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# An easy-to-use liquid chromatography assay for the analysis of lamotrigine in rat plasma and brain samples using microextraction by packed sorbent: Application to a pharmacokinetic study

Sandra Ventura[a,b], Márcio Rodrigues[a,b], Sarah Pousinho[a], Amílcar Falcão[c,d], Gilberto Alves[a,c,e,*]

[a] CICS-UBI—Health Sciences Research Centre, University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal
[b] UDI-IPG—Research Unit for Inland Development, Polytechnic Institute of Guarda, 6300-749 Guarda, Portugal
[c] CNC—Center for Neuroscience and Cell Biology, University of Coimbra, 3004-517 Coimbra, Portugal
[d] Laboratory of Pharmacology, Faculty of Pharmacy, University of Coimbra, Pólo das Ciências da Saúde, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal
[e] Laboratory of Pharmaco-Toxicology—UBIMedical, University of Beira Interior, 6200-284, Covilhã, Portugal

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ABSTRACT

A simple and rapid high-performance liquid chromatography method with diode-array detection (HPLC-DAD) using microextraction by packed sorbent (MEPS) during the sample preparation step was developed and validated to quantify lamotrigine (LTG) in rat plasma and brain samples. MEPS variables such as pH, number of draw-eject cycles, and washing and desorption conditions were optimized. The chromatographic resolution of LTG and chloramphenicol, used as internal standard (IS), was accomplished in less than 5 min on a C18 column, at 35 °C, using an isocratic elution with acetonitrile (13%), methanol (13%) and water-triethylamine (99.7:0.3, v/v; pH 6.0) pumped at a flow rate of 1 mL/min. Detection was performed at 215 nm. Calibration curves were linear over the range of 0.1–20 μg/mL ($r^2 \geq 0.9947$) for LTG in both rat plasma and brain homogenate samples. The intra and interday imprecision did not exceed 8.6% and the intra and interday inaccuracy ranged from −8.1 to 13.5%. LTG was extracted from rat plasma and brain homogenate samples with an average absolute recovery ranging from 68.0 to 86.7%, and its stability was demonstrated in the assayed conditions. No interferences were observed at the retention times of the analyte (LTG) and IS. To the best of our knowledge, this is the first bioanalytical assay that uses MEPS procedure for the determination of LTG not only in rat plasma but also in tissue (brain) samples. This novel method was successfully applied to a preliminary pharmacokinetic study in rats and it seems to be a cost-effective tool to support non-clinical pharmacokinetic-based studies involving LTG treatment.


## 1. Introduction

Lamotrigine (LTG; Fig. 1) is a second-generation antiepileptic drug (AED) exhibiting a broad spectrum of efficacy against several types of epilepsy seizures, and it is also effective as a mood stabilizer agent in bipolar syndromes [1–3].

LTG has a narrow therapeutic range, a large inter-individual variability in its pharmacokinetics and some side effects are concentration-dependent, justifying therapeutic drug monitoring (TDM) in many clinical circumstances. For instance, LTG undergoes extensive metabolism to an inactive glucuronide metabolite, and its own metabolism is characterized by an autoinduction phenomenon that appears to be complete within 2 weeks, resulting in a 17% reduction in LTG serum concentrations [4]. The biotransformation of LTG is also susceptible to heteroinduction and enzyme inhibition. Indeed, the metabolism of LTG is significantly affected by concomitant use of hepatic enzyme inducers such as classic AEDs (carbamazepine, phenytoin, primidone and phenobarbital) and oxcarbazepine, as well as others drugs such as rifampicin, ritonavir, acetaminophen and olanzapine [3–6]. Contraceptives containing estradiol can also reduce the serum concentration of LTG by 50% and in women on oral contraceptives this interaction results in different steady-state LTG concentrations between the days of pill intake compared with the pill-free interval [2,4]. On the other hand, the LTG metabolism is inhibited by valproic acid and

* Corresponding author at: CICS-UBI—Health Sciences Research Centre, University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal.
*E-mail address:* gilberto@fcsaude.ubi.pt (G. Alves).

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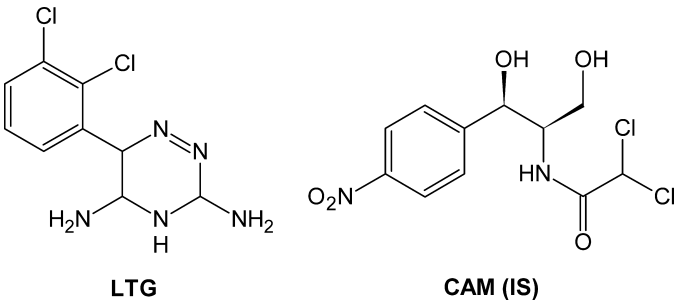


**Fig. 1.** Chemical structure of lamotrigine (LTG) and chloramphenicol (CAM) used as internal standard (IS).

sertraline. In fact, the inhibitory interaction with valproic acid was found to be clinically relevant and smaller doses of LTG as well as a slower titration rate should be used to minimize the risk of side effects [4]. The most serious adverse effect observed within the LTG therapeutic range (2.5–15 μg/mL) is skin rash, probably related to its aromatic ring and the formation of toxic metabolites [7]. Indeed, the incidence of toxic effects is significantly increased when serum or plasma concentrations exceed 15 μg/mL [4].

Over the years, rodents (rats and mice) have been largely employed as whole laboratory animal models to identify new anticonvulsant compounds and to obtain a better understanding of the pharmacokinetics of established AEDs at non-clinical level, and to study their involvement in drug-drug interactions [8–16]. Due to the fact that rodents eliminate most drugs much more rapidly than humans, anticonvulsant doses of AEDs are usually much higher in rodent models of seizures than effective doses in epilepsy patients. In spite of the pharmacokinetic differences observed between species, the effective plasma levels of AEDs are usually similar among rodents and humans [9,17]. Therefore, rodent models can be used to evaluate and predict plasma levels in humans by calculating the corresponding doses that will produce a similar anticonvulsant effect [15].

More specifically, LTG efficacy has been extrapolated from pharmacological studies conducted in rats. However, like other AEDs, LTG needs to cross the blood–brain barrier to exert its therapeutic effect. Thus, the determination of LTG levels in plasma and brain tissue is essential to characterise its pharmacokinetic/pharmacodynamic relationship [17–19]. Likewise, information on the LTG concentrations achieved simultaneously in plasma/serum and brain (biophase) is also determinant to predict the impact of drug-drug or herb-drug interactions involving LTG as the victim (object) drug. Hence, the availability of suitable bioanalytical methodologies to support the measurement of LTG concentrations in these particular biological samples is imperative.

To date, only a few number of high performance liquid chromatography (HPLC) methods coupled to ultraviolet [20–24] or mass spectrometry [25] detection has been reported in literature for the quantification of LTG in rat plasma/serum and brain. However, in those methods, sample preparation has been mainly performed through classic procedures, such as solid-phase extraction [20], protein precipitation [22–25] and/or liquid-liquid extraction [21,23,24]. Nevertheless, in recent years several miniaturized sample preparation techniques have been developed whose importance in bioanalysis has been increasingly recognized, among them is microextraction by packed sorbent (MEPS). In fact, MEPS has been successfully applied to the quantitative analysis of several therapeutic agents, namely antibiotics, antihypertensives, antiarrhytmics, antidepressants, antipsychotics, and even antiepileptic drugs including LTG [26]. Nonetheless, as far as we know, no bioanalytical assay has been developed for the quantification of LTG specifically in rat plasma and brain tissue samples using MEPS. Therefore, the purpose of this work was to develop and validate a novel method for the quantification of LTG in rat plasma and brain homogenate using the innovative MEPS technology in sample preparation.

## 2. Material and methods

### 2.1. Materials and reagents

LTG was kindly provided by Bluepharma (Coimbra, Portugal). Chloramphenicol, used as internal standard (IS; Fig. 1), was purchased from Sigma–Aldrich (St Louis, MO, USA). Methanol and acetonitrile, both of HPLC gradient grade, were purchased from Fisher Scientific (Leicestershire, United Kingdom) and the ultrapure water (HPLC grade, >18 MΩ cm) was prepared by means of a Milli-Q water apparatus from Millipore (Milford, MA, USA). Triethylamine, dihydrogen phosphate dehydrate and di-sodium hydrogen phosphate anhydrous were acquired from Merck KGaA (Darmstadt, Germany) and the 85% *ortho*-phosphoric acid from Fischer Scientific UK. Pentobarbital (Eutasil® 200 mg/ml, Ceva Saúde Animal) used as anaesthetic drug was commercially acquired. MEPS 250 μL syringe and MEPS BIN (barrel insert and needle) containing ~4 mg of solid-phase silica – $C_{18}$ material (SGE Analytical Science, Australia) were supplied by ILC (Porto, Portugal).

### 2.2. Blank rat matrices

Healthy adult male Wistar rats (300–380 g, 10–12 weeks old) were obtained from local certified animal facilities (Faculty of Health Sciences of the University of Beira Interior, Covilhã, Portugal) and were used as source of blank matrices (plasma and brain tissue) required for the validation experiments. For that, rats not subjected to any other treatment were anesthetized with pentobarbital (60 mg/kg) and then decapitated. Blood samples were directly collected into heparinised tubes and after exsanguination the brain was quickly excised. The blood samples were centrifuged at 4000 rpm for 10 min (4 °C) and then the plasma was immediately separated from the blood cells and stored at −20 °C until to be used. The brain tissue was weighed and homogenized in 0.1 M sodium phosphate buffer, pH 5.5 (4 mL/g of tissue) using a Ultraturrax® tissue homogenizer. The brain tissue homogenates were centrifuged at 13,500 rpm for 10 min (4 °C) and the supernatants were collected and stored at −20 °C until used. The animal procedures were conducted in accordance with the European Directive (2010/63/EU).

### 2.3. Stock solutions, calibration standards and quality control samples

The LTG stock solution (1 mg/mL) and working solution (100 μg/mL) were prepared in methanol, and then adequately diluted in water-methanol (50:50; v/v) to afford six different spiking solutions at 0.5, 1, 3.5, 15, 62.5 and 100 μg/mL. Each one of these solutions were used daily for spiking aliquots of blank rat samples (plasma and brain homogenate; 20 μl spiking solution to 80 μl of blank sample) in order to prepare the corresponding calibration standards at six different concentrations (0.1, 0.2, 0.7, 3, 12.5 and 20 μg/mL). The stock solution of the IS was also prepared in methanol (1 mg/mL) and the working solution (250 μg/mL) was obtained after diluting an appropriate volume of the stock solution with water-methanol (50:50; v/v). All solutions were stored at 4 °C and protected from light, except the IS working solution which was daily prepared.

Quality control (QC) samples at four concentration levels were also prepared independently in the same biological matrices, representing the lowest ($QC_{LLOQ}$), low ($QC_1$), medium ($QC_2$) and high