Development of an HPLC method for the determination of diphenidol in plasma and its application in an oral multidose bioequivalence study in a healthy female Mexican population

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Hydrophilic Interaction Liquid Chromatography (HILIC) and Advanced Applications

"Having a printed book in one's hands is always an opportunity for the imagination, and if the book is pleasant and very useful, it's the best of these."

Since Alpert [1] coined the term hydrophilic interaction liquid chromatography (HILIC) to define the highly efficient chromatographic separation of very polar molecules that takes place through the interaction between an aqueous organic mobile phase and a polar stationary phase, the technique has been fine tuned, and accounts of many of its applications have been published.

In this information field, I commend the excellent effort of Perry G Wang (Research Chemist, Office of Regulatory Science, US FDA) and Weixuan He (Associated Director, Product and Process Development, at Meda Pharmaceuticals), who compiled and edited the state-of-the-art in their book *Hydrophilic Interaction Liquid Chromatography (HILIC) and Advanced Applications*, published by CRC Press (Taylor & Francis Group, Boca Raton, FL, USA, 2011).

This is a well-organized piece that takes us through 23 chapters ranging from the most basic concepts up to the very specific applications developed for recognized scientific teams who make contributions to the book.

Chapters 1, 2, 3, 11 and 17 offer the basic concepts for understanding HILIC, comparing diverse aqueous normal-phase chromatographies with true HILIC, explaining the relationship between log k' and percentage organic volume in the mobile phase, using the calculation of log D for choosing internal standards during method development, giving a point of view on ion-exchage chromatography as well as HILIC, exposing the effects of buffer concentration and temperature on HILIC performance, and describing the retention and selectivity of several polar phases.

Although the book is not divided into sections, its organization allows us to realize that chapters are grouped by similar items. Chapters 4, 5 and 6 afford advanced applications in the food and safety field; the separation of proanthocyanidins and other plant polyphenols, the use of HILIC for checking final quality, nutrients and the safety of diverse food products, and toxic analysis episodes during seafood consumption.

Material on environmental applications, included in Chapters 7 and 8, explores the search for pesticides and drugs in wastewater and soil.

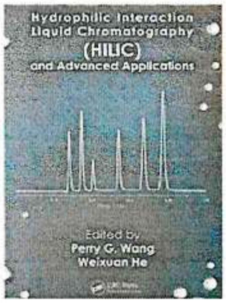
For all of those who love clinical applications, Chapters 9 and 10 delve into the hard work of biological sample processing previous to HILIC and depict the effort of scientists employing this type of chromatography for selecting and quantifying a huge variety of biomarkers for several diseases. Chapter 18 introduces us to the cumbersome analysis of very polar drugs for pharmacokinetic purposes.

In Chapters 12 through 16, five research groups describe their works in the field of pharmaceutical applications. HILIC has become a solution for the analysis of polar impurities during R&D and for the characterization of very polar acid or basic drugs. On these pages, two examples of HILIC-UPLC are shared: analysis of ioversol and of hydrazine-based mildronate.

Two special chapters are included for biochemical applications (Chapters 19 and 21): the separation of very hydrophilic mono- and di-phosporylated nucleotides, and the separation of carbohydrates.

Finally, and to avoid the idea that HILIC is solely for small molecules, Chapters 20, 22 and 23 are presented in the area of proteomics. HILIC has been utilized for the characterization of post-translational protein modifications such as glycosylation and phosporylation.

The great amount of applications included that have coupled HILIC with MS is not surprising, and there are several physicochemical reasons for this. First, the high percentage of organic solvent – usually acetonitrile – used in the mobile phase decreases viscosity, with a consequent decrease in pressure. Thus, high flow-rates can be used, rendering shorter run



Hydrophilic Interaction Liquid Chromatography (HILIC) and Advanced Applications

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Bioequivalence of 250 mg lysine clonixinate tablets after a single oral dose in a healthy female Mexican population under fasting conditions

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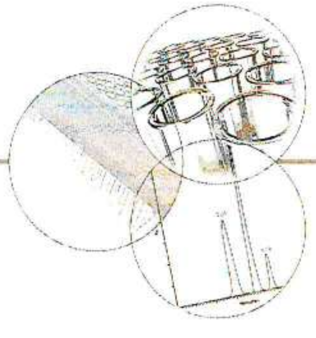
Abstract. Objective: To evaluate the bioequivalence between two 250 mg-tablets of lysine clonixinate, Dorixina Forte® (Siegfried Rhein, México) as reference product, and Prestodol® (Farmaceúticos Rayere, S.A., México) as test formulation. Methods: 26 healthy adult female Mexican volunteers received a single oral dose of 250-mg lysine clonixinate under fasting conditions. The drug was administered following a randomized, two-period, two-sequence, cross-over design. Twelve serial blood samples were collected up to 8 h after dosing, and clonixin (CLX) was measured by ultra-performance liquid chromatography (UPLC) coupled with tandem mass spectrometry. Decimal logarithm values of C_{max} and area under the curve (AUC) were used to construct a classic confidence interval at 90% (90% CI). Bioequivalence was established if 90% CI of mean ratios (test/reference) fall within the 0.8 – 1.25 range. Results: Volunteers formed a homogeneous population in terms of age (27.2 ± 6.3 years), weight (55.9 ± 6.5 kg), height (1.6 ± 0.04 m), and body mass index (BMI) (22.91 ± 2.03 kg/m^2). Reference formulation exhibited the following pharmacokinetics: C_{max} (32.39 ± 8.32 µg/ml); t_{max} (0.64 ± 0.2 h); AUC_{0-8h} (48.92 ± 16.51 µg.h/ml); $t_{1/2}$ (1.3 ± 0.24 h); CL_{app} (5.64 ± 1.99 l/h), and Vd_{app} (10.22 ± 2.9 l). Concerning bioequivalence, 90% CI were: C_{max} (82.32 – 98.79), AUC_{0-t} (94.59 – 106.29), and AUC_{0-inf} (94.61 – 106.42), with a statistical power of > 0.90 at every tested interval. Conclusions: This single-dose study found that both 250-mg immediate-release tablets of lysine clonixinate met the Mexican regulatory criteria for bioequivalence in these volunteers.

Introduction

Clonixin (CLX) is an anti-inflammatory non-steroidal analgesic drug without narcotic effects (NSAIDs) that belongs to the fenamates family; its salt, lysine clonixinate (Chemical Abstracts Society (CAS) registry no. 55837-30-4) is an amorphous white powder soluble in organic solvents. It is used to relieve middle to severe episodes of dental pain [11], dysmenorrhea [3], post-operative pains [2], and migraine [10].

The mechanism of action of CLX relies on the blunting of 5-lipoxygenase with diminishing in the synthesis of the pro-inflammatory 5-HETE [6], and it has also been reported that CLX exerts an inhibitory effect on the expression of nitric oxide synthase (NOS) induced, which participate during inflammation [5]. This selectivity of CLX in 5-lipoxygenase over cyclooxygenases may explain the lack of effect on platelet number and function during its therapeutic use associated with common NSAIDs [9].

Pharmacokinetic data of CLX are succinct. The first attempt to determine the metabolic pathways of CLX in humans employed the tritium-labeled drug [8]. It showed three main metabolites both in plasma and urine: 5-OH-clonixin; 4′-OH-clonixin and 2′-ethoxyclonixin. Erratic values of plasma concentrations have been previously reported following an intravenous (IV) dose of lysine clonixinate solution in 10 children post-surgery [7]. These authors reported a distribution volume of ca. 1.3 l/kg, and a dose-dependent elimination half-life between 30 and 50 min. Serum concentrations were quite similar between IV and oral administration after 45 min post-dose; and areas under the curve were also similar, demonstrating high degree of bioavailability by oral route.



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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.

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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.

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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