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Short communication

LC–MS determination of desmopressin acetate in human skin samples

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Abstract

A sensitive, selective and accurate high-performance liquid chromatography-mass spectroscopy (LC–MS) assay for the determination of desmopressin acetate (1-deamino-8-D-arginine vasopressin, DDAVP) from human skin samples was developed and validated. Pieces of human breast skin were impregnated with DDAVP solutions and DDAVP was extracted with an optimum extraction procedure. The extracted solutions were then analyzed by a LC system, comprising of a Nucleosil® C18 column (CC 125/2, 120-3) and a mobile phase of 0.01% formic acid in a mixture of 1.6 mM ammonium acetate and acetonitrile (33:67, v/v), coupled with electrospray ionization mass spectrometry (ESI-MS). Satisfactory results were obtained with limits of detection and quantification as low as 10 and 40 ng/ml, respectively, and with very good intra- and inter-day repeatability.

Keywords: Desmopressin acetate; LC–MS; Skin samples; Transdermal delivery

1. Introduction

Desmopressin acetate (1-deamino-8-D-arginine vasopressin (DDAVP)), is a synthetic analogue of the neurohypophyseal peptide hormone vasopressin that has been developed through systematic structural modifications [1]. It was obtained by deamination of cysteine in position 1 of vasopressin yielding 3-mercaptopropionic acid (Mpr) and by replacement of L-arginine with D-arginine in position 8 (Scheme 1). DDAVP is used in the treatment of chronic diseases such as neurogenic diabetes insipidus [2] and nocturia enuresis [3]. It has a prolonged antidiuretic effect but lacks pressor activity, and does not cause vasoconstriction and contraction of smooth muscles in the uterus or in the intestine as the natural peptide hormone does.

Several investigators have demonstrated the effective treatment of diabetes insipidus by oral and intranasal administration of DDAVP [4–7]. However, the transportation of the drug through these routes produced large inter-subject variability and the duration of treatment is relatively short. Therefore, the

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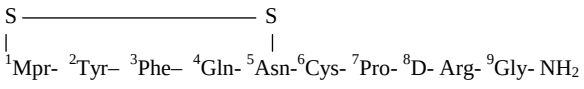

Scheme 1.

search for other routes of drug delivery devoid of such limitations has become essential. Iontophoresis, using pulsed external electrical energy, has recently received attention as a promising technique to enhance the delivery of drug molecules, particularly peptides, across the skin [8–13]. This technique could be of particular benefit for reducing the intersubject variabilities and controlling drug absorption [14,15]. Ionized compounds and low molecular weight molecules are good candidates for iontophoretic delivery [8,16]. Since DDAVP is a small peptide and exists in ionized form at the physiological pH, transdermal iontophoretic delivery may be one possible route for its administration. DDAVP has been evaluated in some studies on transdermal delivery. For example, Kahns et al. [17] reported transdermal delivery of DDAVP using the prodrug approach. The enhanced transdermal absorption of DDAVP by iontophoresis in rats and mice has also been reported [18–20]. These reports suggest that there is a prospect for transdermal delivery of DDAVP.

In order to investigate the potential of the transdermal route for prolonged and controlled delivery of drugs, the development of accurate, selective and sensitive analytical method capable of detecting and quantifying these agents in the different skin layers as well as in plasma is highly essential. A literature survey revealed only very few reports on the analysis DDAVP, such as radioimmunoassay [21,22], high-pressure liquid chromatography [23,24] and capillary electrophoresis [25,26] in pharmaceutical formulations and plasma. No report has been found on the determination of DDAVP in skin samples. Recently, LC–MS has become one of the most widely utilized methods in clinical and pharmaceutical analysis of drugs as it provides extraordinary efficiency of drug detection, characterization and quantification [26,27]. Despite its complexity, the instrument's software permits smooth utility and applications. In addition, it ensures low-level detection and quantification of drugs with high degree of specificity at relatively short time of analysis without a need for complete chromatographic resolution of analytes, which is of great importance for skin samples.

The analysis of drugs from biological samples, such as the skin, where a very minute amount of the analyte may be present, requires the development of effective extraction procedure to completely recover the analyte from the samples. The objectives of this study were, therefore, to develop an effective extraction method for maximum recovery of DDAVP from the skin and to develop an optimum LC–MS method that enables sensitive, selective and accurate determination of DDAVP in the skin extracts.

2. Experimental

2.1. Materials

Methanol, acetonitrile and ethanol were all of HPLC grade and obtained from J.T. Baker (Deventer, The Netherlands). Formic acid and ammonium acetate were supplied by Merck (Darmstadt, Germany). Morphine was from Sigma (Munich, Germany). Desmopressin acetate was kindly donated by Ferring Pharmaceuticals (Copenhagen, Denmark). Human breast skin was generously offered by Dr. J. Wohrlab, Department of Dermatology, Faculty of Medicine, Martin Luther University Halle-Wittenberg.

2.2. Equipment

LC–MS analysis was performed using Finningan LCQ ion-trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA) coupled with an HPLC pump SpectraSystem P4000 equipped with an autosampler AS 3000 and a membrane degasser. The HPLC column used was a Nucleosil® C18 column (CC 125/2, 120-3) purchased from Macherey-Nagel (Düren, Germany).

2.3. Calibration curve

Standard stock solutions of DDAVP were prepared in bidistilled water at a concentration of 100 μg/ml. A piece of human breast skin was extracted with water:methanol (50:50, v/v) and the extract was used as a diluent to prepare a series of dilutions of standard solutions at concentrations of 0.05, 0.1, 0.25, 0.5, 1 and

2 μg/ml. To each of these solutions, a constant amount of morphine solution in methanol was added as an internal standard. A calibration curve was constructed by plotting the ratio of the peak area of DDAVP to that of morphine against the concentration of DDAVP. Peak area measurements were performed by LCQ software.

2.4. Skin sample preparation

Several equal sized pieces of skin, each with an area of 3.1416 cm^2, were cut from human breast skin and placed in 1.5 ml Eppendorf tubes. In each of the tubes, a 20 μl of 0.5, 1 or 5 μg/ml solution of DDAVP in a mixture of methanol and bidistilled water (50:50, v/v) was added. The tubes were kept at $32 \pm 1\,^{\circ}C$ for 300 min, to allow penetration of DDAVP into the skin. They were then placed in a refrigerator for 24 h before being extracted.

2.5. Extraction of DDAVP from the skin samples

Each of the skin samples were extracted with 200 μl of bidistilled water or different compositions of the mixture of bidistilled water and methanol or ethanol after mixing in a vortex mixer for about a minute and in a sonicator for an hour. The samples were then centrifuged at 2500 rpm for 15 min and 100 μl of the supernatant solution was taken for analysis. A constant amount of morphine was added to each of the samples before analysis.

2.6. Chromatographic and mass spectrometric conditions

A 0.01% formic acid in a mixture of 1.6 mM ammonium acetate and acetonitrile (33:67, v/v) at a flow rate of 0.2 ml/min was used as a mobile phase after degassed with helium. In all cases, 10 μl samples were injected.

The mass spectrometer was operated in an electrospray mode with positive ion detection applying an electrospray voltage of 4.5 kV and a heated capillary temperature of 220 °C. The molecular ions at mass to charge ratio (m/z) of 1069.2 and 286.4 for DDAVP and morphine, respectively, were monitored in selected ion monitoring (SIM) mode and analytical data were acquired by LCQ software.

2.7. Assay validation

2.7.1. Linearity

The linearity of the plot of the relative peak area versus the concentration of DDAVP over the concentration range of 0.05–2 μg/ml was tested using linear regression analysis. The regression equation and the correlation coefficient of the calibration curve were determined.

2.7.2. Limits of detection (LOD) and quantification (LOQ)

The limits of detection and quantification were estimated in accordance to the base line noise, considering a signal-to-noise ratio of 1:3 and 1:10, respectively.

2.7.3. Precision and accuracy

The intra- and inter-day precision and accuracy of the measurements were determined by replicate analysis of control samples containing 100, 250 and 500 ng/ml of DDAVP in the water:methanol (50:50, v/v) mixture extract of the breast skin for 3 consecutive days.

3. Results and discussion

With the increasing interest of using pharmacologically active peptides as drugs, developing a highly selective, sensitive and accurate analytical method for the detection and quantification of these agents in biological and pharmaceutical samples has been compulsory. Recently, LC–MS has become one of the most widely utilized methods in the analysis of peptides and proteins. Unlike the classical HPLC-UV, LC–MS permits determination of drugs in mixtures, even without baseline separation.

3.1. Method development

3.1.1. Extraction of DDVAP from skin samples

In the analysis of drugs from biological samples, such as skin, where a very minute amount of the analyte may be present, development of an appropriate extraction procedure to completely recover the analyte from the samples is a fundamental part of the development of a selective, sensitive and accurate analytical method. Thus, in this work, different

Table 1
Percentage recovery of DDAVP upon extraction from the skin samples ($n = 3$)

Concentration of DDAVP in the pieces of skin (μg/ml)	Solvent of extraction	% of DDAVP recovered[a] (S.D.[b])
0.5	Distilled water	53.8 (±4.2)
	Ethanol:distilled water (20:80, v/v)	75.7 (±1.5)
	Ethanol:distilled water (50:50, v/v)	85.4 (±3.6)
	Ethanol:distilled water (80:20, v/v)	78.0 (±2.5)
	Ethanol	70.5 (±2.8)
	Methanol:distilled water (20:80, v/v)	79.2 (±2.2)
	Methanol:distilled water (50:50, v/v)	88.8 (±2.1)
	Methanol:distilled water (80:20, v/v)	83.2 (±1.8)
	Methanol	73.6 (±2.8)
1	Distilled water	58.4 (±3.1)
	Ethanol:distilled water (20:80, v/v)	73.4 (±3.6)
	Ethanol:distilled water (50:50, v/v)	88.5 (±5.2)
	Ethanol:distilled water (80:20, v/v)	80.0 (±3.2)
	Ethanol	72.3 (±1.9)
	Methanol:distilled water (20:80, v/v)	76.4 (±2.8)
	Methanol:distilled water (50:50, v/v)	90.6 (±4.3)
	Methanol:distilled water (80:20, v/v)	82.7 (±2.0)
	Methanol	75.6 (±5.7)
5	Distilled water	55.3 (±2.1)
	Ethanol:distilled water (20:80, v/v)	77.3 (±3.3)
	Ethanol:distilled water (50:50, v/v)	87.7 (±4.4)
	Ethanol:distilled water (80:20, v/v)	76.4 (±1.8)
	Ethanol	69.5 (±4.6)
	Methanol:distilled water (20:80, v/v)	78.1 (±2.8)
	Methanol:distilled water (50:50, v/v)	91.4 (±2.5)
	Methanol:distilled water (80:20, v/v)	81.4 (±3.5)
	Methanol	74.5 (±2.0)

[a] Mean of six determinations.
[b] S.D.: standard deviation.

extraction solvents consisting of methanol or ethanol in bidistilled water at compositions of 0, 20, 50, 80 and 100% were compared based on the percentage of drug recovery. It was observed that 50% methanol and 50% ethanol have the maximum percentage of drug recovery (Table 1). Although there was no significant difference in the extraction ability of the two solvent compositions, the 50% methanol was taken as the optimum extraction solvent due to the fact that better mass spectroscopic sensitivity was achieved in using methanol than ethanol.

3.1.2. LC–MS analysis

A number of combinations of bidistilled water and organic solvents (methanol and acetonitrile) in the presence of different concentrations of ammonium acetate and/or formic acid were tested during method development. The results invariably showed that the intensity of the chromatographic peak for DDAVP was higher in case of using mixtures of water and acetonitrile than that of water and methanol. Addition of ammonium acetate to the former significantly increased the peak intensity. This increase in peak intensity could be attributed to the more complete conversion of the non-ionized molecules of DDAVP into charged molecules that can be detected by the MS detector. In line with the advantage of having the best sensitivity, a mixture of 1.6 mM ammonium acetate:acetonitrile (33:67, v/v) was initially chosen to be the best mobile phase composition. However, with this composition, DDAVP and the internal standard (morphine) were eluted at a very long retention times

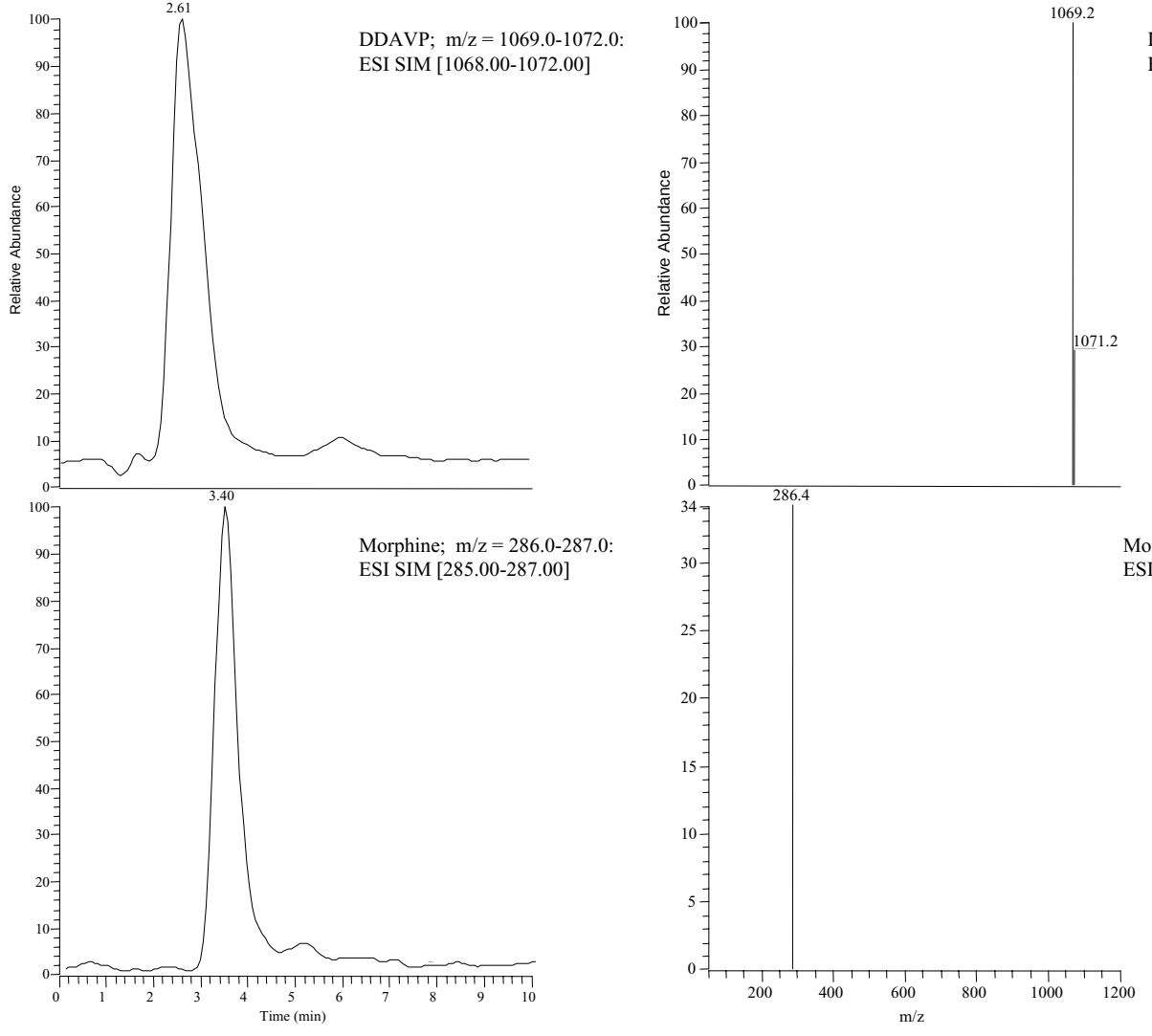

Fig. 1. Chromatogram and mass spectra of DDAVP (0.5 μg/ml) extracted from human breast skin in a mixture of methanol and bidistilled water (50:50, v/v) and morphine (internal standard; 0.2 μg/ml).

(≅10 and 14 min, respectively). In order to detect both DDAVP and morphine at lower retention times with no significant reduction in their peak intensities, we found it important to acidify the mobile phase. Therefore, a mobile phase consisting of 0.01% formic acid in a mixture of 1.6 mM ammonium acetate:acetonitrile (33:67, v/v) that produced peaks of the best intensity and eluted DDAVP and morphine at reasonably shorter time, ≅2.6 and 3.4 min (Fig. 1), respectively, was used.

3.2. Assay validation

3.2.1. Linearity

Initially, 50% methanol in bidistilled water has been used as a diluent for the preparation of standard solutions in the determination of calibration curve. However, it has been observed that in the presence of the skin extract, the intensity of the peaks for both DDAVP and morphine were decreased non-proportionally. Therefore, it has been necessary to use the 50% methanol–water skin extract as a dilution solvent. The calibration curve obtained by plotting the relative peak area against the concentration of standard solutions of DDAVP was linear in the concentration range of 0.05–2 μg/ml. The regression equation was $rPA = 0.039 + 2.14C$ with a correlation coefficient of 0.9998, where rPA (relative peak area) = (peak area of DDAVP)/(peak area of morphine) and C is the concentration of DDAVP. The coefficient of variation of the slope was 1.3%.

3.2.2. Limits of detection and quantification

The limit of detection, based on the 3:1 peak height ratio of DDAVP over noise, was 10 ng/ml and the limit of quantification, considering a signal-to-noise ratio of 1:10, was 40 ng/ml with a relative standard deviation (R.S.D.) of 2.5%.

3.2.3. Precision and accuracy

Method precision was determined by replicated analysis of samples at three different concentration levels of DDVAP. Intra-day repeatability was studied by carrying three measurements for each level on the same day. Inter-day repeatability was obtained by performing three determinations for each concentration on 3 consecutive days. The relative standard deviations ranged from 1.4 to 3.5% for intra-day repeatability and from 2.6 to 4.2% for inter-day repeatability. The accuracy, expressed as % deviation of the nominal concentrations (% DEV), ranged from −4.7 to 2.1%. Results are shown in Table 2. The results indicated good accuracy and precision of LC–MS for the determination of DDAVP at these low levels and suggested the application of the developed method for the analysis of the drug in skin samples.

Table 2
Accuracy and intra- and inter-day precision of LC–MS determination of DDAVP from skin samples

Nominal concentration (ng/ml)	Calculated concentration[a] (ng/ml)	% DEV[b]	% R.S.D.[c]	
			Intra-day	Inter-day
100	95.3	−4.7	3.5	4.2
250	248.6	−0.6	1.4	3.1
500	510.4	2.1	1.8	2.6

[a] Mean of six determinations.
[b] % DEV: percentage of deviation from the nominal value.
[c] % R.S.D.: percentage of relative standard deviation.

4. Conclusions

A selective, sensitive and accurate LC–MS analytical method for the determination of DDAVP from skin samples has been developed and validated. The method has additional advantages of short analysis time, non-tedious sample preparation and the avoidance of complete separation of the analyte from components of the skin. Therefore, the developed method could be of importance in studies aiming at searching appropriate formulations or methods for transdermal administration of DDAVP.

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