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High-performance liquid chromatographic method for the determination of dimethindene in urine

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ABSTRACT

An automated high-performance liquid chromatographic assay, using on-line solid-phase extraction, is described for the determination of dimethindene in urine. The solid-phase extraction of the sample (1000 μl) and the elution of the drug on to the analytical column are performed automatically and concomitantly. The limit of quantification is 5 pmol/ml.

INTRODUCTION

When a drug is applied topically it can cross the skin and reach the systemic circulation. Concentrations of the drug in the blood can therefore give a measure of its percutaneous absorption. The skin is known to be an excellent barrier to the permeation of substances and therefore the amount of a drug found in the blood is often very low. Hence, sensitive analytical methods able to determine the drug are needed.

An enzyme-linked immunosorbent assay (ELISA) has been found to be useful for the determination of the antihistaminic drug dimethindene maleate, N,N-dimethyl-3-[1-(2-pyridinyl)ethyl)]-1*H*-indene-2-ethanamine maleate, in serum at concentrations as low as 0.5 pmol/ml following oral and intravenous administration [1]. For topical administration, serum levels are very low and the ELISA technique is not sensitive enough. However, concentrations of dimethindene found in urine are higher than in the blood and can be determined using high-performance liquid chromatography (HPLC) [2] and gas chromatography [3]. The chromatographic methods were suitable for its determination at the 10 pmol/ml level in urine. However, they involve a tedious liquid–liquid extraction and evaporation–redissolution. In addition, they require deactivation of the glassware with silylating reagents to limit the strong adsorption of dimethindene on the glass. Unfortunately, ELISA cannot be used to determine dimethindene in urine owing to cross-reactions.

Application of automated on-line solid-phase extraction to biopharmaceutical analysis has been shown to be effective [4–7]. In this paper, a fully automated on-line HPLC system, using solid-phase extraction with automatic extraction cartridge exchange, is described.

EXPERIMENTAL

Chemicals

Anhydrous sodium acetate, ammonium sulphate, glacial acetic acid, fuming hydrochloric acid (37%) and sodium hydroxide, all of analytical-reagent grade, were purchased from Merck (Darmstadt, Germany). Ammonium acetate (BioChemika MicroSelect, >99%) was obtained from Fluka (Buchs, Switzerland). HPLC grade methanol and acetonitrile were from Mächler (Basle, Switzerland). Water was doubly distilled.

Dimethindene maleate (mol. mass 408.50) and its metabolite, N-desmethyldimethindene, were ob-

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tained from Zyma (Nyon, Switzerland). All reference solutions were freshly prepared in drug-free urine. A reference stock solution was prepared by dissolving about 20 mg (49 μmol) of either dimethindene maleate or its metabolite in 10.0 ml. Dilute solutions were obtained by successive dilution with the drug-free urine used for the reference solutions.

Apparatus

The chromatographic system consisted of three Model 6000A pumps, a Model 680 gradient/event controller and a Model 710B intelligent sample processor (WISP), all from Waters Assoc. (Milford, MA, USA). A Model OSP-2 on-line sample preparator was obtained from Merck. A Model 783A variable-wavelength UV detector was obtained from Applied Biosystems (Foster City, CA, USA). The WISP was equipped with a 2000-μl fixed loop, a 2.5-ml syringe and a cooling unit set to 10°C. Full details of the system configuration have been described in a previous paper [5].

LiChroCART cartridges (4 mm × 4.0 mm I.D.) from Merck, which were packed with either LiChrospher 100 CN (particle diameter, d_p = 5 μm) or LiChrospher 100 RP-18 (d_p = 5 μm), were used for on-line solid-phase extraction. The analytical column (Suplex pKb-100, d_p = 5 μm) (150 mm × 4.6 mm I.D.) was purchased from Supelco (Bellefonte, PA, USA).

Data acquisition and integration were performed using a Maxima 820 datastation from Waters Assoc.

Chromatography

Extraction and elution on to the analytical column were performed concomitantly by the OSP-2 on-line sample preparator, which was activated by the gradient/event controller following the instructions in Table I.

The HPLC mobile phases used were as follows: (A) mixture of 0.01 *M* sodium acetate and 0.04 *M* ammonium sulphate, adjusted to pH 3.0 with acetic acid–acetonitrile (90:10, v/v); (B) solution of 0.05 *M* ammonium acetate buffer adjusted to pH 7.0 with 1 *M* sodium hydroxide; (C) methanol–water (90:10, v/v). All aqueous solutions used were filtered on Zetapor membranes (pore size 0.22 mm) from Cuno (Meriden, CT, USA). The mobile phases were quickly degassed *in vacuo* prior to use. The flow-rates of the pumps were varied according to the instructions in Table II. The injection volume for samples and standards was 1000 μl. Detection was performed at 258 nm. The run time and the acquisition time were set to 28 min. Typical chromatograms are given in Fig. 1.

TABLE I

EVENT SETTINGS OF THE GRADIENT/EVENT CONTROLLER FOR ON-LINE SAMPLE PREPARATOR ACTIVATION

Time (min)	Event	Action	Comment
0.00	1	On	Valve 1 in position 2
0.00	3	On	Clamp closed (ready for extraction)
0.00	5	Off	Move direction set (left to right)
4.50	1	Off	Valve 1 in position 1(bypass of extraction side, end of washing)
4.60	3	Off	Clamp opened
4.70	4	Pulse	Move (1 cartridge from extraction side to analytical side)
4.80	3	On	Clamp closed (ready for analysis)
5.00	2	On	Valve 2 in position 2 (desorption, start of drug elution)
5.50	1	On	Valve 1 in position 2 (conditioning of a new cartridge)
14.00	2	Off	Bypass of valve 2 (initial setting)

Sample preparation

In order to prevent drug adsorption, the 4-ml WISP vials were directly filled from the 2-l flasks usually used for urine collection in pharmacokinetic studies, without any pipetting device.

TABLE II

FLOW-RATE SETTINGS OF THE GRADIENT/EVENT CONTROLLER

Time (min)	Flow-rate (ml/min)	Eluent A (%)	Eluent B (%)	Eluent C (%)	Curve[a]
Initial	2.20	45	0	55	–
6.00	3.20	62	0	38	11
8.00	3.20	0	62	38	11
14.00	3.20	62	0	38	11
20.00	2.20	45	0	55	11
35.00	0.00	45	0	55	11

[a] Curve 11 : new values are instantaneously set without any flow or eluent gradient.

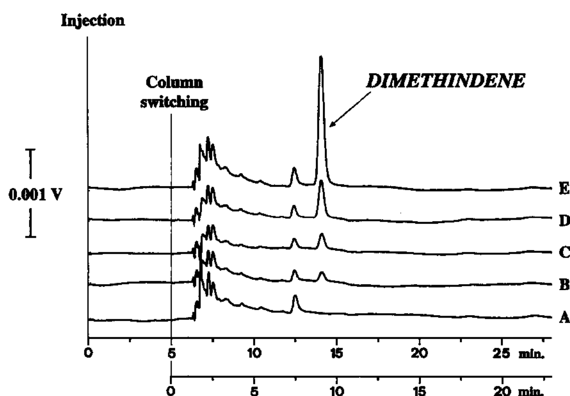

Fig. 1. Typical chromatograms of urine standards: (A) 0.0; (B) 4.9; (C) 12.3; (D) 24.5; (E) 49.0 pmol/ml. For experimental conditions, see text.

Standard preparation

Standard urine samples were prepared by diluting drug-free urine with reference solutions.

Calibration and quantification

Calibration was performed by linear regression analysis of the detector response over the concentration range 4.9–49.0 pmol/ml of dimethindene. The concentration of dimethindene equivalent in each sample (pmol/ml) was calculated using the calibration graphs.

Method validation

The recovery was determined according to ref. 8. The linearity range was checked by the correlation coefficient (r) of a calibration graph in the range 4.9–490 pmol/ml. The linearity of the calibration graphs was checked by their correlation coefficient in the range 4.9–49.0 pmol/ml. The precision was determined in terms of repeatability and reproducibility (n = 6). The accuracy was determined as the percentage deviation between the mean concentration found (n = 6) and the theoretical concentration. The stability of dimethindene under the experimental conditions was investigated.

RESULTS AND DISCUSSION

Solid-phase extraction

Systematic investigations were carried out combining either a cyanopropyl (CN) or an octadecyl (RP-18) reversed-phase extraction cartridge with a CN or a deactivated reversed-phase (Suplex pKb-100) analytical column. The combination of a CN cartridge and a Suplex analytical column was found to give more reproducible results than the other combinations.

Dimethindene could not be extracted onto the short CN cartridges in its fully protonated form, *i.e.*, in acidic medium. However, good extraction efficiency was observed with spiked urine samples using ammonium acetate buffer (pH 7.0). The recovery of dimethindene was found to be higher than 100% (n = 4) for a standard 49.0 pmol/ml urine. This result demonstrated that dimethindene in aqueous solutions (pH 7) is strongly adsorbed on plastic and glassware, as suggested by others [2,3]. Investigations of the adsorption of dimethindene on glass were carried out with dimethindene maleate standards in water and urine. The results showed that, in contrast to water, urine prevented this adsorption. Therefore, all the reference and standard solutions were prepared in drug-free urine.

As shown in Fig. 2, very efficient clean-up was obtained. Almost all of the matrix components were washed out of the cartridge within 4.5 min (see also Table I).

The extraction capacity of the cartridges was found to be higher than 490 pmol/ml. The plot of dimethindene peak height *versus* its concentration in prepared standards was linear in the range 4.9–490 pmol/ml (r = 1.000).

The relative standard deviation for the cartridge-to-cartridge variation of the dimethindene peak height was found to be 1.3% for a standard 49.0

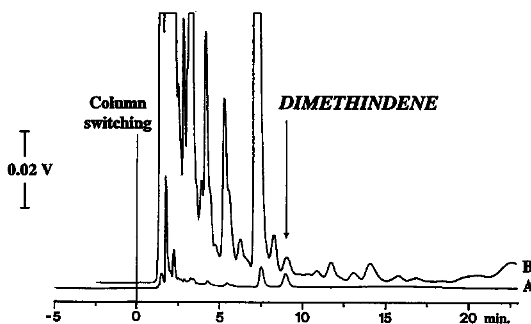

Fig. 2. Clean-up of the same urine sample under the experimental conditions with a washing time of (A) 4.5 and (B) 2.0 min. The time needed for a cartridge move was 0.5 min. For experimental conditions, see text.

TABLE III

PRECISION AND ACCURACY OF DIMETHINDENE ASSAY

Analyses carried out under the described experimental conditions.

Nominal concentration (pmol/ml)	Concentration found (mean ± S.D., n = 6) (pmol/ml)	R.S.D.[a] (%)	Confidence interval of the mean value (P = 95%) (pmol/ml)	Accuracy[b] (%)
Intra-assay variability				
4.9	4.76 ± 0.29	6.0	4.76 ± 0.30	−2.9
12.3	12.21 ± 0.63	5.1	12.21 ± 0.66	−0.3
24.5	24.90 ± 0.88	3.5	24.90 ± 0.93	1.6
49.0	48.89 ± 2.43	5.0	48.89 ± 2.55	−0.3
Inter-assay variability				
4.9	4.82 ± 0.46	9.6	4.82 ± 0.49	−1.7
12.3	12.28 ± 0.78	6.4	12.28 ± 0.82	0.2
24.5	24.89 ± 1.82	7.3	24.89 ± 1.91	1.6
49.0	49.10 ± 1.14	2.3	49.10 ± 1.20	0.2

[a] Relative standard deviation.

[b] Defined as the percentage deviation between the mean concentration found and the theoretical concentration.

pmol/ml urine sample (n = 10). No peak broadening was observed. One cartridge could be used up to 24 times.

Chromatography

Dimethindene was desorbed from the cartridge and eluted onto a deactivated reversed-phase analytical column (Suplex pKb-100) by isocratic elution with a mobile phase consisting of buffers (pH 3.0)–acetonitrile (90:10, v/v). This column was especially designed for the determination of basic compounds such as antihistamines. The dimethindene metabolite N-desmethyldimethindene [2] eluted at the same retention time as dimethindene. The UV spectra of dimethindene and its metabolite, recorded in solution in the mobile phase, showed that their molar absorptivities are identical. As the objective of the study was to determine the amount of drug reaching the systemic circulation, the measured amount of dimethindene included dimethindene plus N-desmethyldimethindene. Under the described experimental conditions, the recoveries of dimethindene and its metabolite were identical.

N-Desmethyldimethindene could be separated from dimethindene ($\alpha_{i,\text{dimethindene}}$ = 0.78) using 0.05 *M* ammonium acetate buffer (pH 7.0)–acetonitrile (77:23, v/v) as the mobile phase, but variations in the N-desmethyldimethindene peak area of 30% were observed. These variations were considered to be unacceptable. In a separate study [9], the concentration of the metabolite in the urine of healthy volunteers, collected after topical administration of dimethindene maleate, was found to be *ca.* 20% of the dimethindene content.

Method validation

The precision and the accuracy were determined over the range 4.9 - 49 pmol/ml. The results (Table III) ranged from 2.3 to 9.6% and from −1.7 to 1.6%, respectively. The parameters of the linear regressed calibration graphs, relating the dimethindene peak height to its concentration in prepared standards, were typically slope = 0.0343 and intercept = 0.26 with a correlation coefficient (r) of 0.999. The linearity range was from 4.9 to 490 pmol/ml of urine (r = 1.000). The limit of quantification for the assay was of the order of 5 pmol/ml. Dimethindene was found to be stable under the experimental conditions for up to 12 h. The relative standard deviation measured for a standard sample of 24.5 pmol/ml analysed every 30 min was 3.9% (n = 23). No drift of peak height was observed.

CONCLUSION

The method developed is suitable for the determi-

nation of dimethindene in urine at the pmol/ml level. It allows fully automated extraction and analysis of urine samples. Detailed validation data demonstrate its reliability.

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