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ORIGINAL ARTICLE

Highly sensitive assay for the determination of therapeutic peptide desmopressin in human plasma by UPLC–MS/MS

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Abstract An analytical method based on ultra-performance liquid chromatography with positive ion electrospray ionization (ESI) coupled with tandem mass spectrometry detection (UPLC–MS/MS) was developed and validated for the determination of therapeutic peptide desmopressin in human plasma. A desmopressin stable labeled isotope (desmopressin d_8) was used as an internal standard. Analyte and the internal standard were extracted from 200 μL of human plasma *via* solid-phase extraction technique using Oasis WCX cartridges. The chromatographic separation was achieved on an Aquity UPLC HSS T3 column by using a gradient mixture of methanol and 1 mM ammonium formate buffer as the mobile phase. The calibration curve obtained was linear ($r^2 \geq 0.99$) over the concentration range of 1.01–200 pg/mL. Method validation was performed as per FDA guidelines and the results met the acceptance criteria. The results of the intra- and inter-day precision and accuracy studies were well within the acceptable limits. The proposed method was successfully applied to pharmacokinetic studies in humans.

1. Introduction

Desmopressin is a synthetic analog of vasopressin, a natural pituitary hormone with antidiuretic properties. The deamination of vasopressin in the N-terminal 1 position and replacement of 8-L-arginine with 8-D-arginine results in the formation of desmopressin. It has a longer duration of antidiuretic activity than that of the natural hormone and is essentially devoid of other associated pharmacological effects such as vasoconstriction and contraction of smooth muscles in the uterus or in the intestine [1–3]. This prolonged and specific antidiuretic effect makes desmopressin useful for managing a number of enuretic

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disorders, including nocturia, primary nocturnal enuresis and central diabetes insipidus [4,5]. The oral administration of desmopressin is shown to be safe and effective for treating central diabetes insipidus and primary nocturnal enuresis [6]. The low doses of desmopressin (0.200–1.20 mg per day) may result in very low plasma concentrations. Therefore, a highly sensitive and selective method for the determination of therapeutic levels during clinical studies is required.

According to the literature, few liquid chromatography/mass spectrometric methods (LC–MS) have been reported for the quantitative determination of desmopressin in skin samples [7], blood plasma samples [8] and in urine samples [9]. Also, an LC–MS method [10] has been reported for the qualitative detection of desmopressin in human plasma samples for doping control purpose. Most of the analytical methods [7,9,10] reported so far were too insensitive and/or not suitable for quantitative determination of desmopressin in human plasma samples for pharmacokinetic/bioequivalence studies. However, a promising method was reported by Nguyen et al. [8] with an LOQ of 2 pg/mL and employs multi-step solid phase extraction involving many stringent method development protocols with a chromatographic run time of >18 min, which may not be favorable for routine drug analysis. This method utilizes a single-quadrupole mass spectrometry with selected-ion monitoring (SRM) mode to detect the precursor ion. But in the present method a triple-quadrupole mass spectrometry (LC–MS/MS) with multiple reaction monitoring (MRM) mode was used to detect both the precursor ion and fragment ion. It shows that the proposed method is highly specific. Moreover, the method reported by Nguyen et al. [8] does not describe the development process, volume of the sample used, various validation experiments, stability studies and suitability for application to pharmacokinetic/bioequivalence studies. The salient features of chromatographic methods developed for desmopressin in human plasma are summarized in Table 1.

This paper presents, for the first time, the complete development and validation of a simple, highly sensitive and selective UPLC-MS/MS method in MRM mode for the quantification of desmopressin in human plasma using desmopressin d_8 as an internal standard (IS). This sensitive method (1.01 pg/mL) requires only 200 μL human plasma for solid-phase extraction (SPE) technique, minimum usage of organic solvents and demonstrates excellent performance in terms of ruggedness with a sample cut off time of 7.0 min. The application of this assay method to a clinical pharmacokinetic study in healthy South Indian male subjects following oral administration of desmopressin is described under fasting condition. The authenticity in the measurement of study data is demonstrated through incurred samples reanalysis.

2. Experimental

2.1. Chemicals and reagents

Reference sample desmopressin ($\geq 97\%$) and the internal standard (IS) desmopressin d_8 ($\geq 97\%$) were purchased from Sigma-Aldrich Limited (Bengaluru, India). LCMS grade methanol was purchased from J.T. Baker (Phillipsburg, USA). Analytical grade ammonium formate, formic acid and acetic acid were purchased from Merck Ltd (Mumbai, India). Water used for the LC–MS/MS analysis was prepared by using Milli Q water purification system procured from Millipore (Bangalore, India). The control human plasma sample was procured from Deccan′s Pathological Lab′s (Hyderabad, India).

2.2. UPLC–MS/MS instrument and conditions

An UPLC system (Waters Corporation, Milford, USA) consisting of an Aquity HSS T3 column (100 mm × 2.1 mm, 1.8 μm; Waters corporation, Milford, USA) equipped with a binary pump and a 96-vial autosampler (Waters, Milford, USA) was used for the study. Aliquots of 20 μL of the processed samples were injected into the column, which was kept at 40 °C. A mobile phase consisting of a mixture of 1 mM ammonium formate buffer (solvent A) and methanol (solvent B) in a gradient proportion was used to separate the analyte from the endogenous components. The gradient program was run from 25% B to 75% B in 3 min and ramped up to 90% B within 0.2 min and held for 1 min and ramped down to initial conditions within 0.25 min and stayed there for 2.0 min. Flow gradient was also performed starting

Table 1 Salient features of LC–MS methods developed for desmopressin in biological samples.

S. no.	Column; mobile phase; flow rate; injection volume	Extraction technique; biological matrix; mean recovery	Detection technique; linear dynamic range/LOD; analytical run time; retention time; application/purpose	Refs.
1	Nucleosil C_{18} (CC 125/2, 120-3); acetonitrile–0.01% formic acid in 1.6 mM ammonium acetate (33:67, v/v); 0.20 mL/min; 10 μL	Extraction with water and methanol/ethanol (50:50); skin; ND	LC–MS; 0.05–2 μg/mL; 10 min; 2.6 min; transdermal	[7]
2	Phenomenex Luna C_{18} (150 mm × 2 mm i.d., 5 μm); methanol–0.05% formic acid, pH 3 (gradient composition); 0.20 mL/min; 50 μL	SPE with Strata-X 8B-S100-TAK C18-E (30 mg) cartridges; blood plasma; 88.67%	LC–MS; 2.00 pg/mL; 18 min; ND; ND	[8]
3	Pyramid–C_{18} (50 mm × 2.1 mm i.d., 1.9 μm); acetonitrile–0.1% formic acid (gradient composition); 0.30 mL/min; ND	SPE with STRATA-XCW (30 mg) cartridges; urine; 103%	LC–MS/MS TOF; 50–2000 pg/mL; 13 min; 5.4 min; doping control	[9]
4	Zorbax 300SB C_{18} (50 mm × 1.0 mm, i.d., 3.5 μm); acetonitrile–0.1% acetic acid–0.01% triflouro acetic acid (gradient composition); 50 μL/min; 30 μL	SPE with Oasis® WCX (60 mg) cartridges; plasma; 40%	LC–MS/MS; 50 pg/mL; 25 min; 10 min; qualitative analysis	[10]
5	Aquity HSS T3 (100 mm × 2.1 mm i.d., 1.8 μm); methanol–1 mM ammonium formate (gradient composition); gradient flow; 20 μL	SPE with Oasis® WCX (30 mg) cartridges; plasma; 77.3%	LC–MS/MS; 1.01-200 pg/mL; 7 min; 3.3 min; pharmacokinetics	PM

ND, no data available; SPE, solid phase extraction; PM, present method.

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