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EVALUATION OF SOME METHODS FOR DETERMINING LAMOTRIGINE FROM BIOLOGICAL FLUIDS AND PHARMACEUTICAL PREPARATIONS

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INTRODUCTION

Epilepsy is a neurological condition, a chronic disease characterized by recurrent episodes of spontaneous disruption of normal brain function, manifested by epileptic seizures. Epileptic seizures are a transient appearance of signs and symptoms due to abnormal neural activity in the brain. (Berg A.T. et al., 2010; Fisher R.S.et et al. 2005). Epilepsy cannot be cured, but seizures can be controlled with medication in about 70% of cases (Kotsopoulos A. I, et al. 2002; Popa C. 1992). Given the distinct characteristics of antiepileptic drugs (AEDs), therapeutic drug monitoring (TDM) can make a significant contribution to the field of epilepsy. (Abend et al. 2010).

Drug treatment varies depending on the type of epilepsy, age, the patient's condition (overweight, pregnant patients), etc. (Munoz A et al. 2007). It must ensure the best possible control of crises with minimal side effects. Therapy will be started immediately, as soon as possible, even after the diagnosis of epilepsy has been established. (Musicco M et al, 1997; Graber & Prince 1999). It is preferable to take a single drug to minimize side effects, drug interaction, and possible teratogenic effects. (Popa C. 1992, Musicco M et al. 1997). This increases the patient's quality of life and reduces the cost of medication. The administration of a single drug can be done when the patient has only one type of seizure. (Munoz A et al. 2007). When the semiology is varied and the crisis cannot be controlled, therapy is resorted to with several drugs, initially with two and then with three antiepileptics of different classes, according to the epileptic syndrome (Cohen et al, 1987; Goa et al, 1993; Johannessen et al. al, 2003).

Many studies analyze the potential of CT for AED, describe the relationships between serum drug concentration, clinical effect and adverse drug reactions for each AED, and the different analytical methods used to quantify the drug in serum. (Fisher 2005; Kotsopoulus et al. 2002). Retrospective and prospective studies were performed on serum drug concentration, oral efficacy, reference concentration range and active metabolites, link between drug concentrations in terms of clinical efficacy and no response. (Benardete & Kriegstein 2002; Berg & shinnar 1997). Measurement of serum medication may play a beneficial role in patient management and individualization of treatment. (Elwes et al. 1998; Johannessen et al. 2003). Standardized studies to assess in particular the relationship: concentration-efficacy-toxicity for recent AEDs are urgently needed.

The selection of AED for a particular type of seizure depends on the type of drug (eg side effects, toxicity, drug interactions), specific to the patient (sex, age, genetics, use of contraception), availability, cost. (Elwes et al. 1998).

Lamotrigine LMT

Antiepileptic drugs work by inhibiting the nerve impulse and prevent its spread from the epileptogenic focus. LMT is a category of antiepileptic drugs that block sodium channels. (*Graber and Prince, 1999*). It may suppress the release of glutamate and aspartate, (*Olney JW et al. 1974*), 2 of the dominant excitatory neurotransmitters in the CNS. (*Reiss and Oles, 1996: X. Li et al. 2008, Munoz A. et al. 2007*). LMT is inactivated in the liver by glucuronidation. (*Alarcon G. 1996*).

In the treatment of epilepsy, anticonvulsant drugs are used to inhibit seizures. LMT is an effective oral antiepileptic in the treatment of focal epilepsy with or without secondary generalization and has been suggested to be effective in the treatment and prophylaxis of affective disorders. (*Cohen AF et al. 1987; Fisher RS et al. 2005; Goa KI et al. 1993*). There is still limited information on therapeutic intervals for monitoring LMT treatment. TDM is an established tool for drug dose adjustment for the patient, avoiding side effects and assessing patient compliance to obtain an optimal clinical response (*Johannessen et al, 2003*).Lamotrigine: [3,5-diamino-6- (2,3-dichlorophenyl) -1,2,4-triazine] is a phenyl-triazine derivative with broad-spectrum anticonvulsant activity. It is a weak lipophilic base, bound to plasma proteins in a proportion of 55%. Extensive metabolism of the LMT molecule occurs in the liver, predominantly by N-glucuronidation by uridine-glucuronyl-transferase isoenzymes. (*Thomas et al. 2010; Rogawski & Loscher 2007*). Metabolites

are not considered to be pharmacologically active. It is an effective drug as adjunctive therapy and as monotherapy for partial and generalized convulsions. It has also been studied for use in the treatment of neurological lesions and as a tranquilizer, as well as in the treatment of bipolar disorder. LMT is effective in preventing or delaying manic, depressive, or rapid cyclical episodes (it is designated a first-line drug for treating depression in bipolar disorder) (*Goa KI et al. 1993; Hussein & Posner 1997*).Oral contraceptives (containing estradiol) and pregnancy can drastically decrease the concentration of serum LMT (*Cohen et al. 1987*). The effect was not observed in extensive LMT molecules in women who also received valproate (*Leviel & Naquet 1977*). Transient decreases in serum concentration have been observed and after surgery in epilepsy, CT is beneficial in preventing early postoperative seizures. Treatment monitoring is also beneficial in completing individual reference intervals and for optimizing individual dosing regimens. (*Silver et al. 1991*). Dose adjustment and monitoring are also required in patients undergoing hemodialysis or in those with severe hepatic impairment. (*Franceschi & Furlann 2005; Goa KI et al. 1993*).LMT should be introduced with slow titrations due to the possibility of severe dermatological reactions, including Stevens-Johnson syndrome and toxic epidermal necrolysis. AED enzymes can halve the half-life (8-20 hours), which leads to lower LMT levels. The half-life increased to 60 hours when LMT was associated with VPA. (*Leviel & Naquet 1977; Silver et al. 1991*). In polytherapy with VPA, its inhibitory effect counteracts the inducing effect of AEDs that induce enzymes (*Hussein & Posner 1997*). The drug combined with metsuximide decreases the concentration of LMT by 70% (*Leviel & Naquet 1977*). Plasma concentrations of LMT were shown to increase by 90% from baseline in pregnancy until the third trimester (*Thomas et al. 2010*), this was not observed in patients receiving concomitant VPA (*Leviel & Naquet 1977; GoaKI et al. 1993*). Therefore, TMD is important in preventing seizures during pregnancy, and dose adjustments are based on minimum serum concentrations at least once a month (*Rogawski & Loscher 2007*).Transplacental transfer of LMT into maternal blood, amniotic fluid, and umbilical cord blood was measured and correlated (*Brodie et al. 1995*). It has been reported that in children and the elderly the clearance of LMT is higher (*Franceschi & Furlan 2005*). TDM is valuable for dose adjustment during pregnancy and for verifying inter-individual variability and drug interactions. Reference concentrations of 2.5-15 mg/L have been suggested in patients receiving therapeutic doses (*Abden et al. 2010; Brodtkorb & Reimers 2008*). The prevalence of toxicity increases significantly at concentrations higher than 15 mg/L (*Cohen et al. 1987*). Monitory of LMT can be complicated by pharmacodynamic interactions (in combination with other antiepileptics such as carbamazepines) (*Brodie et al. 1995; Goa et al. 1993; Ashton et al. 1999*).

MATERIAL AND METHODS FOR DETERMINING LAMOTRIGINE

Several methods for the determination of LMT in serum or plasma have been reported, including: high performance liquid chromatography (HPLC), (*Cheng et al, 2005; Patil and Bodhankar, 2005; Castel-Branco et al, 2001; Croci et al, 2001; Torra et al, 2000; Delmar Cantu et al, 2006*), radioimmunoassay (*Biddlecombe et al. 1990*), capillary electrophoresis (*Shihabi ZK. Oles KS. 1996; Theurillat et al., 2002*), spectrophotometry (*Fadhil and Muhammad, 2014; Mohammed Ishaq et al, 2013; Rajendraprasad et al, 2009*), electrochemical methods (*Hanawa et al, 2018; Dominguez-Renedo et al, 2008; Manjunatha et al, 2009; Hayedeh Bagheri Saeghi et al, 2011*).

Chromatographic determination

The HPLC method was generally chosen for the determination of LMT in biological fluids. It involves a simple isolation procedure before the chromatographic step. The most common procedures are: liquid-liquid extraction (*Croci et al. 2001; Stoforidis A et al. 1999; Castel-Branco et al. 2001; Queiroz et al. 2002; Patil & Bodhankar 2005*), protein precipitation (*Castel-Branco et al. 2001; Talekar et al. 2000; Youssef & Taha 2007*), use of an automatic dialysis system, enriched with sequential traces (*Dreassi et al. 1996*), microextraction of the solid phase out of line (*Patil et al. 2004*), extraction solid phase off-line with Isolute C8 cartridge (*Youssef & Taha 2007*) and the use of a vacuum processor for extraction with Oasis HLB extraction column (*Lensmeyer et al. 1997; Talekar et al. 2000*). These methods require intense work, time consuming and an internal standard. An alternative method of extracting the sample that has generated great interest is to inject the serum directly, using a flow extraction method. (*Vermeij TA. & Edelbroek PM. 2007*). The direct serum injection technique is preferred because the sample pretreatment procedures: time consuming procedures, errors and low recovery risks can be quickly avoided. (*Sadilek et al. 2007; Mullett W. 2007; Kataoka H. 2003*). On the other hand, the internal standard is not necessary, because a small amount of sample is handled. In this way, the combined systems of switching the extraction column with columnar in-line, have proven to be the best solution for the treatment and analysis of complex biological matrix such as human serum (*Cabrera K. 2004*).

In the last 10-15 years, laboratory medicine has presented the introduction and evolution of mass spectrometry in tandem with liquid chromatography (LCMS/MS method). This includes a solid phase

extraction (SPE) technique with a drying and reconstitution step. (*Delmar-Canto et al. 2006; Chandan RS. Et al. 2013*). In this technique, the cation exchange column was used instead of the reverse phase column due to the poor retention of the analyte in this column. The method is useful for therapeutic monitoring of drugs. (*Matar et al. 1998; Kataoka H. 2003; Angelis-Stoforidis P et al. 1999; Bhaskara Reddy et al. 2013; Torra M et al. 2003; Bhaskara Reddy et al. 2013*).

The proposed method was further compared with other published methods and proved to be faster and more selective, requiring a simple sample preparation procedure. The reduced amount of solvent consumed, by increasing the volume of serum introduced in the precolumn, shortens the analysis time to 2 minutes, leading to an average cost effective friendly to the chromatographic procedure. (*Barbosa NR. & Midio AF. 2000; Cabrera K. 2004*). Dosage chromatographic methods have proven to be a very good alternative for the determination of LMT in human serum, useful and suitable for experimental and clinical research as well as for routine monitoring of patients, in order to define the optimal range used in different types of epilepsy: children, adults, the elderly. (*Torra M et al. 2003; Delmar Cantu M et al. 2006; Bhaskara Reddy et al. 2013*).

Determination of spectrophotometric LMT with bromo-phenol blue (BPB)

Three rapid methods with high sensitivity and specificity for the determination of LMT in pure substance and in tablets have been developed. Method A is based on the formation of LMT-blue bromine phenol (BPB) complexes at pH 1.44 +/- 0.01 with λ of 420 nm. Method B and Method C wherein the drug-dye pairs are dissolved in sulfuric acid resulting in the acid form (measured at 420 nm) or the basic form (measurable at 600 nm). (*Vinay et al. 2009; Dreassi et al. 1996*). LMT reacts with BPB, an anionic dye, in an acid medium (pH = 1.44 ± 0.01) and forms a yellow coplex, which is extracted quantitatively by method A. The complex has maximum absorbance at 420 nm. The ion pairs dissolve and dissolve in alcoholic solution of sulfuric acid or alcoholic solution of potassium hydroxide (free dye in protonated form at 420 nm or in deprotonated form at 600 nm). (*Rajendraprasad et al. 2010*).The absorption was determined with a digital spectrophotometer. All 3 methods are selective and 2 of them (B and C) are extremely sensitive, applicable to ng/mL, sensitivity suitable only with fluorescent LC or electrochemical detector. The methods do not interact with excipients and additives. Statistical parameters showed good accuracy and precision. Therefore, these methods will be widely used for the determination of LMT from pharmaceutical to laboratory for routine analysis. (*Najib & Aziz 2013; Rajendraprasad et al. 2010*). The described analysis was processed, the determinations were repeated 7 times in one day in order to study the precision, accuracy, repeatability. The values were lower than 1.4%. Accuracy was determined by mediating the deviation at known concentrations. The BIAS was calculated at each concentration. Selectivity was studied on mixtures. It was found that there was no interference of the inactive ingredients. Robustness and stability were assessed by small changes for two selected variables (water volume and reagent time in method A; ethanolic volume of sulfuric acid/potassium hydroxide and dissociation time in methods B and C) and the effects of the changes were studied on the absorbance of the colored system. The changes had a negligible influence. Intermediate values for accuracy ranged from 1.5 to 2.5% (*Gilman et al. 2001; Dasgupta A. 2012; Najib & Aziz 2013; Vinay et al. 2013; Rajendraprasad et al. 2010*).

The method is applied for the quantification of LMT in tablets. The results are comparable to those obtained in the published methods.

Spectrophotometric determination of LMT in pharmaceutical preparations

The method is based on the reaction between LMT with gold chloride III at pH: 2.5-3.5, with the formation of a yellow colored complex, with maximum absorption at 400 nm. The linear curve is subject to Beer's law in the range of 10 - 160 μg / mL LMT. No interferences of other ingredients were observed. Optimal reagent conditions and other analytical parameters were evaluated. The proposed method has been successfully applied in the determination of LMT in pharmaceutical preparations. It is a direct, simple and highly selective method. (*Mohammed Ishaq et al. 2013; El-Enamy et al. 2010; Frag EA et al. 2012; Rajput & Patel 2004; Vidal E et al. 1999*).

UV spectrophotometric methods for the determination of LMT and CBZ in binary mixtures and urine samples

Four UV spectrophotometric methods have been developed for the determination of antiepileptic drugs from binary mixtures and urine samples without being separated from each other. The first method is based on total absorption according to Beer's Law, the second uses two wavelengths: 304 and 315 nm for CBZ and 0 for LMT; in the same way 282 - 290 nm for LMT and 0 for CBZ; the third method involves the use of the first derivative with amplitude at 308.9 for CBZ and 286.6 for LMT, and the fourth uses the ratio of derivatives. It is not necessary to separate CBZ and LMT from samples. Interferences expected to be present in the urine were removed by adding suppressor solution to both the sample (urine) and the blank. (*Fadhil M. Najib, Muhammad S. Mustafa 2014; Franceschi I. Furlann M 2004; Patel A. Kataria M 2012; Ulu TS. 2011*).

The proposed methods were selective for the simultaneous determination of two drugs in the presence or co-administered antiepileptic. The suppression solution was prepared from the salt of interfering ions. For validation, determination of CBZ and LMT, in mixtures, by the proposed methods, their standards were used. Statistical analysis showed that there were no significant differences between them. (*Fadhil M. Najib, Muhammad S. Mustafa 2014*).

Determination of LMT with carbon paste modified electrode

The procedure was optimized for the determination of dopamine in LMT with carbon paste electrode modified by cyclic voltammetry, with 0.2 M phosphate buffer at pH 7. The carbon paste electrode was modified with LMT and Triton X-100 (TX-100) . This electrode showed a very good sensitivity to dopamine. Increasing TX-100 increases the peak redox current of dopamine. The mechanism of electrocatalytic oxidation has been studied in its entirety. The preparation of the modified electrode is very easy, as well as the recharging by a simple polishing. (*Manjunatha et al. 2009; Wang J. et al. 1993; Sharma NC & Sharma S 2011; Liu-Li-Hong et al. 2012*).

The study found good reproducibility, increased stability of the voltammetric response and a low detection limit for dopamine. High sensitivity, extreme simplicity, fast response and low price make the use of this method a priority. (*Manjunatha et al. 2009*).

Ion-selective electrode with polymeric membrane based on MIP for LMT

Molecularly Imprinted Polymers are constructed of materials with recognizable properties for certain molecules or compounds similar to antibodies, but without experimental restrictions, obtained, isolated and chemically and thermally stable. (*Mosbach K, 1994; Case F, Hand Honeicutt, 1994*).The procedure for synthesizing a MIP is based on the chemical polymerization of a functional monomer (*Fujishima et al, 2005; KiKuchi & Siratori 2004*) and a cross-linking agent in the presence of a molecule used as a template. (*KiKuchi & Siratori 2004*; *Haydeh Bagheri et al. 2011*). The polymer contains sites with a high affinity for template molecules, due to the shape and arrangement of the functional groups of the monomer units. (*Hayedeh Bagheri Sadeghi et al. 2011; Kriz & Masbach 2000*). This polymer contains stes with a high affinity and are used as antibody-like materials for the sensitivity and selectivity, owing to their long-term stability chemicals inertness and insolubility in water and most organic solvents. (*Gholivand et al. 2014; Ugo P et al. 1998; Wang et al. 2004*). The sensor was prepared by mixing thouroughlybMIP, additive, plasticizer in a glass dish. (*Asaik et al, 2016; Haydeh Bagheri et al. 2011*). The imprinted polymer is able to become selective again with the analyte and its similar structures. The performance of the sensor was investigated by measuring values of various LMT solutions. MIPs have several advantages over their biological counterparts: including low cost, ease of preparation, physical and chemical stability. The MIP molecular recognition is based both on the template molecular structure and on the interaction between the print molecule and the imprinted polymer. (*Gupta et al. 2007; Inzelt G. 2003; Ongera et al. 2008*).

Potentiometric procedure was successfully applied for the LMT determination in tablets. The resulting data were statistically compared with the labeled amounts on the tablets and the results demonstrate that the method is comarable with other methods reported for determination of LMT. (*Ugo et al. 1998)*. The analytical applicability of sensor was checked by determination and recovery of LMT in plasma and urine. (*Haydeh Bagheri et al. 2011; Burgoa et al, 2005)*.

Currently, there is an increasing amount of data on MIP electrochemical sensors with capacitive transduction (Haupt et Mosbach, 2000), conductometrics (*Suedee et al, 2000*), amperometrics (*Kriz et Mosbach, 1995*) and voltammetry (*Kirsch et al, 2001; Javanbacht et al. 2008*). Despite the relatively simple transduction of the potentiometric signal, there is little data on molecularly printed potentiometric sensors.

Boron-doped diamond electrodes (electrochemical studies: BDD)

The electrochemical activity of LMT was studied by cyclic voltammetry using BDD (boron-doped diamond) electrodes. The preparation of the electrode was described by *Fujishima et al. 2005*. BDD microelectrodes were prepared by increasing the thin film on the tungsten wire. The preparation details were described by *Asaik et al. 2016*. The size of the conductive peak was less than 200 µm, and the length between: 100 - 200 µm.

All measurements were made at room temperature (25ºC) in a Faraday cage. Cyclic voltamogram (CV) and chronamperogram (CA) were recorded using a potentiostat. Electrochemical measurements were performed with a cell with three electrodes: Ag/AgCl (saturated KCl), used as the reference electrode and a platinum wire used as the measuring electrode. BDD was used as the working electrode was mounted at the end of the cell using a ring.

The geometric area of the working electrode was estimated to be 0.363 cm². Prior to use BDD was pretreated by ultrasonication in 2-propanol for 5 minutes and in pure water for 10 minutes. At the beginning of the work these electrodes are polished with 0.5 µm alumina powder for 10 minutes. The results suggest that, initially, LMT is oxidized, then reduced. Using BDD greatly increases sensitivity. In

the case of the mercury electrode, LOD (detection limit) was estimated at 4.68 mM, but cannot be used for in vivo measurements due to toxicity. (*Burgoa et al. 2005*).

Determination of LMT in Pharmaceuticals with Adsorptive Stripping Voltammetry Using Screen Printed

In these experiments the electrode was modified by depositing a film of mercury on its surface. The peak for determining the LMT occurs at -1.06 V. The deposition potential was set at 0.40V due to the convenient quality of the signal obtained at this value. The electrochemical signal obtained with the mercury-coated electrode is higher than that obtained with the unmodified electrode. The parameters were calculated in terms of reproducibility. The results obtained were compared with those obtained by HPLC (*Matar et al, 1998*), described as a reference technique (24.5 ± 0.8 with modified electrodes compared to 25.2 ± 1.1 mg by HPLC). The LMT solution was prepared by dissolving it in water.Voltametric measurements are made with μAutolab (EcoChemie). Used: pulse amplitude: -62 mV; pulse duration: 500 ms. (*Burgoa Calvo et al. 2005; Beni et al. 2005; Hanawa et al. 2018; Gratteri et al. 1995*). The electrode is easy to prepare, and the measurement results by this method are comparable to those obtained by using HPLC.

Quantitative determination of LMT by linear scanning voltammetry

The method uses the electrochemically activated pencil lead graphite electrode as the working electrode. The electrochemical cell used consists of three electrodes: indicator electrode: graphite electrode type pencil lead; reference electrode: Ag/AgCl electrode and auxiliary electrode: platinum electrode. The indicator electrode (graphite type pencil lead Rotring HB) is the one at which the electrochemical reaction takes place. It is electrochemically activated in buffer (Britton-Robinson), at pH 2.21 by cyclic voltammetry (10 cycles between -0.2 V and +3.0 V), at a scanning speed of 500 mV/s. A new pencil lead was used for each experiment. (*Hanawa et al. 2018*).

The performance parameters of the electrochemical method for determining the LMT that were evaluated are: linearity, limit of detectors and limit of quantification, accuracy, precision, retrieval, selectivity (*Rogawski et al. 2002*).

To demonstrate linearity, linear scanning voltamograms for LMT solutions of different concentrations were recorded (figures 1, 2). Peak current intensities corresponding to lamotrigine oxidation were measured for each of the recorded voltamograms. The detective limit and the quantification limit were determined using the regression data of the calibration line. (*Rogawski & Loscher 2007*).

The accuracy and precision are determined from the average and the coefficient of variation for the concentrations of the analyzed samples. Accuracy is evaluated by repeatability and intermediate accuracy; repeatability was assessed at three concentration levels; the intermediate accuracy was evaluated by comparing the results obtained on different days. (*Rogawski et al. 2002; Saberi et al. 2012; Paolicchi et al. 2012*).

The standard additive method was used to evaluate the lamotrigine content of the pharmaceutical preparation. High sensitivity, extreme simplicity, fast response and low price make the use of this method a priority.

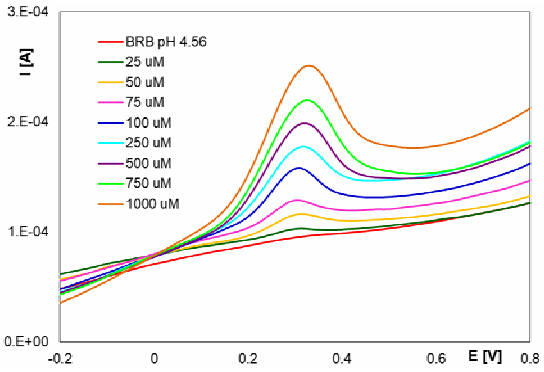

Fig. 1. Linear scanning voltamograms for LMT solutions of different concentrations; pH = 4,56.

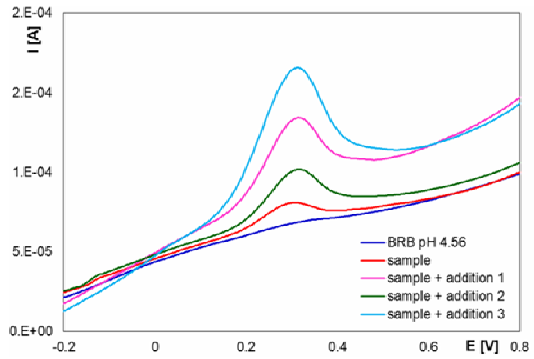

Fig. 2. Linear scanning voltammograms obtained for the sample in BR buffer pH = 4.56 before and after three additions of 0.05 mL stock solution LMT 2×10-2 M using an electrochemically activated pencil lead type graphite electrode (PGE *)

CONCLUSIONS

The pharmacological importance of LMT and its applications in the case of neurological diseases have led to the development of simple, reliable and fast methods for its determination from biological samples.

The choice of the method for determining lamotrigine is imposed by a series of conditions: the

type of sample (serum, plasma, urine, pharmaceutical preparation), routine determination, research; only LMT in the sample or mixed with other antiepileptics (Carbamazepine, valproate, etc.).

The HPLC method has proven to be a very good alternative for the determination of LMT in human serum samples. The serum can be injected directly into the chromatographic system. Reduced solvent consumption, short analysis time (2 minutes), lead to a cost-effective and environmentally friendly chromatographic procedure. It is a simple, useful and suitable method for clinical and experimental research, as well as for routine monitoring. High performance liquid chromatography in tandem with mass spectrometry is a powerful tool in terms of sensitivity, specificity and performance analysis.

Electrochemistry, an active field of modern research, offers some of the most sensitive electroanalytical techniques, stripping voltammetry and differential pulse voltammetry are able to determine the trace concentrations of an electroactive analyte and provide useful information on physical and chemical properties. Cyclic voltammetry has proven to be a very good alternative for the determination of LMT in human serum, useful and suitable for experimental and clinical research as well as for routine monitoring of patients, in order to define the optimal range used in different types of epilepsy: children, adults, the elderly.

For routine determinations, the endowment of the laboratory is essential in choosing the working method.

ABSTRACT

Lamotrigine is a broad-spectrum antiepileptic used as monotherapy or as an adjunct to other antiepileptics for the treatment of partial or generalized tonico-clonic seizures. This drug is also used in the treatment of neurological lesions, bipolar disorder, and other psychiatric disorders.

The choice of the appropriate method for the routine monitoring of the drug administered to the patient with epilepsy in order to individualize the therapy, the difficulty of establishing the dose, the therapeutic field, the fluctuations of the serum concentration in certain physiological conditions (during pregnancy) imposed this study.

The literature presents several methods for determining LMT and metabolites in the biological matrix including: HPLC, gas chromatography with nitrogen-phosphorus detector, mass spectrometry with thermospray chromatography, capillary electrophoresis, radioimmunoassay, immunofluorimetric analysis, UV electrophotometry, methods. In this paper, the focus was on chromatographic and spectrophotometric and electrochemical methods. Electrochemical methods with modified electrodes for LMT dosing are gaining ground lately.

The choice of method aims at: simplicity of analysis, precision, accuracy, sensitivity and selectivity of the method, low analysis time, low costs.

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