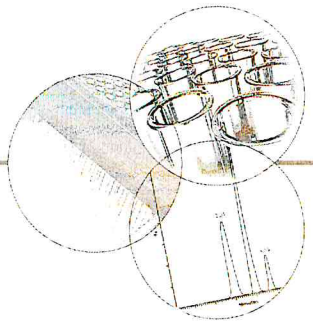
RESEARCH ARTICLE



Development of an ultra-performance liquid chromatography–tandem mass spectrometry micromethod for quantification of lamotrigine in human plasma and its use in a bioequivalence trial

Background: The aim of the present work was to develop a chromatographic technique coupled with mass spectrometry for the measurement of lamotrigine in plasma. Lamotrigine and guanabenz (internal standard) were measured by selected reaction monitoring. The method was validated and applied in a bioequivalence trial on 26 female volunteers. Lamotrigine chewable tablets (100 mg) were administered and monitored for up to 96 h.
Results: The method was linear between 0.05 and 5.0 µg/ml, with acceptable stability, accuracy and precision. Mean maximum plasma concentration was 1.37 µg/ml and was reached at 1.6 h postdose. Elimination half-life was 32.7 h.
Conclusion: Lamotrigine tablets were bioequivalent. Ultra-performance liquid chromatography with tandem mass spectrometry represents a powerful tool in terms of sensitivity, specificity and high-throughput analysis.

Lamotrigine (LMT; 3,5-diamino-6-[2,3-dichlorophenyl]-1,2,4-triazine; **Figure 1**) is an antiepileptic drug that has been used for over 20 years in the clinical treatment of several types of tonic–clonic seizures in adults, and in the Lennox–Gastaut syndrome in children. Recently, LMT has also been utilized in the treatment of bipolar disorders and as a mood stabilizer. The molecular mechanism of LMT effects might lie in inhibition of synaptic release of glutamate by regulating the recovery of inactivated sodium channels [1].

Lamotrigine is a lipophilic weak base (pKa = 5.7) that is totally absorbed from the gastrointestinal tract and metabolized predominantly by glucuronidation. It exhibits an elimination half-life between 24 and 35 h, with a plasma peak time at approximately 2 h. It has been reported that following a single oral dose of 200 mg of LMT, plasma concentrations up to 2.5 µg/ml were reached [2].

Depending on the purpose of the technique (therapeutic drug monitoring in children or in pregnant women, pharmacokinetics or drug–drug interactions), several methods have been developed for LMT quantification in biological fluids. One of the first techniques reported using 50 µl, is based on high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection employing liquid–liquid extraction [3]; however, these authors failed to mention the selectivity of the technique regarding LMT metabolites. Recently, several methods based on HPLC–UV were published, with the inconvenient lack of selectivity associated with this detection type. In addition, some of these methods of LMT extraction involving direct precipitation of plasma proteins have long running times due to the necessity to separate other molecules in the same sample [4,5], or implies the use of solid-phase extraction [6–9]. Recently, an automated, rapid and sensitive HPLC–UV method has been reported using column-switching for online sample cleaning [10]. At present, HPLC coupled with mass spectrometry (MS) has been recognized as the gold standard for measurement of drugs and their metabolites in a very sensitive and specific manner. Initial attempts to measure LMT by HPLC–MS utilized very small amounts of plasma, either by direct precipitation [11] or by solid-phase extraction [12]; nonetheless, these maintained long running times and failed to increase sensitivity due to LMT monitoring in 'single ion monitoring' mode. The coupling of ultra-performance liquid chromatography with tandem MS (UPLC–MS/MS) dramatically increased the bioanalytical possibilities by creating a rapid, sensitive and specific analytical tool.

Thus, the aim of the present work was to develop a method for plasma quantification of LMT by means of the employment of small amounts of sample based on UPLC–MS/MS and in a non-time-consuming fashion, and its further application in a bioequivalence trial.

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ANTIEPILEPTIC DRUG

Compounds used for the clinical management of seizures

UPLC–MS/MS

The analytical platform used in present work, ultra-performance liquid chromatography coupled with tandem mass spectrometry

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