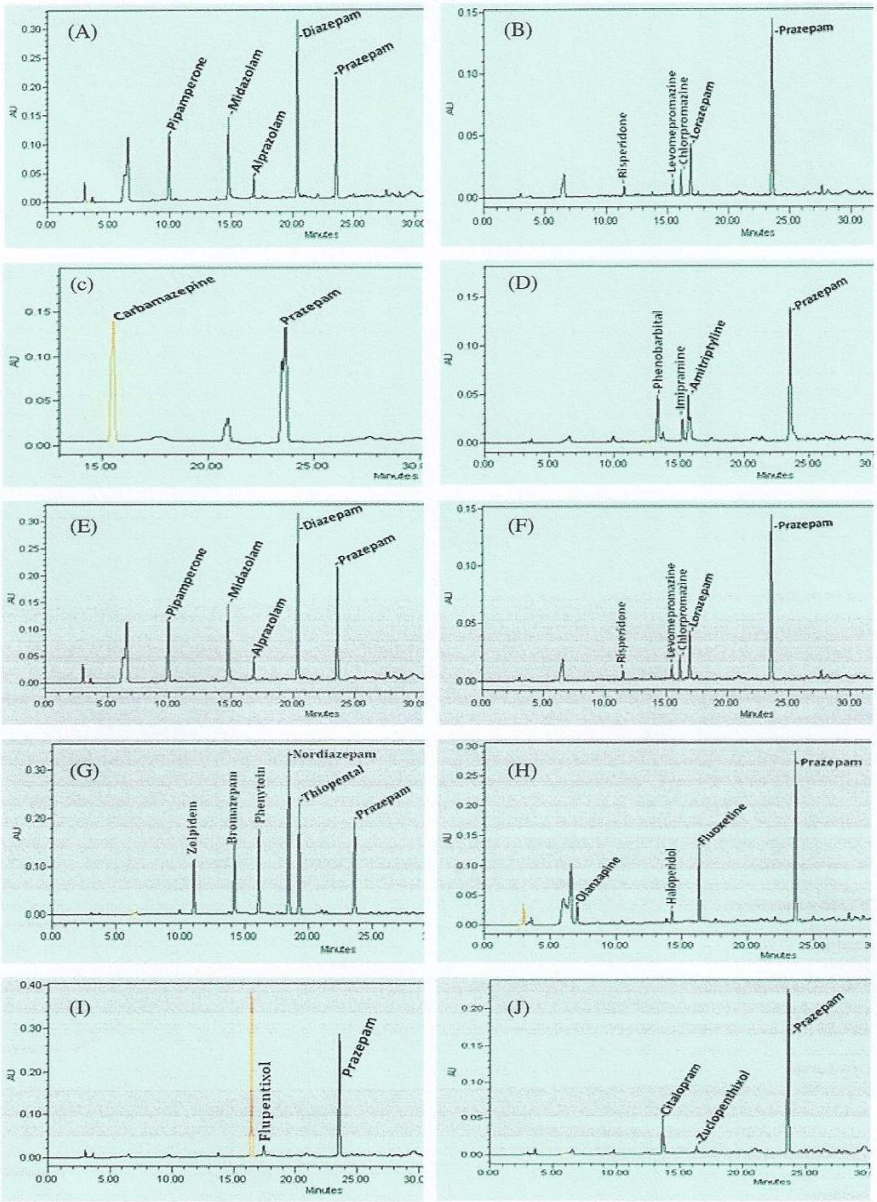

Figure 1. HPLC chromatograms for various molecules

Figure 1 shows HPLC chromatograms obtained with serum containing droperidol 150 ng/mL, clomipramine 300 ng/mL, thiopental 5000 ng/mL (A), sulpiride 1000 ng/mL, phenytoin 20000 ng/mL, clonazepam 100 ng/mL (B), carbamazepine 10000 ng/mL (C), phenobarbital 50000 ng/mL , imipramine 200 ng/mL, amitriptyline 200 ng/mL (D), pipamperone 1000 ng/mL, midazolam 500 ng/mL, alprazolam 100 ng/mL, diazepam 1000 ng/mL (E), risperidone 100 ng/mL, levomepromazine 100 ng/mL, chlorpromazine 200 ng/mL, lorazepam 200 ng/mL (F), zolpidem 500 ng/mL, bromazepam 500 ng/mL, phenytoin 20 ng/mL, nordizepam 1000 ng/mL, thiopental 5000 ng/mL (G), olanzapine 100 ng/mL, haloperidol 50 ng/mL, fluoxetine 500 ng/mL (H), flupentixol 100 ng/mL (I), citalopram 200 ng/mL, and Zuclopenthixol 100 ng/mL(J)

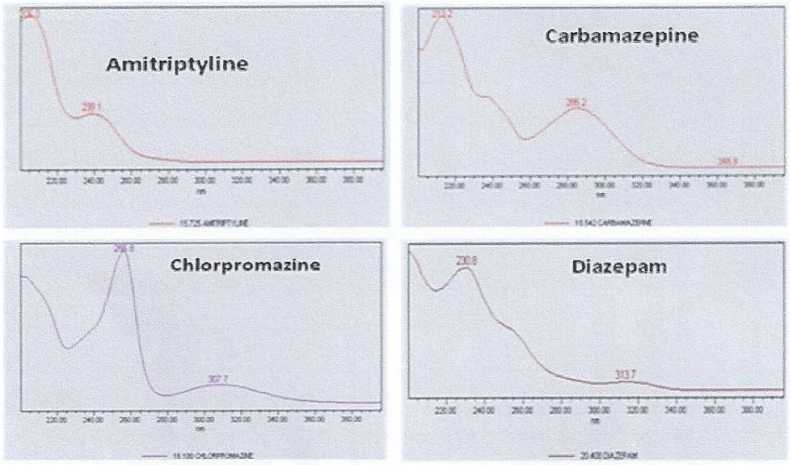

Figure 2. UV-visible spectra of 4 molecules taken as examples between 200 and 400 nm

UV-visible spectra of analytes in the sample were compared to those registered in the library of the method to confirm the real presence of the analyte. A wavelength of maximum absorbance was automatically selected for each molecule (Figure 2).

Linearity

The validated analytical procedure showed a good linearity within tested dosing intervals (Table 1) with determination coefficients of not less than 0.99 for all molecules, i.e. it allowed us to obtain results directly proportional to concentrations of analyte in analyzed samples.

Trueness

The trueness parameter was assessed by calculating the relative bias and the recovery. Calculations were performed using Enoval software and the results are presented in table 2. For all tested drugs the results for relative bias were systematically inferior to 15 %.

Precision

Both intra-assay (repeatability) and inter-assay (intermediate precision) have been assessed during the validation process and obtained results for the relative standard deviation (RSD) are presented in table 2. Thirteen percent was the maximum value for the RSD when we consider both repeatability and intermediate precision.

Accuracy

To assess this validation parameter for the present analytical method, an accuracy profile generated by Enoval software has been used. We set acceptance limits at ± 30% and the risk of having future measurements outside acceptance limits has been set at 17.5%. Figure 3 presents accuracy profiles obtained for various molecules.

Limits of detection and quantification

On one side the signal to noise (S/N) approach was used to determine the LOD and LLOQ and the S/N ratios of 3:1 and 10:1 were considered respectively for the LOD and LLOQ. On the other side, the intersection of the accuracy profile and acceptance limits was considered to determine the upper limits of quantification of the method. Results for both LOD and LOQ are presented in Table 3.

Table 2. Precision and trueness assessment

Analytes	Nominal [ ] (ng/mL)	A		B		Analytes	Nominal [ ] (ng/mL)	A		B	
		A1	A2	B1	B2			A1	A2	B1	B2
Alprazolam	40.00	8.25	10.81	2.50	102.50	Levomepromazine	40.0	5.32	10.47	4.73	104.70
	150.0	4.30	7.99	-0.96	99.04		150.0	3.51	8.50	4.59	104.60
	300.0	3.67	6.87	-4.44	95.56		400.0	1.49	7.51	7.33	107.30
Amitriptyline	60.00	3.00	4.05	4.02	104.00	Lorazepam	80.00	2.98	6.90	-0.6	99.44
	300.0	3.17	4.08	6.94	106.90		300.0	2.52	8.13	-0.30	99.70
	600.0	2.00	2.37	4.06	104.10		800.0	3.88	6.90	0.06	100.10
Bromazepam	150.0	0.92	2.83	-3.78	96.22	Midazolam	200.0	3.08	5.86	-3.06	96.94
	375.0	1.21	2.26	-1.51	98.49		750.0	3.28	6.95	-3.57	96.43
	1250	1.08	2.27	-1.18	98.82		1500	3.90	5.55	-3.06	96.94
Carbamazepine	2500	1.95	5.16	0.58	100.60	Nordiazepam	300.0	0.52	3.97	-1.30	98.70
	20000	3.50	4.70	0.20	100.20		750.0	1.32	2.74	-0.07	99.93
	50000	1.14	4.38	0.44	100.40		2500	0.65	2.32	-1.60	98.40
Chlorpromazine	80.00	1.15	1.72	0.53	100.50	Olanzapine	30.00	9.75	10.27	-7.27	92.73
	300.0	2.65	6.68	6.56	106.60		80.00	6.10	13.21	-1.05	98.95
	800.0	2.60	4.19	4.66	104.70		300.0	9.52	9.52	-4.20	95.80
Citalopram	50.00	2.36	3.06	-0.81	99.19	Phenobarbital	15000	5.83	7.43	2.30	102.30
	400.0	1.94	4.81	6.45	106.50		75000	3.26	3.57	0.73	100.70
	1000	0.85	1.65	1.47	101.50		150000	3.54	5.12	1.27	101.30
Clomipramine	120.0	2.64	3.44	12.90	112.90	Phenytoin	6000	2.51	6.72	11.06	111.10
	400.0	3.48	5.28	3.53	103.50		15000	1.81	4.52	12.84	112.80
	1000	3.38	7.66	-0.54	99.46		50000	2.27	4.49	6.16	106.20
Clonazepam	40.00	2.36	6.14	-1.67	98.33	Pipamperone	400.0	3.77	6.98	-0.81	99.19
	150.0	6.42	7.40	-3.26	96.74		1500	5.13	9.05	-2.79	97.21
	400.0	5.73	7.86	-0.44	99.56		3000	4.27	8.62	-3.79	96.21
Diazepam	400.0	4.76	7.20	0.92	100.90	Risperidone	40.00	4.35	4.35	3.58	103.60
	1500	4.96	8.76	-2.16	97.84		150.0	5.20	8.77	9.27	109.30
	3000	4.63	7.37	-4.03	95.97		400.0	3.90	7.13	7.57	107.60
Droperidol	60.00	3.35	4.49	2.94	102.90	Sulpiride	400	3.16	3.83	5.25	105.30
	200.0	4.25	5.52	5.05	105.10		1500	2.58	2.85	-1.18	98.82
	500.0	1.54	4.95	5.52	105.50		4000	2.99	2.99	0.10	100.10
Fluoxetine	150.0	4.76	6.89	-2.63	97.37	Thiopental	1.500	1.63	3.30	-3.26	96.74
	400.0	1.79	6.11	6.67	106.70		3.750	1.28	2.67	-1.63	98.37
	1500	5.44	7.30	5.90	105.90		12.50	1.92	3.66	-2.21	97.79
Flupentixol	25.00	3.15	4.18	3.84	103.80	Zolpidem	150.0	0.63	3.59	-2.37	97.63
	200.0	4.08	4.08	4.69	104.70		375.0	1.25	2.78	-1.22	98.79
	500.0	2.23	3.12	-0.55	99.45		1250	0.65	2.63	-1.54	98.46
Haloperidol	15.00	8.41	10.20	-6.64	93.36	Zuclopenthixol	25.00	6.25	6.40	1.90	101.90
	40.00	2.72	6.06	-1.02	98.98		200.0	2.82	3.20	4.85	104.80
	150.0	6.25	6.25	-4.09	95.91		500.0	0.91	1.10	-1.37	98.63
Imipramine	60.00	3.05	3.05	-6.20	93.80						
	300.0	2.04	3.15	1.33	101.30						
	600.0	2.67	3.23	-0.51	99.49						

A = Precision, A1 = Repeatability (RSD %), A2 = Intermediate precision (RSD %), B = Trueness, B1 = Relative bias (%), B2 = Recovery (%).

Table 3. Results for limits of quantification and detection of the method against molecule therapeutic windows

Molecules	Therapeutic windows (ng/mL)	LOD (ng/mL)	LLOQ - ULOQ (ng/mL)
Alprazolam	10 - 50	3.0	9 - 300
Amitriptyline	50 - 200	4.8	16 - 600
Bromazepam	80 - 170	2.3	8 - 1250
Carbamazepine	6000 - 12000	20.5	68 - 50000
Chlorpromazine	30 – 300	1.2	4 - 800
Citalopram	20 - 200	3.5	11 - 1000
Clomipramine	100 - 250	11.8	39 - 1000
Clonazepam	20 - 80	3.8	13 - 400
Diazepam	125 - 1500	6.2	20 - 3000
Droperidol	5 – 50	1.8	5 - 500
Fluoxetine	100 - 450	6.7	22 - 1500
Flupentixol	1 - 15	3.6	12 - 500
Haloperidol	5 - 17	2.9	10 - 150
Imipramine	45 - 250	9.0	30 - 600
Levomepromazine	15 – 60	3.6	12 - 400
Lorazepam	20 -250	2.0	6 - 800
Midazolam	80 - 250	3.0	10 - 1500
Nordiazepam	200 - 1800	5.0	16 - 2500
Olanzapine	20 - 80	3.7	12 - 300
Phenobarbital	15000 - 40000	437.0	1459 - 150000
Phenytoin	10000 - 20000	21.0	70 - 50000
Pipamperone	100 – 400	8.8	30 - 3000
Risperidone	20 – 60	5.4	18 - 400
Sulpiride	200 - 1000	11.0	37 - 4000
Thiopental	1000 - 5000	3.5	12 - 12500
Zolpidem	80 - 300	1.8	6 - 1250
Zuclopenthixol	4 - 50	2.9	10 - 500

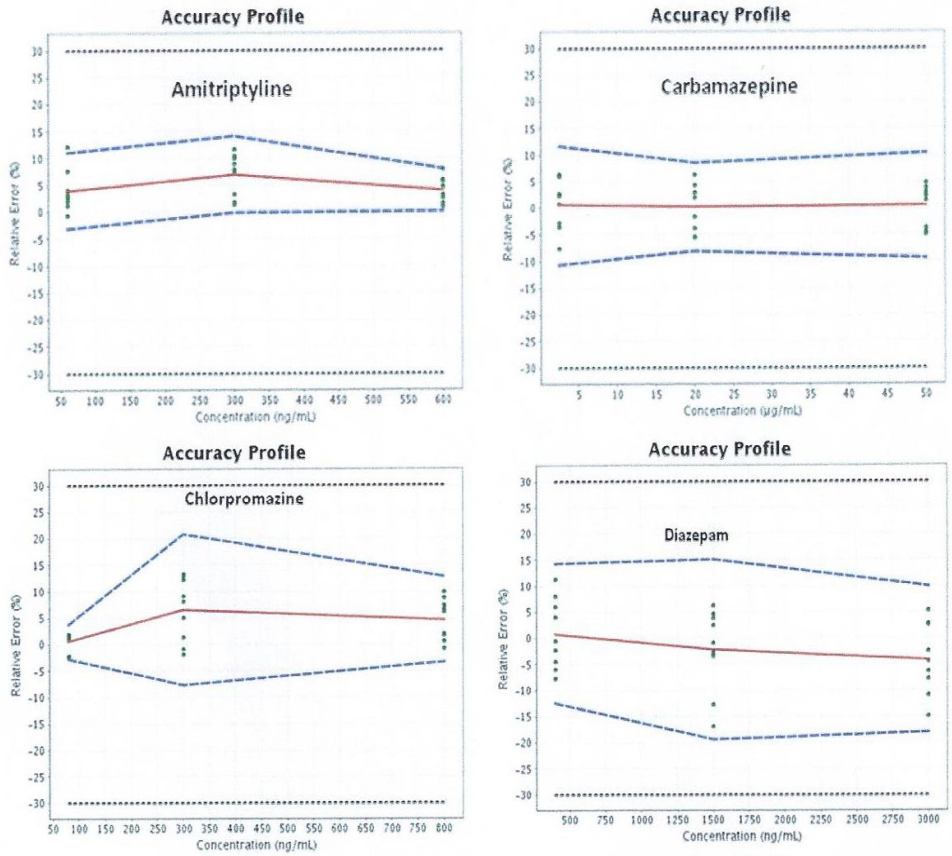

Figure 3. Accuracy profiles for 4 molecules taken as examples

The plain mid line stands for the relative bias, the dashed lines correspond to the β-expectation tolerance limits and the dotted lines represent the acceptance limits. The dots represent the relative error of the back-calculated concentrations and are plotted with respect to their targeted concentrations.

Discussion

According to various regulatory documents for validation of analytical methods including harmonized strategies for validation of quantitative analytical procedures, response function, linearity, selectivity, trueness, precision, accuracy and limits of quantification are validation parameters, commonly verified during the validation process (Hubert et al., 2007_a; Hubert et al., 2007_b). Requirements to be met have been set for a method to be valid. As far as precision is concerned, according to the FDA, among other requirements for a bioanalytical method to be considered as valid, the RSD % should not exceed 15 % except for LLOQ (Rozet et al., 2007; Hubert et al., 2007_a). When we consider both repeatability and intermediate precision for all molecules at all tested concentration levels, the maximum value of the relative standard deviation that we found was 13%. . Despite differences in decision rules observed in various regulatory documents, the accuracy of the method remains so far the main criterion commonly used in deciding the validity of analytical procedures (Rozet et al., 2007; Hubert et al., 2008). According to the validation process used here, the method is considered as valid within the range where the accuracy profile is within acceptance limits. Therefore, the validity of the validated analytical procedure was demonstrated within tested dosing intervals for all molecules. As far as limits of quantification are concerned, both low and upper limits of therapeutic windows of all tested drugs were covered except for haloperidol, flupentixol and zuclopenthixol where the low limits were not covered.

Conclusion

Monitoring plasma concentration levels of psychotropic drugs remains a useful tool for the optimisation of treatment and confirmation of toxicity for these drugs. The aim of this study was to validate an analytical method that could be used in such activities for psychotropic drugs commonly prescribed in Rwanda. A simple and accurate HPLC method allowing simultaneous determination

of several molecules and applicable in routine activities of clinical laboratories, has been successfully validated. Except for haloperidol, flupentixol and zuclopenthixol where it is only applicable for the confirmation of intoxication, the method is suitable for both therapeutic drug monitoring and confirmation of drug poisoning.

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Declaration of interest

The authors report no declarations of interests.

References

Addington, D. (2009). Best practices: improving quality of care for patients with first-episode psychosis. *Psychiatr Serv, 60*, 1164–1166.

Adli, M., Baethge, C., Heinz, A., Langlitz, N. & Bauer, M. (2005). Is dose escalation of antidepressants a rational strategy after a medium-dose treatment has failed? *Eur Arch Psychiatry Clin Neurosci, 55*, 387–400.

Arlenda Home Page, enoval Version V3.0a PROD, Last update: June 30, 2011. Accessed from https://www.arlenda.com.

Bates, D.W. & Gawande, A.A. (2003). Improving safety with information technology. *N Engl J Med, 348*, 2526–2534.

Baumann, P., Hiemke, C., Ulrich, S., Eckermann, G., Gaertern, I., Gerlach, M., Zernig, G. (2004). The AGNP-TDM expert group consensus guidelines: therapeutic drug monitoring in psychiatry. *Pharmacopsychiatry, 37*, 243–265.

Bengtsson, F. (2004). Therapeutic drug monitoring of psychotropic drugs. TDM "nouveau". *Ther Drug Monit, 26*, 145–151.

Brosen, K. (1996). Drug-metabolizing enzymes and therapeutic drug monitoring in psychiatry. *Ther Drug Monit, 18*, 39– 396.

Bugamelli, F.C., Sabbioni, R., Mandrioli, E., Kenndler, F., Albani, M.A. & Raggi, M.A. (2002). Simultaneous analysis of six antiepileptic drugs and two selected metabolites in human plasma by liquid chromatography after solid-phase extraction. *Anal.Chim. Acta, 472*, 1-10.

Cutroneo, P., Beljean M., Tan Luu, P.R. & Siouffi, A.M. (2006). Optimization of the separation of some psychotropic drugs and their respective metabolites by liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis 41*, 333–340.

Hiemke, C. (2008). Clinical utility of drug measurement and pharmacokinetics therapeutic drug monitoring in psychiatry. *Eur J Clin Pharmacol, 64*, 159–166.

Hiemke, C. (2008). Therapeutic drug monitoring in neuropharmacology: does it hold its promises? *Eur Arch Psychiatry Clin Neurosci, 258*, (Suppl 1), 21–27.

Hiemke, C., Baumann, P., Bergemann, N., Conca, A., Dietmaier, O., Egberts, K., Zernig, G. (2011). AGNP Consensus Guidelines for Therapeutic Drug Monitoring in Psychiatry: Update 2011. *Pharmacopsychiatry, 44*, 195–235.

Hubert, P., Nguyen-Huu, J.J., Boulanger, B., Chapuzet, E., Cohen, N., Compagnon, P.A., Rozet E. (2007) Harmonization of strategies for validation of quantitative analytical procedures. A SFSTP proposal-Part III. *Journal of Pharmaceutical and Biomedical Analysis, 45*, 82-96.

Hubert, P., Nguyen-Huu, J.J., Boulanger, B., Chapuzet, E., Cohen, N., Compagnon, P.A., Rozet, E.(2008) Harmonization of strategies for validation of quantitative analytical procedures. A SFSTP proposal-Part IV. *Journal of Pharmaceutical and Biomedical Analysis, 48*, 760-771.

Hubert, P., Nguyen-Huu, J.J., Boulanger, B., Chapuzet. E., Chiap, P., Cohen, N., Rozet, E. (2007). Harmonization of strategies for validation of quantitative analytical procedures. A SFSTP proposal-Part II. *Journal of pharmaceutical and Biomedical Analysis, 45*, 70-81.

Jeffrey, A., Lieberman, J.A., Stroup, S.T., McEvoy, J.P., Swartz, M.S., Rosenheck, R.A., Hsiao J.K. (2005). Effectiveness of Antipsychotic Drugs in Patients with Chronic Schizophrenia. *N Engl J Med, 353*, 1209–1223.

Klotz, U. (2009). Pharmacokinetics and drug metabolism in the elderly. *Drug Metabolism Reviews, 41* (2): 67–76.

Kratzsch, C., Peters, F.T., Kraemer, T., Weber, A. & Maurer H. (2002). Screening, library-assisted identification and validated quantification of fifteen neuroleptics and three of their metabolites in plasma by liquid chromatography/ mass spectrometry with atmospheric pressure ionization. *J. Mass Spectrum., 38*, 283-295.

Malhotra, A.K., Murphy, G.M., Kennedy J.L. (2004). Pharmacogenetics of psychotropic drug response. *Am. J. Psychiatry, 161*, 780–796.

Müller, M. J., Dragicevic, A., Fric, M., Gaertner, I., Grasmäder, K., Härtter, S., ... Hiemke, C. (2003). Therapeutic drug monitoring of tricyclic antidepressants: how does it work under clinical conditions? *Pharmacopsychiatry, 36*, 98–104.

Preskorn, S.H. &Fast, G.A. (1991). Therapeutic drug monitoring for antidepressants: efficacy, safety, and cost effectiveness . *J Clin Psychiatry, 52* (Suppl), 23–33.

Raggi, M.A. (2002). Therapeutic drug monitoring: chemical–clinical correlations of atypical antipsychotic drugs. *Curr. Med. Chem., 9*, 1397–1409.

Rozet, E., Ceccato, A., Hubert, C., Ziemons, E., Oprean, R., Rudaz, S., Hubert, P. (2007). Analysis of recent pharmaceutical regulatory documents on analytical method validation. *Journal of Chromatography A, 1158*, 111-125.

Sanchez de la Torre, C., Martinez, M.A. & Almarza, E. (2005). Determination of several psychiatric drugs in whole blood using capillary gas–liquid chromatography with nitrogen phosphorus detection: comparison of two solid phase extraction procedures. *Forensic Science International, 155*, 193–204.

Sheng, J., Lei, J., Ju, H., Song, C. & Zhang, D. (2010). Rapid ultraviolet monitoring of multiple psychotropic drugs with a renewable microfluidic device. *Analytica Chimica Acta, 679*: 1–6.

Smink, B.E., Brandsma, J.E., Dijkhuizen, A., Lusthof, K. J., Gier, J.J., Egberts, A.C.G. & Uges, D.R.A. (2004). Quantitative analysis of 33 benzodiazepines, metabolites

and benzodiazepine-like substances in whole blood by liquid chromatography–(tandem) mass spectrometry. *Journal of Chromatography B, 811*, 13–20.

Touw, D.J., Neef, C., Thomson, A.H. & Vinks, A.A. (2005). Cost-effectiveness of therapeutic drug monitoring: a systematic review. *Ther Drug Monit, 27*, 10–17.

Trivedi, M.H., Rush, A.J., Bradley, N.G., Stewart J.W., Wisniewski S.R., Warden, D., Howland R. (2005). Maximizing the adequacy of medication treatment in controlled trials and clinical practice: STAR(*)D measurement-based care. *Neuropsychopharmacol, 32*, 2479–2489.

Vecchione, G., Casetta, B., Chiapparino, A., Bertolino, A., Tomaiuolo, M., Cappucci, F., Grandone E. (2012). A reliable and rapid tool for plasma quantification of 18 psychotropic drugs by ESI tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis, 67*(68), 104– 113.

Wang, Q.L., Fan, L.Y., Zhang, W. & Cao, C.X. (2006). Sensitive analysis of two barbiturates in human urine by capillary electrophoresis with sample stacking induced by moving reaction boundary. *Anal. Chim. Acta, 580*, 200–205.

Wilhelm, M., Battista, H.J. & Obendorf, D. (2000). Selective and sensitive assay for the determination of benzodiazepines by high-performance liquid chromatography with simultaneous ultraviolet and reductive electrochemical detection at the hanging mercury drop electrode. *J. Chromatogr. A, 897*, 215–225.

Zhang, J., Heineman, R. & Halsall, H.B. (1999). Capillary electrochemical enzyme immunoassay (CEEI) for phenobarbital in serum. *J. Pharmaceut. Biomed., 19*, 145–152.

CHAPTER III. SERUM CONCENTRATION LEVELS OF PSYCHOTROPIC DRUGS IN RWANDAN PATIENTS

After its validation, the analytical method was applied to determine serum concentration levels of psychotropic drugs in Rwandan patients, with the purpose of identifying eventual problems associated with the lack of therapeutic drug monitoring of these drugs in Rwanda.

III.1 TDM and blood concentration levels in psychopharmacotherapy

Various studies have shown the importance of the measurement of blood concentration levels (TDM) in the optimisation of psychotropic treatment (1, 13, 20, 21). This practice is a requirement when there is a need to individualize drug dose in order to maintain its concentration within a targeted therapeutic range. In fact, patients differ in their ability to absorb, distribute, metabolise and eliminate drugs due to various factors including genetic peculiarities, concurrent disease, age, concomitant medications, etc (1, 13). One should not rely only on conventional drug doses as a great interindividual variability is observed in psychopharmacotherapy. It has also been demonstrated that even when the recommended dose is thoroughly maintained, interindividual variability of pharmacokinetic parameters of a drug may be responsible for an under- or overdosage in 30-50% of patients treated with psychotropic medications (133).

III.2 TDM and psychotropic medication compliance

The measurement of drug blood concentrations is also important in case of suspicion of medication noncompliance. In fact based on the blood concentration level of a drug and its metabolite (s), one can easily conclude on whether the patient is taking her (his) medication properly or not. Knowing that in some cases psychopharmacotherapy could be a lifelong treatment, monitoring blood concentrations is essential to reduce the risk of treatment discontinuation. The latter could be the result of the lack of therapeutic effect due to drug concentrations below therapeutic range but it may also be the result of severe side effects

associated with drug concentrations beyond therapeutic range. Rwandan patients under psychotropic treatment include both inpatients and outpatients. The latter generally receive their treatment on monthly basis, which need a thorough follow up to assure that they take their treatment as required. When there is no improvement of patient clinical state regardless of proper prescription (right dose), medication noncompliance is among suspected causes and without results of blood concentrations it is not easy to conclude in such a situation.

III.3 Psychopharmacotherapy in Rwandan patients

As shown in the chapter 2, psychotropic medications used in Rwanda include mainly tricyclic antidepressants and first generation antipsychotics both known for their frequent adverse effects and the severity of toxicity in case of overdose. Therefore, these drugs should be thoroughly monitored to reduce the risk of toxicity in patients under treatment. As far as antipsychotics are concerned, one should highlight the predominant use of first generation antipsychotics in Rwandan patients compared to western countries. This could be an explanation of a high frequency of extrapyramidal symptoms observed in Rwandan patients. Typical antipsychotics represented 56% of the total cases and 98% of antipsychotic use. Currently around ten atypical antipsychotics are available on the market: clozapine, risperidone, olanzapine, quetiapine, ziprazidone, aripiprazole, paliperidone, asenapine, iloperidone and long-acting paliperidone with the last two drugs used only in USA and Canada. Among these drugs only risperidone and olanzapine were found in Rwanda.

Knowing that psychotropic drugs are not monitored in Rwanda, measuring blood concentration levels of these drugs in Rwandan patients was interesting as it helped to show the real situation of the country as far as psychopharmacotherapy is concerned. Blood samples were collected from 128 patients treated with psychotropic drugs in three referral hospitals of Rwanda: Kigali University Teaching Hospital (CHUK), King Faisal Hospital (KFH) and

Ndera Neuropsychiatric Hospital (HNP-NDERA). The study population consisted of patients with at least 1 week of treatment prior to blood collection. Both males and females were included in our study with females representing 54% of participants aged from 12 to 68 years, with a mean age of 31years. Details of information about participants are provided in appendix 5. Blood collected from patients were immediately centrifuged to keep serum samples refrigerated. Sample analysis was realised in the Laboratory of Clinical, Forensic, Environmental and Industrial Toxicology of the University Teaching Hospital of Liège (Belgium) and results are presented in the publication 2.

For cases where prescribed dose and patient weight were available, analytical results were compared to plasma concentrations calculated using these parameters to identify possible misdosing cases and results are presented in the appendix 6.

III.4 Psychotropic medication side effects in Rwandan patients

Various side effects have been reported by our patients and in general they were not different from those commonly reported for psychotropic drugs. Among our study population, the most commonly reported side effects were drowsiness (32%), dysarthria (25%), amnesia (23%), asthenia (15%), dizziness (13%), and fatigue (10%). Side effects have been reported in all cases regardless of drug plasma concentration levels. In supratherapeutic cases, reported side effects were not enough to conclude about drug overdoses and the conclusion was made more difficult by the treatment combination observed in most of cases. The table 7 presents various adverse effects observed amongst participants and their respective rates.

III.5 Risk of drug-drug interactions in Rwandan patients under psychotropic treatment

Among the study population, polymedication cases were observed in 91 patients representing 71% of the total population, and at least one drug-drug interaction was predictable in 74% of

these patients. The appendix 7 shows possible drug-drug interactions that were predictable among the study population.

Table 7. Reported adverse effects among the study population

Adverse effects	**Frequency**	**%**
Amnesia	30	23.0
Anorexia	2	1.6
Arm paralysis	6	4.7
Asthenia	19	14.8
Back pain	2	1.6
Difficulty walking	2	1.6
Dizziness	16	12.5
Drowsiness	41	32.0
Dysarthria	32	25.0
Erection disorders	2	1.6
Excessive appetite	2	1.6
Excessive saliva secretion	7	5.5
Eye pain	2	1.6
Fatigue	13	10.2
Headache	6	4.7
Insomnia	3	2.3
Leg paralysis	2	1.6
Neck stiffness	5	3.9
Reasoning disorders	3	2.3
Sedation	2	1.6
Sleeplessness	2	1.6
Stomach-ache	3	2.3
Trembling hands	3	2.3
Visual disorders	2	1.6
Weight gain	6	4.7
Others	13	10.2

PUBLICATION 2

Determination of blood concentration levels of psychotropic medications in Rwandan patients.

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Original Paper

Determination of blood concentration levels of psychotropic medications in Rwandan patients

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Objectives: In Rwanda, no therapeutic monitoring of psychotropic drugs is done. This results in difficult treatment optimisation and exposition to a high risk of toxicity and drug ineffectiveness for patients under treatment. This study aimed to determine blood concentration levels of psychotropic drugs in Rwandan patients and identify problems associated with the lack of therapeutic drug monitoring (TDM) of these drugs.
Methods: The analysis was performed on 1 ml of serum sample using prazepam as internal standard. Regarding the step of sample preparation, we used a liquid–liquid extraction with a mixture of organic solvents: diethyl ether/dichloromethane/hexane/*n*-amyl alcohol (50/30/20/0.5:V/V). A Waters Alliance 2695 was used for analysis. The chromatography was run on a Symmetry C8 column and as mobile phase acetonitrile and phosphate buffer (pH 3.8) were used.
Results: Concerning the results, serum samples from 128 patients were analysed. Twenty-one different psychotropic drugs belonging to various pharmacological classes were detected and quantified. Analytical results were put into three categories based upon therapeutic reference ranges (TRR) of various drugs: subtherapeutic, therapeutic and supratherapeutic. For a total of 237 analyses, results within TRR represented 46% while 47 and 8% of results were, respectively, below and above TRR.
Conclusion: It was therefore concluded that patients under psychotropic treatment in Rwanda are exposed to both the risk of drug ineffectiveness and the risk of toxicity (54%) with only 46% of results within the TRR. Consequently, TDM is needed to optimise psychotropic treatment in Rwandan patients.

Keywords: Psychotropic drugs, Drug ineffectiveness, Drug toxicity, Rwandan patients

Introduction

Psychotropic drugs were introduced in 1950s and now around 130 molecules are available.[1] Even though these drugs are essential and effective for treatment of many psychiatric disorders, for many patients, therapeutic outcomes are still far from satisfactory.[2,3] The large variation in individual susceptibility to psychotropic drug treatment remains a critical problem in the management of serious psychiatric disorders.[4]

During more than five decades, clinical research has been focussed on developing new drugs forgetting the improvement of administration ways of available ones.[5,6] In order to tackle this challenge, now there is a growing evidence that patients may substantially benefit from improving the way the available medications are administered.[7] To adjust the dosage of prescribed medications according to the characteristics of individual patient, therapeutic drug monitoring (TDM) plays a major role.[1] The latter refers to the measurement of drug levels that, with appropriate clinical pharmacological interpretation, will directly affect prescribing procedures.[8,9] It has been used to individualise drug therapy since the early 1970s.[8] The considerable interindividual variability in the pharmacokinetic properties of drugs is the main reason of using TDM for optimisation of psychopharmacotherapy.[1]

Due to genetic peculiarities, concurrent disease, age, concomitant medications, etc., patients differ in their ability to absorb, distribute, metabolise and eliminate drugs to the extent that at the same dose a more than 20-fold

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interindividual variation in the drug's steady state concentration can be observed.[10–14]

In psychopharmacotherapy, it has been demonstrated that incidence of undesirable effects is often dose-related and for at least some psychotropic drugs, the same correlation has been observed for therapeutic effects and plasma levels.[10,15] This obviously demonstrates the importance of TDM use for dose adjustment of psychotropic drugs. Moreover, TDM has the potential to improve the cost-effectiveness of psychopharmacotherapy.[8,16]

Pregnant women, children, elderly patients, individuals with intelligence disabilities, patients with known or suspected genetically determined pharmacokinetic abnormalities or individuals with pharmacokinetically relevant comorbidities may predominantly benefit from TDM in psychiatry. However, TDM must be adequately integrated into the clinical treatment process in order to potentially benefit from it.[1]

Rwandan population experienced the atrocities caused by different events and especially the 1994 genocide. Due to these events, many Rwandans remained with sequels including trauma, depression and other psychological harms. Research has shown that over 20% of the Rwandan population has symptoms of post traumatic stress, and 50% of these people live in extreme depression, due to the effects of the genocide.[17]

If some psychological problems directly related to the genocide can be managed using psychological counselling, others require the use of psychotropic drugs. Apart from genocide-related problems, other mental illnesses requiring the use of psychotropic drugs are found among Rwandans as well. To the best of our knowledge, so far in Rwanda, no control of plasma concentration levels is done to optimise the treatment with these drugs and thus reduce the risk of ineffectiveness and toxicity in patients. This prompted us to undertake a study aiming to determine the concentrations of psychotropic drugs in blood of Rwandan patients in order to identify possible problems that could be associated to the lack of TDM activities for these drugs. Blood samples were collected in three referral hospitals of Rwanda: Kigali University Teaching Hospital (CHUK), King Faisal Hospital (KFH) and Ndera Neuropsychiatric Hospital (HNP-NDERA) and then analysed in the Laboratory of Clinical, Forensic, Environmental and Industrial Toxicology of CHU-Liege (Belgium).

Materials and Methods

Study population

The study involved 128 patients under treatment with psychotropic drugs in referral hospitals of Rwanda for at least 1 week prior to blood collection. The females represented 54% of participants aged from 12 to 68 with a mean age of 31 years. The number of psychotropic drugs taken by patient varied between 1 and 4 with an average of two drugs. For a total of 128 participants, 74 (57.8%) were from Ndera Neuropsychiatric Hospital, 53 (41.4%) from Kigali University Teaching Hospital and 1 (0.8%) from King Faisal Hospital. The study was approved by the Rwanda National Ethics Committee (RNEC) with the registration number of No. 349/RNEC/2012, and an informed consent was obtained from each participant or legal guardian.

Blood collection

Blood samples were collected in tubes free of anticoagulant, centrifuged and serum was stored at (20°C before analysis. Blood was collected with respect to TDM guidelines[1,18] and four blood collection tubes equivalent to around 16ml of whole blood were collected once from each participant. Sample transportation was done respecting the cold chain in order to assure sample stability.

Sample preparation and analysis

An HPLC-DAD technique previously validated and used in routine was used for sample analysis.[19,20] The analysis was performed on 1ml of serum sample using prazepam as internal standard. For sample preparation step prior to HPLC analysis, we used a liquid–liquid extraction with a mixture of organic solvents: diethyl ether/dichloromethane/hexane/*n*-amyl alcohol (50/30/20/0.5: V/V). A Waters Alliance 2695 was used for analysis. The chromatography was run on a Symmetry C8 column and as mobile phase acetonitrile and phosphate buffer (pH 3.8) were used. For results validation, quality control samples were used.

Chemicals and reagents

Compounds used as reference standards were purchased from various suppliers. Amitriptyline, carbamazepine and clomipramine have been obtained from LGC GmbH (Luckenwalde, Germany), clonazepam and midazolam from Roche (Brussels, Belgium), citalopram, flupentixol and zuclopenthixol from Lundbeck (Brussels, Belgium), chlorpromazine, diazepam, fluoxetine, olanzapine, phenobarbital and zolpidem from Cerilliant (Texas, USA), haloperidol, pipamperone and risperidone from Janssen-Cilag (Antwerp, Belgium), levomepromazine and sulpiride from Sanofi-Aventis (Diegem, Belgium), phenytoin from Kela Pharma (Sint Niklaas, Belgium) and thiopental from Inresa (Freiburg, Germany). Prazepam was purchased from Certa (Braine-l'Alleud, Belgium). Sodium carbonate and sodium dihydrogenophosphate were, respectively, purchased from Merck (Darmstadt, Germany) and J. T. Baker (Deventer, the Netherlands). Acetonitrile was purchased from Lab Scan (Dublin, Ireland), *n*-amyl alcohol and dichloromethane from J. T. Baker (Deventer, the Netherlands), *n*-hexane and methanol from Lab Scan (Sowinskeigo, Poland), diethyl ether and acetonitrile supra gradient from Biosolve (Valkenswaard, the Netherlands). All organic solvents

were certified for HPLC use. Blank human serum was purchased from CHU-Liege blood bank.

Chromatographic conditions

The used chromatographic system consisted of a Waters Alliance 2695 Separations Module (Zellik, Belgium), equipped with a quaternary, low-pressure mixing pump, a degassing line and a thermostated autosampler and coupled to a 2996 photodiode array detector from Waters too. The HPLC instrument was piloted by Empower® software (Waters), which was used also for data processing (area integration, calculation and plotting of chromatograms). A Symmetry® C8 analytical column (4.6×250mm) packed with 5µm diameter particles (Waters), together with a guard column (4.6×20mm) packed with identical material were used for separation performed at 30°C. An injection volume of 40µl, a carousel temperature of 25°C and a run time of 45min were fixed. The mobile phase consisted of acetonitrile and sodium dihydrogenophosphate buffer (pH 3.8) used in gradient elution mode. UV–visible spectra were recorded in the range of 200–400nm.

Statistical analysis

For data comparison, Chi-square tests were used and the *P*-values were considered to conclude about the significance of differences. We used a confidence level of 95% with a significance level (alpha) of 0.05. Thus, the difference between compared data was considered as significant where the *P*-value was <0.05.

Results

Overall results for sample analysis

Twenty-one different molecules belonging to various pharmacological classes were detected and quantified with a total of 237 analyses. Analytical results obtained for the study population are presented in Tables 1–4. Results have been presented into results drug by drug and results by pharmacological classes and both classified into three categories: subtherapeutic, therapeutic and supratherapeutic, respectively, for results below, within and above therapeutic reference range (TRR). For TRRs, we used values found in the literature.[1,3,21] Tables 1 and two show, respectively, results of analysis drug by drug and by pharmacological classes.

Table 1 Overall results drug by drug

Drugs	Therapeutic windows (ng/ml)	Total analyses	Subtherapeutic results	Therapeutic results	Supratherapeutic results
Amitriptyline	80–200	11	8	3	0
Carbamazepine	4000–12000	44	6	35	3
Chlorpromazine	30–300	27	23	4	0
Citalopram	50–110	7	2	3	2
Clomipramine	175–450	4	3	0	0
Clonazepam	20–70	1	0	1	0
Diazepam	125–1500	3	2	1	0
Fluoxetine	120–500	4	2	1	1
Flupentixol	1–10	11	7	4	0
Haloperidol	1–10	54	22	27	5
Levomepromazine	30–160	36	16	17	3
Midazolam	80–250	1	1	0	0
Olanzapine	20–80	2	1	1	0
Phenobarbital	10000–40000	10	3	5	2
Phenytoin	10000–20000	6	5	1	0
Pipamperone	100–400	1	0	1	0
Risperidone	20–60	1	1	0	0
Sulpiride	200–1000	2	2	0	0
Thiopental	1000–5000	4	0	3	1
Zolpidem	80–150	7	7	0	0
Zuclopenthixol	4–50	1	0	0	1
	Total	237	111	108	18
	Percentage	100	46.8	45.6	7.6

Table 2 Overall results by pharmacological classes

	Subtherapeutic results		Therapeutic results		Supratherapeutic results		Total	
Pharmacological classes	*n*	%	*n*	%	*n*	%	*n*	%
Antidepressants	15	57.7	8	30.8	3	11.5	26	100
Antiepileptics	11	22.0	36	72.0	3	6.0	50	100
Antipsychotics	72	53.3	54	40.0	9	6.7	135	100
Barbiturates	3	21.4	8	57.2	3	21.4	14	100
Benzodiazepines	10	83.3	2	16.7	0	0	12	100
Total	111	46.8	108	45.6	18	7.6	237	100

Table 3 Results by number of drugs per patient

Number of psychotropic drugs per patients	Subtherapeutic cases		Therapeutic cases		Supraherapeutic cases		Total	
	n	%	*n*	%	*n*	%	*n*	%
1	20	40.0	27	54.0	3	6.0	50	100
2	46	46.9	41	41.8	11	11.2	98	100
3	39	48.1	38	46.9	4	4.9	81	100
4	6	75.0	2	25	0	0	8	100
Total	111	46.8	108	45.6	18	7.6	237	100

Table 4 Comparison of results for two main sample collection sites

Pharmacological classes	Total results		Subtherapeutic results		Therapeutic results		Supratherapeutic results	
	CHUK	NDERA	CHUK (%)	NDERA (%)	CHUK (%)	NDERA (%)	CHUK (%)	NDERA (%)
Antidepressants	18	9	55	78	39	0	6	22
Antiepileptics	24	28	33	14	67	75	0	11
Antipsychotics	15	119	80	49	20	44	0	7
Barbiturates	12	1	8	100	67	0	25	0
Benzodiazepines	2	9	50	89	50	11	0	0
Overall	71	166	45	47	49	45	6	8

For a total of 237 analyses, only 108 results representing 46% were found within TRR, while 47 and 8% of total results were, respectively, below and above TRR.

Antidepressants, antiepileptics, antipsychotics, barbiturates and benzodiazepines were pharmacological classes of psychotropic drugs found in analysed samples. Antipsychotics also known as neuroleptics were the most represented class with 57% of total analyses, while benzodiazepines were the least represented with only 5% of total analyses. Though unexpected concentrations were observed in all classes, statistical tests performed on results showed a significant difference in results from various pharmacological classes ($P=0.0001$). Antiepileptics were found to be more adjusted and this can partially be explained by the fact that this class included phenytoin and carbamazepine both with relatively large TRRs. Overall results were also presented by number of psychotropic drugs per patient (Table 3). Even though a relatively high rate of therapeutic results were observed in case of monotherapy, a statistical analysis of results showed no significant difference in analytical results as far as the number of drugs per patient is concerned ($P=0.32$).

Comparison of results from the two main sites of blood collection

On one side, we had Ndera Neuropsychiatric Hospital (HNP-NDERA), a specialised hospital in mental illness treatment in Rwanda, representing 58% of participants and 70% of total analyses. In this hospital, our participants were mainly inpatients. On the other side, there was Kigali University Teaching Hospital (CHUK), one of the four referral hospitals of Rwanda with a mental health department. This hospital represented 41% of our participants with 30% of total results. The participants from this hospital were mainly outpatients. The results showed that among participants from HNP-NDERA, only 45% of results were within TRR. Results below and above therapeutic range represented, respectively, 47 and 8% of total results. The class of antipsychotics was the most represented with 72% of total analyses. The risk of toxicity was high in antidepressants (22%) while a high rate of results within TRR was observed with antiepileptics (75%). Among participants from CHUK, the results from sample analysis showed 49% of total results within TRR, 45% below TRR and 6% above TRR. Antiepileptics represented 34% of total analyses with only about 3% for benzodiazepines. A high risk of drug ineffectiveness was observed with antipsychotics (80%) and the risk of toxicity was high with barbiturates (25%).

The results from the two main sample collection sites were compared to see if there would be a difference in the two hospitals; a referral university teaching hospital with mainly outpatients on one side, and a neuropsychiatric hospital with mainly inpatients on the other side. The results are compiled in Table 4. Though the results from the two hospitals showed big similarities, we observed more therapeutic values in CHUK.

In an attempt to identify the possible reasons of unexpected concentrations, analytical results have been compared to expected plasma concentrations calculated using prescribed doses (Supplementary Material 1). This helped us to identify cases where misdosing, medication noncompliance or other reasons could explain the obtained results.

To calculate the expected plasma concentrations, the following equation has been used $Cp_{SSAv} = \frac{F \times Dose}{CL \times \tau}$.

Cp_{SSav}, F, CL and τ are, respectively, the average plasma concentration at the steady state, drug bioavailability, drug clearance and the dosing interval.[22] Plasma concentration calculations have been made in cases where necessary information including patient weight and doses were available. The application of the above formula was possible for 84 patients with a total of 137 cases. Considering analytical results, 64 (47%) cases were below TRR (TRR), 66 (48%) within TRR and 7 (5%) above TRR while according to calculated concentrations, the results below, within and above TRR represented, respectively, 52 (38%), 70 (51%) and 15 (11%). On one side, among 64 results below TRR, calculation of expected plasma concentrations revealed underdosing problems in 29 cases (45%) while in 35 cases (55%), noncompliance, drug interactions and other reasons could be an explanation to the obtained results. On the other side, overdosing problems have been identified in three of seven cases of results above TRR and other reasons including interindividual variability and drug interactions could explain other cases. In general, among 137 cases where the calculation of plasma concentration was possible, misdosing cases represented 23%.

Discussion

A poor medication compliance in patients under treatment with psychotropic drugs and especially antidepressants was reported.[23–27] This could be the case for participants of the present study where a large group of patients had concentrations below TRR yet treated with a standard dose. However, poor medication compliance is not the only possible reason of getting subtherapeutic concentrations; drug interactions could also lead to the same results, as polymedication was observed in many of our participants (71%). Nevertheless, as far as polymedication is concerned, statistical analysis of results showed no significant association between analytical results and the number of drugs taken by a patient ($P=0.32$). Knowing that there is no systematic quality control for psychotropic drugs used in Rwanda, the use of counterfeit drugs can also be responsible for such findings. Many other reasons including poor absorption from gastrointestinal tract and extensive metabolism for drugs affected by genetic polymorphisms can also lead to lower than expected concentrations. Regardless of their cause, subtherapeutic concentrations lead to drug ineffectiveness or poor clinical response at least for drugs with demonstrated concentration–effect relationship.[1,28]

In the same way, many reasons including poor metabolism and elimination, drug interactions, etc., could explain supratherapeutic concentrations observed in our participants. In psychopharmacotherapy, high blood concentrations of drugs are often associated with severe adverse effects (type A adverse reactions).[1,29] So, patients with concentrations above TRR representing 8% of our participants were exposed to severe side effects and toxicity of various drugs, which could result into medication noncompliance. This is particularly the case for treatment with antidepressants where side effects have been reported to be among the main reasons of treatment discontinuation especially during the first 6weeks of treatment.[24] Among patients under antidepressant treatment in the present study, 11% were found with serum concentrations above TRR.

Among our participants, eight patients were taking concomitantly antiretroviral drugs. Even though obtained results did not allow to conclude on that, drug interactions were possible wherever HIV treatment was specified. In fact, in all these cases, the combinations contained on one hand nevirapine (CYP450 inhibitor) and on the other hand carbamazepine, phenobarbital or phenytoin (CYP450 inducers).

A standard dose especially in oral administration does not always guarantee an effective concentration. For psychotropic drugs, even when the recommended dose is thoroughly maintained, interindividual variability of pharmacokinetic parameters is responsible for an under- or overdosage in 30–50% of patients.[30] Actually, without ignoring other factors that could be involved, this can partially explain the findings of the present study, where with standard doses, only 46% of results were found within TRR. Considering that standard doses and TRRs used were in most of the cases determined in populations predominantly made up of Caucasian people,[31,32] abnormal drug concentrations may also be the result of possible ethnic differences in metabolising psychotropic drugs. For example, the phenotyping of CYP2D6 (enzyme responsible for the metabolism of many of psychotropic agents) has shown differences in expression of this enzyme when Caucasians are compared to Black Africans and Orientals. The frequency of poor metabolizers was found to be lower in the latter two populations (1–2%) compared to Caucasian people (7%).[33] This actually means that as far as CYP2D6 substrates are concerned, with respect to TRRs, relatively higher doses may be required in Rwandan population (black Africans).

Though we did not find a study where serum levels have been determined for all classes of psychotropic drugs, when we consider a study carried out in Germany on antidepressants,[34] the rate of therapeutic serum levels was found to be 44% in patients treated without TDM and higher in TDM group (58%). When we consider antidepressants only, the rate of therapeutic serum levels in our patients was only 31%.

Study limitations

The main limitation of our study was the lack of some clinical information about our patients. Detailed information about diagnoses and treatment outcomes were not

collected while needed to make suitable conclusions about our findings. Actually, this information was not found in patients' files and in most cases, patients were not able to explain correctly about their cases. In most of cases reference products for active metabolites were not available, reported concentrations were only for parent drugs. Another limitation of our study was that reference ranges used could vary depending on various factors including co-medication and indications for drugs with various indications. As information about drug indications was lacking for our patients, it was not easy to make suitable interpretation of obtained results.

Conclusion

In order to optimise psychotropic treatment and thus reduce the risk of medication discontinuation, TDM remains an essential tool. The results of the present study showed that patients under psychotropic treatment in Rwanda are exposed to both the risk of drug ineffectiveness (47%) and the risk of toxicity (8%), with only 46% of results within the TRR. Various factors including medication non-compliance, dose misadjustment, and many others not easily identifiable without TDM may lead to such results. A prospective study dealing at the same time with laboratory results and treatment outcomes is needed to show exactly the real situation in Rwandan patients and demonstrate the need of TDM in optimising psychotropic treatment in Rwanda.

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Disclaimer statements

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References

1 Hiemke C, Baumann P, Bergemann N, Conca A, Dietmaier O, Egberts K, *et al.* AGNP consensus guidelines for therapeutic drug monitoring in psychiatry: update 2011. Pharmacopsychiatry. 2011;44:195–235.
2 Addington D. Best practices: improving quality of care for patients with first-episode psychosis. Psychiatr Serv. 2009;60:1164–6.
3 Baumann P, Hiemke C, Ulrich S, Gaertner I, Rao ML, G, *et al.* Therapeutic monitoring of psychotropic drugs, an outline of the AGNP-TDM expert group consensus guideline. Ther Drug Monit. 2004;26:167–70.
4 Malhotra AK, Murphy GM, Kennedy JL. Pharmacogenetics of psychotropic drug response. Am J Psychiatry. 2004;161:780–96.
5 Preskorn SH. CNS drug development: part I: the early period of CNS drugs. J Psychiatr Pract. 2010;16:334–9.
6 Preskorn SH. CNS drug development: part II: advances from the 1960s to the 1990s. J Psychiatr Pract. 2010;16:413–5.
7 Bates DW, Gawande AA. Improving safety with information technology. N Engl J Med. 2003;348:2526–34.
8 Touw DJ, Neef C, Thomson AH, Vinks AA. Cost-effectiveness of therapeutic drug monitoring: a systematic review. Ther Drug Monit. 2005;27:10–17.
9 Baumann P, Hiemke C, Ulrich S, Eckermann G, Gaertner I, Gerlach M, *et al.* The AGNP-TDM expert group consensus guidelines: therapeutic drug monitoring in psychiatry. Pharmacopsychiatry. 2004;37:243–65.
10 Bengtsson F. Therapeutic drug monitoring of psychotropic drugs. TDM "nouveau". Ther Drug Monit. 2004;26:145–51.
11 Hiemke C. Therapeutic drug monitoring in neuropharmacology: does it hold its promises? Eur Arch Psychiatry Clin Neurosci. 2008;258(Suppl 1):21–7.
12 Hiemke C. Clinical utility of drug measurement and pharmacokinetics therapeutic drug monitoring in psychiatry. Eur J Clin Pharmacol. 2008;64:159–66.
13 Jaquenoud Sirot E, Knezevic B, Morena GP, Harenberg S, Oneda B, Crettol S, *et al.* ABCB1 and cytochrome P450 polymorphisms: clinical pharmacogenetics of clozapine. J Clin Psychopharmacol. 2009;29:319–26.
14 Klotz U. Pharmacokinetics and drug metabolism in the elderly. Drug Metab Rev. 2009;41(2):67–76.
15 Raggi MA. Therapeutic drug monitoring: chemical-clinical correlations of atypical antipsychotic drugs. Curr Med Chem. 2002;9:1397–409.
16 Preskorn SH, Fast GA. Therapeutic drug monitoring for antidepressants: efficacy, safety, and cost effectiveness. J Clin Psychiatry. 1991;52:23–33.
17 Munyendamutsa N. Plus de 20% des Rwandais sont traumatisés par le Génocide. Rwanda News Agency, 2010, April 7. Available from http://www.rnanews.com/index.php?option=com_content&view=article&id=3111:plus-de-20-des-rwandais-sont-traumatises-par-le-genocide&Itemid=47.
19 Gaillard Y, Pépin G. Use of high performance liquid chromatography with photodiode array UV detection for creation of a 600-compound library: application to forensic toxicology. J Chromatogr A. 1996;763:149–63.
20 Hahirwa I, Charlier C, Denooz R, Karangwa C. Validation of analytical method for the determination in serum of psychotropic drugs commonly prescribed in Rwanda by HPLC-DAD. Acta Clin Belg. 2013;68(6):479.
21 Wu HBA. Tietz clinical guide to laboratory tests. 4th edn. St-Louis, MO: Saunders; 2006.
22 Bauer LA. Applied clinical pharmacokinetics. 2nd ed. University of Washington, Schools of Pharmacy and Medicine; Seattle, 2008.
23 Demyttenaere K. Risk factors and predictors of compliance in depression. Eur Neuropsychopharmacol. 2003;13:69–75.
24 Mitchell PB. Therapeutic drug monitoring of psychotropic medications. Br J Pharmacol. 2001;52:45–54.
25 Akerblad AC, Bengtsson F, Ekselius L, von Knorring L. Effects of an educational compliance enhancement programme and therapeutic drug monitoring on treatment adherence in depressed patients managed by general practitioners. Int Clin Psychopharmacol. 2003;18:347–54.
26 Beasley CM Jr, Stauffer VL, Liu-Seifert H, Taylor CC, Dunayevich E, Davis JM. All-cause treatment discontinuation in schizophrenia during treatment with olanzapine relative to other antipsychotics: an integrated analysis. J Clin Psychopharmacol. 2007;27:252–8.
27 Lingam R, Scott J. Treatment non-adherence in affective disorders. Acta Psychiatr Scand. 2002;105:164–72.
28 Charlier C, Pinto E, Ansseau M, Plomteux G. Relationship between clinical effects, serum drug concentration, and concurrent drug interactions in depressed patients treated with citalopram, fluoxetine, clomipramine, paroxetine or venlafaxine. Hum Psychopharmacol. 2000;15(6):453–9.
29 Katon W, Cantrell CR, Sokol MC, Chiao E, Gdovin JM. Impact of antidepressant drug adherence on comorbid medication use and resource utilization. Arch Intern Med. 2005;165:2497–503.
30 Pfuhlmann B, Gerlach M, Burger R, Gonska S, Unterecker S, Jabs B, *et al.* Therapeutic drug monitoring of tricyclic antidepressants in everyday clinical practice. J Neural Transm Suppl. 2007;72:287–96.

31 Frackiewicz EJ, Jhee SS, Shiovitz TM, editors. Ethnicity in drug development and therapeutics. Cambridge: Cambridge University Press; 2011.
32 Ulrich S, Neuhof S, Braun V, Meyer FP. Therapeutic window of serum haloperidol concentration in acute schizophrenia and schizoaffective disorder. Pharmacopsychiatry. 1998;31(5):163–9.
33 Brosen K. Drug-metabolizing enzymes and therapeutic drug monitoring in psychiatry. Ther Drug Monit. 1996;18(4):393–6.
34 Müller MJ, Dragicevic A, Fric M, Gaertner I, Grasmäder K, Härtter S, *et al.* Therapeutic drug monitoring of tricyclic antidepressants: how does it work under clinical conditions? Pharmacopsychiatry. 2003;36(3):98–104.

CHAPTER IV. ANALYTICAL METHOD TRANSFER IN RWANDA

The final target of this work was to initiate in Rwanda therapeutic drug monitoring activities for psychotropic drugs. Knowing that equipments used in such activities are available in Rwanda, the transfer of a validated method to Rwandan laboratories was enough to start carrying out such activities in this country. In fact, one of the important steps of the present work consisted in transferring in Rwanda the analytical method developed and validated in the Laboratory of Clinical, Forensic, Environmental and Industrial Toxicology of the University Teaching Hospital-Liège (Belgium). The method to be transferred can be applied in therapeutic drug monitoring activities, but also in other situations requiring the determination of blood concentration levels of psychotropic drugs. The Laboratory of Analysis of Foodstuffs, Drugs, Water and Toxics of the University of Rwanda was chosen as the receiving laboratory for the analytical method transfer. Taking into account the difference between the two laboratories as far as analytical customs and equipments are concerned, among various approaches used in analytical method transfer, revalidation of the method in the receiving laboratory was adopted.

Considering the working environment in Rwanda and the time allocated to this work, among 27 molecules for which the transferred method had been previously validated in Belgium, 10 molecules representing almost 90% of cases in Rwandan patients, were concerned by the analytical validation realised in Rwanda in the framework of the method transfer. Results of the transfer of the analytical method to the Laboratory of Analysis of Foodstuffs, Drugs, Water and Toxics-University of Rwanda were subject to the publication 3 submitted to Rwanda Journal Series I: Medicine and Health Sciences.

PUBLICATION 3

The transfer of a High Performance Liquid Chromatography with Diode Array Detection method for the determination in serum of psychotropic drugs: Method revalidation

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(*Submitted to Rwanda Journal Series I: Medicine and Health Sciences*)

The transfer of a High Performance Liquid Chromatography with Diode Array Detection method for the determination in serum of psychotropic drugs

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Abstract

Background: The relevance of the determination of blood concentration levels of psychotropic drugs has been demonstrated in Rwanda. However, due to the lack of appropriate analytical techniques, such activities are not carried out in this country. **Objective:** The aim of this work was to transfer to a Rwandan laboratory via revalidation, a High Performance Liquid Chromatography based method applicable for the determination in serum of psychotropic drugs commonly prescribed in Rwanda. **Method:** A liquid-liquid extraction using prazepam as internal standard was used for sample preparation. A chromatographic separation was performed on a Symmetry C8 analytical column, using acetonitrile and a phosphate buffer as mobile phase. The method was validated with respect the total error concept as decision criterion. **Results:** The validated method was linear over tested dosing intervals with a coefficient of determination greater than 0.99 for all analytes. The precision was good with RSD between 1.3 and 15.6% and the trueness ranged between 87 and 109%. The accuracy of the method was demonstrated as well. **Conclusion:** The method allowing a simultaneous determination in serum of several psychotropic drugs was successfully validated and thus transferred in the Laboratory of Analysis of Food Stuffs, Drugs, Water and Toxics (Rwanda).

Keywords: Method transfer, HPLC/DAD, psychotropic drugs, serum.

1. Introduction

In Rwanda psychotropic drugs are used not only in treatment of usual mental diseases but also in management of some psychological problems directly related to the history of the country, especially to the 1994 Genocide against Tutsi. So far in Rwanda, the determination of blood concentration levels of these drugs is not done regardless of the case. However, the need to carry out such activities in Rwanda has been demonstrated (Hahirwa, Charlier, Karangwa & Denooz, 2015). Equipments that can be used to carry out such activities are now available but the lack of suitable analytical techniques to be used remains a problem. Therapeutic drug monitoring of psychotropic medications is carried out in routine in the Laboratory of Toxicology of the University Teaching Hospital of Liège (Belgium) and a transfer of the method used in such activities to the Laboratory of Analysis of food Stuffs, Drugs, Water and Toxics (Rwanda) was envisaged.

The analytical method transfer (Fontenay, 2008; Dewé et al., 2007; Scypinski, Roberts, Oates & Etse, 2002; Kaminski, Schepers & Wätzig, 2010) consists in transferring an analytical procedure from a laboratory, where it was originally developed and validated or where it is in routine use (sender), to a new laboratory (receiver) for its application in routine. In fact, the transfer process starts with the decision of transferring a validated analytical method to the receiving laboratory and ends with the official qualification of the latter by the sending laboratory. The purpose of the analytical method transfer is therefore, to qualify the receiver to use the analytical procedure. The results obtained by the receiving laboratory after being qualified will thus be reliable (Fontenay, 2008; Scypinski et al., 2002; Kaminski et al., 2010; Klingle et al., 2001; Rozet et al., 2008).

The transfer process constitutes the last step for the analytical method to be used in routine in the receiving laboratory. The process includes physical transfer of the analytical method from the sender to the receiver which must warrant its ability to implement the method by obtaining

accurate results (Rozet et al., 2008; Rozet et al., 2009). Analytical method transfer assessment is now required in validation protocol of regulatory agencies such as the Food and Drug Administration (Rozet et al., 2008; USP, 2014; Schepers & Wätzig, 2005).

The most common approaches for analytical method transfer are comparative testing, covalidation involving two or more laboratories, revalidation and transfer waiver (Scypinski et al., 2002; USP, 2014; Ermer, Limberger, Lis & Wätzig, 2013; Agut, Caron, Giordano, Hoffman & Ségalini, 2011). The comparative testing approach implies the analysis of the same predetermined samples by both the sending and the receiving laboratories. To carry out such analysis, a preapproved transfer protocol providing the details of the analytical method, the samples to use and predetermined acceptance criteria is required (Scypinski et al., 2002; USP, 2014). For the transfer by covalidation, the laboratory performing the validation is qualified to use the method and the receiving laboratory is involved as part of the validation team generating data for the assessment of reproducibility. Like in comparative testing, a preapproved transfer or validation protocol with details of the method and acceptance criteria is necessary (USP, 2014; Scypinski & Young, 2011). Another acceptable approach to transfer a validated analytical technique consists in its revalidation or partial validation by the receiving laboratory. When the two laboratories do not share the same environment (validation standards, analytical customs and equipments), revalidation may constitute an efficient approach for the method transfer (Rozet et al., 2009; Agut et al., 2011).

Under certain circumstances, the receiving laboratory can start using the method without going through the formal transfer process and this is commonly known as transfer waiver approach. In fact, this means that the receiving laboratory is considered to be qualified to use the method; therefore the comparison of interlaboratory data is not required. Below are some situations where the transfer waiver can be justified:

- the composition of the new products to be analysed is similar to that of an existing product already analysed by a technique with which the receiving laboratory is already familiar;
- the analytical method to transfer is described in one or more pharmacopeial compendia and has not been changed;
- the transfer concerns a method which is the same or very similar to a method already in use in the receiving laboratory;
- changes involved in the new method do not substantially affect the ability to use it (e.g., changes in sample preparation or in calculation formulas);
- when there is a movement of the personnel in charge of development, validation, or routine analysis from the transferring laboratory to the receiving one (Fontenay, 2008; Scypinski et al., 2002; Rozet et al., 2008; Scypinski & Young, 2011; Lin, Wenkwi & Weng, 2011).

The determination of psychotropic drugs in biological samples is relevant in various situations including therapeutic drug monitoring, detection of intoxications and forensic cases. In clinical practice, the determination of blood concentration levels of psychotropic drugs is relevant for the optimisation of treatment with these drugs as they are associated with a great interindividual variability in clinical response (Malhotra, Murphy & Kennedy, 2004; Vecchione et al., 2012). Sometimes a poorly adapted dosing of these drugs can worsen the patient status due to their eventual toxicity and this is particularly the case for tricyclic antidepressants, barbiturates and first generation antipsychotics. Moreover, due to their widespread use, psychotropic drugs are frequently involved in cases of deliberate and accidental poisoning (Sanchez de la Torre, Martinez & Almarza, 2005; Smink et al., 2004).

The objective of this study was to transfer to the Laboratory of Analysis of food Stuffs, Drugs, Water and Toxics (Rwanda) an analytical method based on High Performance Liquid Chromatography coupled to a Diode Array Detection (HPLC/DAD) used in the determination

of psychotropic drugs in serum and validated in Laboratory of Clinical, Forensic, Environmental and Industrial Toxicology, University Teaching Hospital-Liège (Belgium).

Among various approaches used in analytical method transfer, revalidation of the method by the receiving laboratory was adopted. The transfer concerned ten psychotropic drugs most commonly prescribed in Rwanda: carbamazepine, chlorpromazine, citalopram, diazepam, flupentixol, haloperidol, levomepromazine, phenobarbital, phenytoin, and zolpidem. The validation process aims to appreciate the performance of the method and evaluate by experimentation if the method meets the expected requirements (Rozet, 2007; Hubert et al., 2007a; Hubert et al., 2007b; Hubert et al., 2008). Selectivity, response function, linearity, trueness, precision, accuracy and limits of quantification and detection are validation parameters that were verified during the validation process.

2. Materials and methods

With regard to the method validated in Liège (Hahirwa, Charlier, Denooz & Karangwa, 2013), the same technique was kept for the preparation of standard solutions and the sample preparation process. The difference between the previous validation and the present one is mainly the change in chromatographic systems. The HPLC system used in Liège consisted of a Waters Alliance 2695 Separations Module coupled to a 2996 photodiode array detector, while in Rwanda an Agilent 1200 Series coupled to a G1315D diode array detector was used.

2.1 Chemicals and reagents

Carbamazepine, citalopram, chlorpromazine, haloperidol, levomepromazine, phenobarbital, phenytoin and zolpidem used as reference standards were purchased from LGC GmbH (Luckenwalde, Germany), while diazepam and flupentixol were respectively purchased from Cerilliant (Texas, USA) and Lundbeck (Brussels, Belgium). Prazepam used as internal

standard was purchased from Certa (Braine-l'Alleud, Belgium). Acetonitrile, methanol, sodium carbonate and sodium dihydrogenophosphate were all purchased from Merck (Darmastadt, Germany); dichloromethane and n-hexane from Sigma-Aldrich Chemie GmBH (Steinheim, Germany); n-Amyl alcohol from BDH Laboratory supplies (Poole, England) and diethyl ether from Scharlab S.L. (Sentmenat, Spain). All organic solvents were certified for HPLC use. Blank human serum was obtained from the Rwanda National Transfusion Center.

2.2 Chromatographic conditions

The HPLC system used consisted of an Agilent 1200 Series (Agilent Technologies, Böblingen, Germany) made of a G1311A quaternary solvent pump, a G1322A solvent degasser, a G1329A automated sampler and a G1316A column compartment. For the detection a G1315D diode array detector was used. The HPLC instrument was piloted by ChemStation software (Agilent Technologies). A Symmetry® C8 analytical column (4.6mm×250mm) packed with 5µm diameter particles (Waters, Zellik, Belgium) was used for separation performed at 30°C. An injection volume of 40 µL, a sample temperature of 25°C, a column temperature of 30°C and a run time of 45 min were fixed. The mobile phase consisted of acetonitrile and sodium dihydrogenophosphate buffer 43.5 mM, pH 3.8 used in gradient elution mode (table 1). UV–visible spectra were recorded at 205 nm (chlorpromazine, citalopram, phenobarbital and zolpidem), 213 nm (carbamazepine, diazepam and haloperidol) and 230 nm (flupentixol, levomepromazine and phenytoin).

Table 1. Mobile phase gradient

Time (min)	Flow (mL/min)	Acetonitrile (%)	Phosphate buffer (%)
0	1.0	13.0	87.0
9.0	1.0	35.0	65.0
28.0	1.5	80.0	20.0
30.0	1.5	80.0	20.0
31.0	1.5	13.0	87.0
32.0	1.0	13.0	87.0
45.0	1.0	13.0	87.0

2.3. Solutions

Standard stock solutions were prepared by dissolution or dilution of various compounds with methanol. Stock solutions were refrigerated between 2 and 8°C. Calibration and validation standard samples were prepared by spiking the blank serum with an adequate amount of standard stock solutions. Calibration standard samples were prepared in duplicates on three consecutive days at six levels of concentration (table 2). Validation standard samples were prepared in triplicates on three consecutive days at 8 levels of concentration (table 3). Sodium carbonate 1M and sodium dihydrogenophosphate buffer 43.5 mM were prepared by dissolving an adequate amount of these compounds in bidistilled water. The pH of the buffer solution was adjusted to 3.8 using phosphoric acid.

Table 2. Levels of concentration (ng/mL) for calibration standard samples

	L_1	L_2	L_3	L_4	L_5	L_6
Carbamazepine (TRR: 6000 - 12000)	1000	2000	5000	10000	25000	50000
Chlorpromazine (TRR: 30 - 300)	20	50	100	200	500	1000
Citalopram (TRR : 50 - 110)	10	25	50	100	250	500
Diazepam (TRR : 125 - 1500)	100	200	500	1000	2500	5000
Flupentixol (TRR : 1 - 10)	5	10	25	50	125	250
Haloperidol (TRR : 1 - 10)	5	10	25	50	125	250
Levomepromazine (TRR : 30 - 160)	10	20	50	100	250	500
Phenobarbital (TRR: 10000 - 40000)	5000	12500	25000	50000	125000	250000
Phenytoin (TRR: 10000 - 20000)	5000	12500	25000	50000	125000	250000
Zolpidem (TRR: 80 - 150)	20	50	100	200	500	1000

Caption: TRR-*Therapeutic reference range (in ng/mL)*

Table 3. Levels of concentration (ng/mL) for validation standard samples

	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8
Carbamazepine (TRR: 6000 - 12000)	20	50	200	500	750	2500	10000	40000
Chlorpromazine (TRR: 30 - 300)	2	4	8	12	16	20	60	800
Citalopram (TRR : 50 - 110)	1	2	4	6	8	10	30	400
Diazepam (TRR : 125 - 1500)	10	20	40	60	80	100	750	4000
Flupentixol (TRR : 1 - 10)	0.5	1	2	3	4	5	37.5	200
Haloperidol (TRR : 1 - 10)	0.5	1	2	3	4	5	37.5	200
Levomepromazine (TRR : 30 - 160)	1	2	4	6	8	10	75	400
Phenobarbital (TRR: 10000 - 40000)	50	200	1000	2000	3000	5000	15000	200000
Phenytoin (TRR: 10000 - 20000)	50	200	1000	2000	3000	5000	15000	200000
Zolpidem (TRR: 80 - 150)	2	4	8	12	16	20	60	800

2.4. Sample preparation

One hundred microliters of internal standard (prazepam 10 mg/L) were added to 1 mL of serum. Then, 500 µL of sodium carbonate 1M were added in order to increase the sample ionic strength and put the analytes in their unionized form and thus facilitate their transfer to the organic phase. This mixture was extracted with 5 mL of a mix of organic solvents: diethyl ether/dichloromethane/hexane/n-amyl alcohol (50/30/20/0.5: V/V/V/V). After shaking during 15 min and centrifuging during 10 min at 2000 rounds/min, 3.5 mL of the supernatant were picked up and evaporated to dryness under nitrogen flow at 40°C and reconstituted with70 µL of a mix of acetonitrile and bidistilled water (50/50: V/V). The recovery mix was then transferred into an Eppendorf tube and centrifuged for 5 min. Afterward, the supernatant was analysed by HPLC.

2.5. Method validation

2.5.1. Validation parameters assessed

1°) Selectivity

Retention times and UV-visible spectra were parameters used to assess the selectivity of detection of the method. This parameter refers to the extent to which the method can determine the particular analyte (s) in a complex mixture without interference from other components of the mixture. In other words, the selectivity of an analytical method is its ability to discriminate between the analytes and interfering compounds (Rozet, 2007; Hubert et al., 2007a).

2°) Response function

To assess this parameter, calibration standards prepared in duplicates at six levels of concentration on three consecutive days were used. The response function of an analytical procedure stands for the relationship existing, within a specified range, between the response (signal) and the concentration (quantity) of analyte in the sample (Rozet, 2007; Hubert et al., 2007a).

3°) Linearity

The linearity of an analytical method refers to the relationship between introduced quantity (concentration) and the concentration back-calculated from the calibration curve. This criterion shows the ability of the method within a specified range, to obtain results directly proportional to concentrations of analyte in samples (Rozet, 2007; Hubert et al., 2007a). To assess this parameter, the determination coefficients of plots of introduced quantities against calculated concentrations were considered. Slopes and intercepts were considered as well.

4°) Precision

The precision of an analytical procedure is a validation parameter that provides information on random error. It is defined as the closeness of agreement between series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions (Hubert et al., 2007a). To assess this parameter relative standard deviation (RSD %) was considered. Both repeatability and intermediate precision were assessed.

5°) Trueness

The trueness refers to the closeness of agreement between conventionally accepted value or reference value and the average value obtained from a large series of tested results. This parameter giving information on systematic error is usually expressed in terms of bias, relative bias or recovery (Rozet, 2007). The trueness of the present method was assessed based on relative bias and recovery.

6°) Accuracy

The accuracy of an analytical method refers to the closeness of agreement between the test result and the value accepted either as the reference value or conventional true value. In fact, this parameter expresses the total error related to test result (random and systematic errors) or the sum of precision and trueness of an analytical method (Rozet, 2007; Kratzsch, Peters, Kraemer, Weber & Maurer, 2002). Accuracy profiles of various molecules were generated by Enoval V3.0 software (Arlenda, 2013).

7°) Limits of detection and quantification

Low and upper limits of quantification (LLOQ and ULOQ) of an analytical procedure are respectively the lowest quantity and the highest quantity of analyte in the sample that can

accurately be quantitatively determined. The limit of detection (LOD) of a method is the lowest amount of analyte in a sample that can be detected (Rozet, 2007; Hubert et al., 2007a; Kratzsch et al., 2002). The assessment of LOD and LLOQ was based on the results of bias and coefficient of variation as well as UV-visible spectra of various molecules at different levels of concentration, while the intersection of tolerance limits and acceptance limits was considered for the upper limit of quantification.

2.5.2. Validation process

Validation process was carried out according to the general guidelines for validation of analytical methods (Rozet, 2007; Hubert et al., 2007a; Hubert et al., 2007b; Hubert et al., 2008).

To evaluate the response function relationship of the method, calibration standard samples were prepared in duplicates on three consecutive days at six levels of concentration. Calibration curves were obtained by plotting ratios of analyte peak area over internal standard peak area versus the analyte concentrations in spiked samples.

To evaluate the linearity, precision, trueness, uncertainty of measurement, accuracy and the upper limits of quantification of the method, three levels of concentration were prepared in triplicates on three consecutive days. Results were processed according to the total error concept with the Enoval V3.0 software. To determine the LLOQ and LOD, five levels of concentration below therapeutic reference ranges were prepared. The upper limit of quantification of the method was determined by the intersection of the accuracy profile and acceptance limits.

3. Results

3.1. Selectivity

To assess the selectivity of the method, retention times and UV spectra (Fig. 1) were parameters used. As shown in the chromatograms (Fig. 2), the method allowed simultaneous separation of several molecules and generated peaks with good resolution. However, it was not possible to separate simultaneously molecules with relatively very close or same retention times. To prevent possible coelution once in the same run, such molecules were put into different groups during the validation process.

UV-visible spectra registered in the library of the method and those of analytes in the sample were compared to confirm the real presence of the analytes.

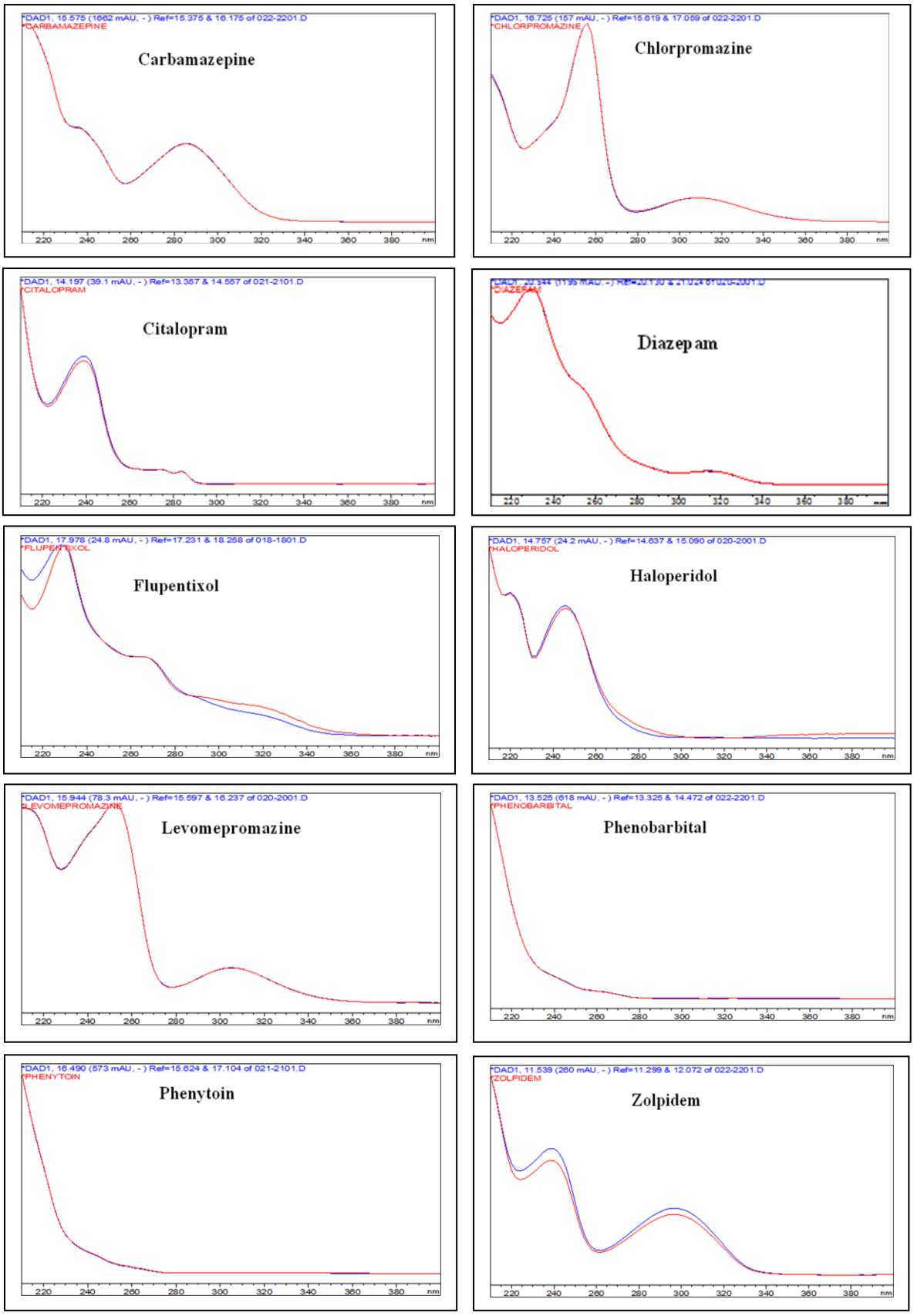

Figure 1. UV-visible spectra of various analytes (blue) vs. library reference spectra (red)

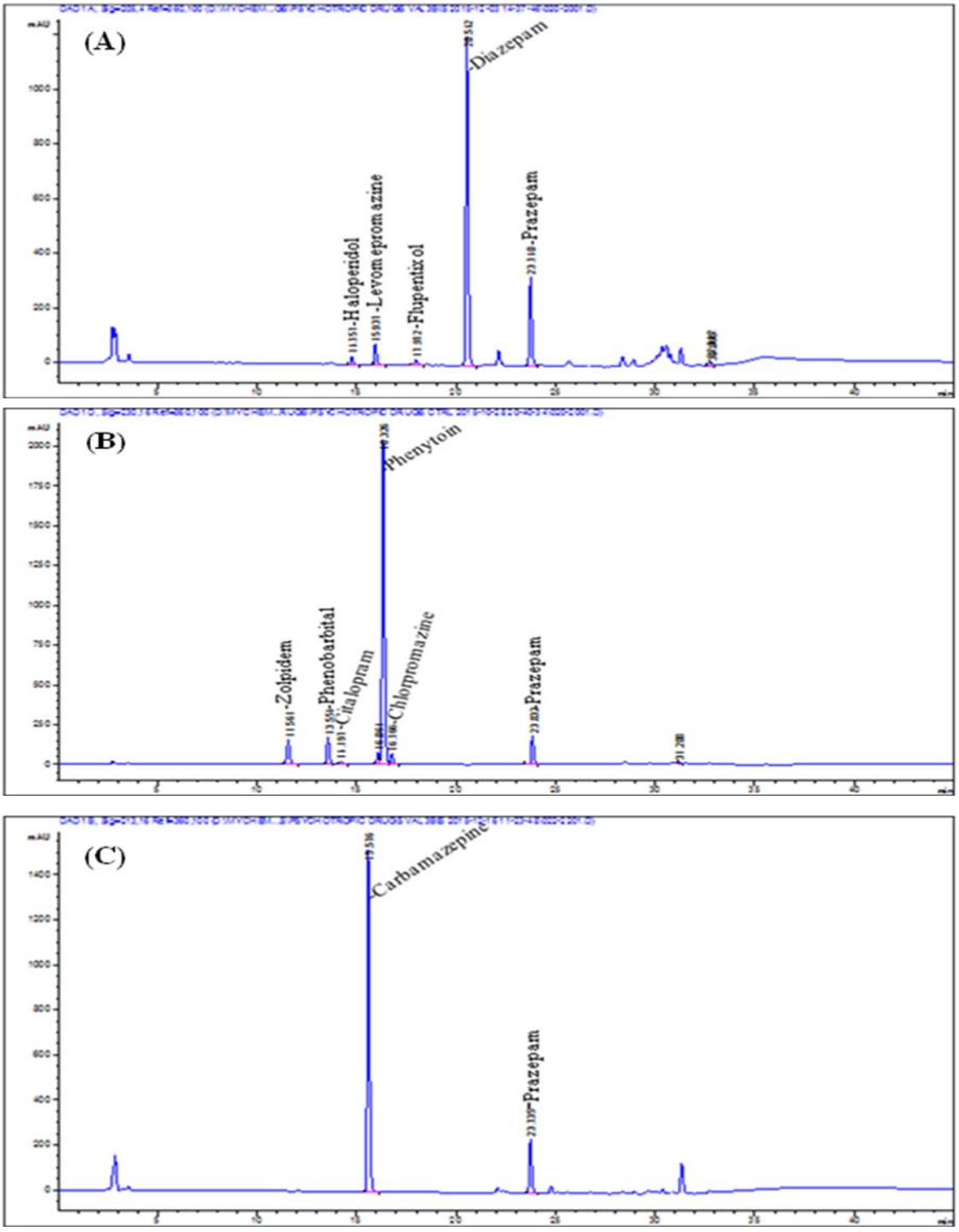

Figure 2. HPLC chromatograms of various analytes

The figure 2 presents HPLC chromatograms obtained with a serum spiked with diazepam 4000 ng/mL, haloperidol 200 ng/mL, flupentixol 200 ng/mL, levomepromazine 200 ng/mL (A), citalopram 400 ng/mL, chlorpromazine 800 ng/mL, phenobarbital 200000 ng/mL, phenytoin 200000 ng/mL, zolpidem 800 ng/mL (B) and carbamazepine 40000 ng/mL (C).

3.2. Response function

To assess the relationship between signal and analyte concentration, calibration curves made of six levels of concentration prepared in duplicates (table 2) on three consecutive days were used. A linear model was used for all analytes. The coefficient of determination was > 0.99 for all molecules. These curves were then used for the determination of analyte concentrations in validation samples.

3.3. Linearity

The present analytical method showed a good linearity over the whole concentration range investigated (table 3) with determination coefficients greater than 0.99, a slope value close to 1 and an intercept close to 0 for all molecules, i.e. it gave results directly proportional to concentrations of analyte in samples. The figure 3 presents the results for the linearity of the method.

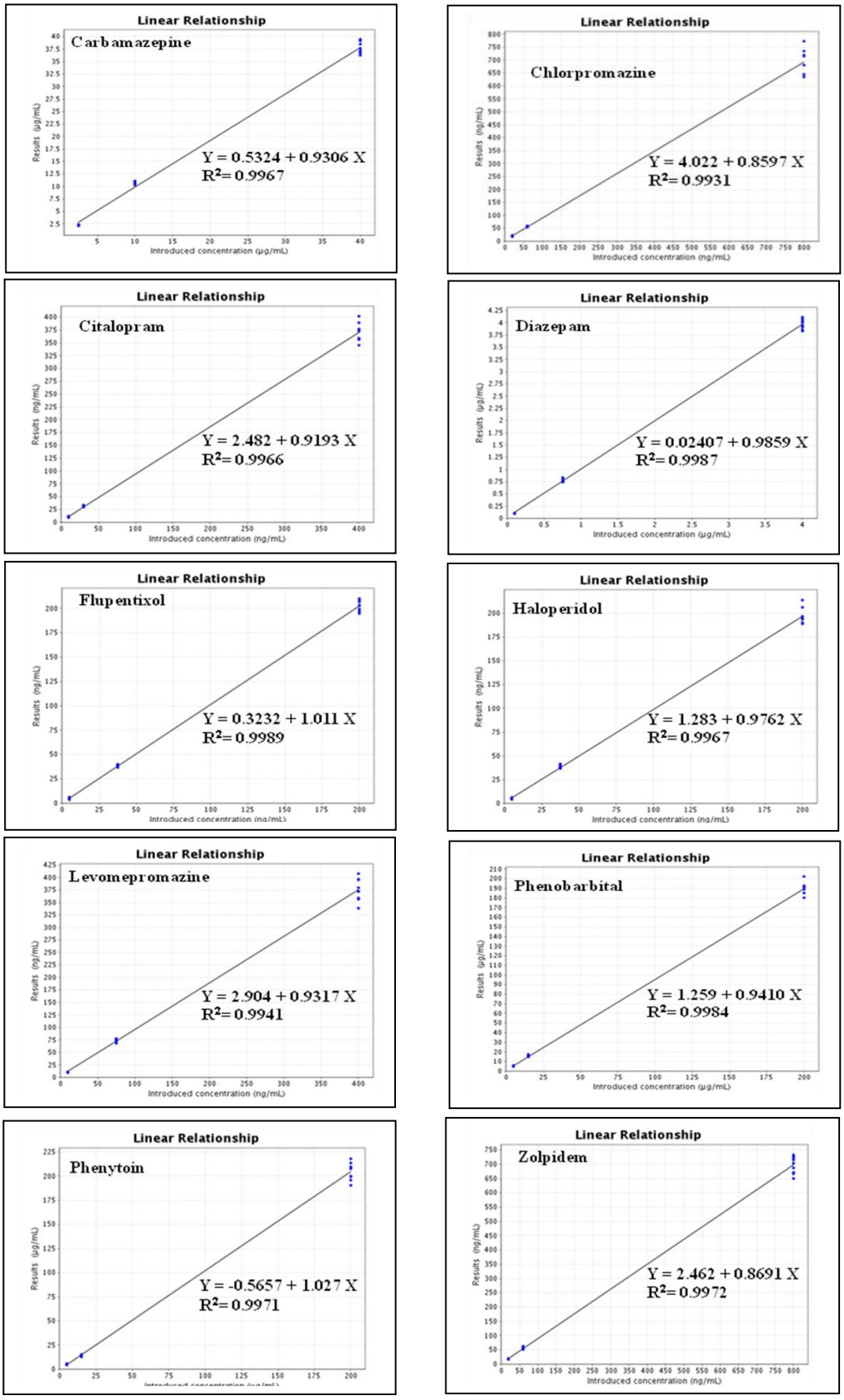

Figure 3. Linear functions of various analytes

3.4. Precision

During the validation process both repeatability (intra-assay) and intermediate precision (inter-assay) were assessed. Relative standard deviations (RSD) were calculated and results are presented in table 4. Taking into consideration both repeatability and intermediate precision for all molecules, results for RSD varied between 1.3 and 15.6%.

Table 4. Precision and trueness assessment

Analytes	Nominal [ ] (ng/mL)	Precision		Trueness	
		Repeatability (RSD %)	Intermediate precision (RSD %)	Relative bias (%)	Recovery (%)
Carbamazepine	2500	3.44	4.60	-10.17	90
	10000	2.27	2.29	6.05	106
	40000	1.31	3.27	-5.99	94
Chlorpromazine	20	6.45	8.97	-0.17	100
	60	3.27	3.27	-5.13	95
	800	3.70	6.45	-13.53	87
Citalopram	10	9.23	10.77	0.67	101
	30	3.92	3.92	5.85	106
	400	4.38	4.38	-7.47	93
Diazepam	100	7.66	7.66	1.33	101
	750	2.17	5.62	5.30	105
	4000	2.39	2.39	-0.91	99
Flupentixol	5	15.65	15.65	8.00	108
	37.5	2.38	2.38	1.84	101
	200	1.85	2.82	1.22	101
Haloperidol	5	10.02	14.92	3.11	103
	37.5	4.21	4.76	4.27	104
	200	2.94	4.55	-1.84	98
Levomepromazine	10	7.91	7.91	4.00	104
	75	4.16	4.24	-0.04	100
	400	3.04	6.13	-6.19	94
Phenobarbital	5000	5.79	5.79	8.55	109
	15000	5.79	6.18	6.26	106
	200000	3.04	3.04	-5.29	95
Phenytoin	5000	9.37	10.02	1.23	101
	15000	4.51	4.51	-4.59	95
	200000	4.41	4.54	2.38	102
Zolpidem	20	5.84	5.84	-9.278	91
	60	1.89	6.85	-6.000	94
	200	3.72	3.72	-12.79	87

3.5. Trueness

Relative bias and recovery were calculated to assess the trueness of the method. Enoval software was used to perform calculations and the results are presented in table 4. As can be seen from results, the relative bias varied between 0.2 and 12.8% while the recovery ranged from 87 to 109% for all analytes.

3.6. Accuracy

Accuracy profiles generated by Enoval software were used to assess the accuracy of present analytical method. The acceptance limits and the β-expectation tolerance interval were respectively set at ± 30% and 82.5%. Accuracy profiles of various molecules are presented by the figure 4. As shown in this figure, the tolerance limits remained within the acceptance limits on the whole investigated concentration range for all analytes exception made for low concentrations of haloperidol and flupentixol.

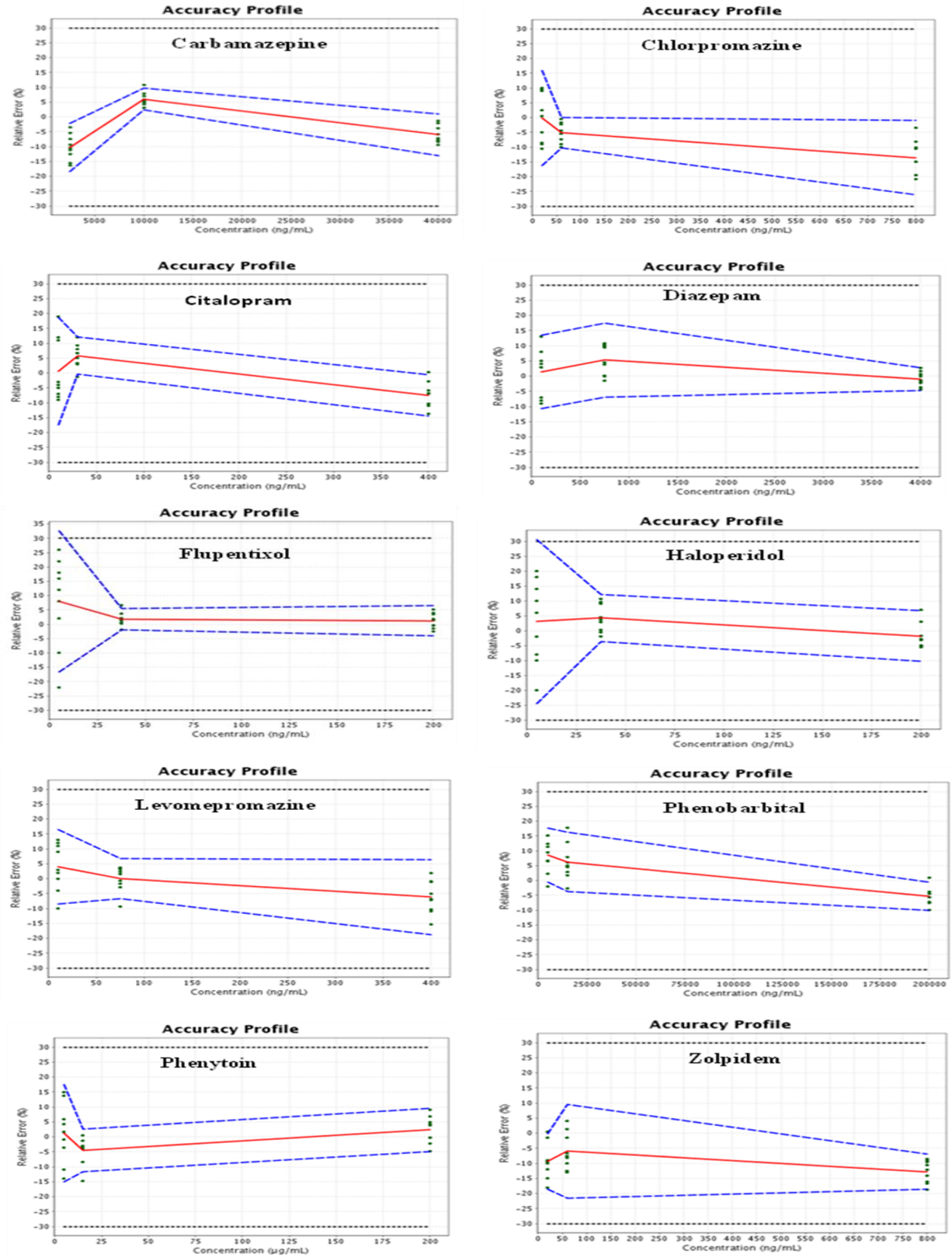

Figure 4. Accuracy profiles of various analytes

Captions: *Relative bias (—), β-expectation tolerance limits (---), acceptance limits (....), relative back-calculated concentrations (.)*

3.7. Limits of detection and quantification

A level of concentration with a UV-spectrum matching the one in the method library but for which the relative bias and/or CV exceeded 20% was considered for LOD, while the same conditions for spectrum with relative bias and CV less than 20% were considered for the LLOQ. For the upper limits of quantification, the intersection of tolerance limits and acceptance limits was considered. Results for limits of quantification and detection are compiled in table 5.

Table 5. Limits of quantification and detection of the method

Molecules	**Therapeutic windows (ng/mL)**	**LOD (ng/mL)**	**LLOQ - ULOQ (ng/mL)**
Carbamazepine	6000 - 12000	20	750 - 40000
Chlorpromazine	30 - 300	8	16 - 800
Citalopram	50 - 110	8	10 - 400
Diazepam	125 - 1500	5	20 - 4000
Flupentixol	1 - 10	5	8 - 200
Haloperidol	1 - 10	5	6 - 200
Levomepromazine	30 - 160	6	10 - 400
Phenobarbital	15000 - 40000	50	5000 - 200000
Phenytoin	10000 - 20000	50	5000 - 200000
Zolpidem	80 - 150	2	6 - 800

4. Discussion

The method transferred to Rwanda was previously validated in Belgium. Changes in chromatographic systems (from Waters to Agilent HPLC) and materials used in sample preparation but also difference in analytical customs between the two laboratories are the main reasons for having chosen revalidation of the method in Rwanda as efficient approach for analytical method transfer. When his approach is used, the decision about the transferability of the method is based on acceptance criteria of analytical validation, i.e. a successful validation by the receiving laboratory means a successful method transfer (Scypinski & Young, 2011). In fact, when revalidation is used as approach for the analytical

method transfer, the receiving laboratory is deemed qualified to use the method up on the completion of validation process (Scypinski et al., 2002; Scypinski & Young, 2011).

Response function, linearity, selectivity, trueness, precision, accuracy and limits of quantification are validation parameters commonly verified for analytical validation of a quantitative method (Hubert et al., 2007a; Hubert et al., 2007b; Hubert et al., 2008) and requirements for a method to be valid have been set. According to the FDA for example, a good precision of a bioanalytical method is demonstrated by a RSD not exceeding 15 %, except for LLOQ where a RSD of up 20% can be tolerated (Hubert et al., 2007a; Hubert et al., 2007b). Considering both repeatability and intermediate precision for all molecules, the present method meets this requirement and thus showed a good precision. Regardless of differences that can be observed in decision rules when different regulatory documents are considered, the accuracy of the method remains so far a validation parameter commonly used to assess the validity of analytical method (Hubert et al., 2007a; Hubert et al., 2008). When accuracy profiles are used as decision tools, the method is valid within the range where the tolerance limits are within acceptance limits. As shown by the figure 4, the validity of this method was demonstrated on the whole concentration range investigated for all analytes except haloperidol and flupentixol as far as minimal therapeutic concentrations are considered. As far as limits of quantification are concerned, compared to results obtained in Belgium (Hahirwa et al., 2013), a subtle difference in LOD and LOQ was observed and this could be the result of the difference in approaches used to determine these limits; signal to noise approach was used in Belgium while in Rwanda peaks, relative bias and CVs were considered. In both cases low limits of quantification were inferior to low limits of therapeutic reference ranges exception made to flupentixol and haloperidol due to their low therapeutic reference ranges.

5. Conclusion

In case of revalidation as approach for the analytical method transfer, the receiving laboratory is qualified to use the method up on the completion of the validation process. As it was the case in Belgium, all validation parameters assessed in Rwanda demonstrated the validity of the present method for the determination of psychotropic drugs in serum. The coefficients of variation did not exceed 15% for all concentration levels investigated and the accuracy of the method was demonstrated over investigated concentration ranges. Therefore, this method originating from Belgium was successfully transferred in Rwanda trough revalidation. The transferred method, useful for therapeutic drug monitoring and detection of intoxications as well, can now be applied in routine activities of the Laboratory of Analysis of food Stuffs, Drugs, Water and Toxics. To the best of our knowledge, this is the first transfer realised in Rwanda for such bioanalytical method.

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Declaration of interest

The authors report no declarations of interests.

References

Agut, C., Caron, A. Giordano, C., Hoffman D. & Ségalini, A. (2011). Transfer of analytical procedures: A panel of strategies selected for risk management, with emphasis on an integrated equivalence-based comparative testing approach. *J. Pharm. Biomed. Anal, 56,* 293-303.

Arlenda Home Page, enoval Version V3.0b PROD, last update: August 22, 2013. Accessed from https://www.arlenda.com/enoval3.0 on December 28, 2015.

Dewé, W., Govaets, B., Boulanger, B., Rozet, E., Chiap, P. & Hubert, P. (2007). Using total error as decision criterion in analytical method transfer. *Chemometr Intell Lab Syst, 85,* 262-268.

Ermer, J., Limberger, M., Lis, K. & Wätzig, H. (2013). The transfer of analytical procedures. *J. Pharm. Biomed. Anal, 85,* 262–276.

Fontenay, G. (2008). Analytical method transfer: New descriptive approach for acceptance criteria definition. *J. Pharm. Biomed. Anal, 46,* 104-112.

Hahirwa, I., Charlier, C., Denooz, R. & Karangwa, C. (2013). Validation of analytical method for the determination in serum of psychotropic drugs commonly prescribed in Rwanda by HPLC-DAD. *Acta Clinica. Belgica, 68,* 479.

Hahirwa, I., Charlier, C., Karangwa, C. & Denooz R. (2015). Determination of blood concentration levels of psychotropic medications in Rwandan patients. *Acta Clinica Belgica, 70*(6), 425-431.

Hubert, P., Nguyen-Huu, J.J., Boulanger, B., Chapuzet, E., Cohen, N., Compagnon, P.A., ... Rozet E. (2007). Harmonization of strategies for validation of quantitative analytical procedures. A SFSTP proposal-Part III. *Journal of Pharmaceutical and Biomedical Analysis, 45,* 82-96.

Hubert, P., Nguyen-Huu, J.J., Boulanger, B., Chapuzet, E., Cohen, N., Compagnon, P.A., ... Rozet, E. (2008). Harmonization of strategies for validation of quantitative analytical procedures. A SFSTP proposal-Part IV. *Journal of Pharmaceutical and Biomedical Analysis, 48,* 760-771.

Hubert, P., Nguyen-Huu, J.J., Boulanger, B., Chapuzet. E., Chiap, P., Cohen, N., ... Rozet, E. (2007). Harmonization of strategies for validation of quantitative analytical procedures. A SFSTP proposal-Part II. *Journal of pharmaceutical and Biomedical Analysis, 45,* 70-81.

Kaminski, L., Schepers, U. & Wätzig, H. (2010). Analytical method transfer using equivalence tests with reasonable acceptance criteria and appropriate effort: Extension of the ISPE concept, *J. Pharm. Biomed. Anal, 53,* 1124–1129.

Klingle, R., Khan-Malek, R., Snikeris, F., Munden, P., Agut, C. & Bauer, M. (2001). A unified approach for design and analysis of transfer studies for analytical methods. *Drug Inf J, 35,* 1271-1288.

Kratzsch, C., Peters, F.T., Kraemer, T., Weber, A. & Maurer, H. (2002). Screening, library-assisted identification and validated quantification of fifteen neuroleptics and three of their metabolites in plasma by liquid chromatography/mass spectrometry with atmospheric pressure ionization. *J. Mass Spectrum., 38,* 283-295.

Lin, J.Z., Wenkwi, L. & Weng, N. (2011). Capsule review on bioanalytical method transfer: opportunities and challenges for chromatographic methods. *Bioanalysis 3,* 57-66.

Malhotra, A.K., Murphy, G.M. & Kennedy J.L. (2004). Pharmacogenetics of psychotropic drug response. *Am. J. Psychiatry, 161,* 780–796.

Rozet, E., Ceccato, A., Hubert, C., Ziemons, E., Oprean, R., Rudaz, S., ... Hubert, P. (2007). Analysis of recent pharmaceutical regulatory documents on analytical method validation. *Journal of Chromatography A, 1158,* 111-125.

Rozet, E., Dewé, W., Morello, R., Chiap, P., Lecomte F., Ziemons, E., ... Hubert, P. (2008). Risk-based approach for the transfer of quantitative methods: Bioanalytical applications. *J. Chromatogr. A, 1189,* 32–41.

Rozet, E., Dewé, W., Ziemons, E., Bouklouze, A., Boulanger, B. & Hubert P. (2009). Methodologies for the transfer of analytical methods: A review. *J. Chromatogr. B, 877,* 2214–2223.

Sanchez de la Torre, C., Martinez, M.A. & Almarza, E. (2005). Determination of several psychiatric drugs in whole blood using capillary gas–liquid chromatography with nitrogen phosphorus detection: comparison of two solid phase extraction procedures. *Forensic Science International, 155*, 193-204.

Schepers, U. & Watzig, H. (2005). Application of the equivalence test according to a concept for analytical method transfers from the International Society for Pharmaceutical Engineering (ISPE). *J. Pharm. Biomed. Anal*, *39,* 310-314.

Scypinski, S. & Young, J. (2011). Analytical methodology transfer-Analytical Research & Development. New Brunswick, New Jersey, USA: Bristol-Myers Squibb Company.

Scypinski, S., Roberts, D., Oates, M. & Etse, J. (2002). Pharmaceutical Research and Manufacturers Association Acceptable Analytical Practice for Analytical Method Transfer. *Pharmaceutical Technology*, *64*, 84-88.

Smink, B.E., Brandsma, J.E., Dijkhuizen, A., Lusthof, K. J., Gier, J.J., Egberts, A.C.G. & Uges, D.R.A. (2004). Quantitative analysis of 33 benzodiazepines, metabolites and benzodiazepine-like substances in whole blood by liquid chromatography–(tandem) mass spectrometry. *Journal of Chromatography B, 811*, 13–20.

USP (2014). USP-37: General Information Chapter ‹1224›: Transfer of analytical procedures. Rockville, Maryland, USA: United States Pharmacopeia.

Vecchione, G., Casetta, B., Chiapparino, A., Bertolino, A., Tomaiuolo, M., Cappucci, F., ... Grandone E. (2012). A reliable and rapid tool for plasma quantification of 18 psychotropic drugs by ESI tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, *67*(68), 104-113.

CONCLUSIONS AND PERSPECTIVES

Psychotropic drugs are associated with a considerable interindividual variability in their pharmacokinetic properties, which demonstrates the relevance of TDM in the optimisation of treatment with these drugs. Genetic peculiarities, concurrent disease, age and concomitant medications are among other factors that affect patients' ability to absorb, distribute, metabolise and eliminate psychotropic drugs, resulting in a great variation in blood concentration levels of these drugs. To adjust the dosage of prescribed medications according to the characteristics of individual patient, TDM plays a major role. In fact, one should not pretend optimizing psychotropic treatment without TDM, especially when the treatment involves tricyclic antidepressants, typical antipsychotics, barbiturates or other drugs requiring TDM. Regardless of the drug class, TDM is recommended whenever medication noncompliance is suspected, when there is a lack of therapeutic effects at usual doses, but also in case of potential drug-drug interactions.

In psychopharmacotherapy, incidence of undesirable effects is often dose-related and for a large number of psychotropic drugs, the same correlation is observed for therapeutic effects and blood concentration levels. Therefore, dose adjustment is often required for treatment optimisation and adequate dose adjustment should be based on blood concentration level of drugs rather than on simple routine patient assessment.

In addition to TDM, measuring blood concentration levels of psychotropic drugs may be relevant in other situations including intoxication detection and forensic cases. This practice can also play a paramount role in quality control of drugs used in countries like Rwanda where quality control tests are limited to visual inspection in most cases. In fact, Rwanda is not spared from the trafficking of poor quality drugs including counterfeit drugs representing around 10% of drugs sold worldwide, a rate that can exceed 30% in some parts of Africa and Asia (134, 135).

Even though detailed information about the use of psychotropic in Rwanda is not easy to get, according to the Mental health department of the Ministry of health, cases of mental problems that could involve the use of psychotropic drugs in Rwanda were 64,038 (around 5% of the total Rwandan population) in 2015 and 10,909 new cases were recorded between June 2014 and July 2015. As far as Rwanda is concerned, it is almost impossible to optimise treatment with psychotropic medications, knowing that no determination of blood concentration levels is conducted neither for TDM purpose nor for any other reason.

To solve such a problem, various steps were taken. In first phase, psychotropic drugs commonly prescribed in Rwanda were identified in order to develop a suitable analytical method that could be applied in carrying out TDM and detection of intoxications involving these drugs. The identification of concerned drugs was realised through a survey on the use of psychotropic drugs conducted in Rwandan referral hospitals and other institutions involved in management of these drugs. Based on the results of this survey, twenty seven molecules belonging to various pharmacological classes of psychotropic drugs were selected: alprazolam, amitriptyline, bromazepam, carbamazepine, chlorpromazine, citalopram, clomipramine, clonazepam, diazepam, droperidol, fluoxetine, flupentixol, haloperidol, imipramine, levomepromazine, lorazepam, midazolam, nordiazepam, olanzapine, phenobarbital, phenytoin, pipamperone, risperidone, sulpiride, thiopental, zolpidem and zuclopenthixol.

In the second phase, an analytical technique that can be applied in the determination of selected drugs was validated. This technique described in the publication 1 consists in a high performance liquid chromatography with a diode array detection suitable for a simultaneous determination of various psychotropic drugs in serum. The sample preparation process is relatively simple as it involves a liquid-liquid extraction requiring simple laboratory materials.

The analytical column used in this technique allows the separation of several psychotropic drugs and a simultaneous quantification of various molecules which is particularly interesting especially in the case of polymedication. This HPLC/DAD method was validated according to the FDA criteria for the 27 molecules selected. In general, this technique is suitable for both therapeutic drug monitoring and detection of intoxications, but for drugs with low therapeutic reference ranges, this method can be used only for the detection of intoxications and this is the case for haloperidol, flupentixol and zuclopenthixol. For these drugs, a more sensitive technique is thus required to adequately carry out therapeutic drug monitoring.

The third phase of this work consisted in the determination of blood concentration levels of psychotropic drugs in Rwandan patients under psychotropic treatment, with the purpose of identifying problems that could be associated with the lack of TDM for these drugs in Rwanda.

Blood samples collected with respect to TDM conditions from 128 patients under psychotropic treatment in Rwanda, were analysed in the Laboratory of Clinical, Forensic, Environmental, and Industrial Toxicology of the University Teaching Hospital of Liège. As can be seen in the publication 2, only 46% of analytical results were found within therapeutic reference ranges with results below and above therapeutic ranges representing respectively 47% and 8% of analytical results. Knowing that the study population included both inpatients and outpatients, medication noncompliance, drug-drug interactions and drug misdosing were possible explanations to plasma concentrations out of therapeutic reference ranges representing 54% of total cases. However, in addition to mentioned reasons of unexpected concentrations, one should not ignore the potential impact of interindividual variability of pharmacokinetic parameters, at least for some cases.

The determination of plasma concentrations based on drug doses, patients' characteristics as well as pharmacokinetic parameters of various drugs revealed potential drug misdosing cases in 23% of total cases. Therefore, other cases of plasma concentrations out of therapeutic reference ranges could be the result of medication noncompliance, drug-drug interactions or other factors. Polymedication cases represented 71% of the total population and drug-drug interactions were predictable in 74% of these patients.

Antidepressants, antipsychotics, antiepileptics, barbiturates and benzodiazepines were classes of 21 different psychotropic drugs found in analysed samples. Serum concentrations out of therapeutic reference ranges were found in all classes. Talking about pharmacological classes of psychotropic drugs, it is important to highlight the predominant use of typical antipsychotics in Rwandan patients. Typical antipsychotics are part of drugs for which TDM is highly recommended and they represented 98% of antipsychotic medications representing 54% of total cases in the study population. These drugs known for their frequent adverse effects and their severe toxicity in case of overdose were involved in 50% of supratherapeutic cases. However, to conclude about overdose cases based on reported side effects was not easy, as the same effects were observed in subtherapeutic, therapeutic and supratherapeutic cases.

Analytical results of blood samples collected from Rwandan patients under treatment with psychotropic medications demonstrated the need of therapeutic drug monitoring for the optimisation of psychotropic treatment. To carry out TDM in Rwanda, an appropriate analytical method is required. The fourth phase of this work was to transfer in Rwanda an analytical method that can be applied in carrying out such activities. The analytical method transfer was realized by revalidation of the method in the receiving laboratory and this for ten molecules most commonly prescribed in Rwanda. The difference between the two

laboratories in terms of analytical customs and equipments was the main reason to choose revalidation of the method as a suitable approach for the method transfer. Taking into account the working conditions in Rwanda and the time allocated to this work, the revalidation of the method involved only 10 of the 27 drugs. However, knowing that selected drugs represented around 90% of cases in Rwandan patients and that the validation on both sides (Belgium and Rwanda) involved the same persons, these drugs were enough to assess the transferability of the method. As described in the publication 3, a method based on high performance liquid chromatography with diode array detection for the determination of psychotropic drugs in serum, was successfully transferred from the Laboratory of Clinical, Forensic, Environmental and Industrial Toxicology/University Teaching Hospital-Liège to the Laboratory of Analysis of Foodstuffs, Drugs, Water and Toxic/University of Rwanda.

The transferred method is suitable for both therapeutic drug monitoring and detection of intoxications.

In conclusion, TDM remains an essential tool for optimizing of psychotropic treatment and reducing the risk of medication discontinuation. As revealed by the analysis of serum samples from Rwandan patients under treatment with psychotropic drugs, these patients are exposed to a high risk of drug ineffectiveness (47%) and drug toxicity (8%) as well. Results within therapeutic reference ranges were found in only 46% of total cases. This could be the result of medication noncompliance, drug-drug interactions, dose misadjustment, poor quality of used drugs, or simply the result of interindividual variability in pharmacokinetic properties of psychotropic medications used. Regardless of the reason, taking into consideration the rate of drug concentrations out of therapeutic reference ranges (54%), the need to determine plasma levels for psychotropic treatment optimisation in Rwanda, is obviously demonstrated. The main reason of not carrying out TDM in Rwanda is the lack of structure and validated method

than can be applied in such activities. The analytical method transferred in the Laboratory of Analysis of Foodstuffs, Drugs, Water and Toxics can be applied to initiate in Rwanda activities of therapeutic drug monitoring and detection of intoxications for psychotropic medications.

The present study can be usefully completed on one side, by a study considering simultaneously laboratory results and treatment outcomes, in order to clearly demonstrate the relevance of TDM in psychotropic treatment in Rwanda. On the other side, a pharmacogenetic study of psychotropic drugs in Rwandan population would be interesting. This study could actually help to identify possible groups' differences in metabolism of these drugs and thus verify the reliability of standard doses as well as therapeutic reference ranges used as far as Rwandan population is concerned.

The analytical method transfer should be done for the remaining drugs and in as many laboratories as possible in Rwandan referral hospitals. The development of other more sensitive techniques allowing the determination of both drugs and their active metabolites is also necessary, to make possible the TDM of all psychotropic drugs used in Rwanda including those with low therapeutic reference ranges.

Treatment optimisation cannot be obtained by simply measuring drug plasma level, it rather requires in addition, a suitable result interpretation as well as an adequate medical decision. Though imperfect, our work definitely constitutes a big contribution to the initiation in Rwanda of therapeutic drug monitoring and related activities required for the optimisation of psychotropic treatment. Patients under treatment with psychotropic drugs in Butare University Teaching Hospital will be the first to benefit from TDM in the near future. TDM activities will then be integrated into routine activities of the National Reference Laboratory and other referral hospital laboratories countrywide.

REFERENCES

1. Baumann P, Hiemke C, Ulrich S, et al. The AGNP-TDM expert group consensus guidelines: therapeutic drug monitoring in psychiatry. *Pharmacopsychiatry* 2004; 37: 243-265.

2. Touw DJ, Neef C, Thomson AH &Vinks AA. Cost-effectiveness of therapeutic drug monitoring: a systematic review. *Ther Drug Monit* 2005; 27: 10–17.

3. Kang JS & Lee MH. Overview of Therapeutic Drug Monitoring. *Korean J Intern Med* 2009; 24: 1-10.

4. Dasgupta A. *Hand book of drug monitoring methods: Therapeutics and drugs of abuse*. Humana Press Inc. 999 Riverview Drive, Suite 208, Totowa, New Jersey 07512, 2008.

5. Wallemacq EP. Therapeutic monitoring of immunosuppressant drugs. Where are we? *Clinical Chemistry and Laboratory Medicine* 2005; 42(11): 1204-1211.

6. Michel M, Wallemacq P, König J et al. Therapeutic monitoring of mycophenolate Mofetil in organ transplant recipients. *Clinical Pharmacokinetics* 2002; 41(5): 319-327.

7. Wallemacq P. Therapeutic drug monitoring of imatinib. Rev Bras Hematol Hemoter 2011; 33(4): 250-8.

8. Marquet P & Kintz P. Therapeutic drug monitoring of high-dose buprenorphine: why and how? *Annales de Toxicologie Analytique*, vol. XVI, n° 4, 2004.

9. Marquet P. Clinical application of population pharmacokinetic methods developed of immunosuppressive drugs. *Ther Drug Monit*. 2005; 27(6): 727-732.

10. Sauvage FL, Gaulier JM, Lachatre G & Marquet P. A fully automated turbulent-flow liquid chromatography- tandem Mass spectrometry technique for monitoring antidepressants in human serum. *Ther Drug Monit*. 2006; 28(1): 123-130.

11. Saint-Marcoux F, Ludovic SF & Marquet P. Current role of LC-MS in therapeutic drug monitoring. *Anal Bioanal Chem* 2007; 388: 1327-1329.

12. Shenfield GM. Therapeutic drug monitoring beyond 2000. *Br J Clin Pharmacol*

2001; 52 (Suppl 1): 3S-4S.

13. Hiemke C, Baumann P, Bergemann N, Conca A, et al. AGNP Consensus Guidelines for Therapeutic Drug Monitoring in Psychiatry: Update 2011. *Pharmacopsychiatry* 2011; 44:195–235.

14. Bengtsson F. Therapeutic drug monitoring of psychotropic drugs. TDM "nouveau". *Ther Drug Monit* 2004; 26:145–151.

15. Hiemke C. Therapeutic drug monitoring in neuropharmacology: does it hold its promises? *Eur Arch Psychiatry Clin Neurosci* 2008; 258 (Suppl 1): 21–27.

16. Hiemke C. Clinical utility of drug measurement and pharmacokinetics therapeutic drug monitoring in psychiatry. *Eur J Clin Pharmacol* 2008; 64:159–166.

17. Jaquenoud SE, Knezevic B, Morena GP et al. ABCB1 and cytochrome P450 polymorphisms: clinical pharmacogenetics of clozapine. *J Clin Psychopharmacol* 2009; 29:319 – 326.

18. Klotz U. Pharmacokinetics and drug metabolism in the elderly. *Drug Metabolism Reviews* 2009; 41(2):67–76.

19. Raggi MA. Therapeutic drug monitoring: chemical–clinical correlations of atypical antipsychotic drugs, *Curr. Med. Chem.* 2002; 9:1397–1409.

20. Charlier C, Pinto E, Ansseau M & Plomteux G. Relationship between clinical effects, serum drug concentration, and concurrent drug interactions in depressed patients treated with citalopram, fluoxetine, clomipramine, paroxetine or venlafaxine. *Hum. Psychopharmacol. clin. Exp. 2000*; 15: 453-459.

21. Charlier C, Pinto E, Ansseau M & Plomteux G. Venlafaxine: the relationship between dose, plasma concentration and clinical response in depressive patients. *Journal of psychopharmacology* 2002; 16(4): 369-372.

22. Charlier C, Ansseau M, Gougnard T et al. Le monitoring thérapeutique des

antidépresseurs. *Rev Med Liège* 1997; 52(5): 336-344.

23. Conca A, Schmidt E, Pastore M et al. Therapeutic drug monitoring in Italian psychiatry. *Pharmacopsychiatry* 2011; 44: 259-262.
24. Zernig G, Lechner T, Kramer-Reinstadler K et al. What the clinician still has to be reminded of. *Ther Drug Monit* 2004; 26: 582.
25. Chen P, Tanasijevic MJ, Schoenenberger RA et al. A computer-based intervention for improving the appropriateness of antiepileptic drug level monitoring. *Am J Clin Pathol* 2003; 119: 432-438.
26. Anderson IM, Ferrier IN, Baldwin RC et al. Evidence-based guidelines for treating depressive disorders with antidepressants: a revision of the 2000 British Association for Psychopharmacology guidelines. *J Psychopharmacol* 2008; 22: 343-396.
27. Buchanan RW, Kreyenbuhl J, Kelly DL et al. Schizophrenia Patient Outcomes Research Team (PORT). The 2009 schizophrenia PORT psychopharmacological treatment recommendations and summary statements. *Schizophr Bull* 2010; 36: 71-93.
28. Falkai P, Wobrock T, Lieberman J et al. World Federation of Societies of Biological Psychiatry (WFSBP) guidelines for biological treatment of schizophrenia, Part 1: acute treatment of schizophrenia. *World J Biol Psychiatry* 2005; 6: 132-191.
29. Falkai P, Wobrock T, Lieberman J et al. World Federation of Societies of Biological Psychiatry (WFSBP) guidelines for biological treatment of schizophrenia, part 2: long-term treatment of schizophrenia. *World J Biol Psychiatry* 2006; 7: 5-40.
30. Goodwin GM. Consensus Group of the British Association for Psychopharmacology. Evidence-based guidelines for treating bipolar disorder: revised second edition-recommendations from the British Association for Psychopharmacology. *J Psychopharmacol* 2009; 23: 346-388.
31. Grunze H, Vieta E, Goodwin GM. The World Federation of Societies of Biological

Psychiatry (WFSBP) guidelines for the biological treatment of bipolar disorders: update 2009 on the treatment of acute mania. *World J Biol Psychiatry* 2009; 10: 85-116.

32. Haberstroh J, Hampel H, Pantel J. Optimal management of Alzheimer's disease patients: Clinical guidelines and family advice. *Neuropsychiatr Dis Treat* 2010; 6: 243-253.
33. Sartorius N, Baghai TC, Baldwin DS et al. Antidepressant medications and other treatments of depressive disorders: a CINP Task Force report based on a review of evidence. *Int J Neuropsychopharmacol* 2007; 10 (Suppl 1): S1-S207.
34. Zimmerman NP, Hickie IB, McGorry PD. Guidelines for youth depression: time to incorporate new perspectives. *Med J Aust* 2010; 193: 557.
35. Mistretta V & Charlier C. Ce que les psychiatres devraient connaitre sur l'intérêt du dosage des antidépresseurs dans la pratique clinique. *Acta Psychiatrica Belgica* 2012; 112(1): 45-51.
36. Åkerblad AC, Bengtsson F, Ekselius L et al. Effects of an educational compliance enhancement programme and therapeutic drug monitoring on treatment adherence in depressed patients managed by general practitioners. *Int Clin Psychopharmacol* 2003; 18: 347-354.
37. Beasley CM, Stauffer VL, Liu-Seifert H et al. All-cause treatment discontinuation in schizophrenia during treatment with olanzapine relative to other antipsychotics: an integrated analysis. *J Clin Psychopharmacol* 2007; 27: 252-258.
38. Lingam R & Scott J. Treatment non-adherence in affective disorders. *Acta Psychiatr Scand* 2002; 105: 164-172.
39. Kane JM, Leucht S, Carpenter D et al. The expert consensus guideline series. Optimizing pharmacologic treatment of psychotic disorders. Introduction: methods, commentary, and summary. *J Clin Psychiatry* 2003; 12 (Suppl): 5-19.
40. Meijer WE, Bouvy ML, Heerdink ER et al. Spontaneous lapses in dosing during chronic

treatment with selective serotonin reuptake inhibitors. *Br J Psychiatry* 2001; 179: 519-522.

41. Åkerblad AC, Bengtsson F, Holgersson M et al. Identification of primary care patients at risk of nonadherence to antidepressant treatment. *Patient Prefer Adherence* 2008; 2: 379-386.
42. Byerly MJ, Thompson A, Carmody T et al. Validity of electronically monitored medication adherence and conventional adherence measures in schizophrenia. *Psychiatr Serv* 2007; 58: 844-847.
43. Katon W, Cantrell CR, Sokol M S et al. Impact of antidepressant drug adherence on comorbid medication use and resource utilization. *Arch Intern Med* 2005; 165: 2497-2503.
44. Sajatovic M, Velligan DI, Weiden PJ et al. Measurement of psychiatric treatment adherence. *J Psychosom Res* 2010; 69: 591-599.
45. Velligan DI, Lam YW, Glahn DC et al. Defining and assessing adherence to oral antipsychotics: a review of the literature. *Schizophr Bull* 2006; 32: 724-742.
46. Weiden PJ, Kozma C, Grogg A et al. Partial compliance and risk of rehospitalization among California Medicaid patients with schizophrenia. Psychiatr Serv 2004; 55: 886-891.
47. Ministry of Health of Rwanda. https://hmis.moh.gov.rw/hmis, accessed on October 5, 2015.
48. Ban AT. Pharmacotherapy of mental illness - A historical analysis. *Prog. Neuro-Psychopharmacol. & Biol. Psychiat.* 2001; 25: 709-727.
49. Chlorpromazine chemical structure, available at *http://www.chemspider.com/Chemical-Structure.2625.html*, accessed on 08/09/2015.
50. Janicak PG, Marder SR & Pavuluri MN. *Principles and practice of psychopharmacotherapy.* 5th ed. Lippincott Williams & Wilkins, 2001 Market Street, Philadelphia, PA 19103 USA, 2011.

51. Coutts RT & Urichuk LJ. Polymorphic cytochromes P450 and drugs used in psychiatry. *Cell Mol Neurobiol.* 1999; 19: 325-354.

52. Greenblatt DJ, von Moltke LL & Shader RI. The importance of presystemic extraction in clinical psycho-pharmacology. *J Cin Psychopharmacol.* 1996; 16: 417-419.

53. Vgontzas AN, Kales A, Bixler EO. Benzodiazepines side effects: role of pharmacokinetics and pharmacodynamics. *Pharmacology.* 1995; 51: 205-223.

54. De Vane CL & Pollock BG. Pharmacokinetics consideration of antidepressant use in the elderly. *J Clin Psychiatry.* 1999; 60 (suppl 20): 38-44.

55. Klotz U. Effect of age on pharmacokinetics and pharmacodynamics in man. *Inter J Pharmacol Ther.* 1998; 36: 581-585.

56. Perry JP, Alexander B, Liskow BI & DeVane LC. *Psychotropic drug handbook*. 8th ed. Lippincott Williams & Wilkins, 351 West Camden Street, Baltimore, MD 21201, 2007.

57. Bertelsen KM, Venkatakrishnan K, Von Moltke LL et al. Apparent mechanism-based inhibition of human CYP2D6 in vitro by paroxetine: comparison with fluoxetine and quinidine. *Drug Metab Dispos* 2003; 31: 289–293.

58. Zhou SF. Polymorphism of human cytochrome P450 2D6 and its clinical significance: Part I. *Clin Pharmacokinet* 2009; 48: 689–723.

59. Zhou SF. Polymorphism of human cytochrome P450 2D6 and its clinical significance: Part II. *Clin Pharmacokinet* 2009; 48: 761–804.

60. Zhou SF, Liu JP & Chowbay B. Polymorphism of human cytochrome P450 enzymes and its clinical impact. *Drug Metab Rev* 2009; 41: 89–295.

61. Court MH. Interindividual variability in hepatic drug glucuronidation: studies into the role of age, sex, enzyme inducers, and genetic polymorphism using the human liver bank as a model system. *Drug Metab Rev* 2010; 42: 202–217.

62. Barski OA, Tipparaju SM & Bhatnagar A. The aldo-keto reductase superfamily and its

role in drug metabolism and detoxification . *Drug Metab Rev* 2008; 40: 553–624

63. Beedham C, Miceli JJ & Obach RS. Ziprasidone metabolism, aldehyde oxidase, and clinical implications. *J Clin Psychopharmaco*l 2003; 23: 229–232.
64. Breyer-Pfaff U & Nill K . Carbonyl reduction of naltrexone and dolasetron by oxidoreductases isolated from human liver cytosol. *J Pharm Pharmacol* 2004; 56: 1601–1606.
65. Benedetti M S, Whomsley R, Poggesi I et al. Drug metabolism and pharmacokinetics. *Drug Metab Rev* 2009; 41: 344–390.
66. Gervasini G, Carrillo JA & Benitez J. Potential role of cerebral cytochrome P450 in clinical pharmacokinetics: modulation by endogenous compounds. *Clin Pharmacokinet* 2004; 43: 693–706.
67. Meyer RP, Gehlhaus M, Knoth R et al. Expression and function of cytochrome p450 in brain drug metabolism. *Curr Drug Metab* 2007; 8: 297–306.
68. Klotz U. Pharmacokinetics and drug metabolism in the elderly. *Drug Metab Rev* 2009; 41: 67–76.
69. Aichhorn W, Marksteiner J, Walch T et al. Influence of age, gender, body weight and valproate comedication on quetiapine plasma concentrations. *Int Clin Psychopharmacol* 2006; 21: 81–85.
70. Aichhorn W, Weiss U, Marksteiner J et al. Influence of age and gender on risperidone plasma concentrations. *J Psychopharmacol* 2005; 19: 395–401.
71. Aichhorn W, Whitworth AB, Weiss ME et al. Second-generation antipsychotics: Is there evidence for sex differences in pharmacokinetic and adverse effect profiles? *Drug Saf* 2006; 29: 587–598.
72. Soldin P & Mattison DR. Sex differences in pharmacokinetics and pharmacodynamics. *Clin Pharmacokinet* 2009; 48: 143–157.

73. Hirchfeld RM & Vornik LA. Newer antidepressants: review of efficacy and safety of escitalopram and duloxetine. J Clin Psychiatry. 2004; 65(suppl 4): 46-52.

74. Stahl SM. *Stahl's Essential Psychopharmacology: Neuroscientific basis and practical applications*. 4th ed. Cambridge University Press, New York, 2013.

75. Ebert U. Basic Mechanisms of Psychotropic Drugs. *Epilepsia* 2002; 43(Suppl. 2): 2–7.

76. Strange PG. Antipsychotic Drugs: Importance of dopamine receptors for mechanisms of therapeutic actions and side effects. *Pharmacol Rev* 2001; 53: 119–133.

77.http://park12.wakwak.com/~pharma1/textbook/Antipsychotic/Antipsychotic-e.html accessed on August 07, 2015.

78. Horacek J, Bubenikova-Valesova V, Kopecek M et al. Antipsychotic Drugs and the Neurobiology of Schizophrenia. *CNS Drugs* 2006; 20(5): 389-409.

79. Pickford M. Antipsychotic drug overdose. *Emergency nurse* 2000; 7(9): 17-22.

80. Minns AB & Clarck RF. Toxicology and overdose of atypical antipsychotics. *Am J Emerg Med* 2012; 43(12): 906-913.

81. Dubois D. Toxicology and overdose of atypical antipsychotic medications in children: does new necessarily mean safer? *Curr Opin Pediatr* 2005; 17(2): 227-33.

82. Tan HH, Hoppe J & Heard K. A systematic review of cardiovascular effects following atypical antipsychotic overdose. *Am J Emerg Med* 2009; 27: 607-16.

83. Burns MJ. The pharmacology and toxicology of atypical antipsychotic agents. *J Toxicol Clin Toxicol* 2001; 39: 1-14.

84. Kendler KS & Gardner CO. Boundaries of major depression: An evaluation of DSM-IV criteria. *Am J Psychiatry*. 1998; 155: 172–177.

85. Kendler KS, Myers J & Zisook S. Does bereavement-related major depression differ from major depression associated with other stressful life events? *Am J Psychiatry*. 2008; 165: 1449-1455.

86. Ustun TB, Ayuso-Mateos JL, Chatterji S et al. Global burden of depressive disorders in the year 2000. *Br J Psychiatry*. 2004; 184: 386-392.

87. Tournier M, Grolleau A, Cougnard A et al. Factors associated with choice of psychotropic drugs used for intentional overdose. *Eur Arch Psychiatry Clin Neurosci.* 2009; 259(2): 86-91.

88. Hawton K, Bergen H, Simkin J et. al. Toxicity of antidepressants: rates of suicide relative to prescribing and non-fatal overdose. *Br J Psychiatry.* 2010; 196: 354-358.

89. Charler C, Broly F, Lhermitte M et al. Polymorphisms in the CYP 2D6 gene: Association with plasma concentrations of fluoxetine and paroxetine. *Therapeutic drug Monitoring* 2003; 25(6): 738-742.

90. Kerr GW, McGoffie AC & Wilkie S. Tricyclic antidepressant overdose: a review. *Emerg Med J.* 2001; 18: 236-241.

91. Bormann J. The "ABC" of GABA receptors. *Trends Pharmacol Sci.* 2000; 21: 16-19.

92. https://www.google.be/search?q=mechanism+of+action+of+barbiturates+and+ benzodiazepin.

93. Weinberger DR. Anxiety at the frontier of molecular medicine. *N Engl J Med.* 2001; 344(16): 1247-1249.

94. Dawson GR, Collinson N & Atack JR. Development of subtype selective $GABA_A$ modulators. *CNS spectr.* 2005; 10: 21-27.

95. De Leon J. Incorporating pharmacogenetics into clinical practice: reality of a new tool in psychiatry. Current issues in clinical implementation. *CNS Spectr* 2006; 11 (Suppl 3): 8–12.

96. Evans WE & Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. *Science* 1999; 286: 487–491.

97. Mrazek DA. Psychiatric pharmacogenomic testing in clinical practice. *Dialogues Clin*

Neurosci 2010; 12: 69–76.

98. Deville M, Denooz R & Charlier. Toxicité induite par les antidépresseurs: Etat des lieux. *Acta Psychiatrica Belgica* 2013; 113(2): 17-21.

99. Bergmann TK, Bathum L & Brøsen K. Duplication of CYP2D6 predicts high clearance of desipramine but high clearance does not predict duplication of CYP2D6. *Eur J Clin Pharmacol* 2001; 57: 123–127.

100. De Leon J, Susce MT, Pan RM et al. The CYP2D6 poor metabolizer phenotype may be associated with risperidone adverse drug reactions and discontinuation. *J Clin Psychiatry* 2005; 66: 15–27.

101. De Leon J. Glucuronidation enzymes, genes and psychiatry. *Int J Neuropsychopharmacol* 2003; 6: 57–72.

102. Valdes RJ, Payne DA & Linder MW (eds.). *Laboratory medicine practice guidelines and recommendations for laboratory analysis and application of pharmacogenetics to clinical practice*. Washington, DC: National Academy of Clinical Biochemistry, 2010.

103. Faber MS, Jetter A & Fuhr U. Assessment of CYP1A2 activity in clinical practice: why, how, and when? *Basic Clin Pharmacol Toxicol* 2005; 97: 125–134.

104. Ferrari A, Bertolotti M, Dell UA et al. Serum time course of naltrexone and 6ß-naltrexol levels during long term treatment in drug addicts. *Drug Alcohol Depend* 1998; 52: 211–220.

105. Zullino DF, Delessert D, Eap CB et al. Tobacco and cannabis smoking cessation can lead to intoxication with clozapine or olanzapine. *Int Clin Psychopharmacol* 2002; 17: 141–143.

106. Abbott NJ, Patabendige AA, Dolman DE et al. Structure and function of the blood-brain barrier. *Neurobiol Dis* 2010; 37: 13–25.

107. Choong E, Dobrinas M, Carrupt PA et al. The permeability P-glycoprotein: a focus on

enantioselectivity and brain distribution. *Expert Opin Drug Metab Toxicol* 2010; 6: 953–965.

108. Dutheil F, Jacob A, Dauchy S et al. ABC transporters and cytochromes P450 in the human central nervous system: influence on brain pharmacokinetics and contribution to neurodegenerative disorders. *Expert Opin Drug Metab Toxicol* 2010; 6: 1161–1174.

109. Fenner KS, Troutman MD, Kempshall S et al. Drug-drug interactions mediated through P-glycoprotein: clinical relevance and in vitro-in vivo correlation using digoxin as a probe drug. *Clin Pharmacol Ther* 2009; 85: 173–181.

110. Lee CA, Cook JA, Reyner EL et al. P-glycoprotein related drug interactions: clinical importance and a consideration of disease states. *Expert Opin Drug Metab Toxicol* 2010; 6: 603–619.

111. Uhr M, Tontsch A, Namendorf C et al. Polymorphisms in the drug transporter gene ABCB1 predict antidepressant treatment response in depression. *Neuron* 2008; 57: 203–239.

112. Ujiie Y, Fukasawa T, Yasui-Furukori N et al. Rifampicin markedly decreases plasma concentration and hypnotic effect of brotizolam. *Ther Drug Monit* 2006; 28: 299–302.

113. Gunes A, Spina E, Dahl ML et al. ABCB1 polymorphisms influence steady-state plasma levels of 9-hydroxyrisperidone and risperidone active moiety. *Ther Drug Monit* 2008; 30: 628–633.

114. Roberts RL, Joyce PR, Mulder RT et al. A common P-glycoprotein polymorphism is associated with nortriptyline-induced postural hypotension in patients treated for major depression. *Pharmacogenomics J* 2002; 2: 191–196.

115. Bauer LA. *Applied Clinical pharmacokinetics*. 2nd ed. University of Washington, Schools of Pharmacy and Medicine; Seattle, 2008.

116. Hermida J, Paz E, Tutor JC. Clozapine and norclozapine concentrations in serum and

plasma samples from schizophrenic patients. *Ther Drug Monit* 2008; 30: 41-45.

117. Heller S, Hiemke C, Stroba G et al. Assessment of storage and transport stability of new antidepressant and antipsychotic drugs for a nationwide TDM service. *Ther Drug Monit* 2004; 26: 459-461.
118. Denooz R, Mercerolle M, Lachâtre G & Charlier C. Ultra-Performance Liquid Chromatography–Tandem Mass Spectrometry method for the determination of bupropion and its main metabolites in human whole blood. *Journal of Analytical Toxicology* 2010; 34: 280-286.
119. Greiner C, Hiemke C, Bader W et al. Determination of citalopram and escitalopram together with their active main metabolites desmethyl (es-) citalopram in human serum by column-switching high performance liquid chromatography (HPLC) and spectrophotometric detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 2007; 848: 391–394.
120. Härtter S, Weigmann H, Hiemke C. Automated determination of reboxetine by high-performance liquid chromatography with column-switching and ultraviolet detection. *J Chromatogr B Biomed Sci Appl* 2000; 740: 135–140.
121. Waldschmitt C, Vogel F, Maurer C et al. Measurement of duloxetine in blood using high-performance liquid chromatography with spectrophotometric detection and column switching. *Ther Drug Monit* 2007; 29: 767–772.
122. Kirschbaum KM, Müller MJ, Zernig G et al. Therapeutic monitoring of aripiprazole by HPLC with column-switching and spectrophotometric detection. *Clin Chem* 2005; 51: 1718–1721.
123. Sachse J, Härtter S, Hiemke C. Automated determination of ziprasidone by HPLC with column switching and spectrophotometric detection. *Ther Drug Monit* 2005; 27: 158–162.

124. Sachse J, Härtter S, Weigmann H et al. Automated determination of amisulpride by liquid chromatography with column switching and spectrophotometric detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 2003; 784: 405–410.

125. Kirchherr H, Kühn-Velten WN. Quantitative determination of forty eight antidepressants and antipsychotics in human serum by HPLC tandem mass spectrometry: a multi-level, single-sample approach. *J Chromatogr B* 2006; 843: 100-113.

126. Schulz M, Schmoldt A. Therapeutic and toxic blood concentrations of more than 800 drugs and other xenobiotics. *Pharmazie* 2003; 58: 447-474.

127. High-Performance Liquid Chromatography [HPLC] System. Available at http://www.waters.com/waters/en_US/How-Does-High-Performance-Liquid-Chromatography-Work? Accessed on October 15, 2015.

128. Rouessac F, Rouessac A & Ourisson G, 2000. *Analyse chimique: Méthodes et techniques instrumentales modernes, 5^{e} édition*. Dunod, Paris, 430 p.

129. Hubert P, Nguyen-Huu JJ, Boulanger B et al. Harmonization of strategies for validation of quantitative analytical procedures. A SFSTP proposal-Part III. *Journal of Pharmaceutical and Biomedical Analysis* 2007; 45: 82-96.

130. Hubert P, Nguyen-Huu JJ, Boulanger B et al. Harmonization of strategies for validation of quantitative analytical procedures. A SFSTP proposal-Part IV. *Journal of Pharmaceutical and Biomedical Analysis* 2008; 48: 760-771.

131. Hubert P, Nguyen-Huu JJ, Boulanger B et al. Harmonization of strategies for validation of quantitative analytical procedures. A SFSTP proposal-Part II. *Journal of pharmaceutical and Biomedical Analysis* 2007; 45: 70-81.

132. Rozet E, Ceccato A, Hubert C et al. Analysis of recent pharmaceutical regulatory documents on analytical method validation. *Journal of Chromatography A* 2007; 1158: 111-125.

133. Pfuhlmann B, Gerlach M, Burger R et al. Therapeutic drug monitoring of tricyclic antidepressants in everyday clinical practice. *J Neural Transm* 2007; [Suppl72]: 287–296.

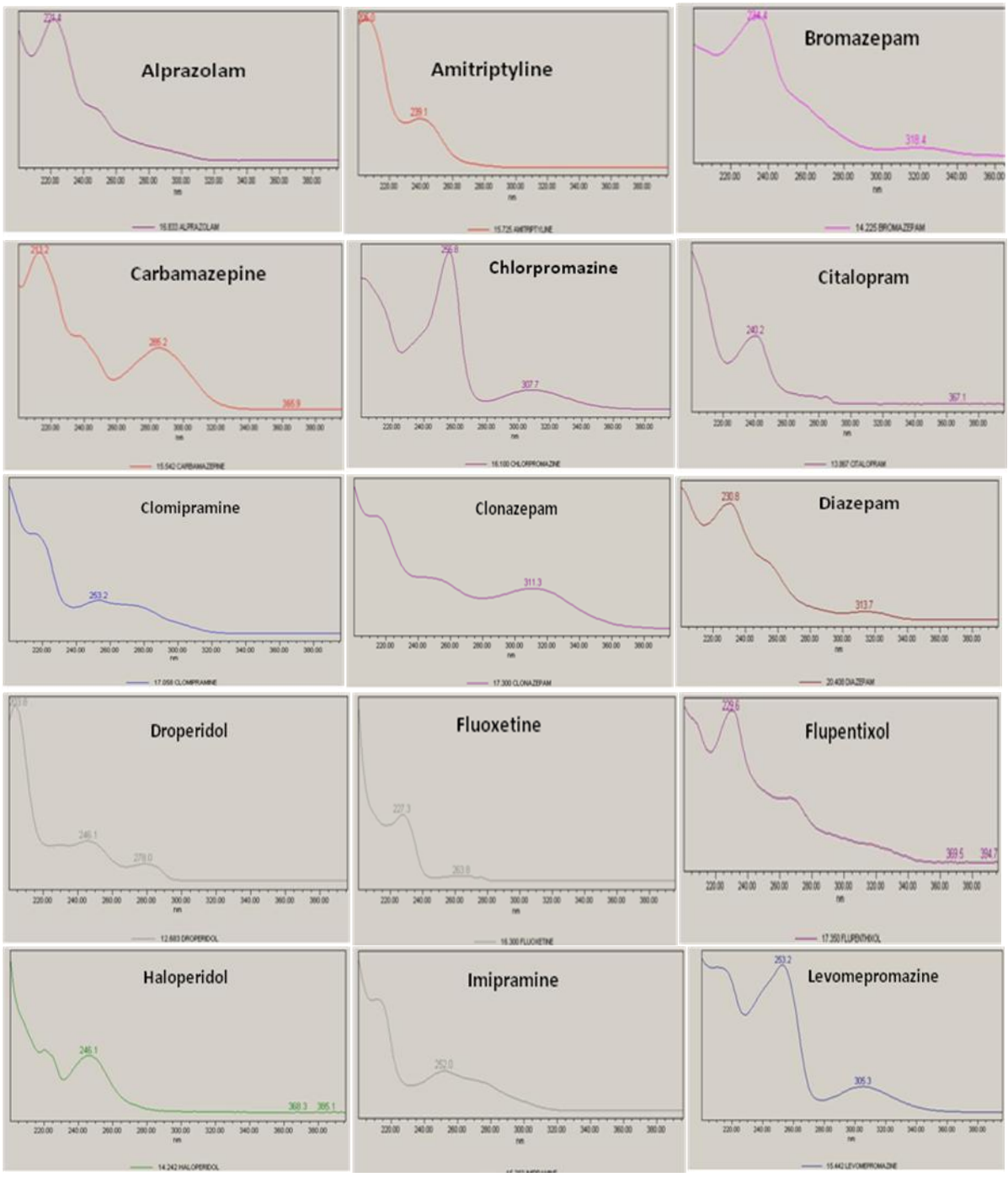
134. Sanofi-Aventis, Press Pack: Drug Counterfeiting (2008). Available on http://ec.europa.eu/internal_market/indprop/docs/conf2008/wilfried_roge_en.pdf. Accessed on March 19, 2016.

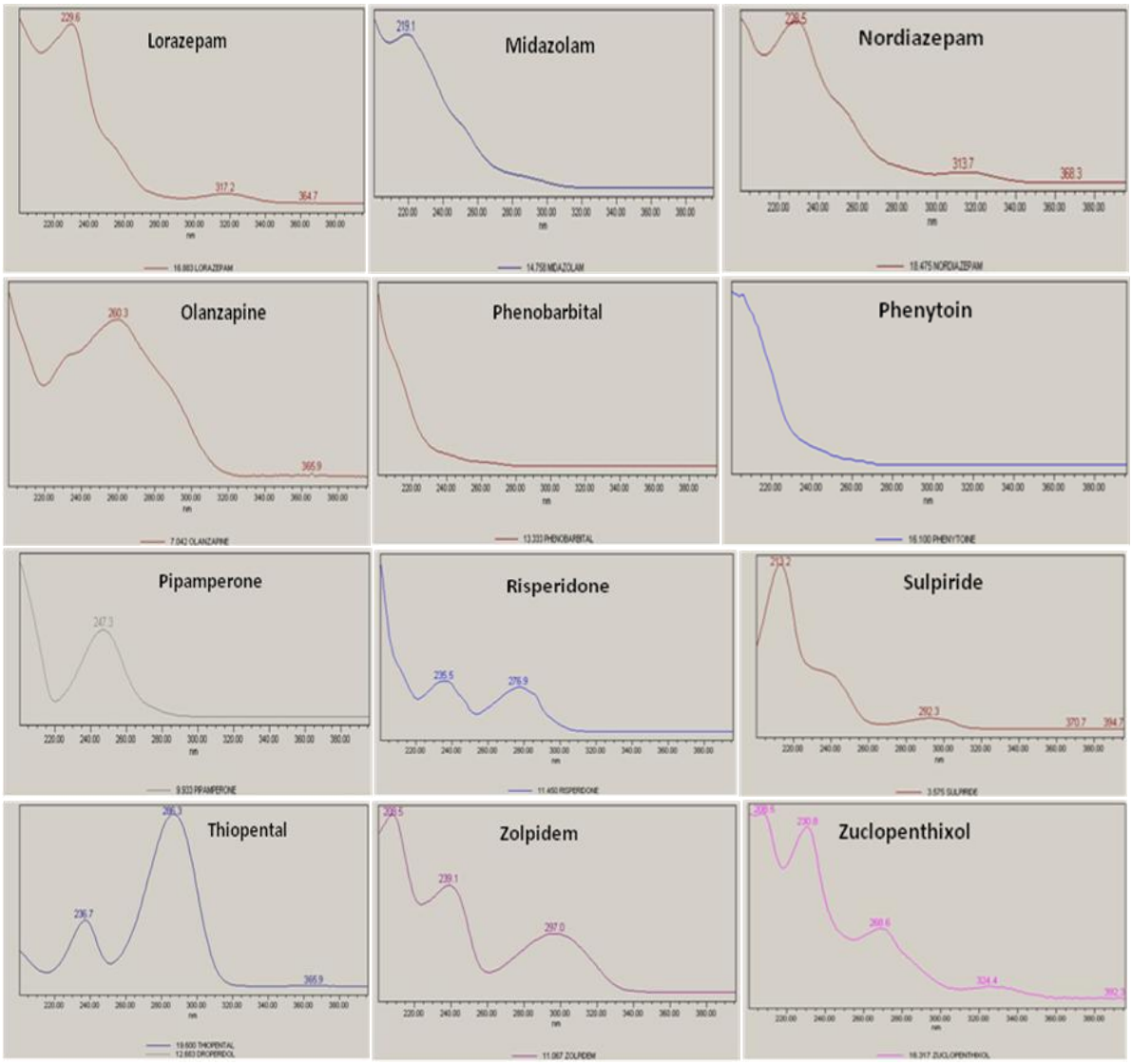
135. Habyalimana V, Mbinze JK, Tshilombo NK et al. Analytical Tools and Strategic Approach to Detect Poor Quality Medicines, Identify Unknown Components, and Timely Alerts for Appropriate Measures: Case Study of Antimalarial Medicines. *American Journal of Analytical Chemistry* 2015, 6: 977-994.

APPENDICES

Appendix 1

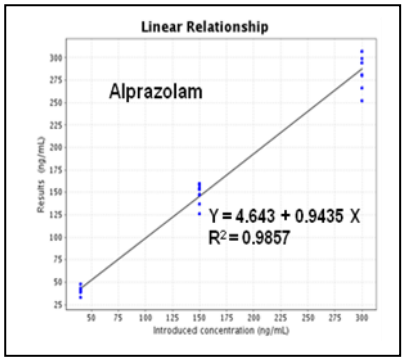
UV-visible spectra of various compounds between 200 and 400 nm

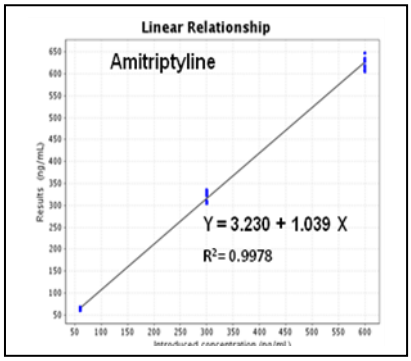


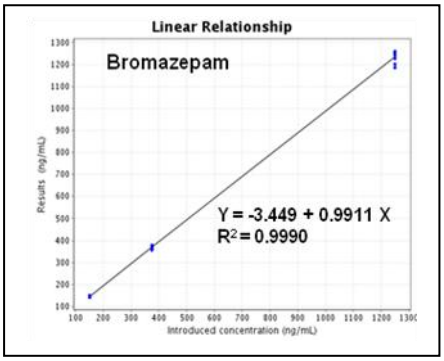

Lorazepam
229.6
317.2
364.7
Midazolam
219.1
Nordiazepam
228.5
313.7
368.3
Olanzapine
260.3
365.9
Phenobarbital
Phenytoin
Pipamperone
247.3
Risperidone
236.5
276.9
Sulpiride
213.2
292.3
370.7
394.7
Thiopental
236.7
286.3
365.9
Zolpidem
208.5
239.1
297.0
Zuclopenthixol
208.5
230.8
268.6
324.4

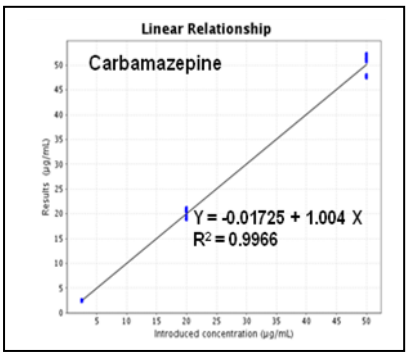
Appendix 2

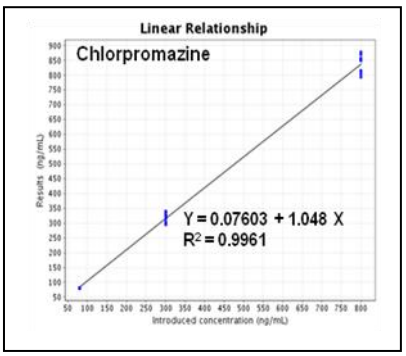
Analytical validation: Linearity

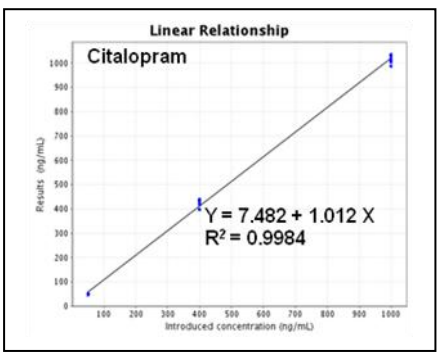


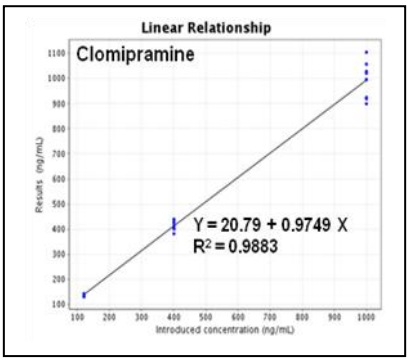


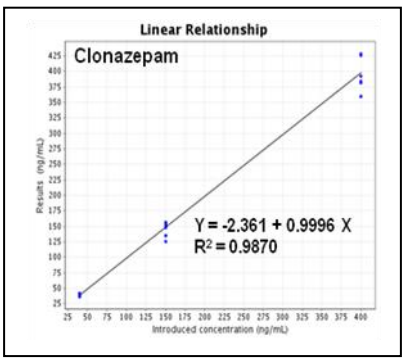


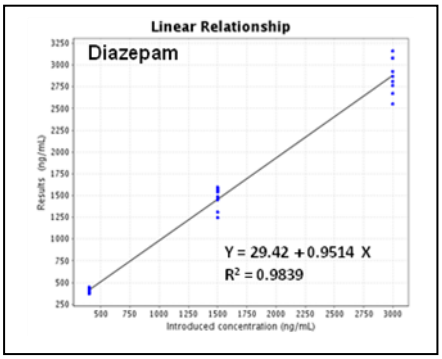


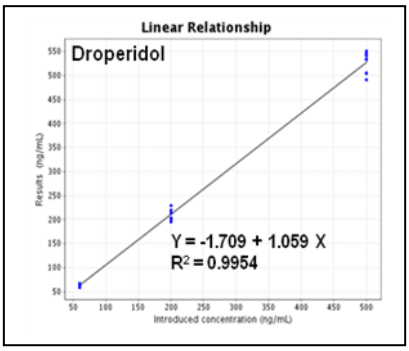


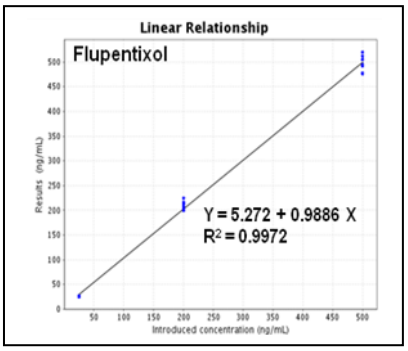


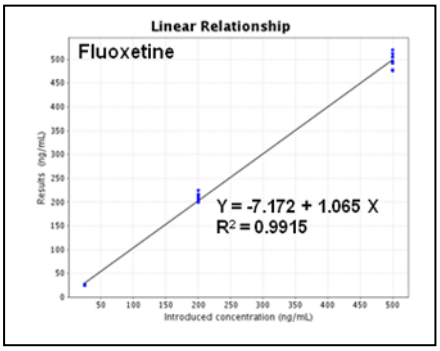


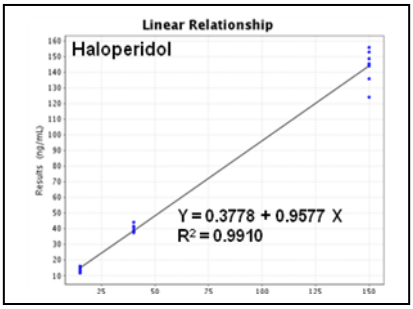


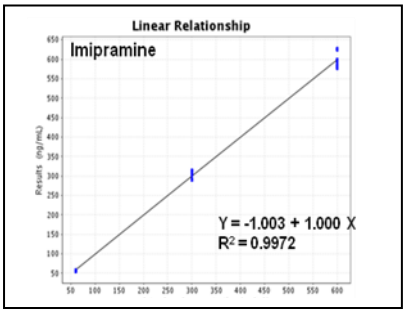


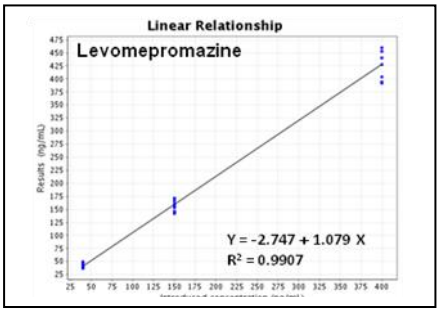


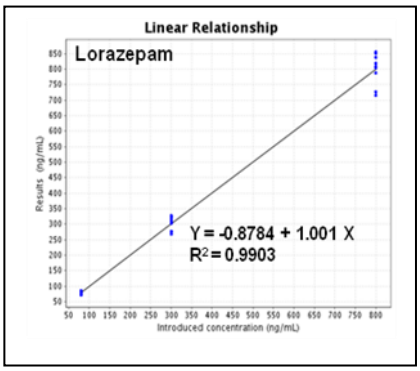


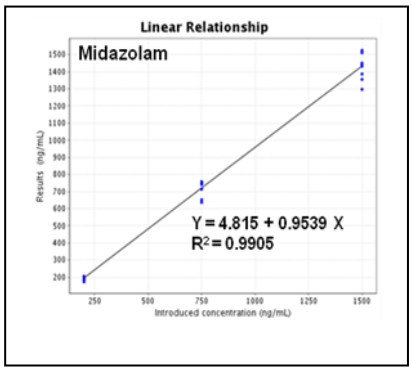


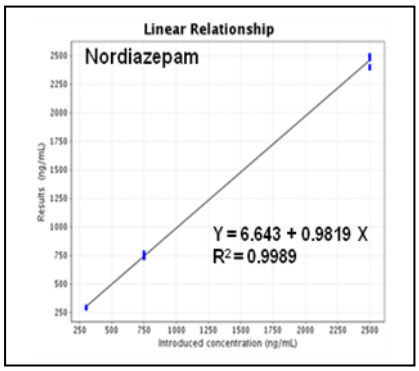


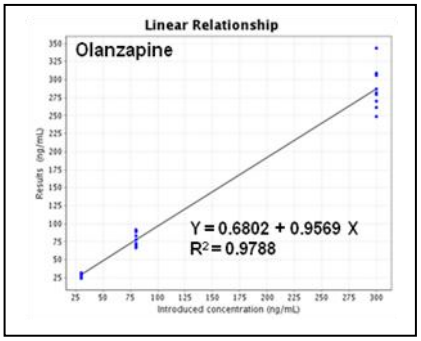


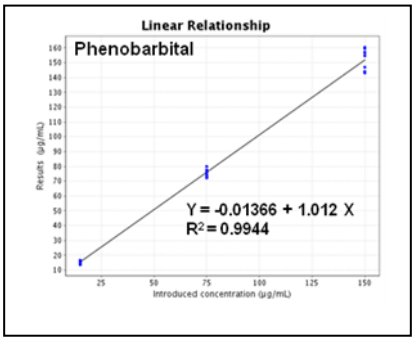


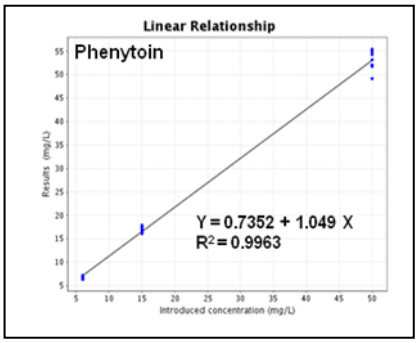

Linear Relationship
Lorazepam
Y = -0.8784 + 1.001 X
R² = 0.9903
Results (ng/mL)
Introduced concentration (ng/mL)

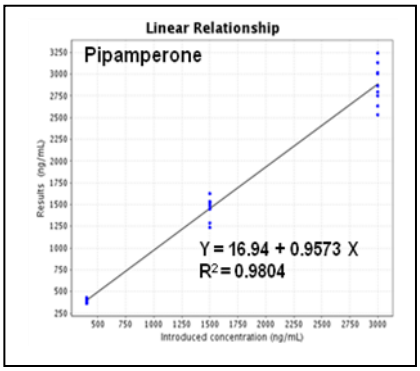

Linear Relationship
Midazolam
Y = 4.815 + 0.9539 X
R² = 0.9905
Results (ng/mL)
Introduced concentration (ng/mL)

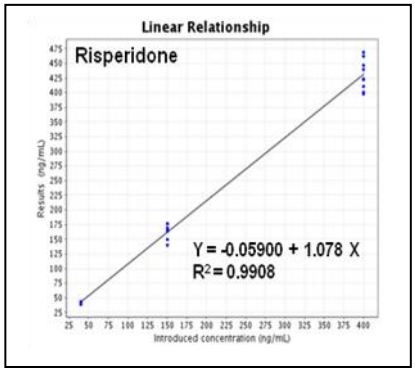

Linear Relationship
Nordiazepam
Y = 6.643 + 0.9819 X
R² = 0.9989
Results (ng/mL)
Introduced concentration (ng/mL)

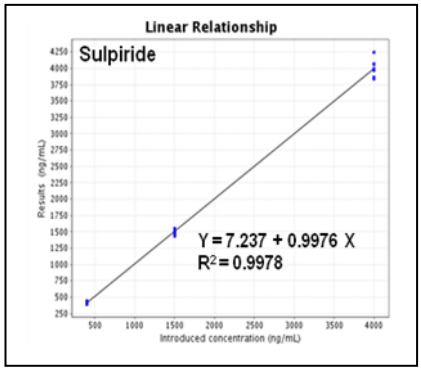

Linear Relationship
Olanzapine
Y = 0.6802 + 0.9569 X
R² = 0.9788
Results (ng/mL)
Introduced concentration (ng/mL)

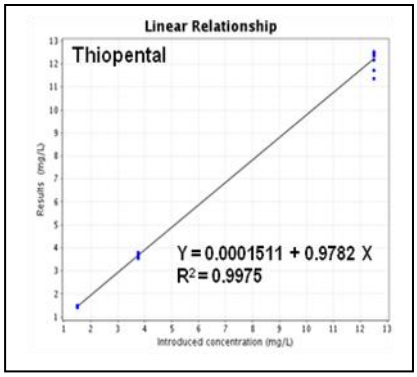

Linear Relationship
Phenobarbital
Y = -0.01366 + 1.012 X
R² = 0.9944
Results (µg/mL)
Introduced concentration (µg/mL)

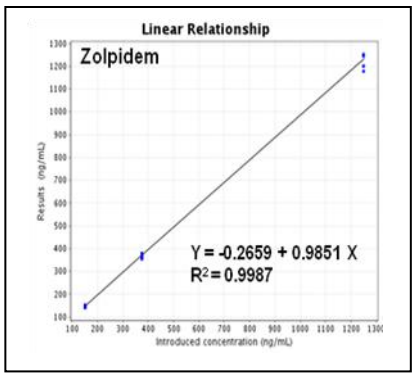

Linear Relationship
Phenytoin
Y = 0.7352 + 1.049 X
R² = 0.9963
Results (mg/L)
Introduced concentration (mg/L)

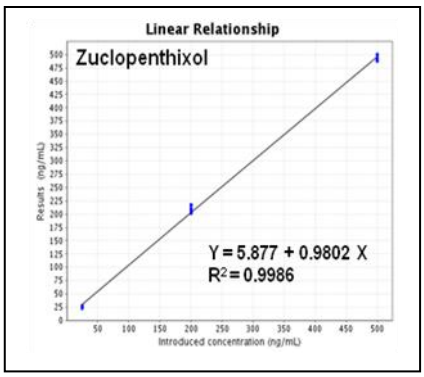

Linear Relationship
Pipamperone
Y = 16.94 + 0.9573 X
R² = 0.9804
Results (ng/mL)
Introduced concentration (ng/mL)


Linear Relationship
Risperidone
Y = -0.05900 + 1.078 X
R² = 0.9908
Results (ng/mL)
Introduced concentration (ng/mL)


Linear Relationship
Sulpiride
Y = 7.237 + 0.9976 X
R² = 0.9978
Results (ng/mL)
Introduced concentration (ng/mL)


Linear Relationship
Thiopental
Y = 0.0001511 + 0.9782 X
R² = 0.9975
Results (mg/L)
Introduced concentration (mg/L)


Linear Relationship
Zolpidem
Y = -0.2659 + 0.9851 X
R² = 0.9987
Results (ng/mL)
Introduced concentration (ng/mL)


Linear Relationship
Zuclopenthixol
Y = 5.877 + 0.9802 X
R² = 0.9986
Results (ng/mL)
Introduced concentration (ng/mL)

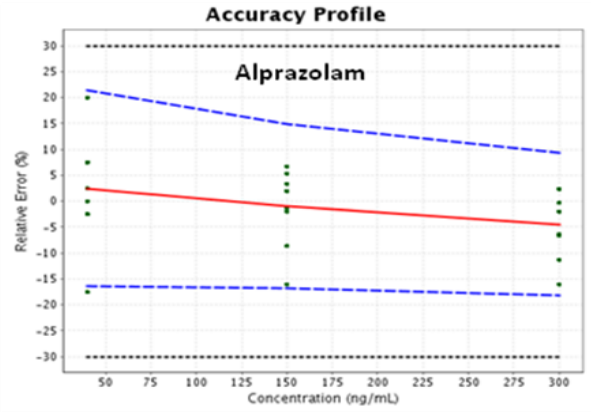
Appendix 3

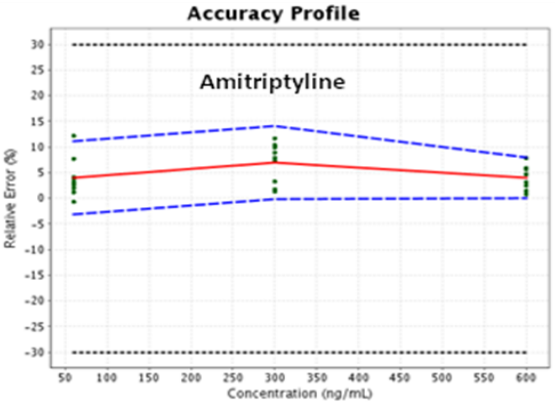
Analytical validation: Relative expanded measurement uncertainty

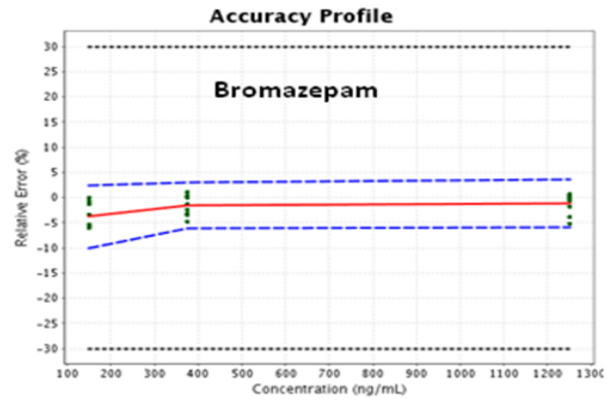
Analytes	Target [ ] (ng/mL)	Uncertainty (%)	Analytes	Target [ ] (ng/mL)	Uncertainty (%)
Alprazolam	40.00	9.15	**Levomepromazine**	40.0	23.65
	150.0	6.20		150.0	19.34
	300.0	5.32		400.0	17.28
Amitriptyline	60.00	8.92	**Lorazepam**	80.00	15.69
	300.0	8.93		300.0	18.62
	600.0	5.15		800.0	15.52
Bromazepam	150.0	6.48	**Midazolam**	200.0	13.23
	375.0	5.09		750.0	15.76
	1250	5.15		1500	12.28
Carbamazepine	2500	11.78	**Nordiazepam**	300.0	9.15
	20000	10.35		750.0	6.20
	50000	10.05		2500	5.32
Chlorpromazine	80.00	3.82	**Olanzapine**	30.00	21.86
	300.0	15.22		80.00	29.97
	800.0	9.36		300.0	20.07
Citalopram	50.00	6.70	**Phenobarbital**	15000	16.26
	400.0	10.96		75000	7.66
	1000	3.71		150000	11.35
Clomipramine	120.0	7.53	**Phenytoin**	6000	15.33
	400.0	11.74		15000	10.29
	1000	17.41		50000	10.14
Clonazepam	40.00	14.01	**Pipamperone**	400.0	15.72
	150.0	15.98		1500	20.34
	400.0	17.34		3000	15.50
Diazepam	400.0	16.02	**Risperidone**	40.00	9.16
	1500	19.69		150.0	19.66
	3000	16.45		400.0	16.04
Droperidol	60.00	9.89	**Sulpiride**	400	8.32
	200.0	12.09		1500	5.45
	500.0	11.34		4000	6.31
Fluoxetine	150.0	15.26	**Thiopental**	1.500	7.46
	400.0	14.00		3.750	6.05
	1500	16.06		12.50	8.27
Flupentixol	25.00	9.18	**Zolpidem**	150.0	8.28
	200.0	8.60		375.0	6.30
	500.0	6.89		1250	6.03
Haloperidol	15.00	22.19	**Zuclopenthixol**	25.00	13.54
	40.00	13.77		200.0	6.91
	150.0	13.18		500.0	2.39
Imipramine	60.00	6.42			
	300.0	7.03			
	600.0	7.03			

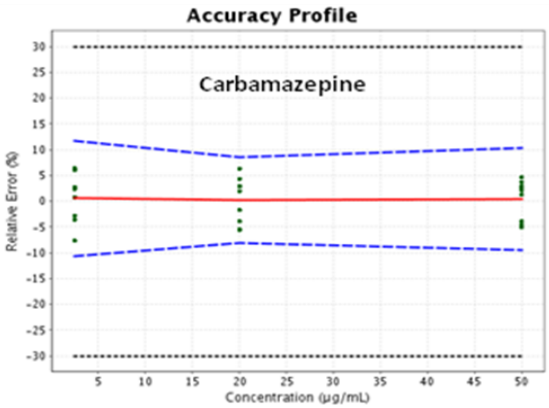
Appendix 4

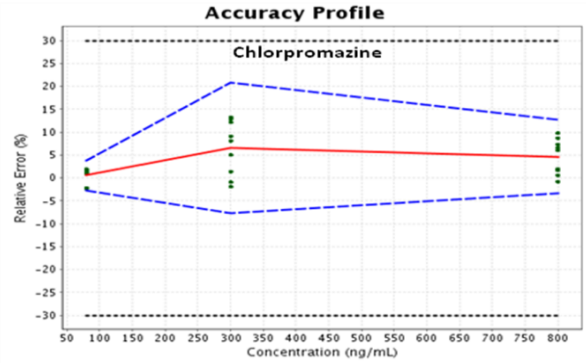
Analytical validation: Accuracy profiles of various compounds

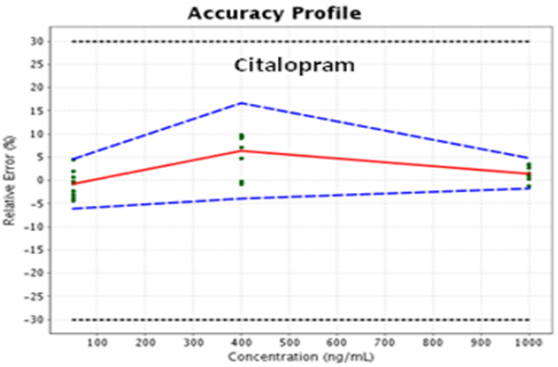


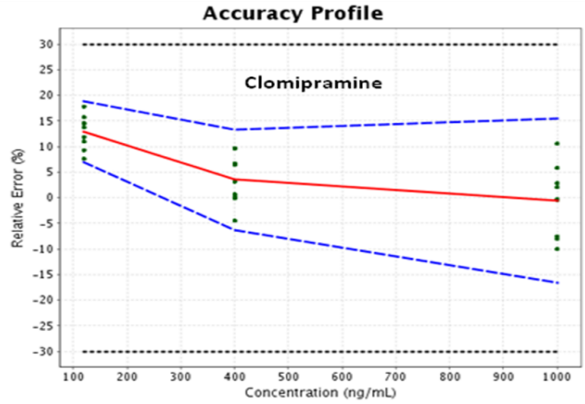


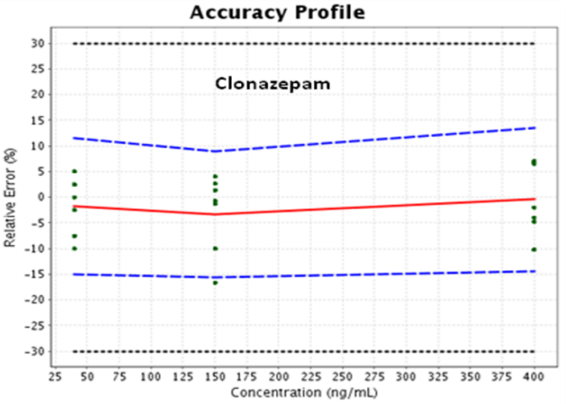


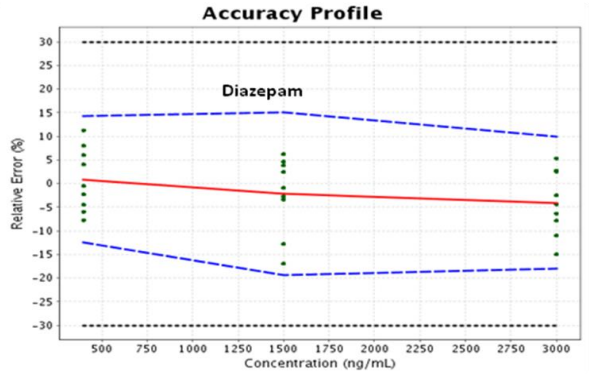


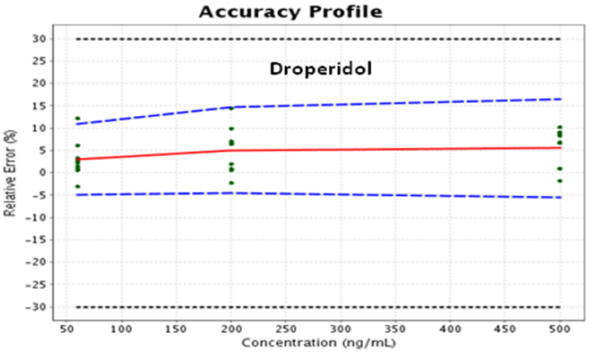


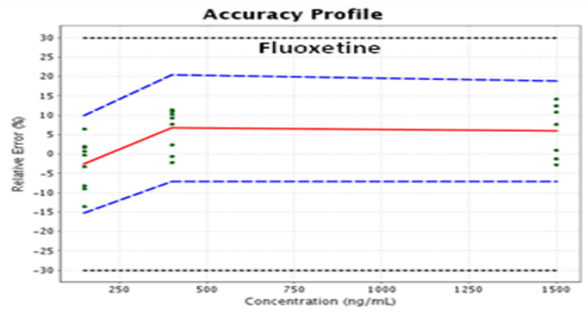


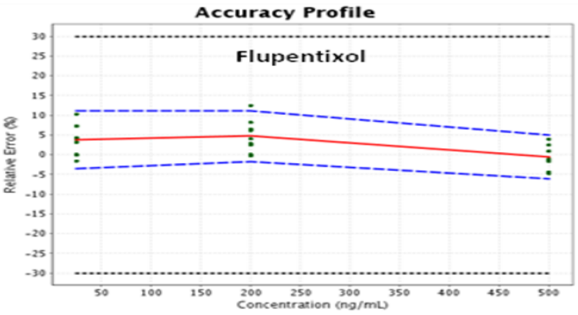

Accuracy Profile
Clomipramine
Relative Error (%)
Concentration (ng/mL)

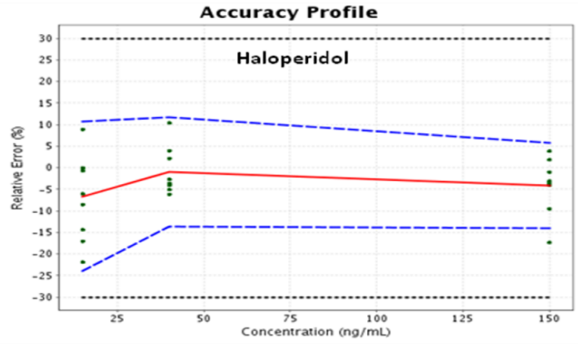

Accuracy Profile
Clonazepam
Relative Error (%)
Concentration (ng/mL)

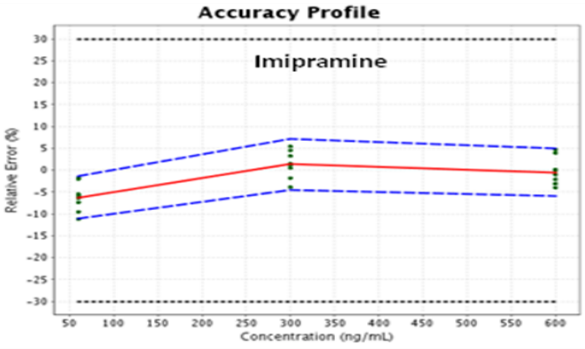

Accuracy Profile
Diazepam
Relative Error (%)
Concentration (ng/mL)

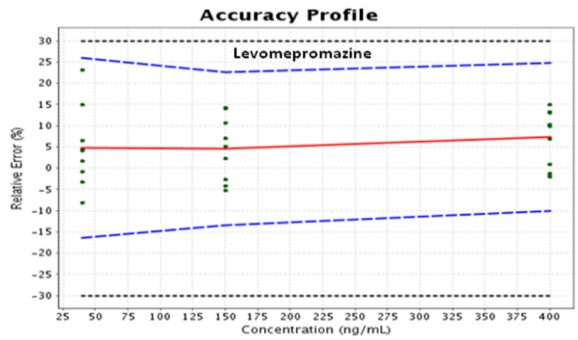

Accuracy Profile
Droperidol
Relative Error (%)
Concentration (ng/mL)

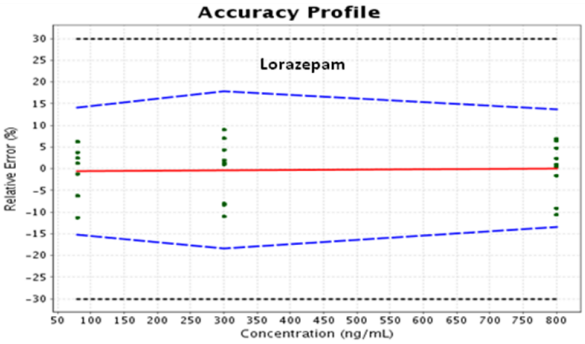

Accuracy Profile
Fluoxetine
Relative Error (%)
Concentration (ng/mL)

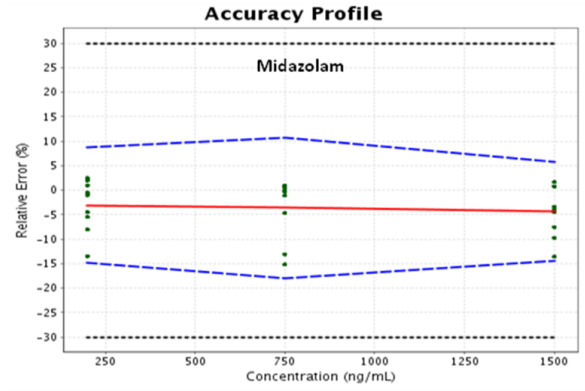

Accuracy Profile
Flupentixol
Relative Error (%)
Concentration (ng/mL)

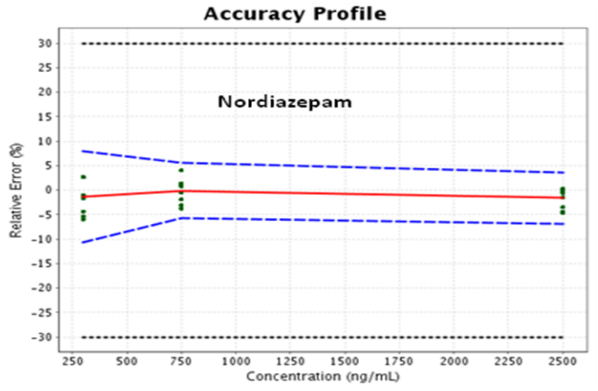

Accuracy Profile
Haloperidol
Relative Error (%)
Concentration (ng/mL)

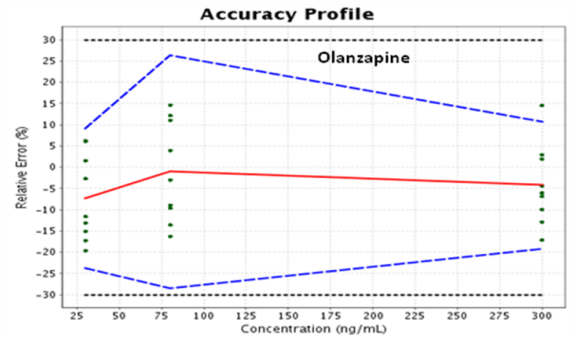

Accuracy Profile
Imipramine
Relative Error (%)
Concentration (ng/mL)

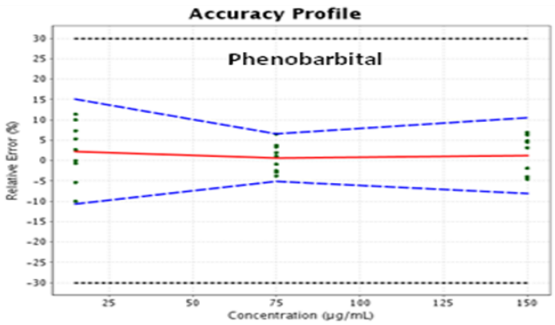

Accuracy Profile
Levomepromazine
Relative Error (%)
Concentration (ng/mL)

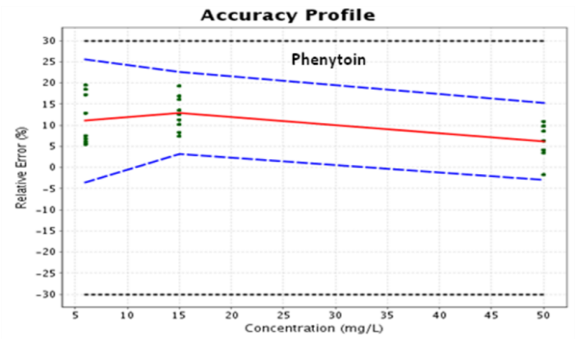

Accuracy Profile
Lorazepam
Relative Error (%)
Concentration (ng/mL)

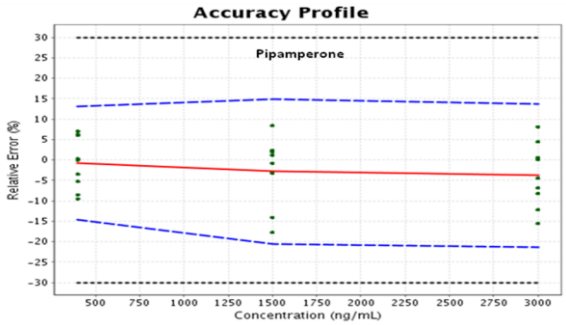

Accuracy Profile
Midazolam
Relative Error (%)
Concentration (ng/mL)

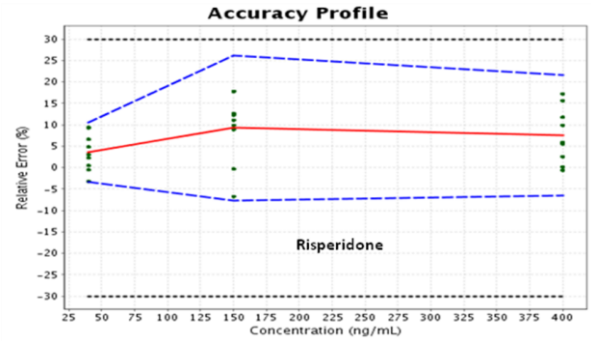

Accuracy Profile
Nordiazepam
Relative Error (%)
Concentration (ng/mL)

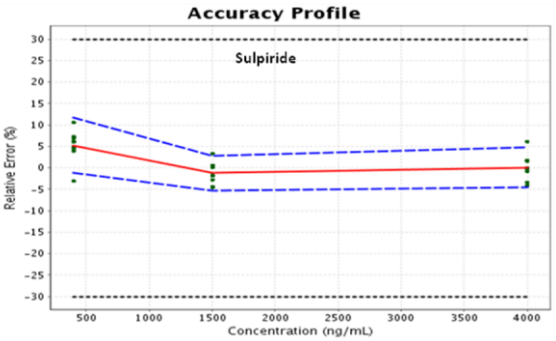

Accuracy Profile
Olanzapine
Relative Error (%)
Concentration (ng/mL)

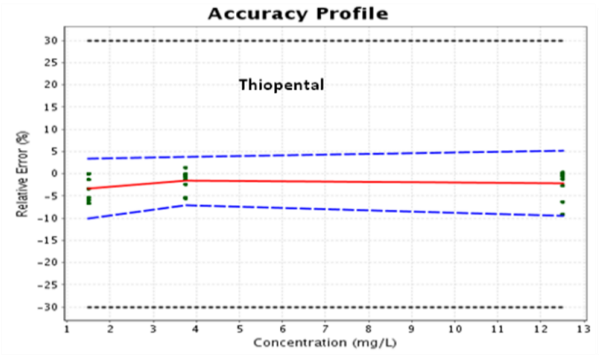

Accuracy Profile
Phenobarbital
Relative Error (%)
Concentration (µg/mL)

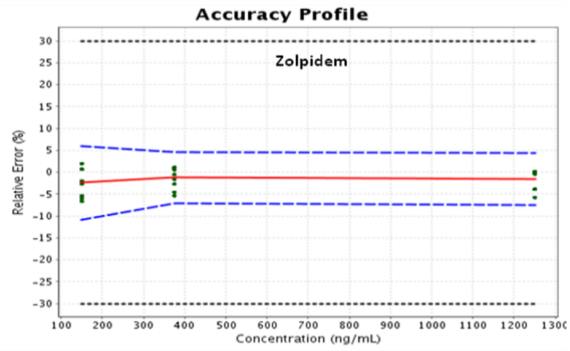

Accuracy Profile
Phenytoin
Relative Error (%)
Concentration (mg/L)

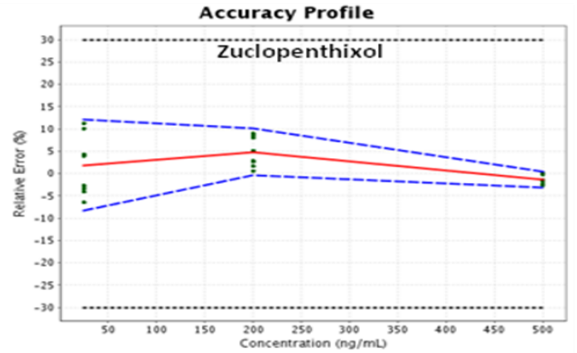

Accuracy Profile
Pipamperone
Relative Error (%)
Concentration (ng/mL)


Accuracy Profile
Risperidone
Relative Error (%)
Concentration (ng/mL)


Accuracy Profile
Sulpiride
Relative Error (%)
Concentration (ng/mL)


Accuracy Profile
Thiopental
Relative Error (%)
Concentration (mg/L)


Accuracy Profile
Zolpidem
Relative Error (%)
Concentration (ng/mL)


Accuracy Profile
Zuclopenthixol
Relative Error (%)
Concentration (ng/mL)

Appendix 5

Analytical results and patients information

Patients codes	Psychotropic Medications	Doses	Treatment length	Analytical results (ng/mL)	Interpretation	Other concomitant Medications	Adverse reactions
N/001	Diazepam Zuclopenthixol Carbamazepine	10 mg/day 150 mg/day 400 mg/day	7 days	52 76.4 6 900	Subtherapeutic Supratherapeutic Therapeutic	Not reported	Asthenia, Amnesia, dysarthria
N/002	Haloperidol Levomepromazine Carbamazepine	10 mg/day 200 mg/day 800 mg/day	14 days	2.7 18.3 11 900	Therapeutic Subtherapeutic Therapeutic	Not reported	Drowsiness
N/003	Haloperidol Carbamazepine Chlorpromazine	100 mg (LAI) 800 mg/day 200 mg/day	1 month	<LLOQ 109 <LLOQ	Subtherapeutic Subtherapeutic Subtherapeutic	Not reported	Not reported
N/004	Haloperidol Carbamazepine Levomepromazine	10 mg/day 400 mg/day 100 mg/day	1 month	5 8 500 48.1	Therapeutic Therapeutic Therapeutic	Not reported	Asthenia, drowsiness, dysarthria
N/005	Haloperidol Carbamazepine Levomepromazine	10 mg/day 800 mg/day 200 mg/day	7 days	<LLOQ 15 300 26.3	Subtherapeutic Supratherapeutic Subtherapeutic	Not reported	Asthenia, drowsiness, dysarthria
N/006	Haloperidol Carbamazepine Phenobarbital	10 mg/day 800 mg/day 100 mg/day	8 days	6.7 10 500 4 800	Therapeutic Therapeutic Subtherapeutic	Not reported	Not reported
N/007	Haloperidol Levomepromazine	10 mg/day 100 mg/day	7 days	14.2 53.8	Supratherapeutic Therapeutic	Not reported	Asthenia, Excess of saliva
N/008	Haloperidol Levomepromazine Carbamazepine	10 mg/day 100 mg/day Not reported	9 days	<LLOQ <LLOQ 391	Subtherapeutic Subtherapeutic Subtherapeutic	Not reported	Not reported
N/009	Haloperidol	100 mg (LAI)	7 days	2	Therapeutic	Not reported	Not reported

	Carbamazepine	400 mg/day		8 900	Therapeutic		
N/010	Haloperidol Chlorpromazine	100 mg (LAI) 200 mg/day	2 months	<LLOQ 47.3	Subtherapeutic Therapeutic	Not reported	Not reported
N/011	Citalopram Flupentixol	40 mg/day 4 mg/day	7 days	161.6 <LLOQ	Supratherapeutic Subtherapeutic	Not reported	Trembling hands
N/012	Haloperidol Levomepromazine	100 mg (LAI) 25 mg/day	7 days	<LLOQ 17.3	Subtherapeutic Subtherapeutic	Not reported	Not reported
N/013	Haloperidol Levomepromazine	100 mg/day 100 mg/day	2 months	<LLOQ 93.2	Subtherapeutic Therapeutic	Not reported	Asthenia, dysarthria
N/014	Risperidone Diazepam	4 mg/day 10 mg/day	7 days	12.3 492	Subtherapeutic Therapeutic	Not reported	Not reported
N/015	Flupentixol Amitriptyline	2 mg/day 25 mg/day	7 days	<LLOQ <LLOQ	Subtherapeutic Subtherapeutic	Not reported	Not reported
N/016	Haloperidol Chlorpromazine Carbamazepine	100 mg (LAI) 100 mg/day 400 mg/day	2 months	1.8 <LLOQ 11 900	Therapeutic Subtherapeutic Therapeutic	Not reported	Dysarthria
N/017	Haloperidol Carbamazepine Levomepromazine	100 mg (LAI) 800 mg/day 100 mg/day	2 months	<LLOQ 11 200 132.5	Subtherapeutic Therapeutic Therapeutic	Not reported	Amnesia, dry mouth
N/018	Haloperidol Carbamazepine Levomepromazine	5 mg/day 400 mg/day 100 mg/day	2 months	1.1 5 600 <LLOQ	Therapeutic Therapeutic Subtherapeutic	Not reported	Drowsiness
N/019	Haloperidol Chlorpromazine	10 mg/day 100 mg/day	2 months	16.7 129.8	Supratherapeutic Therapeutic	Not reported	Head swelling sensation
N/020	Haloperidol Chlorpromazine	5 mg/day 100 mg/day	1 month	6.2 <LLOQ	Therapeutic Subtherapeutic	Not reported	Asthenia, drowsiness, amnesia, dizziness, tongue paralysis
N/021	Haloperidol Chlorpromazine Carbamazepine	5 mg/day 200 mg/day 400 mg/day	1 month	<LLOQ <LLOQ 9 300	Subtherapeutic Subtherapeutic Therapeutic	Not reported	Incontinence, insomnia

N/022	Haloperidol Carbamazepine Levomepromazine	10 mg/day 400 mg/day 100 mg/day	1 month	<LLOQ 14 700 <LLOQ	Subtherapeutic Supratherapeutic Subtherapeutic	Not reported	Weight gain
N/023	Haloperidol Levomepromazine	100 mg (LAI) 200 mg/day	2 months	<LLOQ 125.1	Subtherapeutic Therapeutic	Lamivudine, nevirapine tenofovir, bactrim	Trembling arms neck pain
N/024	Pipamperone Chlorpromazine	40 mg/day 100 mg/day	1 month	195.2 <LLOQ	Therapeutic Subtherapeutic	Not reported	Trembling arms , anorexia, dysarthria
N/025	Levomepromazine Haloperidol	100 mg/day 10 mg/day	1 month	180.3 <LLOQ	Supratherapeutic Subtherapeutic	Not reported	Asthenia, dysarthria
N/026	Chlorpromazine	100 mg/day	1 month	37.2	Therapeutic	Not reported	Drowsiness, dysarthria, arm paralysis
N/027	Haloperidol Chlorpromazine	5 mg/day 100 mg/day	1 month	13.3 14.8	Supratherapeutic Subtherapeutic	Not reported	Trembling arms , insomnia
N/028	Haloperidol Levomepromazine Chlorpromazine Carbamazepine	10 mg/day 100 mg/day Not reported Not reported	3 months	5.8 <LLOQ 13.1 1 200	Therapeutic Subtherapeutic Subtherapeutic Subtherapeutic	Not reported	Asthenia, drowsiness, insomnia
N/029	Haloperidol Chlorpromazine	100 mg (LAI) 200 mg/day	5 months	<LLOQ 13	Subtherapeutic Subtherapeutic	Not reported	Neck stiffness
N/030	Haloperidol Levomepromazine Carbamazepine	10 mg/day 100 mg/day Not reported	1 month	8.6 45.2 545	Therapeutic Therapeutic Subtherapeutic	Not reported	Drowsiness, Asthenia
N/031	Haloperidol Chlorpromazine Carbamazepine	10 mg/day 100 mg/day 400 mg/day	2 months	5.9 <LLOQ 8 000	Therapeutic Subtherapeutic Therapeutic	Not reported	Drowsiness, dysarthria
N/032	Haloperidol Chlorpromazine	10 mg/day 10 mg/day	2 months	5.7 <LLOQ	Therapeutic Subtherapeutic	Not reported	Weight gain, Asthenia, Amnesia
N/033	Haloperidol Chlorpromazine	10 mg/day 100 mg/day	1 month	7.9 8.9	Therapeutic Subtherapeutic	Not reported	Asthenia, dizziness

N/034	Haloperidol Levomepromazine	10 mg/day 100 mg/day	3 months	11.3 103.7	Supratherapeutic Therapeutic	Not reported	Drowsiness, dysarthria back pain
N/035	Carbamazepine Levomepromazine	600 mg/day 300 mg/day	14 days	11 700 173	Therapeutic Supratherapeutic	Not reported	Neck stiffness, dizziness, drowsiness, excessive salivation
N/037	Levomepromazine Carbamazepine Zolpidem	400 mg/day 400 mg/day 10 mg/day	7 days	70.5 7 200 <LLOQ	Therapeutic Therapeutic Subtherapeutic	Not reported	Drowsiness, dysarthria
N/038	Haloperidol Chlorpromazine	10 mg/day 200 mg/day	7 days	<LLOQ 6.5	Subtherapeutic Subtherapeutic	Not reported	Dysarthria, Asthenia, arm stiffness
N/039	Chlorpromazine	100 mg/day	7 days	28.8	Subtherapeutic	Not reported	Drowsiness
N/040	Haloperidol Levomepromazine Carbamazepine	10 mg/day 200 mg/day 400 mg/day	1 month	<LLOQ 30.5 8 900	Subtherapeutic Therapeutic Therapeutic	Not reported	Dysarthria
N/041	Flupentixol	6 mg/day	7 days	4.8	Therapeutic	Not reported	Dysarthria, salivation excessive
N/042	Haloperidol Levomepromazine	15 mg/day 100 mg/day	7 days	3.7 17	Therapeutic Subtherapeutic	Not reported	Drowsiness, dysarthria, Asthenia
N/043	Haloperidol Chlorpromazine	10 mg/day 100 mg/day	7 days	4 <LLOQ	Therapeutic Subtherapeutic	Not reported	Neck stiffness, dysarthria, salivation excessive
N/044	Haloperidol Chlorpromazine Zolpidem	5 mg/day 100 mg/day 10 mg/day	1 month	1.4 13 <LLOQ	Therapeutic Subtherapeutic Subtherapeutic	Not reported	Not reported
N/045	Haloperidol Chlorpromazine	10 mg/day 100 mg/day	10 days	5 <LLOQ	Therapeutic Subtherapeutic	Not reported	Drowsiness, salivation excessive palpitations
N/046	Haloperidol Levomepromazine	10 mg/day 100 mg/day	7 days	<LLOQ 37.8	Subtherapeutic Therapeutic	Not reported	Neck stiffness, drowsiness difficulty walking
N/047	Haloperidol Levomepromazine	15 mg/day 300 mg/day	2 months	8.6 164.6	Therapeutic Supratherapeutic	Not reported	Dysarthria, Asthenia
N/048	Haloperidol Levomepromazine	10 mg/day 200 mg/day	8 days	5.9 90.9	Therapeutic Therapeutic	Not reported	Not reported

N/049	Haloperidol Levomepromazine	10 mg/day 400 mg/day	12 days	<LLOQ 61.7	Subtherapeutic Therapeutic	Not reported	drowsiness, excessive appetite
N/050	Flupentixol Zolpidem	6 mg/day 10 mg/day	10 days	<LLOQ 5	Subtherapeutic Subtherapeutic	Not reported	Drowsiness, dysarthria, arm rigidity
N/051	Haloperidol Levomepromazine	5 mg/day 200 mg/day	7 days	7.2 60.4	Therapeutic Therapeutic	Lamivudine, Nevirapine Stavudine	Difficulty walking, dysarthria
N/052	Haloperidol Levomepromazine	10 mg/day 200 mg/day	10 days	14.4 79.5	Supratherapeutic Therapeutic	Not reported	Drowsiness
N/053	Flupentixol Citalopram	6 mg/day 20 mg/day	8 months	8.5 21.5	Therapeutic Subtherapeutic	Not reported	Rigidity of arms and legs, dysarthria,
N/054	Carbamazepine Levomepromazine Haloperidol	400 mg/day 300 mg/day 10 mg/day	15 days	13 600 113.4 6.4	Supratherapeutic Therapeutic Therapeutic	Not reported	Drowsiness, Impotence, Asthenia
N/055	Carbamazepine Zolpidem Levomepromazine Haloperidol	400 mg/day 10 mg/day 200 mg/day 10 mg/day	21 days	8 100 <LLOQ 27.6 <LLOQ	Therapeutic Subtherapeutic Subtherapeutic Subtherapeutic	Not reported	Drowsiness, dysarthria
N/056	Carbamazepine Levomepromazine Haloperidol	400 mg/day 200 mg/day 15 mg/day	2 months	8 000 14.3 1	Therapeutic Subtherapeutic Therapeutic	Not reported	Drowsiness, dysarthria
N/057	Haloperidol Chlorpromazine	10 mg/day 200 mg/day	18 days	5.2 <LLOQ	Therapeutic Subtherapeutic	Not reported	Vision disorders, dizziness, drowsiness, paralysis of arms, legs and neck
N/058	Haloperidol Chlorpromazine	10 mg/day 200 mg/day	15 days	1.2 <LLOQ	Therapeutic Subtherapeutic	Not reported	Paralysis of arms and legs
N/059	Flupentixol	6 mg/day	22 days	6.7	Therapeutic	Not reported	Sedation, dysarthria
N/060	Carbamazepine	600 mg/day	11 days	5 200	Therapeutic	Not reported	Dizziness, dysarthria
N/061	Carbamazepine Levomepromazine	400 mg/day 200 mg/day	18 days	8 500 8.5	Therapeutic Subtherapeutic	Not reported	Drowsiness

	Haloperidol	15 mg/day		2.1	Therapeutic		
N/062	Carbamazepine Haloperidol	1000 mg/day 10 mg/day	19 days	8 600 1,1	Therapeutic Therapeutic	Not reported	Dysarthria
N/063	Haloperidol Chlorpromazine	10 mg/day 200 mg/day	20 days	7 14.9	Therapeutic Subtherapeutic	Not reported	Asthenia, dysarthria, excessive salivation
N/064	Carbamazepine Levomepromazine Haloperidol	400 mg/day 200 mg/day 10 mg/day	24 years	6 700 109.2 <LLOQ	Therapeutic Therapeutic Subtherapeutic	Not reported	Drowsiness, dysarthria, excessive salivation
N/065	Amitriptyline Levomepromazine Zolpidem	75 mg/day 200 mg/day 20 mg/day	10 days	54.7 <LLOQ 17	Subtherapeutic Subtherapeutic Subtherapeutic	ARVs	Drowsiness, asthenia
N/066	Citalopram Flupentixol	40 mg/day 4 mg/day	20 days	7.7 <LLOQ	Subtherapeutic Subtherapeutic	Not reported	Dysarthria, excessive salivation dizziness
N/067	Carbamazepine Levomepromazine Haloperidol	600 mg/day 100 mg/day 5 mg/day	21 days	9 700 <LLOQ <LLOQ	Therapeutic Subtherapeutic Subtherapeutic	Not reported	Drowsiness, unceasing head movement, excessive fear
N/068	Carbamazepine Levomepromazine	600 mg/day 200 mg/day	12 days	10 200 67.3	Therapeutic Therapeutic	Not reported	Dysarthria, excessive salivation dizziness, vision disorders
N/069	Flupentixol Zolpidem	6mg/day 10 mg/day	1 month	<LLOQ 4	Subtherapeutic Subtherapeutic	Not reported	Dysarthria
N/070	Clomipramine	100 mg/day	22 days	13.1	Subtherapeutic	Not reported	Dysarthria, sedation, abdominal pain
N/071	Sulpiride	500 mg/day	7 days	75.5	Subtherapeutic	Not reported	Drowsiness, sedation
N/072	Clonazepam Citalopram	4 mg/day 40 mg/day	3 months	33 209.2	Therapeutic Supratherapeutic	Not reported	Dysarthria, trembling arms, difficulty walking, scalp pain
N/073	Clomipramine Zolpidem Flupentixol	100 mg/day 20 mg/day 6 mg/day	1 month	116 <LLOQ 7.9	Subtherapeutic Subtherapeutic Therapeutic	Not reported	Not reported
N/074	Sulpiride	100 mg/day	5 months	38.8	Subtherapeutic	Not reported	Drowsiness, Asthenia,

							weight gain
N/075	Clomipramine	50 mg/day	9 months	87.5	Subtherapeutic	Not reported	Erection disorders, Asthenia, difficulty to move jaws
S/003	Amitriptyline	25 mg/day	2 months	14.7	Subtherapeutic	ARVs	Not reported
S/004	Clomipramine	25 mg/day	4 months	<LLOQ	Subtherapeutic	Not reported	Not reported
S/005	Carbamazepine	1000 mg/day	8 years	7 900	Therapeutic	Not reported	Stomachache, amnesia, fatigue
S/008	Carbamazepine	200 mg/day	3 years	4 500	Therapeutic	Not reported	Fatigue, drowsiness, dizziness pain after bath
S/009	Phenobarbital	300 mg/day	4 years	42 100	Supratherapeutic	Not reported	Amnesia, drowsiness, dizziness
S/010	Phenobarbital Diazepam	50 mg/day 5 mg/day	6 years	<LLOQ <LLOQ	Subtherapeutic Subtherapeutic	Not reported	Not reported
S/011	Olanzapine Levomepromazine	2.5 mg/day 25 mg/day	4 years	<LLOQ 27.68	Subtherapeutic Subtherapeutic	Not reported	Amnesia, tongue self-biting erection disorders
S/012	Phenobarbital	150 mg/day	3 months	29 100	Therapeutic	Not reported	Not reported
S/014	Phenytoin Carbamazepine	300 mg/day Not reported	5 months	<LLOQ 7 600	Subtherapeutic Therapeutic	Not reported	Amnesia
S/015	Flupentixol Carbamazepine Levomepromazine	1 mg/day 200 mg/day 25 mg/day	2 years	<LLOQ 3 400 <LLOQ	Subtherapeutic Subtherapeutic Subtherapeutic	Not reported	Slowed reasoning
S/016	Carbamazepine Haloperidol Levomepromazine	400 mg/day 25 mg/day 25 mg/day	2 months	9 600 2.5 <LLOQ	Therapeutic Therapeutic Subtherapeutic	Not reported	Fatigue, visual disorders, eye pains
S/018	Citalopram	90 mg/day	2 years	95.1	Therapeutic	Not reported	drowsiness
S/019	Flupentixol Carbamazepine Chlorpromazine	20 mg (LAI) 200 mg/day 50 mg/day	8 years	<LLOQ 5 700 <LLOQ	Subtherapeutic Therapeutic Subtherapeutic	Not reported	Not reported
S/021	Phenobarbital	100 mg/day	12 years	25	Therapeutic	Not reported	Retrograde amnesia, fatigue
S/022	Phenobarbital	600 mg/day	8 years	6 500	Subtherapeutic	Not reported	Amnesia, drowsiness, dizziness, fatigue
S/023	Carbamazepine	400 mg/day	6 years	6 700	Therapeutic	Not reported	Amnesia, drowsiness, fatigue

S/024	Haloperidol	5 mg/day	5 years	<LLOQ	Subtherapeutic	Not reported	Fatigue
S/026	Carbamazepine	400 mg/day	2 years	5 500	Therapeutic	Not reported	Amnesia, headache, dizziness,
S/028	Phenytoin Amitriptyline	800 mg/day 50 mg/day	18 years	13 000 < LLOQ	Therapeutic Subtherapeutic	ARVs	Amnesia, headache, dizziness, fatigue
S/029	Phenobarbital Chlorpromazine	100 mg/day 25 mg/day	4 years	38 100 < LLOQ	Therapeutic Subtherapeutic	Not reported	Thinking disorders
S/032	Carbamazepine Chlorpromazine	400 mg/day 25 mg/day	8 years	6 200 < LLOQ	Therapeutic Subtherapeutic	Not reported	Awakening tiredness, amnesia
S/033	Chlorpromazine	75 mg/day	1 month	< LLOQ	Subtherapeutic	Not reported	Amnesia, sleeplessness
S/034	Amitriptyline	50 mg/day	3 years	7.4	Subtherapeutic	Not reported	Fatigue, amnesia
S/036	Amitriptyline	50 mg/day	5 months	142.6	Therapeutic	Losartan Levothyroxine	Amnesia, weight gain
S/037	Haloperidol	2.5 mg/day	8 years	< LLOQ	Subtherapeutic	Not reported	Amnesia, dizziness
S/038	Olanzapine	5 mg/day	9 months	22.6	Therapeutic	Not reported	Weight gain
S/039	Citalopram	20 mg/day	12 years	55.3	Therapeutic	Not reported	Amnesia, drowsiness
S/040	Amitriptyline	75 mg/day	2 years	107.2	Therapeutic	Not reported	Not reported
S/041	Amitriptyline Phenytoin	25 mg/day Not repoted	5 months	< LLOQ 4 400	Subtherapeutic Subtherapeutic	Not reported	Amnesia, drowsiness, dizziness back pain, weight gain
S/043	Carbamazepine	600 mg/day	2 years	8 700	Therapeutic	Not reported	Amnesia, faint
S/044	Carbamazepine	400 mg/day	1 year	6 200	Therapeutic	Not reported	Amnesia, drowsiness, fatigue
S/045	Carbamazepine	400 mg/day	2 months	5 400	Therapeutic	Not reported	drowsiness
S/046	Carbamazepine Phenobarbital	600 mg/day 100 mg/day	14 years	5 700 41 900	Therapeutic Supratherapeutic	Not reported	Acne
S/047	Phenobarbital	150 mg/day	2 years	29 600	Therapeutic	Not reported	Stomachache, amnesia
S/048	Fluoxetine	40 mg/day	2 months	644.9	Supratherapeutic	Fluticasone Betadine	Amnesia, sleeplessness, anorexia lack of concentration
S/049	Fluoxetine	40 mg/day	1 month	415.3	Therapeutic	ARVs	Paralysis, drowsiness, headache, pain of the neck and eyes
S/050	Fluoxetine	40 mg/day	8 days	< LLOQ	Subtherapeutic	Not reported	Headache, stomachache

							drowsiness
S/051	Carbamazepine	200 mg/day	4 months	3 900	Subtherapeutic	Vit B	Headache, amnesia, drowsiness
S/052	Fluoxetine	20 mg/day	2 years	< LLOQ	Subtherapeutic	Lamivudine, Stavudine Nevirapine	Headache, dizziness, drowsiness
S/053	Amitriptyline	50 mg/day	12 days	37.7	Subtherapeutic	Not reported	Amnesia, drowsiness, fatigue, dizziness
S/054	Amitriptyline	25 mg/day	5 months	8.7	Subtherapeutic	Not reported	Not reported
S/055	Phenytoin Carbamazepine	400 mg/day 300 mg/day	14 years	7 100 5 400	Subtherapeutic Therapeutic	Not reported	Amnesia
S/056	Phenytoin	200 mg/day	3 months	2 700	Subtherapeutic	Not reported	Stomachache, burning sensation in the head
S/057	Carbamazepine	600 mg/day	6 years	10 800	Therapeutic	Not reported	Amnesia
S/058	Haloperidol Chlorpromazine	2.5 mg/day 50 mg/day	1 year	< LLOQ 33.8	Subtherapeutic Therapeutic	ARVs	Fatigue, drowsiness
S/059	Amitriptyline	50 mg/day	2 months	84.9	Therapeutic	Not reported	Amnesia, drowsiness, dizziness
S/060	Citalopram	20 mg/day	3 years	62.2	Therapeutic	Not reported	Tongue burn
S/061	Phenobarbital	100 mg/day	8 years	10 300	Therapeutic	Not reported	Not reported
S/062	Phenytoin	100 mg/day	3 years	2 900	Subtherapeutic	Not reported	Amnesia, drowsiness, fatigue, excessive hunger, intellectual capacity decrease
CK/001	Thiopental	200 mg (UD)	Anesthetic	5 700	Supratherapeutic	Perfalgan	Not reported
CK/002	Thiopental	300 mg (UD)	Anesthetic	2 900	Therapeutic	Insuline	Not reported
CK/003	Thiopental	400 mg (UD)	Anesthetic	4 000	Therapeutic	Cefotaxime Tramadol Paracetamol	Not reported
CK/004	Thiopental	500 mg (UD)	Anesthetic	4 200	Therapeutic	Cloxacilline Nevirapine Bactrim	Not reported
KF/001	Midazolam	5 mg/hr (infusion)	7 days	18	Subtherapeutic	Morphine Paracetamol	Not reported

						Diclofenac	

Appendix 6

Analytical results vs. Calculated concentrations

PC	Psychotropic medications	DD (mg)	AR (ng/mL)	RI	CAL Cp_{ss} (ng/mL)	RI	PW (Kg)	F	CL (L/h/kg)
N/035	Carbamazepine	600	11 700	Therap	5 123.0	Therap	61	0.80	0.064
	Levomepromazine	300	173	Supra	267.5	Supra	61	0.50	0.383
N/037	Levomepromazine	400	70.5	Therap	362.6	Supra	60	0.50	0.383
	Carbamazepine	400	7 200	Therap	3 472.2	Sub	60	0.80	0.064
	Zolpidem	10	<LLOQ	Sub	18.7	Sub	60	0.70	0.26
N/038	Haloperidol	10	<LLOQ	Sub	6.0	Therap	63	0.65	0.72
	Chlorpromazine	200	6.5	Sub	117.2	Therap	63	0.45	0.508
N/039	Chlorpromazine	100	28.8	Sub	61.5	Therap	60	0.45	0.508
N/040	Haloperidol	10	<LLOQ	Sub	5.4	Therap	70	0.65	0.72
	Levomepromazine	200	30.5	Therap	155.4	Therap	70	0.50	0.383
	Carbamazepine	400	8 900	Therap	2 976.2	Sub	70	0.80	0.064
N/041	Flupentixol	6	4.8	Therap	6.6	Therap	72	0.47	0.249
N/042	Haloperidol	15	3.7	Therap	6.7	Therap	84	0.65	0.72
	Levomepromazine	100	17	Sub	64.8	Therap	84	0.50	0.383
N/043	Haloperidol	10	4	Therap	4.2	Therap	89	0.65	0.72
	Chlorpromazine	100	<LLOQ	Sub	41.5	Therap	89	0.45	0.508
N/044	Haloperidol	5	1.4	Therap	2.6	Therap	72	0.65	0.72
	Chlorpromazine	100	13	Sub	51.3	Therap	72	0.45	0.508
	Zolpidem	10	<LLOQ	Sub	15.6	Sub	72	0.70	0.26
N/045	Haloperidol	10	5	Therap	5.5	Therap	68	0.65	0.72
	Chlorpromazine	100	<LLOQ	Sub	54.3	Therap	68	0.45	0.508
N/046	Haloperidol	10	<LLOQ	Sub	6.3	Therap	60	0.65	0.72
	Levomepromazine	100	37.8	Therap	90.7	Therap	60	0.50	0.383
N/047	Haloperidol	15	8.6	Therap	9.4	Therap	60	0.65	0.72
	Levomepromazine	300	164.6	Supra	272.0	Supra	60	0.50	0.383
N/048	Haloperidol	10	5.9	Therap	6.3	Therap	60	0.65	0.72
	Levomepromazine	200	90.9	Therap	181.3	Supra	60	0.50	0.383
N/049	Haloperidol	10	<LLOQ	Sub	5.2	Therap	72	0.65	0.72
	Levomepromazine	400	61.7	Therap	302.2	Supra	72	0.50	0.383
N/051	Haloperidol	5	7.2	Therap	4.5	Therap	42	0.65	0.72
	Levomepromazine	200	60.4	Therap	259.0	Supra	42	0.50	0.383
N/052	Haloperidol	10	14.4	Supra	8.4	Therap	45	0.65	0.72
	Levomepromazine	200	79.5	Therap	241.8	Supra	45	0.50	0.383
N/054	Carbamazepine	400	13 600	Supra	3 930.8	Sub	53	0.80	0.064
	Levomepromazine	300	113.4	Therap	307.9	Supra	53	0.50	0.383
	Haloperidol	10	6.4	Therap	7.1	Therap	53	0.65	0.72
N/055	Carbamazepine	400	8 100	Therap	3 019.3	Sub	69	0.80	0.064
	Zolpidem	10	<LLOQ	Sub	16.3	Sub	69	0.70	0.26
	Levomepromazine	200	27.6	Sub	157.7	Therap	69	0.50	0.383
	Haloperidol	10	<LLOQ	Sub	5.5	Therap	69	0.65	0.72

N/056	Carbamazepine	400	8 000	Therap	2540.7	Sub	82	0.80	0.064
	Levomepromazine	200	14.3	Sub	132.7	Therap	82	0.50	0.383
	Haloperidol	15	1	Therap	6.9	Therap	82	0.65	0.72
N/057	Haloperidol	10	5.2	Therap	6.6	Therap	57	0.65	0.72
	Chlorpromazine	200	<LLOQ	Sub	129.5	Therap	57	0.45	0.508
N/058	Haloperidol	10	1.2	Therap	6.0	Therap	63	0.65	0.72
	Chlorpromazine	200	<LLOQ	Sub	117.2	Therap	63	0.45	0.508
N/059	Flupentixol	6	6.7	Therap	6.7	Therap	70	0.47	0.249
N/060	Carbamazepine	600	5 200	Therap	5 208.3	Therap	60	0.80	0.064
N/061	Carbamazepine	400	8 500	Therap	3 205.1	Sub	65	0.80	0.064
	Levomepromazine	200	8.5	Sub	167.4	Supra	65	0.50	0.383
	Haloperidol	15	2.1	Therap	8.7	Therap	65	0.65	0.72
N/062	Carbamazepine	1000	8 600	Therap	7 233.8	Therap	72	0.80	0.064
	Haloperidol	10	1.1	Therap	5.2	Therap	72	0.65	0.72
N/063	Haloperidol	10	7	Therap	7.5	Therap	50	0.65	0.72
	Chlorpromazine	200	14.9	Sub	147.6	Therap	50	0.45	0.508
N/064	Carbamazepine	400	6 700	Therap	2 777.8	Sub	75	0.80	0.064
	Levomepromazine	200	109.2	Therap	145.1	Therap	75	0.50	0.383
	Haloperidol	10	<LLOQ	Sub	5.0	Therap	75	0.65	0.72
N/065	Amitriptyline	75	54.7	Sub	27.1	Sub	75	0.45	0.693
	Levomepromazine	200	<LLOQ	Sub	145.1	Therap	75	0.50	0.383
	Zolpidem	20	17	Sub	29.9	Sub	75	0.70	0.26
N/066	Citalopram	40	7.7	Sub	78.5	Therap	60	0.80	0.283
	Flupentixol	4	<LLOQ	Sub	5.2	Therap	60	0.47	0.249
N/067	Carbamazepine	600	9 700	Therap	5 208.3	Therap	60	0.80	0.064
	Levomepromazine	100	<LLOQ	Sub	90.7	Therap	60	0.50	0.383
	Haloperidol	5	<LLOQ	Sub	3.1	Therap	60	0.65	0.72
N/068	Carbamazepine	600	10 200	Therap	4807.7	Therap	65	0.80	0.064
	Levomepromazine	200	67.3	Therap	167.4	Supra	65	0.50	0.383
N/069	Flupentixol	6	<LLOQ	Sub	7.3	Therap	65	0.47	0.249
	Zolpidem	10	4	Sub	17.3	Sub	65	0.70	0.26
N/070	Clomipramine	100	13.1	Sub	113.2	Sub	50	0.50	0.368
N/071	Sulpiride	500	75.5	Sub	1 613.8	Supra	60	0.33	0.071
N/074	Sulpiride	100	38.8	Sub	276.7	Therap	70	0.33	0.071
N/075	Clomipramine	50	87.5	Sub	37.7	Sub	75	0.50	0.368
S/003	Amitriptyline	25	14.7	Sub	13.8	Sub	49	0.45	0.693
S/004	Clomipramine	25	<LLOQ	Sub	18.1	Sub	78	0.50	0.368
S/005	Carbamazepine	1000	7 900	Therap	6 853.1	Therap	76	0.80	0.064
S/008	Carbamazepine	200	4 500	Therap	1 225.5	Sub	85	0.80	0.064
S/009	Phenobarbital	300	42 100	Supra	57 870.4	Supra	54	1.00	0.004
S/010	Phenobarbital	50	<LLOQ	Sub	14 076.6	Therap	37	1.00	0.004
	Diazepam	5	<LLOQ	Sub	268.1	Therap	37	1.00	0.021
S/011	Olanzapine	2.5	<LLOQ	Sub	3.4	Sub	75	0.87	0.357
	Levomepromazine	25	27.68	Sub	18.1	Sub	75	0.50	0.383
S/012	Phenobarbital	150	29 100	Therap	21 114.9	Therap	74	1.00	0.004
S/014	Phenytoin	300	<LLOQ	Sub	840.7	Sub	42	1.00	0.354

S/015	Flupentixol	1	<LLOQ	Sub	1.1	Therap	70	0.47	0.249
	Carbamazepine	200	3 400	Sub	1 488.1	Sub	70	0.80	0.064
	Levomepromazine	25	<LLOQ	Sub	19.4	Sub	70	0.50	0.383
S/016	Carbamazepine	400	9 600	Therap	3 858.0	Sub	54	0.80	0.064
	Haloperidol	25	2.5	Therap	17.4	Supra	54	0.65	0.72
	Levomepromazine	25	<LLOQ	Sub	25.2	Sub	54	0.50	0.383
S/018	Citalopram	90	95.1	Therap	212.0	Supra	50	0.80	0.283
	Carbamazepine	200	5 700	Therap	1 554.7	Sub	67	0.80	0.064
	Chlorpromazine	50	<LLOQ	Sub	27.5	Sub	67	0.45	0.508
S/021	Phenobarbital	100	25	Therap	14 881.0	Therap	70	1.00	0.004
S/022	Phenobarbital	600	6 500	Sub	89 285.7	Supra	70	1.00	0.004
S/023	Carbamazepine	400	6 700	Therap	2604.2	Sub	80	0.80	0.064
S/024	Haloperidol	5	<LLOQ	Sub	2.9	Therap	65	0.65	0.72
S/026	Carbamazepine	400	5 500	Therap	6 510.4	Therap	32	0.80	0.064
S/028	Phenytoin	800	13 000	Therap	1272.5	Therap	74	1.00	0.354
	Amitriptyline	50	<LLOQ	Sub	18.3	Sub	74	0.45	0.693
S/029	Phenobarbital	100	38 100	Therap	14 269.4	Therap	73	1.00	0.004
	Chlorpromazine	25	<LLOQ	Sub	12.6	Sub	73	0.45	0.508
S/032	Carbamazepine	400	6 200	Therap	3 472.2	Sub	60	0.80	0.064
	Chlorpromazine	25	<LLOQ	Sub	15.4	Sub	60	0.45	0.508
S/033	Chlorpromazine	75	<LLOQ	Sub	42.6	Therap	65	0.45	0.508
S/034	Amitriptyline	50	7.4	Sub	20.8	Sub	65	0.45	0.693
S/036	Amitriptyline	50	142.6	Therap	16.7	Sub	81	0.45	0.693
S/037	Haloperidol	2.5	<LLOQ	Sub	1.6	Therap	60	0.65	0.72
S/038	Olanzapine	5	22.6	Therap	7.8	Sub	65	0.87	0.357
S/039	Citalopram	20	55.3	Therap	47.1	Sub	50	0.80	0.283
S/040	Amitriptyline	75	107.2	Therap	36.9	Sub	55	0.45	0.693
S/041	Amitriptyline	25	<LLOQ	Sub	9.7	Sub	70	0.45	0.693
S/043	Carbamazepine	600	8 700	Therap	5 681.8	Therap	55	0.80	0.064
S/044	Carbamazepine	400	6 200	Therap	3 592.0	Sub	58	0.80	0.064
S/045	Carbamazepine	400	5 400	Therap	3 019.3	Sub	69	0.80	0.064
S/046	Carbamazepine	600	5 700	Therap	4 807.7	Therap	65	0.80	0.064
	Phenobarbital	100	41 900	Supra	16 025.6	Therap	65	1.00	0.004
S/047	Phenobarbital	150	29 600	Therap	31 250.0	Therap	50	1.00	0.004
S/048	Fluoxetine	40	644.9	Supra	76.1	Sub	52	0.80	0.337
S/049	Fluoxetine	40	415.3	Therap	76.1	Sub	52	0.80	0.337
S/050	Fluoxetine	40	<LLOQ	Sub	70.7	Sub	56	0.80	0.337
S/051	Carbamazepine	200	3 900	Sub	1 211.2	Therap	86	0.80	0.064
S/052	Fluoxetine	20	<LLOQ	Sub	40.4	Sub	49	0.80	0.337
S/053	Amitriptyline	50	37.7	Sub	22.5	Sub	60	0.45	0.693
S/054	Amitriptyline	25	8.7	Sub	11.3	Sub	60	0.45	0.693
S/055	Phenytoin	400	7 100	Sub	627.7	Sub	75	1.00	0.354
	Carbamazepine	300	5 400	Therap	2 083.3	Sub	75	0.80	0.064
S/056	Phenytoin	200	2 700	Sub	523.1	Sub	45	1.00	0.354
S/057	Carbamazepine	600	10 800	Therap	6 009.6	Therap	52	0.80	0.064
S/058	Haloperidol	2.5	<LLOQ	Sub	1.6	Therap	57	0.65	0.72

	Chlorpromazine	50	33.8	Therap	32.4	Therap	57	0.45	0.508
S/059	Amitriptyline	50	84.9	Therap	21.8	Sub	62	0.45	0.693
S/060	Citalopram	20	62.2	Therap	34.6	Sub	68	0.80	0.283
S/061	Phenobarbital	100	10 300	Therap	13 354.7	Therap	78	1.00	0.004
S/062	Phenytoin	100	2 900	Sub	168.1	Sub	70	1.00	0.354

Appendix 7

Polymedications and possible drug-drug interactions among the study population

Patients codes	Psychotropic medications	Other concomitant medications	Predictable DDIs	DDI effects
N/001	Diazepam Zuclopenthixol Carbamazepine	Not reported	Yes	Diazepam [] decreased Zuclopenthixol [] decreased
N/002	Haloperidol Levomepromazine Carbamazepine	Not reported	Yes	Haloperidol [] increased or decreased
N/003	Haloperidol Carbamazepine Chlorpromazine	Not reported	Yes	Haloperidol [] increased Chlorpromazine [] increased
N/004	Haloperidol Carbamazepine Levomepromazine	Not reported	Yes	Haloperidol [] increased or decreased
N/005	Haloperidol Carbamazepine Levomepromazine	Not reported	Yes	Haloperidol [] increased or decreased
N/006	Haloperidol Carbamazepine Phenobarbital	Not reported	Yes	Haloperidol [] decreased Carbamazepine [] decreased
N/007	Haloperidol Levomepromazine	Not reported	Yes	Haloperidol [] increased
N/008	Haloperidol Levomepromazine Carbamazepine	Not reported	Yes	Haloperidol [] increased or decreased
N/009	Haloperidol Carbamazepine	Not reported	Yes	Haloperidol [] decreased
N/010	Haloperidol Chlorpromazine	Not reported	Yes	Haloperidol [] increased Chlorpromazine [] increased
N/011	Citalopram Flupentixol	Not reported	No	-
N/012	Haloperidol Levomepromazine	Not reported	Yes	Haloperidol [] increased
N/013	Haloperidol Levomepromazine	Not reported	Yes	Haloperidol [] increased
N/014	Risperidone Diazepam	Not reported	No	-
N/015	Flupentixol Amitriptyline	Not reported	No	-
N/016	Haloperidol Chlorpromazine Carbamazepine	Not reported	Yes	Haloperidol [] increased Chlorpromazine [] increased

N/017	Haloperidol Carbamazepine Levomepromazine	Not reported	Yes	Haloperidol [] increased
N/018	Haloperidol Carbamazepine Levomepromazine	Not reported	Yes	Haloperidol [] increased or decreased
N/019	Haloperidol Chlorpromazine	Not reported	Yes	Haloperidol [] increased Chlorpromazine [] increased
N/020	Haloperidol Chlorpromazine	Not reported	Yes	Haloperidol [] increased Chlorpromazine [] increased
N/021	Haloperidol Chlorpromazine Carbamazepine	Not reported	Yes	Haloperidol [] increased or decreased Chlorpromazine [] increased
N/022	Haloperidol Carbamazepine Levomepromazine	Not reported	Yes	Haloperidol [] increased or decreased
N/023	Haloperidol Levomepromazine	Lamivudine, nevirapine tenofovir, bactrim	Yes	Haloperidol [] decreased
N/024	Pipamperone Chlorpromazine	Not reported	No	-
N/025	Levomepromazine Haloperidol	Not reported	Yes	Haloperidol [] increased
N/027	Haloperidol Chlorpromazine	Not reported	Yes	Haloperidol [] increased Chlorpromazine [] increased
N/028	Haloperidol Levomepromazine Chlorpromazine Carbamazepine	Not reported	Yes	Haloperidol [] increased or decreased Chlorpromazine [] increased
N/029	Haloperidol Chlorpromazine	Not reported	Yes	Haloperidol [] increased Chlorpromazine [] increased
N/030	Haloperidol Levomepromazine Carbamazepine	Not reported	Yes	Haloperidol [] increased or decreased
N/031	Haloperidol Chlorpromazine Carbamazepine	Not reported	Yes	Haloperidol [] increased Chlorpromazine [] increased
N/032	Haloperidol Chlorpromazine	Not reported	Yes	Haloperidol [] increased Chlorpromazine [] increased
N/033	Haloperidol Chlorpromazine	Not reported	Yes	Haloperidol [] increased Chlorpromazine [] increased
N/034	Haloperidol Levomepromazine	Not reported	Yes	Haloperidol [] increased
N/035	Carbamazepine Levomepromazine	Not reported	No	-
N/037	Levomepromazine Carbamazepine	Not reported	Yes	Zolpidem [] decreased

	Zolpidem			
N/038	Haloperidol Chlorpromazine	Not reported	Yes	Haloperidol [] increased Chlorpromazine [] increased
N/040	Haloperidol Levomepromazine Carbamazepine	Not reported	Yes	Haloperidol [] increased or decreased
N/042	Haloperidol Levomepromazine	Not reported	Yes	Haloperidol [] increased
N/043	Haloperidol Chlorpromazine	Not reported	Yes	Haloperidol [] increased Chlorpromazine [] increased
N/044	Haloperidol Chlorpromazine Zolpidem	Not reported	Yes	Haloperidol [] increased Chlorpromazine [] increased
N/045	Haloperidol Chlorpromazine	Not reported	Yes	Haloperidol [] increased Chlorpromazine [] increased
N/046	Haloperidol Levomepromazine	Not reported	Yes	Haloperidol [] increased
N/047	Haloperidol Levomepromazine	Not reported	Yes	Haloperidol [] increased
N/048	Haloperidol Levomepromazine	Not reported	Yes	Haloperidol [] increased
N/049	Haloperidol Levomepromazine	Not reported	Yes	Haloperidol [] increased
N/050	Flupentixol Zolpidem	Not reported	No	-
N/051	Haloperidol Levomepromazine	Lamivudine, Nevirapine Stavudine	Yes	Haloperidol [] decreased
N/052	Haloperidol Levomepromazine	Not reported	Yes	Haloperidol [] increased
N/053	Flupentixol Citalopram	Not reported	No	-
N/054	Carbamazepine Levomepromazine Haloperidol	Not reported	Yes	Haloperidol [] increased or decreased
N/055	Carbamazepine Zolpidem Levomepromazine Haloperidol	Not reported	Yes	Haloperidol [] increased or decreased Zolpidem [] decreased
N/056	Carbamazepine Levomepromazine Haloperidol	Not reported	Yes	Haloperidol [] increased or decreased
N/057	Haloperidol Chlorpromazine	Not reported	Yes	Haloperidol [] increased Chlorpromazine [] increased
N/058	Haloperidol Chlorpromazine	Not reported	Yes	Haloperidol [] increased Chlorpromazine [] increased
N/061	Carbamazepine	Not reported	Yes	Haloperidol [] increased or

	Levomepromazine Haloperidol			decreased
N/062	Carbamazepine Haloperidol	Not reported	Yes	Haloperidol [] decreased
N/063	Haloperidol Chlorpromazine	Not reported	Yes	Haloperidol [] increased Chlorpromazine [] increased
N/064	Carbamazepine Levomepromazine Haloperidol	Not reported	Yes	Haloperidol [] increased or decreased
N/065	Amitriptyline Levomepromazine Zolpidem	Antiretrovirals	Yes	Amitriptyline [] increased Zolpidem [] increased
N/066	Citalopram Flupentixol	Not reported	No	-
N/067	Carbamazepine Levomepromazine Haloperidol	Not reported	Yes	Haloperidol [] increased or decreased
N/068	Carbamazepine Levomepromazine	Not reported	No	-
N/069	Flupentixol Zolpidem	Not reported	No	-
N/072	Clonazepam Citalopram	Not reported	No	-
N/073	Clomipramine Zolpidem Flupentixol	Not reported	No	-
S/003	Amitriptyline	Antiretrovirals	Yes	Amitriptyline [] increased
S/010	Phenobarbital Diazepam	Not reported	Yes	Haloperidol decreased
S/011	Olanzapine Levomepromazine	Not reported	Yes	Olanzapine [] increased
S/014	Phenytoin Carbamazepine	Not reported	Yes	Phenytoin [] decreased Carbamazepine [] decreased
S/015	Flupentixol Carbamazepine Levomepromazine	Not reported	No	-
S/016	Carbamazepine Haloperidol Levomepromazine	Not reported	Yes	Haloperidol [] increased or decreased
S/019	Flupentixol Carbamazepine Chlorpromazine	Not reported	No	Flupentixol [] decreased
S/028	Phenytoin Amitriptyline	Antiretrovirals	Yes	Amitriptyline [] decreased
S/029	Phenobarbital Chlorpromazine	Not reported	No	-

S/032	Carbamazepine Chlorpromazine	Not reported	No	-
S/036	Amitriptyline	Losartan Levothyroxine	No	-
S/041	Amitriptyline Phenytoin	Not reported	Yes	Amitriptyline [] decreased
S/046	Carbamazepine Phenobarbital	Not reported	Yes	Carbamazepine [] decreased
S/048	Fluoxetine	Fluticasone Betadine	No	-
S/049	Fluoxetine	Antiretrovirals	Yes	Fluoxetine [] increased
S/051	Carbamazepine	Vit B	No	-
S/052	Fluoxetine	Lamivudine, Stavudine Nevirapine	Yes	Fluoxetine [] increased
S/055	Phenytoin Carbamazepine	Not reported	Yes	Phenytoin [] decreased Carbamazepine [] decreased
S/058	Haloperidol Chlorpromazine	Antiretrovirals	Yes	Haloperidol [] increased Chlorpromazine [] increased
CK/001	Thiopental	Perfalgan	No	-
CK/002	Thiopental	Insulin	No	-
CK/003	Thiopental	Cefotaxime Tramadol Paracetamol	No	-
CK/004	Thiopental	Cloxacilline Nevirapine Bactrim	No	-
KF/001	Midazolam	Morphine Paracetamol Diclofenac	No	-