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QMS *VERSUS* HPLC-UV FOR LAMOTRIGINE PLASMA MONITORING

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ABSTRACT: Lamotrigine (LTG), a synthetic anticonvulsant, may lead to therapy failure or toxicity risks due to the wide inter and intra individual pharmacokinetic variations. Those wide variations in LTG require its therapeutic drug monitoring (TDM). Our study aims to compare the QMS method with the HPLC-UV which is considered as a reference method for TDM of LTG. The study was conducted in the Clinical Pharmacology Department of the National Centre of Pharmacovigilance during a period of nine months. It included 37 samples from 37 patients treated with LTG. The samples were monitored by HPLC-UV and by PETIA. Regression analysis performed with data yielded to the equation LTGPETIA = 0.7701 LTGHPLC + 1.475 with a Pearson correlation r = 0.804 (P<0.0001). Bias mean between the two methods was 13.45%. In conclusion, the turbidometric technique (QMS) is comparable to the HPLC-UV method in the interval of therapeutic concentration. Under low concentrations, the QMS technique is less precise.

INTRODUCTION: Lamotrigine (LTG) is a synthetic anticonvulsant that was first introduced in 1994 in the United States, as an adjunct therapy for partial seizures [1]. It is a phenyltriazine anti-convulsant that blocks voltage-sensitive sodium channels and inhibits the release of excitatory neurotransmitters. Currently, LTG is widely prescribed as a monotherapy for partial seizures for both adults and children, and as a therapy for bipolar disorder.

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Furthermore, it was demonstrated as being efficient against the drop attacks associated with the Lennox-Gastaut syndrome [2, 3]. Despite its efficiency, LTG may lead to therapy failure or toxicity risks due to the wide intra- and inter-individual pharmacokinetic variations. LTG is rapidly absorbed by the intestine; this absorption will not be affected when eating. The maximum plasmatic concentration is from 1.4 to 4.8 h after the drug administration, and the plasma protein binding is approximately 55% [4, 5].

The LTG is metabolized in the liver by the uridine diphosphate glucuronosyl transferase (UDP). This metabolism is a N2-glucuronidation [6]. The co-administration with other anti-epileptic drugs affects the LTG metabolism, such as valproic acid, which is an enzyme inhibitor that increases the

LTG half-life. However, carbamazepine and phenytoin induce the LTG metabolism, decreasing its half-life [7]. The wide intra- and inter-patient variations in LTG require its therapeutic drug monitoring (TDM) in order to ensure the efficiency of the drug with minimal side effects risk. LTG can be monitored by High Performance Liquid Chromatography (HPLC) [8] or by Quantitative Microsphere System (QMS).

HPLC is a precise and accurate method for LTG monitoring. However, a procedure of extraction of LTG from plasma is needed. QMS is a particle-enhanced turbidimetric immunoassay method (PETIA) developed by Seradyn and successfully adapted to Hitachi 911. [9] This simple method for drug monitoring may be an alternative to HPLC for the TDM of LTG for patients treated by this drug.

In literature, only one study compared the two methods and concluded that turbidometric technique (QMS) is comparable to the HPLC-UV method. This study aims to compare the QMS method to HPLC-UV which is considered as a reference method for TDM of LTG.

METHODS:

Patients and Samples: This study was conducted in the Clinical Pharmacology Department of the National Centre of Pharmacovigilance during nine months, from February to October 2015. It included 37 samples from 37 patients treated with LTG and addressed to our department for TDM of LTG. Blood samples were taken just before the next drug intake (trough concentration C_0). The samples (5 ml per patient) were collected from peripheral veins in EDTA tubes. The samples were monitored by the QMS method, then stored at -20 °C until being monitored by the HPLC-UV.

Method Analysis:

HPLC-UV: The HPLC procedures were carried out as described below [10].

Briefly, 200 mL of the plasma sample was pipetted into labelled disposable polypropylene Eppendorf tubes, to which 50 mL of the IS solution (barbital sodium) was added. After vortex-mixing, 200 mL of 10% acetic acid and 6 mL of the extraction solution (64% diethylether + 36% dichloro-methane) were added for protein precipitation. The tubes were mixed in a vertical shaker for 10 min, centrifuged at 3000 g for 10 min at 4 °C, and the organic layer was transferred into clean 5 mL tubes and evaporated to dryness under azote flux at 50 °C. The residue was dissolved in 100 µL of the mobile phase (75% of KH_2PO_4 + 25% of CH_3CN), and 50 µL were analyzed by the HPLC-UV. The reverse-phase HPLC conditions consisted of chromatographic separation using RP18 column (5 µm, 25 cm × 4.6 mm) at 60 °C with a mobile phase of 75% KH_2PO_4, 25% CH_3CN pumped at a flow rate of 0.8 mL/min, and a UV detection at a wavelength of 210 nm. LTG calibrator samples, from 0 to 50 mg/L (0, 2, 10, 20, 50 mg/L), were used to establish the calibration curve in order to determine the concentration of unknown samples.

Particle Enhanced Turbidimetric Immunoassay (PETIA): The LTG reagent used for the PETIA method was provided by Thermo Fisher. The assay is based on a competition for binding to the antibody binding sites between LTG present in patient sample and the drug coated on microparticles. When LTG is not found in the patient sample, the LTG-coated microparticle reagent (R2) is rapidly agglutinated in the presence of the anti-LTG antibody (R1). The agglutination rate of the particles is directly proportional to the absorbance change rate, spectrophotometrically measured. When a sample containing LTG is added, the agglutination reaction is partially inhibited, slowing the rate of absorbance change [9]. The agglutination inhibition was monitored at a wavelength of 700 nm [11]. Monitorage was made using Indiko® analyser developed by Thermoficher Scientific. Two hundred microliter of the sample were mixed with 120 µL of R1, and then incubated for 5 min. Then, 120 µL of R2 were added and incubated for 1 min.

During every analysis, 3 controls, provided by the manufacturer, were added: one at low concentration in the range (1.6-2.4 µg/mL), one at medium concentration (12-18 µg/mL), and one at high concentration (20-30 µg/mL). If the control will be found beyond the interval, corrective procedures should be conducted.

Statistical Analysis: Cross-validation between the two bioanalytical methods was performed with the consideration of the HPLC-UV assay as the reference method and the immunoassay as the test.

According to the FDA Guidelines for bioanalytical methods [12], the assay is considered acceptable if imprecision at each concentration will be less than 20% for both within-day and between-day variability and inaccuracy ± 15%. The analysis was conducted with Microsoft Excel® and Graph pad®. A regression analysis was performed between LTG concentrations, obtained with HPLC-UV and PETIA. The scatter of the method comparison data was visualized according to Bland-Altman approach [13] with the mean percentage difference represented as a straight line for the comparison between the two assays. Results are illustrated using the mean values, standard deviation, bias, and coefficient of variation (CV %).

RESULTS:

Validation Study: The HPLC method is linear from 2 to 50 µg/mL range (r= 0.99). Detection (LOD) and quantification limits (LOQ) were 0.07 µg/mL and 0.21 µg/mL, respectively. Within-day coefficients of variation were 13.37 to 16 % and day-to-day coefficients of variation were 15.68 to 16.63 % at three quality controls (QCs) 2, 10 and 40 µg/mL [10].

TABLE 1: WITHIN-DAY AND DAY TO DAY CVS OF PETIA'S LTG QUALITY CONTROLS (QCS)

LTG concentration (µg/mL)	2	15	25
Within-day			
Mean ± SD	2.1 ± 0.1	13.6 ± 1.2	24.2 ± 2.5
CV%	6.1	8.9	10.5
Day-to-day			
Mean ± SD	2.4 ± 0.2	16.2 ± 0.4	26.4 ± 3.6
CV%	6.4	2.5	13.5

The PETIA method is linear from 2 µg/mL to 40 µg/L, its limit of detection is 2 µg/mL and the correlation factor is estimated by 0.99. Results from the PETIA validation study for LTG are reported in **Table 1**. Within-day and day-to-day coefficients of variation measured on LTGQCs were less than 5.6%, while inaccuracy was less than 14.6%.

HPLC-UV Results: Thirty seven samples were monitored by HPLC-UV. Mean concentration was 4.79 ± 0.72 µg/mL. The minimal concentration was 0.8 µg/mL while the maximum was 21.08 µg/mL.

PETIA Results:

Quality Controls (QCs): The low QC (n=6) had mean concentration of 2 ± 0.29 µg/mL and biases ≤ 20%. The control limit was (1.6-2.4 µg/mL). The medium QC (n=6) had mean concentration of 12.73 ± 0.43 µg/mL and biases ≤20%. The control limit was (12-18 µg/mL). The high QC (n=6) had mean concentration of 21.03 ± 3.34 µg/mL and biases ≤20%. The control limit was (20-30 µg/mL).

Determination of Blood Concentration: The same 37 samples were monitored by PETIA. Mean concentration was 5.17 ± 3.2 µg/mL. The minimal concentration was 1.2 µg/mL and the maximal one was 16.1 µg/mL.

Methods Comparison: Regression analysis performed with this data yielded to the equation LTGPETIA= 0.7701 LTGHPLC + 1.475 with a Pearson correlation r = 0.804 (P<0.0001). The bias was 19.3% (50-40.13%). The bias was 47.51% for concentrations less than 2 µg/mL and 13.47% for concentration >2 µg/mL. The Bland-Altman approach showed that the LTG concentrations measured with the QMS, exceeded those determined with the HPLC, by 13.06% (95% CI: 72.44%-46.32%).

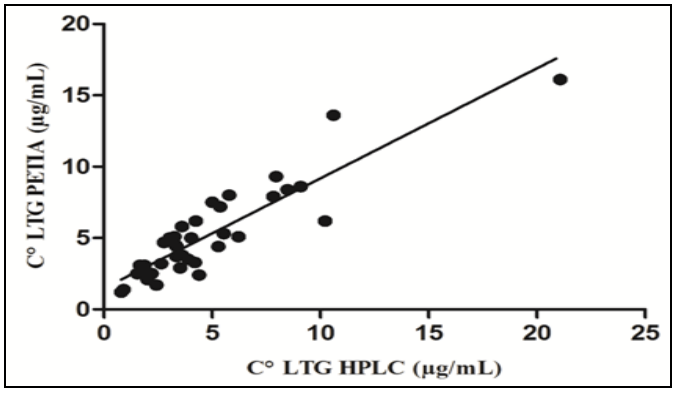

FIG. 1: REGRESSION CURVE BETWEEN THE TWO METHODS

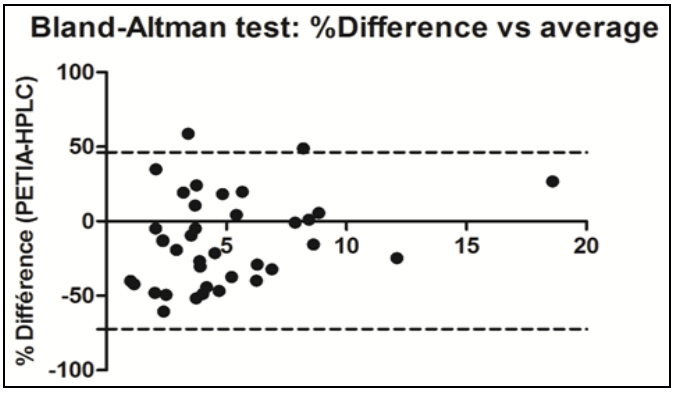

FIG. 2: BLAND-ALTMAN TEST: POURCENTAGE OF DIFFERENCE *vs*. AVERAGE

DISCUSSION AND CONCLUSION: The LTG is a second generation antiepileptic characterized by a wide intra- and inter- individuals variability. Its TDM is necessary; it is fulfilled through the measurement of trough plasmatic concentrations [14]. HPLC-UV is an accurate method but needs an extended step of extraction followed by the injection in the HPLC-UV system [15]. In our study, we compared results of LTG plasma concentrations obtained from a new immunoassay method (PETIA) using Indiko® analyser to HPLC-UV method. Thirty seven samples were analyzed. All samples were provided from patients treated by LTG for epilepsy and bipolar disorder. The blood level of LTG measured by HPLC-UV method varied from 0.8 to 21.08 µg/mL (mean 4.79 ± 0.72 µg/mL). The study of Magnolon and al published in 2012 [11] showed blood levels varying from 0.6 to 16.3 µg/mL for a group of 17 patient treated for epilepsy.

PETIA method consists on a competitive inhibition of agglutination between the microspheres covered with LTG in the reagent and the LTG present in the blood sample. This automatic method does not need extraction, but has a risk of interference with endogenous and exogenous molecules [11]. The plasma levels of LTG using PETIA method in our study varied from 1.2 to 16.1 µg/mL (mean 5.17 ± 3.2 µg/mL). In Magonolon and al analysis, the results varied from 0.83 to 13.88 µg/mL [11]. Comparison of the two method showed a correlation coefficient r equal to 0.804 with a linear regression relationship of LTGPETIA = 0.7701 LTGHPLC + 1.475. Bias mean between the two methods was 13.45%.

In literature, only one study published in 2015 [16] including 61 samples for the determination of the LTG trough plasmatic concentration in epileptic patients, compared PETIA method to HPLC-UV method. They found a positive correlation between the two methods with a Pearson coefficient of 0.968. In our study, the Bland-Altman approach showed that PETIA method overestimated LTG concentrations by 13.06% (95% CI: 46.32%-72.44%). Overestimation was 47.51% when LTG concentrations were less than 2 µg/mL (n=7), and 13.47% for concentrations above 2 µg/mL (n=30). Our results are in accordance with those of Baldelli *et al.* [16]

In fact, they found that LTG concentrations measured with QMS exceeded those determined by HPLC-UV by 15.6%; (95% CI: (0.108-0.182 mg/L); SE, 0.0186). They found also that the mean percent bias between QMS and HPLC-UV methods for LTG data is different according to the range of drug concentration. They found that for concentrations less than 2 mg/L, the bias was 49.5 ± 8.2%, for those ranging from 2 µg/mL to 4.9 µg/mL, it was 18.1 ± 13.4%, whereas in the range 5-8,9 µg/mL, mean bias was 15.4 ± 8.5% (n=22). Many factors may explain this difference. For the QMS, the use of polyclonal antibody induces a cross reaction with multiple LTG metabolites: N-2 glucuronide, N-2 methyl and N-2 oxyde [17, 18] and that causes the over estimation.

In conclusion, the turbidometric technique (QMS) is comparable to the HPLC-UV method in the interval of therapeutic concentration. Under low concentrations, the QMS technique is less precise. It could be therefore, considered as a viable alternative to HPLC-UV method for usual LTG monitoring in the clinical practice.

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CONFLICT OF INTEREST: The authors have no conflicts of interest that are directly relevant to the content of this manuscript.

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