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Application of HILIC stationary phase to determination of dimethindene maleate in topical gel

L. Matysová, L. Havlíková*, R. Hájková, A. Krivda, P. Solich

Department of Analytical Chemistry, Faculty of Pharmacy, Charles University, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic

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ABSTRACT

A novel high performance liquid chromatography method for the determination of dimethindene maleate in pharmaceutical gel using hydrophilic interaction liquid chromatography (HILIC) with UV detection was developed and validated.

Following optimal conditions for the analysis of dimethindene maleate were used: analytical column SeQuant ZIC®–HILIC (50 mm × 2.1 mm, 5 μm), and mobile phase consisted of a mixture of acetonitrile and aqueous solution of acetic acid (25 mM) and ammonium acetate (2.5 mM) (87.5:12.5, v:v). The analysis time was less than 3 min at a flow rate of 0.3 ml min^{-1}. UV detection was performed at 258 nm. The method was validated and system suitability parameters were evaluated. The method is suitable for application for routine determination of dimethindene maleate in topical pharmaceutical preparation.

1. Introduction

Dimethindene maleate (Fig. 1), chemically *N*,*N*-dimethyl-2-{3-[(RS)-1-(pyridine-2-yl)ethyl]-1H-inden-2-yl}ethanamin-(*Z*)-butendioate, is derivate of fenindene reported to be strong antagonist of histamine on H1 receptors. Dimethindene decreases hyper permeability of capillaries during reactions of early sensitivity. It reduces prurience and irritation by different eruptions [1,2].

Hydrophilic interaction liquid chromatography (HILIC) is a technique suitable for separation of very polar, basic and hydrophilic compounds. HILIC is the alternative as the elution order is likewise inverted to RPLC (reversed phase liquid chromatography). This means that solutions that have little or no retention on RPLC columns generally show strong retention on HILIC columns. The HILIC technique thus bears similarities with traditional NPLC (normal phase liquid chromatography), but with the important difference that HILIC employs semi-aqueous mobile phases [3]. Alpert suggests that the mechanism of HILIC implicates partitioning between a water-enriched layer partially immobilised on the hydrophilic stationary phase and the less polar mobile phase [4]. A chromatographic system for HILIC chromatography is essentially instrumental identical to RPLC systems. Because of the similarities to RPLC in mobile phase compositions, the same routines for preparation of, for example, samples and wash solutions could be applied also for HILIC. Typical eluents for HILIC consist of 40-97% acetonitrile in water or a volatile buffer. HILIC columns contain a stationary phase that is hydrophilic and quite often also charged, at least in some region of the pH-scale. Compounds separated on the column interact with the stationary phase, and are generally more strongly retained the more hydrophilic the compound is [3].

The application of HILIC stationary phase to the area of pharmaceutical analysis has a raising potential. Recently HILIC approach has been applied for the analysis of polar molecules [4], peptides, nucleotides, nucleosides and amino acids [5–8], cytostatics [9] and ascorbic acid [10]. A universal HPLC method using HILIC mode for the simultaneous determination of 33 commonly used pharmaceutical counter ions was presented [11]. Dimethindene maleate is an official substance (European Pharmacopoeia—PhEur VI and in the Czech Pharmacopoeia–ČL 2005 for example). It is determined by nonaqueous titrimetry. Several analytical methods for dimethindene determination were published.

Dimethindene has been determined in different biological matrices using HPLC method [2,12,13]. High performance liquid chromatography and electrophoresis were used in a study of metabolism of dimethindene; dimethindene and its main metabolites were analysed [14]. The up-to-date method used gas chromatography in connection with mass fragmentographic method for determination of dimethindene in human plasma [15]. The simple gas chromatographic method for the determination of dimethindene in human urine and serum was also described [16].

Capillary zone electrophoresis (CZE) on-line coupled with capillary isotachophoresis (CITP) was used for the determination of antihistaminic drugs in urine samples, including dimethindene [17]. For the determination of dimethindene and its metabolite in

* Corresponding author. Tel.: +420 495067265; fax: +420 495067164.
E-mail address: lucie.havlikova@faf.cuni.cz (L. Havlíková).

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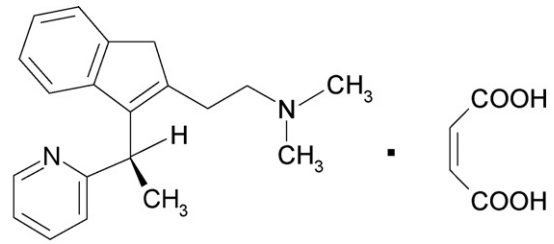
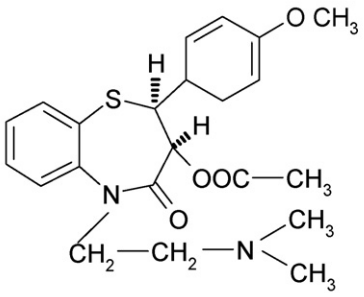


Dimethindene maleate



. HCl

Diltiazem hydrochloride

Fig. 1. Chemical structures—active substance (dimethindene maleate) with the internal standard (diltiazem hydrochloride).

human urine capillary electrophoresis with cyclodextrines as chiral additives was used [18]. In the next paper CE with run buffers containing different cyclodextrines to resolve dimethindene and four possible metabolites into their enantiomers in one run was used [19]. A comparison of capillary electrophoresis with NMR study of enantioseparation of dimethindene was described [20].

A combination of methods for the determination of biological activity of the enantiomers of dimethindene was used in last mentioned analysis. The methods were: CE, NMR, electrospray ionisation mass spectrometry and X-ray crystallography [21].

The aim of this study was to develop and validate a novel simple LC method for determination of dimethindene in topical gel based on HILIC. Dimethindene maleate is a polar basic substance and the analysis of these substances is more difficult by convention HPLC. HILIC stationary phase is suitable solution for the analysis of such polar basic substances.

2. Experimental

2.1. Reagents

Reference standards compounds used in this study—standard of dimethindene maleate and diltiazem hydrochloride (internal standard) were obtained from Herbacos–Bofarma Ltd. (Bochemie Group, Pardubice, Czech Republic), along with substances, which were tested as potential internal standard—methylparaben, propylparaben, ketoprofene, nimesulide, terbinafine hydrochloride. The substances, which were tested as internal standard too, were obtained from Sigma–Aldrich Co. (Prague, Czech Republic)—imidazole, gallic acid and from IVAX (Opava, Czech Republic)—ambroxole hydrochloride, procaine hydrochloride.

Acetonitrile, methanol and acetic acid for HPLC were obtained from Sigma–Aldrich Co. (Prague, Czech Republic). Phosphoric acid 85% and ammonium acetate were obtained from Merck (Darmstadt, Germany). Gel sample and its placebo were supplied from Herbacos–Bofarma Ltd. (Bochemie Group, Pardubice, Czech Republic). The deionised water was purified by a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Chromatographic system

Analysis was performed using Shimadzu LC-2010 C system (Shimadzu, Kyoto, Japan) with built-in UV–VIS detector. The built-in autosampler was conditioned at 25 °C. Chromatographic software Class VP 6.13 was used for data collection and processing. The analytical column ZIC®–HILIC (50 mm × 2.1 mm I.D., 5 μm particle size) was purchased by SeQuant (Umeå, Sweden). The optimal mobile phase for the analysis of dimethindene maleate was a mixture of acetonitrile and aqueous solution of acetic acid (25 mM) and ammonium acetate (2.5 mM) (87.5:12.5, v:v). The injection volume was 10 μl, isocratic elution was performed at a flow rate of 0.3 ml min^{-1} at 25 °C, UV detection was accomplished at 258 nm.

2.3. Reference standard solutions preparation

Standard solution was prepared by dissolving of active substance and internal standard in acetonitrile. The final concentrations of the sample or reference standards were 25 μg ml^{-1} of dimethindene maleate and 10 μg ml^{-1} of internal standard diltiazem hydrochloride.

2.4. Sample preparation

Isolation procedure based on methods for analysis of topical preparations routinely used in the control laboratory was used. An accurately weighed portion of pharmaceutical gel corresponding to 500 μg of dimethindene maleate (about 0.5 g) was transferred into a 50 ml centrifuge tube and supplemented with 20.00 ml of internal standard (10 μg ml^{-1} solution of diltiazem hydrochloride in acetonitrile). The mixture was placed into the ultrasonic bath for 15 min and then centrifuged at 1300 × *g* for 15 min. A volume of 10 μl of supernatant was injected into the column and analysed by HPLC.

Identification of peaks in the gel samples was based on the comparison of retention times of compounds in standard solutions.

3. Results and discussion

3.1. Method development and optimisation

The analysis of polar basic compounds often occurring in the pharmaceuticals is still a challenge. HILIC is the alternative method becoming recently more widely used.

Analysis of dimethindene maleate was accomplished at 258 nm according to the absorption spectrum of dimethindene maleate standard solution.

Analytical column SeQuant ZIC®–HILIC (50 mm × 2.1 mm; 5 μm) was used for purposes of this study.

Isocratic mode and binary mobile phase composed of 5 mM ammonium acetate buffer and acetonitrile were tested. The active substance eluted as dimethindene and maleic acid. The composition of the mobile phase and the flow rate were changed to find optimal conditions for separation of these components. The maleic acid eluted with the void volume and was not retained on HILIC column.

The effect of pH value of ammonium acetate buffer on the separation was evaluated. 5 mM ammonium acetate buffer pH value from 3.0 to 8.0 (pH value was adjusted using acetic acid and/or ammonium hydroxide) and acetonitrile in different volume ratios were tested. With the growing pH value the retention of dimethindene maleate was increased. Higher pH than 4.0 induced the peak tailing. The tested amount of organic modifier was 80-95% in 2.5% increments using 5 mM ammonium acetate pH 3.0–4.0 as the aqueous part of the mobile phase. Higher level of acetonitrile increased the retention of dimethindene; dimethindene was well separated from the maleic acid. The method using 90–97% ace-

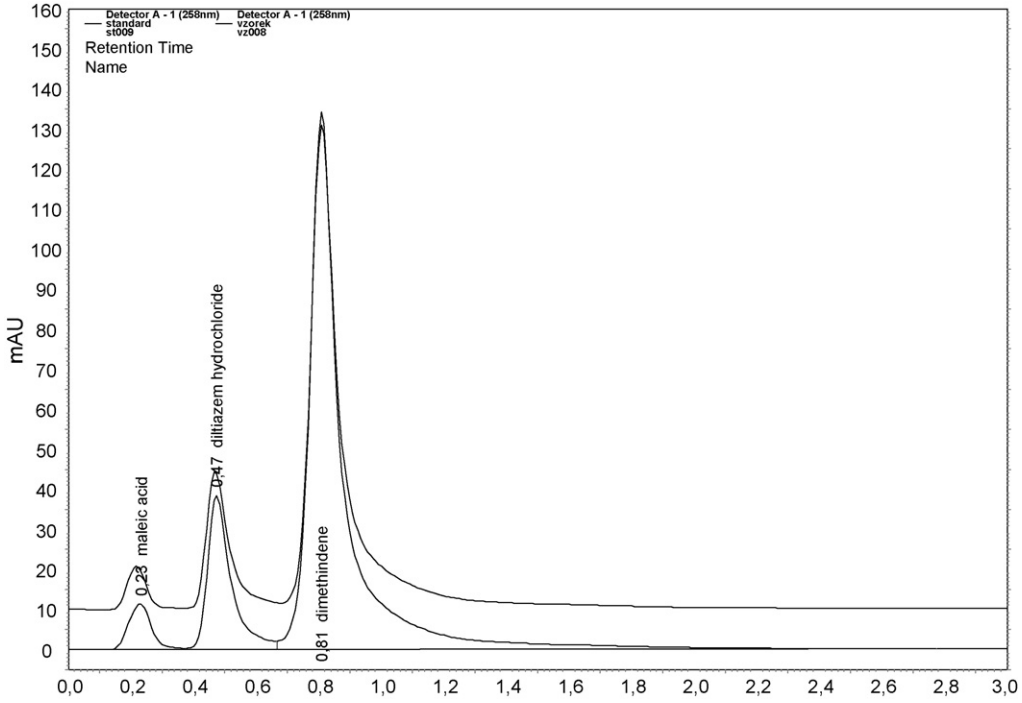


Fig. 2. Chromatogram of standard solution of dimethindene maleate (25 μg ml^{-1}) and internal standard (diltiazem hydrochloride, 10 μg ml^{-1}), lower trace; chromatogram of sample solution (topical gel), upper trace, UV detection at 258 nm; mobile phase: acetonitrile–aqueous solution of acetic acid (25 mM) and ammonium acetate (2.5 mM) (87.5:12.5, v:v); flow rate 0.3 ml min^{-1}; ZIC®–HILIC analytical column 50 mm × 2.1 mm I.D.; 5 μm particle size.

tonitrile was not convenient because it gave dimethindene peak of high peak tailing. Lower acetonitrile level eluted maleic acid and dimethindene not well separated. The concentration of ammonium acetate 2.5–20 mM gave the same retention for dimethindene maleate. A suitable composition of the mobile phase was acetonitrile and aqueous solution of a mixture of acetic acid (25 mM) and ammonium acetate (2.5 mM) (82.5:17.5, v:v) pH 3.70. The flow rate of the mobile phase was tested in the range from 0.15 to 0.5 ml min^{-1}.

The method of internal standard was used for data calculation. The amount of an active substance was calculated using the ratio of the areas of an active substance and internal standard. Several chemicals were tested (for example gallic acid, imidazole, procaine hydrochloride, ambroxol hydrochloride). Diltiazem hydrochloride was separated from void volume and from all analysed components and was chosen for the purpose of the analysis. The void volume was estimated by monitoring the first signal disturbance upon injection for the HILIC column.

The final optimal composition of the mobile phase was a mixture of acetonitrile and aqueous solution of acetic acid (25 mM) and ammonium acetate (2.5 mM) (87.5:12.5, v:v). The separation was performed using the analytical column ZIC®–HILIC SeQuant (Umeå, Sweden) 5 μm particle size and the flow rate of 0.3 ml min^{-1}. The injection volume was 10 μl. The analysis time for all compounds was less than 3 min. The chromatogram of the standard reference solution as well as a real sample (after isolation procedure) is shown in Fig. 2.

3.2. Method validation

The aim of method validation is to demonstrate the method suitability for its intended purpose as stated in ICH guideline Q1A(R2) [22]. The optimised method was partially validated by a standard procedure to evaluate validation characteristics (accuracy, linearity and system suitability test).

Accuracy (% of recovery, % of R.S.D.) using placebo samples spiked with standard solution was investigated. Comparison of real sample concentration and determined concentration was 98.65% and 0.38% for recovery and R.S.D., respectively. Linearity (described by equation of linear curve and corresponding correlation coefficient) using six calibration levels for dimethindene maleate (from 50 to 150% concentration levels) was determined. The method of linear regression for data evaluation was used. Peak area ratios of standard compound and internal standard were plotted against theoretical concentrations of standards. System suitability parameters for verification of the system performance were measured and calculated. All important characteristics including repeatability (standard deviations of retention time and area), peak resolution, theoretical plate number and peak asymmetry (symmetry factor)

Table 1
Method validation results for the active substance dimethindene.

	Dimethindene	Limits
SST		
Theoretical plates[a]	893	$N > 750$
Asymmetry[a]	1.71	
Resolution[a]		
Maleic acid–diltiazem hydrochloride	2.21	$R_{ij} > 1.5$
Diltiazem hydrochloride–dimethindene	2.07	$R_{ij} > 1.5$
Repeatability-t_r[b] (retention time)	0.68	R.S.D. < 1%
Repeatability-A[b] (area)	0.81	R.S.D. < 1%
Validation		
Linearity[d] (correlation coefficient)	0.99998	$R > 0.9990$
Linearity[d] (equation)	$y = 4.131E+5 \times -3.08E+4$	
Slope	$4.131E+5 \pm 1.4E+3$	
Intercept	$3.08E+4 \pm 3.9E+3$	
Accuracy[c] [% R.S.D.]	0.38	R.S.D. < 5%
Accuracy[c] [% recovery]	98.65	100 ± 5%

[a] Made in six replicates.
[b] Made in six replicates.
[c] Six samples injected three times each.
[d] At 1.25, 1.875, 2.50, 2.875, 3.50, and 4.00 μg ml^{-1} ranges, three replicates.

were calculated according to the European Pharmacopoeia [23]. The samples of standard solution injection in six replicates were measured and calculated. The results of the method validation, system suitability test parameters, and the required limits are tabulated in Table 1.

4. Conclusions

HILIC was firstly introduced for the determination of dimethindene maleate in topical gel. Diltiazem hydrochloride was used as internal standard. The separation was carried out using ZIC®–HILIC analytical column (50 mm × 2.1 mm I.D.; 5 μm, SeQuant, Umeå, Sweden) and mobile phase consisting of acetonitrile and aqueous solution of acetic acid (25 mM) and ammonium acetate (2.5 mM) (87.5:12.5, v:v) at a flow rate of 0.3 ml min^{-1}. UV detection was accomplished at 258 nm.

Analyses were performed on Shimadzu LC-2010 C system (Shimadzu, Kyoto, Japan) with built-in UV–vis detector. The built-in autosampler was conditioned at 25 °C. Chromatographic software Class VP 6.13 was used for data collection and processing.

The total analysis time was less than 3 min. Method has been validated and the results were found to be suitable for routine analyses. This method was successfully applied for the identification, quantitative analysis, homogeneity tests and stability tests of dimethindene maleate in a topical gel.

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