VALIDATION OF ANALYTICAL METHOD FOR THE DETERMINATION IN SERUM OF PSYCHOTROPIC DRUGS COMMONLY PRESCRIBED IN RWANDA BY HPLC-DAD AND ANALYSIS OF SERUM SAMPLES FROM RWANDAN PATIENTS

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INTRODUCTION

A considerable interindividual variability in clinical response to psychotropic drug treatment is observed (1). As 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 interindividual variation in the medication's steady state concentration in the body may result (2-4). Monitoring blood concentration levels of these drugs remains a valuable and essential tool for optimization of treatment with these drugs (5).

Currently in Rwanda, no determination of psychotropic drugs in patient blood is done. This makes difficulty the optimization of treatment with these drugs and exposes patients to a high risk of toxicity. This prompted us to undertake a study aiming to validate an analytical technique for the determination in serum of psychotropic drugs most commonly used 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.

MATERIAL AND METHODS

EXTRACTION PROCEDURE

Serum	1 mL
IS (Prazepam 10 mg/L)	100 µL
Na_2CO_3 1M	500 µL
Extraction mixture	5 mL

- Shake 10 minutes
- Centrifuge 5 min at 3000 turns/min
- Pick up 3.5 mL of the supernatant
- Evaporate to dryness under nitrogen

Acetonitrile/H_2O (50/50)	70 µL

- Put the 70 µL into eppendorf tubes
- Centrifuge 5 min
- Put the supernatant in vial (HPLC Waters)

CHROMATOGRAPHIC CONDITIONS

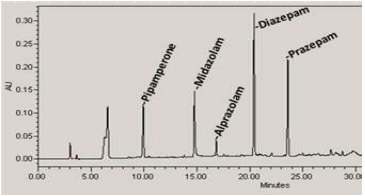
- ✓ Waters Alliance 2695 Separation Module
- ✓ Symmetry® C8 column
- ✓ Mobile phase: - Acetonitrile (ACN)
  - Phosphate buffer pH 3.8 (Ph.B)
- ✓ Injection volume: 40 µL
- ✓ Run time : 45 min
- ✓ Detection wavelength: 200 and 400 nm

Result processing: Enoval® software

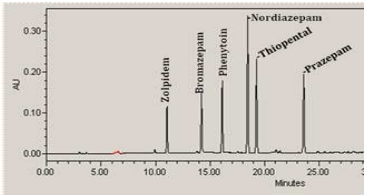
Time (min)	Flow (mL/min)	% ACN	% Ph.B
0	1.0	13.0	87.0
9.0	1.0	35.0	65.0
28.0	1.5	80.0	20.0
30.0	1.5	80.0	20.0
31.0	1.5	13.0	87.0
32.0	1.0	13.0	87.0
45.0	1.0	13.0	87.0

RESULTS

The developed method was linear over tested dosing intervals with coefficient of determination of at least 0.99 for all molecules. The method showed good selectivity, precision and accuracy. The RSD % and the relative bias respectively ranged from 0.5 to 13.2 % and from 0.06 to 12.9 %, while the recovery varied between 92.7 and 112.9 %. The accuracy of the method was demonstrated over selected dosing intervals. Peaks with good resolution were obtained and hereafter some of chromatograms are presented.



Chromatogram of serum spiked with pipamperone 1 µg/mL, midazolam 0.5 µg/mL, alprazolam 0.1 µg/mL and diazepam 1 µg/mL



Chromatogram of serum spiked with bromazepam 0.5 µg/mL, nordizepam 1 µg/mL, phenytoin 20 µg/mL, thiopental 5 µg/mL and zolpidem 0.5 µg/mL

Overall results

Drugs	Therapeutic windows	Total cases	Subtherapeutic cases	Therapeutic cases	Supratherapeutic cases
Amitriptyline	80 - 200 ng/mL	12	9	3	0
Carbamazepine	4 - 12 µg/mL	46	7	36	3
Chlorpromazine	30 - 300 ng/mL	26	22	4	0
Citalopram	50 - 110 ng/mL	7	2	3	2
Clomipramine	175 - 450 ng/mL	4	4	0	0
Clonazepam	20 - 70 ng/mL	1	0	1	0
Diazepam	125 - 1500 ng/mL	3	2	1	0
Fluoxetine	120 - 500 ng/mL	4	2	1	1
Flupentixol	1 - 10 ng/mL	11	7	4	0
Haloperidol	1 - 10 ng/mL	54	21	28	5
Levomepromazine	30 - 160 ng/mL	36	16	17	3
Midazolam	80 - 250 ng/mL	1	1	0	0
Olanzapine	20 - 80 ng/mL	2	1	1	0
Phenobarbital	10 - 40 µg/mL	9	2	5	2
Phenytoin	10 - 20 µg/mL	6	5	1	0
Pipamperone	100 - 400 ng/mL	1	0	1	0
Risperidone	20 - 60 ng/mL	1	1	0	0
Sulpiride	200 - 1000 ng/mL	2	2	0	0
Thiopental	1 - 5 µg/mL	4	0	3	1
Zolpidem	80 - 150 ng/mL	7	7	0	0
Zuclopenthixol	4 - 50 ng/mL	1	0	0	1
	Total	238	111	109	18
	Percentage		46,6%	45,8%	7,6%

Analysis of serum samples from rwandan patients under treatment with psychotropic drugs

Sample collection sites : Kigali University Teaching Hospital (CHUK), Ndera Neuropsychiatric Hospital (HNP -NDERA) and King Faisal Hospital (KFH)

Study population characteristics

Characteristics		Overall	CHUK	HNP-NDERA	KFH
Number of patients		128	53	74	1
Sex	F	54%	66%	46%	0%
	M	46%	34%	54%	100%
Age	Min	12	12	16	65
	Max	68	68	56	65
	Mean	31	32	31	65
Psychotropic drugs/patient	Min	1	1	1	1
	Max	4	4	4	1
	Mean	2	1.5	2.4	1

Results by pharmacological classes comparing two main sites of sample collection

CHUK (referral hospital) versus HNP-NDERA (specialised neuropsychiatric hospital)

Pharmacological classes	Total cases		Subtherapeutic cases		Therapeutic cases		Supratherapeutic cases	
	CHUK	NDERA	CHUK	NDERA	CHUK	NDERA	CHUK	NDERA
Antidepressants	18	9	55%	78%	39%	0%	6%	22%
Antiepileptics	32	29	28%	17%	66%	73%	6%	10%
Barbiturates	4	-	8%	-	67%	-	25%	-
Benzodiazepines	2	9	50%	89%	50%	11%	0%	0%
Neuroleptics	15	119	80%	49%	20%	44%	0%	7%
Overall	71	166	45%	47%	49%	45%	6%	8%

CONCLUSION

A simple and sensitive HPLC-DAD method has been validated for the determination in serum of selected psychotropic drugs. Except for haloperidol, flupentixol and zuclopenthixol where it is only applicable for confirmation of intoxication, the method is suitable for both therapeutic drug monitoring and confirmation of intoxication. Sample analysis has shown that the risk of ineffectiveness in patients under psychotropic treatment is higher (47%) than the risk of toxicity (8%), with only 46% of results within the optimal therapeutic window. The situation is quite similar in both compared sites and this obviously demonstrates the need of therapeutic drug monitoring for these drugs in Rwanda.

References: (1) Malhotra A. K. et al., Pharmacogenetics of psychotropic drug response. *Am. J. Psychiatry 2004; 161: 780–796.* (2) Bengtsson, F., Therapeutic drug monitoring of psychotropic drugs. TDM "nouveau". *Ther Drug Monit 2004; 26: 145–151.* (3) Klotz, U., Pharmacokinetics and drug metabolism in the elderly. *Drug Metabolism Reviews 2009; 41 (2): 67–76.* (4) Brosen, K., 1996. Drug-metabolizing enzymes and therapeutic drug monitoring in psychiatry. *Ther Drug Monit 1996; 18: 39– 396 ;* (5) Hiemke C. et al., AGNP Consensus Guidelines for Therapeutic Drug Monitoring in Psychiatry: Update 2011. *Pharmacopsychiatry 2011; 44: 195–235.*