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Determination of loperamide in human plasma and saliva by liquid chromatography–tandem mass spectrometry

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ABSTRACT

A simple and sensitive liquid chromatography–tandem mass spectrometric method for quantification of loperamide in human plasma and saliva was developed and validated, and then successfully applied in pharmacokinetic clinical study to investigate and correlate bioavailability of Imodium® 2 mg quartet tablet dose in both human plasma and saliva. Loperamide with labeled internal standard was extracted from its biological matrix by methanol as protein direct precipitant in single extraction step. Adequate chromatographic separation for analytes from plasma and saliva matrices was achieved using ACE C18 (50 mm × 2.1 mm, 5 μm) column, eluted by water/methanol/formic acid (30:70:0.1%, v/v), delivered isocratically at constant flow rate of 0.75 ml/min. The method validation intends to investigate specificity, sensitivity, linearity, precision, accuracy, recovery, matrix effect and stability according to European guideline, and partial validation was applied on saliva, specificity, matrix effect, recovery, sensitivity, within and between day precision and accuracy. The calibration curve was linear through the range of 20–3000 pg/ml in both plasma and saliva using a 50 μl sample volume. The partial validation sections outcome in saliva was so close to those in plasma. The within- and between-day precisions were all below 8.7% for plasma and below 11.4% for saliva. Accuracies ranged from 94 to 105% for both matrices. In this study, 26 healthy volunteers participated in the clinical study, and 6 of gave their saliva samples in addition to plasma at the same time schedule. The pharmacokinetic parameters of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$, T_{max} and $T_{1/2}$ in both plasma and saliva were calculated and correlated.

1. Introduction

Imodium® (loperamide hydrochloride – Fig. 1), chemically 4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]-N,N-dimethyl-2,2-diphenylbutanamide hydrochloride ($C_{29}H_{33}ClN_2O_2$) [1]. Loperamide is a synthetic piperidine derivative and opioid agonist with anti-diarrheal activity for oral use [2]. It acts on the mu-receptors in the intestinal mucosa and this leads to a decrease in gastrointestinal motility by decreasing the circular and longitudinal smooth muscle activity of the intestinal wall. This slows intestinal transit and allows for more water and electrolyte absorption from the intestines [3,6]. It is approved for the control of diarrhea symptoms and it is also available without a prescription. Loperamide works by a number of different mechanisms of action that decrease peristalsis and fluid secretion, resulting in longer gastrointestinal transit time and increased absorption of fluids and electrolytes from the gastrointestinal tract [4]. Loperamide has potential therapeutic usefulness as a peripherally selective topical or local opiate anti-hyperalgesic agent that lacks many of the side effects associated with opiate administration [5]. In humans, following oral administration of loperamide solid formulations, about 50% of the administered loperamide is absorbed in the gastrointestinal tract, absorbance occurring within 1 h in high bounding percent to plasma proteins (95%) [8], at maximum concentration time of 6 h and half-life around 11 h [9], but only a little intact drug (1%) reaches systemic circulation, due to its extensive first pass metabolism [6,7]. Loperamide determination in human body makes challenging work in bioavailability and bioequivalence research field, due to its low availability in plasma and low doses [6,7], in pharmaceutical industries and in vitro studies, loperamide has been determined by HPLC easily [10–14], and more sensitive methods were required for in vivo determinations, as in rats by HPLC–UV [15] and HPLC–ECD [16]. In human body there were many determinant studies of loperamide by using LC-tandem MS [17–20], but more sensitive bioanalytical methods with easier

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sample extraction are still required for determination of low doses in human plasma. Loperamide has been extracted by several techniques like liquid–liquid extraction [18,19] and solid phase extraction [17] except that protein direct precipitation is still not reported in the literatures. In this study we established a new bioanalytical method with single extraction step to determine loperamide in human plasma and saliva by using LC–MS/MS, and then was applied in pharmacokinetic clinical study, where the correlation of drug plasma levels vs. saliva levels in human body becomes a very important subject in bioequivalence investigations [25], and such correlation has a particular importance in pharmacokinetic studies, especially to introduce a salivary excretion classification system [25,26].

2. Experimental

2.1. Chemicals and reagents

Loperamide hydrochloride (purity as loperamide hydrochloride = 100%) was obtained from Toronto Research Chemical (TRC) Inc. (Canada); loperamide-d6 (purity as loperamide-d6 = 99.7%) was also obtained from TRC Inc. The blank plasma sample was harvested from donors and collected by the blood bank. Plasma was obtained by centrifugation of blood treated with sodium heparin. LC/MS-quality for deionized water, acetonitrile, methanol and formic acid were purchased from Merck (Darmstadt, Germany).

2.2. Instrumentation

The LC–MS/MS was composed from API 3200 Mass Spectrometer protected by a built-in waste/detector switcher valve, attached to Agilent 1200HPLC Computer System, Windows XP, SP3, and Analyst 1.5.2 software for data management system.

2.2.1. HPLC conditions

Chromatographic conditions were identical for plasma and saliva samples. The elution system was derived from methanol:water (70:30%, v/v) acidified by 0.1% formic acid, delivered isocratically through an analytical column (ACE, C18, with dimensions of 50 mm × 2.1 mm, 5 μm) at a flow rate of 0.75 ml/min under column oven temperature maintained at 50 °C. The auto sampler tray temperature was set at 4 °C and the injection volume was 5 μl.

2.2.2. Mass spectrometric conditions

The mass spectrum machine was optimized to get the highest intensity for loperamide, with nitrogen gas 1 flow = 30, gas 2 = 80, curtain gas = 14, ion spray voltage = 2500 V, drying temperature = 550 °C, orifice temperature = 100 °C under normalized collision energy of 25%. The mass spectrometer was operated in a positive ion mode.

2.3. Standard solutions

Stock solutions of 1.0 mg/ml for loperamide and loperamide-d6 internal standard (IS) were prepared separately in methanol, and these solutions were stored in a refrigerator (4–8 °C). These solutions were further diluted to give appropriate working solutions used to prepare the calibration curves, and similarly, a new loperamide stock solution was diluted to prepare quality control working solutions.

2.3.1. Standard calibration curves and quality control samples

Standard calibration concentrations for loperamide were prepared in human pooled analyte-free plasma by spiking 50 μl from working serial solution for each level into 950 μl of plasma in single dilution step to yield final concentrations of 20, 40, 100, 250, 500, 1000, 2000 and 3000 pg/ml for loperamide. The constructed concentration dynamic range of the calibration curve was proposed on the basis of pharmacokinetic parameters (bioavailability and maximum concentration) of loperamide in human plasma [24], to attain the European and US FDA guidelines requirements with regard to LLOQ and QC values [21,22]. Similarly, quality control samples were prepared in human pooled analyte-free plasma at concentration of 20 pg/ml as LLOQ, 60 pg/ml (QC low), 1500 pg/ml (QC mid) and 2400 pg/ml (QC high). The lowest concentration for calibration curve was considered LLOQ. All the calibration standard and QC plasma samples were divided into aliquots and stored in deep freezer at -40 ± 5 °C until analysis. Calibration curve was constructed from a blank sample (an analyte-free plasma sample, processed without an IS), a zero sample (an analyte-free plasma, processed with IS) and eight non-zero samples covering the total range including LLOQ.

2.4. Sample preparation

Protein direct precipitation is the drug extraction procedure for each of plasma and saliva sample by adding 100 μl acetonitrile (after spiking with 30 μl from 3.0 ng/ml loperamide-d6) to 50 μl plasma sample in an Eppendorf tube; the mixture was vortex-mixed for 30 s using a Vibrax Type VX-Z, VXR Basic Vortexer (IKA-Werke GmbH & Co., Staufen, Germany) and then centrifuged using Multitude Sigma1-15 (Sigma, Germany) for 15 min at 14,000 rpm (14,680 × g). The supernatant was transferred to an auto-sampler micro-vial, and then 5 μl was injected into the analytical column.

2.5. Bioanalytical method validations

In human plasma, the method was validated in concordance with the European FDA guideline [22], taking into consideration the United States FDA guideline requirements [21]. Both guidelines were considered as protocols for all validation sections. The validation was performed in order to evaluate the method in terms of specificity, carryover, sensitivity, calibration curve (linearity of response), accuracy, precision, dilution integrity, matrix effect, recovery and stability. In this study the bioanalytical method was also partially validated on human saliva in terms of specificity, matrix effect, recovery, sensitivity, within and between day precision and accuracy.

2.5.1. Specificity and carryover

The specificity of the method was evaluated by screening 6 individual sources of the appropriate blank matrix, which were individually analyzed as replicates and evaluated for interference with comparison to LLOQ. Carryover effect was evaluated to ensure that the rinsing solution used to clean the injection needle is able to avoid any carry forward of injected sample in the subsequent runs. Specificity test was also applied on 6 individual sources of the appropriate saliva blank. Normally the absence of interfering components is accepted where the response is less than 20% of the lower limit of quantification for the analyte and 5% for the internal standard in both plasma and saliva.

2.5.2. Linearity, accuracy and precision

The accuracy and precision of within-run evaluations were determined by running analytical batch containing 6 replicates from LLOQ and each level of QC with calibration curve included blank and zero, separately for each of plasma and saliva. Between-run linearity, accuracy and precision were determined by running three sets of within-run batches in three separate days, and each precession run was from freshly spiked eight levels constructing