

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

Innocent Hahirwa^{1,2*}, Corinne Charlier¹, Charles Karangwa², Raphaël Denooz¹

¹Laboratory of Clinical, Forensic, Environmental and Industrial Toxicology, CHU-Liege, 4000 Liege, Belgium

²Laboratory of Analysis of Foodstuffs, Drugs, Water and Toxics, University of Rwanda, School of Medicine and Pharmacy, 117 Huye, Rwanda

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 et 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.,

*Corresponding author: ihahirwa@student.ulg.ac.be, i.hahirwa@ur.ac.rw

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 (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) 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 1 and 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 Na₂CO₃ 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 simultaneous 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.

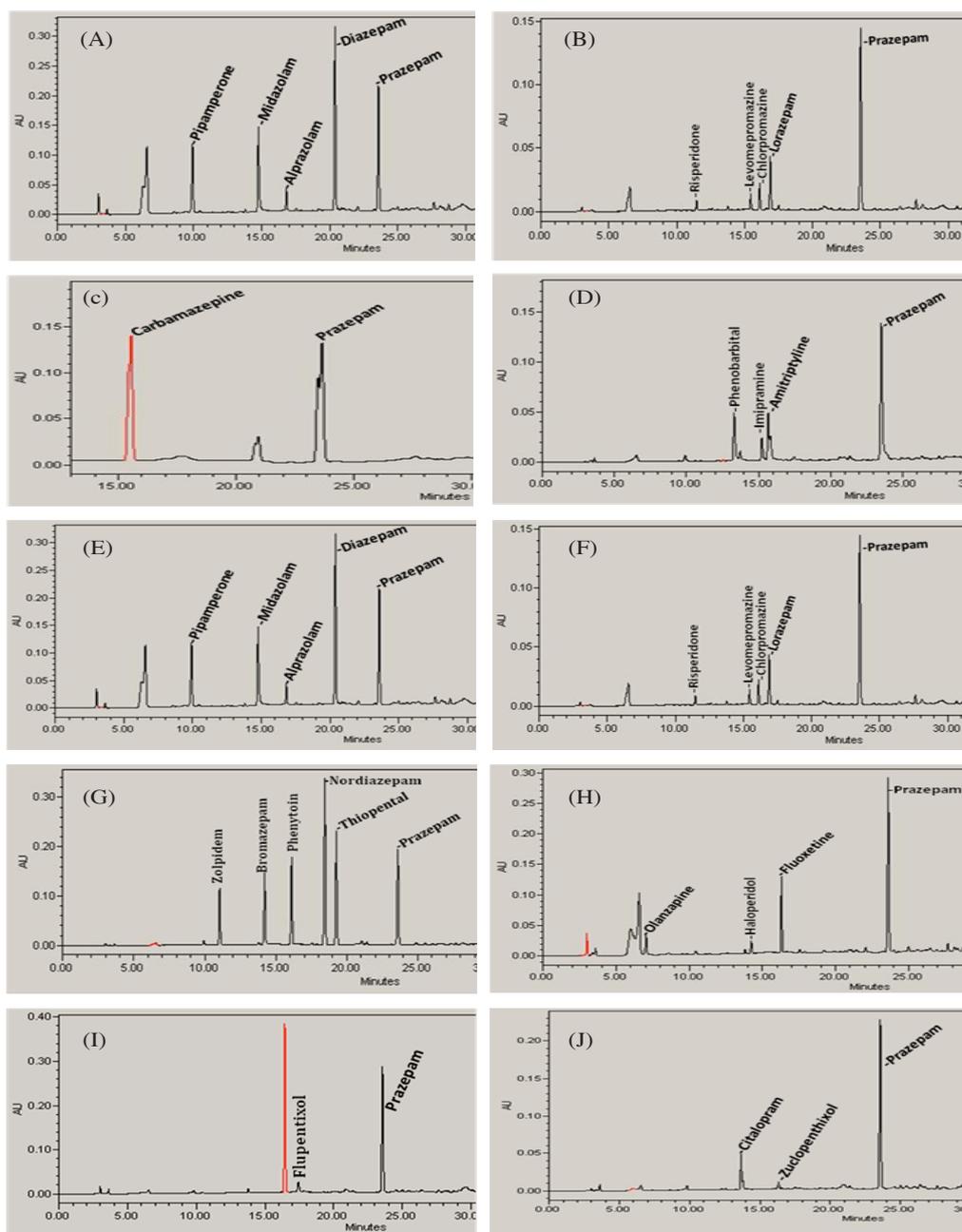


Figure 1. HPLC chromatograms for various molecules

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)

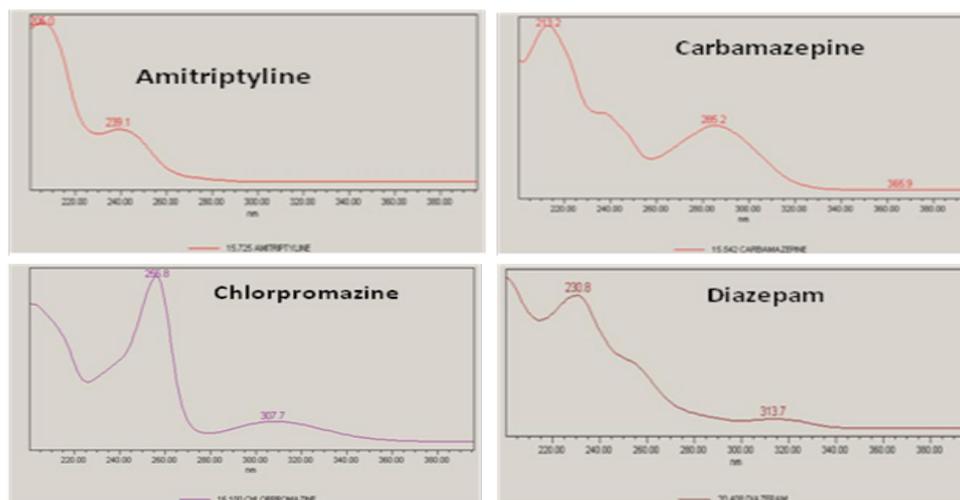


Figure 2. UV-visible spectra of 4 molecules taken as examples between 200 and 400 nm

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 $\pm 30\%$ 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

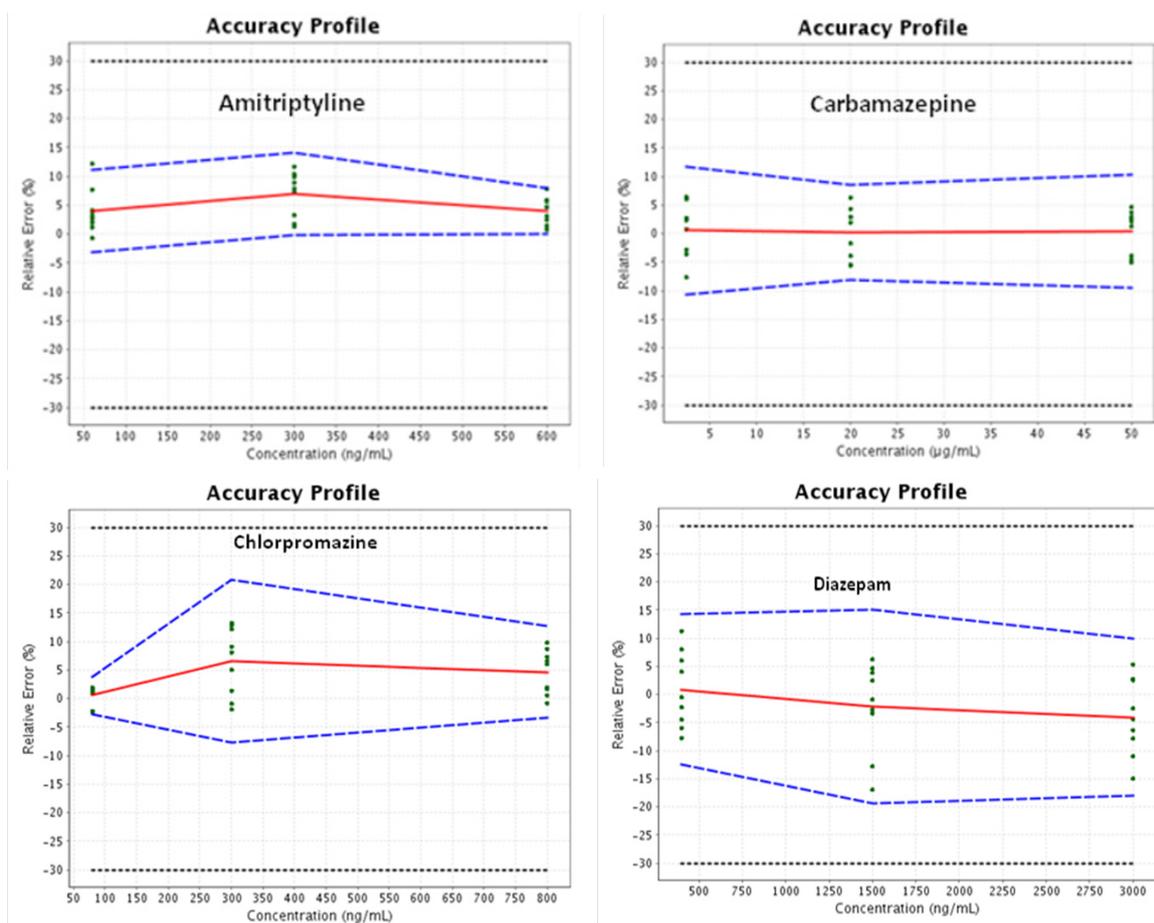


Figure 3. Accuracy profiles for 4 molecules taken as examples

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.

Acknowledgment

We gratefully acknowledge the financial support of the Belgian Technical Cooperation.

Declaration of interest

The authors report no declarations of interests.

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