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Short communication

Determination of dimethindene in human tears by high-performance liquid chromatography

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Abstract

A high-performance liquid chromatographic method is described for the determination of dimethindene in human tears. The tear samples were diluted in a 0.01 *M* hydrochloric acid–*n*-propanol mixture to prevent the irreversible adsorption of dimethindene. The diluted samples were directly injected into the chromatographic system to avoid sample pretreatment. The validation data demonstrate that the method is specific, precise and accurate within the calibration range of 12 to 1000 ng/ml dimethindene free base. © 1998 Elsevier Science B.V.

Keywords: Tears; Dimethindene

1. Introduction

Dimethindene maleate is a potent H_1-receptor antagonist of histamine [1]. Unchanged dimethindene has been determined in biological fluids such as plasma and urine either by various chromatographic techniques [2–4] or by means of an enzyme-linked immunosorbent assay (ELISA) [5]. No method has been described for the determination of dimethindene in human tears.

This paper presents an HPLC assay for the quantification of dimethindene in human tears. As dimethindene has been found to be unavoidably adsorbed on glassware, silanized glassware and plasticware [2–4,6], the aim of this study was to develop a fast and reliable HPLC method for the determination of dimethindene in minute tear volumes without sample pretreatment.

2. Experimental

2.1. Chemicals

Dimethindene maleate, *N*,*N*-dimethyl-3-[1-(2-pyridinyl)ethyl)]-1*H*-indene-2-ethanamine maleate was purchased from USP (USP Convention, Rockville, MA, USA). Methanol and acetonitrile were of HPLC grade from Rathburn (Walkerburn, UK). Water was purified by means of a Milli-Q device from Millipore (Millipore, Le Mont, Switzerland). All other reagents were of analytical grade from E. Merck (Darmstadt, Germany).

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2.2. Tear samples

Tear samples were collected in left and right eyes of subjects after instillation of either the vehicle or the vehicle containing the test article. The sample volume (about 4 μl) was first accurately measured and then added to 100 μl of 0.01 *M* HCl-*n*-propanol 95:5 (v/v) mixture placed into a 1.5-ml polypropylene Eppendorf tube. The vortexed samples were stored frozen at −20°C until analysis.

2.3. Chromatographic equipment and conditions

The HPLC system consisted of a Model 616 pump, a Model 600S controller, an autosampler Model 717plus equipped with a temperature-controlled rack set at 6°C, a Model 486 UV detector and a Maxima 825 data station, all from Waters Assoc. (Milford, MA, USA). The eluents were degassed by means of a GasTorr Model GT-104 degassing unit (Omnilab, Chavannes-des-Bois, Switzerland). HPLC separation was achieved using a CN column Nucleosil CC-5-CN, 125×4.0 mm I.D., dp=5 μm (Macherey Nagel, Oensingen, Switzerland) eluted isocratically in the reversed-phase mode. The mobile phase was 0.05 *M* (NH_4^+) ammonium phosphate buffer pH 5.5–acetonitrile (60:40, v/v). The flow-rate of the mobile phase was 1.2 ml/min. The detection wavelength was set at 258 nm. The injected volume was 52 μl.

2.4. Sample preparation

The thawed diluted tear samples were quickly centrifuged. They were transferred to polypropylene micro-vials by means of a suitable plastic pipette. The vials were placed in the temperature-controlled autosampler rack.

2.5. Standard solutions

The stock and the working solutions of dimethindene were prepared in 0.01 *M* HCl–*n*-propanol (95:5, v/v). The solutions were stable for 1 month at 2°C.

2.6. Calibration curves

A typical calibration curve was constructed with seven blank tear samples spiked with various amount of the appropriately diluted working solution. The calibration curves covered the range 0 to 1000 ng/ml of dimethindene free base. The calibration curves were obtained by plotting the peak height of the dimethindene peak versus the nominal concentrations expressed as ng/ml dimethindene free base. Response weighting ($1/conc^2$) was applied. The equations were calculated using linear regression.

2.7. Method validation

Blank tear samples spiked with 60.0 and 600 ng/ml were used as controls. The specificity of the assay was investigated using 13 blank tear samples from different subjects. The intra-day precision and accuracy of the method were evaluated by repetitive analysis of control samples (n=8 for each level). The inter-day precision and accuracy data were obtained assaying the controls on different days (n=18). The precision and the accuracy were defined as the relative standard deviation and as the deviation from the theoretical nominal concentration, respectively. The linearity data were obtained by means of calibration curves (n=4). The limits of quantification and of detection were defined as the lowest amount detectable with a signal to noise ratio of 10 and 2, respectively. The stability of dimethindene at room temperature as well as in the autosampler rack at 6°C and following freeze–thaw cycles were investigated using the controls.

3. Results and discussion

3.1. Assay

Mixtures containing inorganic acids and either C_3 or C_4 alcohols have been found to prevent the irreversible adsorption of dimethindene [6]. As a consequence, the tear samples were diluted in 0.01 *M* hydrochloric acid–*n*-propanol mixture to prevent the adsorption of dimethindene. In order to avoid both liquid–liquid extraction [2,3] and solid-phase extraction [4], direct injection of diluted tear samples

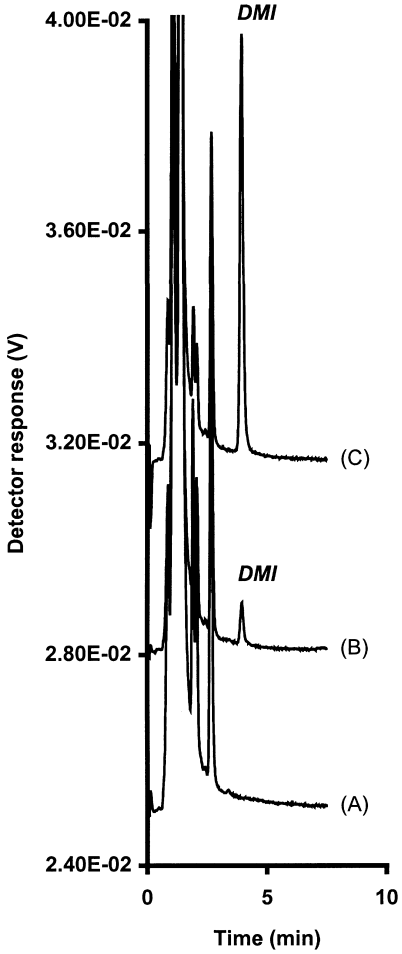

Fig. 1. Typical chromatograms obtained for a blank tear sample (A), and control samples spiked with 60 ng/ml (B) and 600 ng/ml (C) dimethindene base. The samples were assayed according to the recommended procedure. DMI=dimethindene.

onto a regular CN column eluted isocratically in the reversed-phase mode was investigated. Under the recommended experimental conditions, no exogenous and no endogenous compounds from both the vehicle and the dilution mixture interfere with dimethindene. The typical chromatograms obtained for a blank and the control tear samples are shown in Fig. 1.

3.2. Method validation

Intra-day and inter-day precision and accuracy data are shown in Table 1. The results obtained ranged from 0.5 to 1.2% and from −0.3 to 3.5%, respectively. These values were 3.8 to 4.0 and −1.6 to −2.5% for the inter-day precision and accuracy, respectively. The linearity range was 12.0 to 1000 ng/ml. The typical calibration curves equations gave a mean slope of $7.28 \cdot 10^{-2} \pm 1.88 \cdot 10^{-3}$ (R.S.D.= 2.6%), a mean intercept value of 0.6±0.7 and correlation coefficients greater than 0.9996. The limit of quantification was found to be 12.0 ng/ml of dimethindene free base. Taking into account the dilution performed during the sampling, this concentration corresponded to a concentration of about 312 pg/μl in the undiluted tear sample actually collected in the eye. The limit of detection was about 2.4 ng/ml corresponding to 0.4 pmol dimethindene base on column. The typical chromatograms obtained for tear samples collected from two subjects after installation of the drug for which concentrations close to those limits were found, and a blank tear sample are given in Fig. 2. The control samples were found to be stable for 16 h at room temperature

Table 1
Precision and accuracy of dimethindene assay

Nominal concentration (ng/ml)	Concentration found Mean±S.D. (ng/ml)	R.S.D. (%)	Confidence interval of the mean value (P=95%) (ng/ml)	Deviation (%)
Intra-assay variability (n=8)				
60.0	62.1±0.7	1.2	62.1±0.6	3.5
600.0	598.2±3.2	0.5	598.2±2.7	−0.3
Inter-assay variability (n=18)				
60.0	58.5±2.2	3.8	58.5±1.1	−2.5
600.0	590.6±23.4	4.0	590.6±11.8	−1.6

Control samples were assayed according to the recommended procedure. R.S.D.=relative standard deviation. The concentrations are expressed as dimethindene free base.

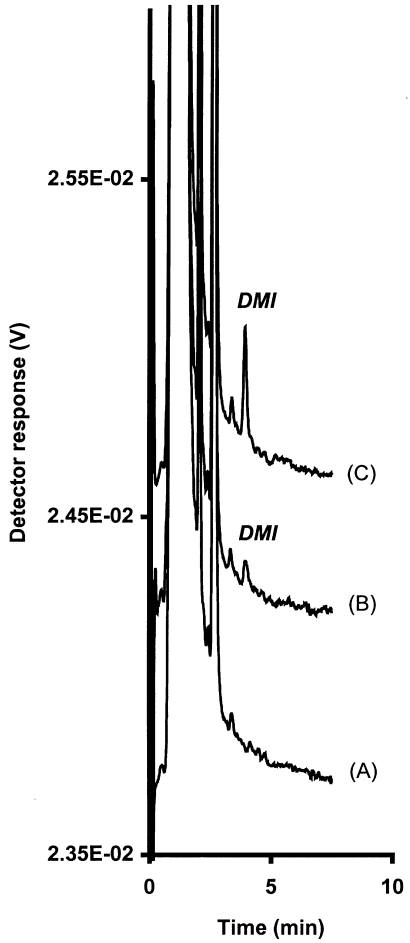

Fig. 2. Typical chromatograms obtained for a blank tear sample (A) and for tear samples collected from human subjects after drug administration for which dimethindene base concentrations were found to be near the limit of quantification (B: 5.6 ng/ml, C: 27.2 ng/ml). The samples were assayed according to the recommended procedure. DMI=dimethindene.

(deviation≤8.4%, $n=3$ for each level) and for 43 h in the autosampler rack (deviation≤7.1%, $n=3$ for each level). No noticeable effect (<2%) on dimethindene concentration in controls were observed after up to 3 freeze–thaw cycles.

4. Conclusion

The developed method is suitable for the determination of dimethindene in tear samples. The samples, which were diluted in a hydrochloric acid–propanol mixture to prevent an irreversible adsorption of dimethindene, could be injected directly in the HPLC system. The validation data demonstrate its reliability.

Acknowledgements

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