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(2*S*,3*S*)-Dicyclohexyl tartrate as mobile phase additive for the determination of the enantiomeric purity of (*S*)-atropine in tablets

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ABSTRACT

A chromatographic method for the determination of the enantiomeric purity of (*S*)-atropine is presented and has been applied to the analysis of tablets. The (2*S*,3*S*)-enantiomer of the chiral selector, dicyclohexyl tartrate, was used as selector in order to elute the impurity, (*R*)-atropine, before the main component, (*S*)-atropine.

INTRODUCTION

The enantioselective action of atropine was reported in 1904 by Cushny [1]. Later the anticholinergic activity of the two enantiomers was demonstrated in several papers, always with a higher potency for the (*S*)-enantiomer [also called (−)-hyoscyamine] [2]. Some of the drugs containing atropine marketed today contain the racemate, while others consist only of the (*S*)-enantiomer.

Methods are needed for monitoring the enantiomeric purity of (*S*)-atropine, and some reversed-phase chromatographic methods are now available for direct enantioselectivity analysis. Armstrong *et al.* [3] have achieved enantioselectivity using a β-cyclodextrin bonded phase for atropine and analogues. However, the separation factor (α) for atropine was not sufficient to obtain baseline resolution ($\alpha = 1.04$). High selectivity ($\alpha = 3.42$) was on the other hand reported using α_1-acid glycoprotein as the chiral stationary phase [4]. The elution of the (*S*)-enantiomer before the (*R*)-form and the low capacity of the protein column make the method less suitable for the analysis of (*R*)-atropine as an impurity in (*S*)-atropine. A third approach is the use of an enantiomeric form [in ref. 5 the (2*R*,3*R*)-form] of dicyclohexyl tartrate (DCHT) as a mobile phase additive in reversed-phase chromatography where the support is porous graphitic carbon (Hypercarb). The reproducibility of the method is good, which makes it suitable for routine analysis.

This study demonstrates how the retention order of the enantiomeric forms of a solute can be controlled by the choice of enantiomer of the selector (DCHT). This possibility can give particular advantages in the quantitation of enantiomeric impurities.

EXPERIMENTAL

Chemicals

Racemic atropine sulphate salt, (*S*)-atropine hemisulphate salt and tropic acid were obtained from Sigma (St. Louis, MO, USA). Homatropine and (−)-scopolamine bromide were purchased from Merck (Darmstadt, Germany). Tropine and (*R*)- and (*S*)-mandelic acid were bought from Fluka (Buchs, Switzerland). (1*R*,2*S*)-Norephedrine hydrochloride salt was from Serva (Heidelberg, Germany). (1*S*,2*R*)-Norephedrine hydrochloride salt and (*R*)- and (*S*)-phenyl-1,2-ethanediol were from Janssen (Beerse, Belgium); (*R*)- and (*S*)-ethyl mandelate were from Aldrich (Milwaukee, WI, USA). Belladonine, apoatropine and atropic acid were kind gifts from Astra (Södertälje, Sweden). (2*S*,3*S*)-DCHT was synthesized according to the procedure

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previously described [6] for its optical enantiomer, (2*R*,3*R*)-DCHT, after exchanging (2*R*,3*R*)-tartaric acid for (2*S*,3*S*)-tartaric acid. $[\alpha]_D^{22} = -13.3$ (c = 1.0 g/100 ml, methanol).

Apparatus

The chromatographic system consisted of a Beckman 114 M pump (Fullerton, CA, USA), a Rheodyne 7120 injector (Berkeley, CA, USA) and a Spectromonitor 3100 detector (Milton Roy, Riviera Beach, CA, USA) connected to a Nelson 2600 chromatography data system (Nelson Analytical, Cupertino, CA, USA) via a Model 762 interface.

Two analytical Hypercarb columns (Shandon, UK) (100 mm × 4.7 mm I.D.) were coupled in series. The columns and solvent reservoir were kept at 25 ± 0.1°C with a Heto type 02 pt 923 TC water bath (Birkerød, Denmark).

Chromatographic technique

The columns were equilibrated with 500 ml of phosphate buffer pH 2.8 before introducing the mobile phase containing the chiral selector. The breakthrough curve for the selector was recorded at 195 nm and was used to calculate the amount of selector adsorbed onto the support. The determination of atropine enantiomers was performed at 214 nm with a mobile phase consisting of 0.25 m*M* (2*S*,3*S*)-DCHT in phosphate buffer pH 2.8. The flow-rate was 0.4 ml/min and the mobile phase was not recirculated.

Calibration graph

A calibration graph was used for the analysis of the total concentration of atropine in the samples. Injection of racemic atropine gave two peaks of equal area, and the possibility that the (*S*)- and (*R*)-enantiomers, owing to interaction with the selector, would give a significant difference in UV absorbance could be excluded. Standard solutions of (*S*)-atropine (three different weighings) dissolved in the mobile phase in the concentration range $2 \cdot 10^{-7}$ to $5 \cdot 10^{-5}$ *M* were prepared. (The concentrations used were $2 \cdot 10^{-7}$, $5 \cdot 10^{-7}$, $1 \cdot 10^{-6}$, $5 \cdot 10^{-6}$, $1 \cdot 10^{-5}$, $2 \cdot 10^{-5}$, $3 \cdot 10^{-5}$ and $5 \cdot 10^{-5}$ *M*.) The areas of the chromatographic peaks were recorded by the Nelson 2600 system using a time constant of 6 s. The calibration graph was constructed by plotting the peak area *versus* the concentration of the standards. The validity of the graph was checked regularly by injecting a standard solution of a concentration close to the samples.

Assay procedure

The same batch of commercially available (*S*)-atropine depot tablets, 0.2 mg, was stored for six years in glass bottles sealed with plastic lids, under three different conditions: 25°C; 30°C and 30% relative humidity; and 37°C.

The dissolution of tablets was performed as follows [7]. Ten tablets were dissolved in 25 ml of acetonitrile and put in an ultrasonic bath for 5 min. A 25-ml aliquot of phosphate buffer pH 2.8 was added to the solution and left for another 5 min in the ultrasonic bath. Phosphate buffer pH 2.8 was added to a total volume of about 200 ml. The liquid phase was filtered through a glass fibre filter (Whatman 934-AH) into a volumetric flask of 250 ml and phosphate buffer was added to volume. A 20-μl aliquot of the sample solution was injected into the chromatographic system.

RESULTS AND DISCUSSION

Control of the retention order

The detectability is improved by a low retention, and in studies of impurities the main component is often used in rather high concentrations which might give overloading effects, manifested as an increased tailing. The detectability of an impurity is as a rule improved if it is eluted before the main peak and not after, when the tailing of the main peak will make the definition of the baseline difficult.

The previously used [5] selector, (2*R*,3*R*)-DCHT, gave an elution of (*S*)-atropine before (*R*)-atropine. For the determination of (*R*)-atropine as an impurity, the retention order should be reversed. (2*S*,3*S*)-DCHT was thus used as chiral selector. Table I presents the retention order and capacity factor of some enantiomeric solutes using the two enantiomers of the selector.

Mobile phase

Besides racemization, (*S*)-atropine can be degradad by hydrolysis and dehydration. Dehydration gives apoatropine, which may be dimerized to belladonine. The products of hydrolysis are tropine, tropic acid and atropic acid [8].

TABLE I

CONFIGURATION OF DICYCLOHEXYL TARTRATE AND RETENTION ORDER OF SOLUTES

Mobile phase: 0.25 m*M* DCHT in phosphate buffer pH 2.8; amount of DCHT adsorbed: 0.132 mmol/g.

Solute	(2*S*,3*S*)-DCHT			(2*R*,3*R*)-DCHT		
		k'_1	α		k'_1	α
2-amino-1-[spiro-(cyclopentane-1,1'-inden)-3'-yl]ethanol		15.9	1.60		15.9	1.75
Norephedrine	(1*S*,2*R*)	0.24	1.18	(1*R*,2*S*)	0.13	1.22
Mandelic acid	(*S*)	1.67	1.08	(*R*)	2.60	1.08
Ethyl mandelate	(*S*)	5.27	1.12	(*R*)	6.13	1.13
1-Phenyl-1,2-ethanediol	(*S*)	1.51	1.07	(*R*)	1.43	1.08
Atropine	(*R*)	1.39	1.18	(*S*)	1.42	1.21

As the main aim was to study enantiomeric purity, chromatographic conditions under which the determination of (*R*)- and (*S*)-atropine is unaffected by the degradation products had to be found. Previous studies [5] with (2*R*,3*R*)-DCHT as selector have shown that the enantioselectivity of atropine is almost independent of pH in the interval pH 2–5, whereas an increase in retention is observed with increasing pH. The acids on the other hand showed a decreased retention with increasing pH. Therefore the analysis was performed at pH 3 to facilitate the separation of atropine from tropic acid. In Fig. 1a the resolution of (−)-scopolamine, racemic homatropine, racemic atropine and racemic tropic acid is demonstrated. Tropine does not absorb UV radiation at 214 nm and was not injected. A chromatogram of all solutes except atropine is presented in Fig. 1b. No peaks from apoatropine, atropic acid or belladonine could be detected within 2 h.

Precision in determination of enantiomeric purity

The standard solutions contained a total concentration of atropine enantiomers of about $2.5 \cdot 10^{-5}$ *M*. They were prepared from (*S*)-atropine spiked with racemic atropine to contain (*R*)-atropine in amounts corresponding to 1–10% of the total concentration. The chromatographic system could be used for over 200 injections, but there was a continuous increase in retention (and decrease in peak height), and thus the areas of the peaks were used for quantitation. The increase in capacity factor between the first injection and injection No. 200 was 37%, and as stated in ref. 5 this is probably due to hydrolysis of the selector, giving an acid which may act as a counterion. The precision of the determination of impurity in the range 1–10% is given in Table II. A calibration graph over the interval $2 \cdot 10^{-7}$ to $5 \cdot 10^{-5}$ *M* was used for the determination of the concentration in the main peak and in the impurity. The graph was linear ($y = 4 \cdot 10^{-8} + 1.829x$) with a correlation coefficient (r^2) of 0.999. As can be seen in Table II the precision of the determination im-

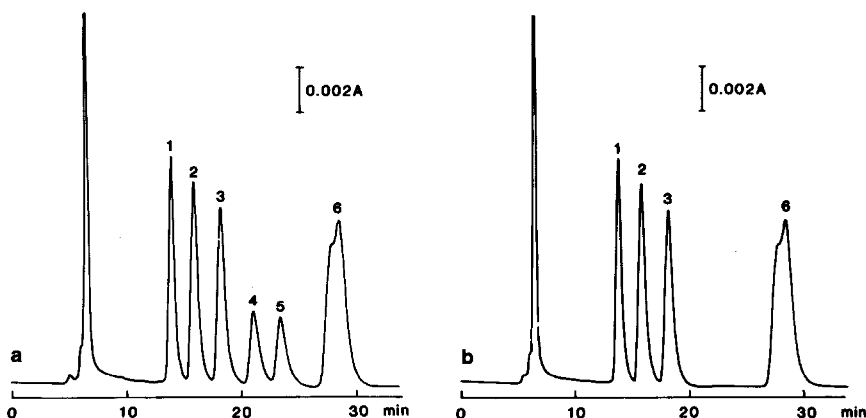

Fig. 1. Separation of atropine and analogues. For chromatographic conditions see Experimental section. (A) Solutes: 1 = (−)-scopolamine; 2 = (*R*)-homatropine; 3 = (*S*)-homatropine; 4 = (*R*)-atropine; 5 = (*S*)-atropine; 6 = racemic tropic acid. (B) Injection lacking atropine.

TABLE II

DETERMINATION OF ENANTIOMERIC PURITY

For chromatographic conditions see Experimental section. Samples: two different weighings of (*S*)-atropine and two of racemic atropine were used to prepare stock solutions. By mixing the stock solutions in different proportions, two series of samples were prepared. One of the series is marked with an asterisk in the table. The data were obtained on an intra-assay basis.

Proportion of (*R*)-atropine (%)			Number of injections
Added	Found	R.S.D.	
0.98*	0.92	4.3	5
1.11	1.00	9.5	15
2.00	1.95	5.3	6
2.83*	2.72	4.4	7
3.33	3.25	4.0	8
5.53	5.55	3.1	7
5.55*	5.35	1.5	8
9.99*	9.79	1.9	7
10.9	10.7	0.5	9

proved with increasing proportion of the impurity.

The total amount of atropine was determined by adding the areas for the main peak and the impurity. The total concentration of atropine found in the analysis was close to that expected (Table III).

In order to determine the minimum detectable concentration of (*R*)-atropine (after 200 injections), a standard curve was made for peak height *versus* concentration of (*R*)-atropine for six different concentrations in the interval 0.118–2.43 μM ($y = 8.6 + 3.1 \cdot 10^8 x$; $r^2 = 0.999$). The minimum detectable concentration, giving a peak height three times the baseline noise, was extrapolated to 59 nM, *i.e.* when injecting 20 μl of a 25 μM solution of atropine the presence of 0.3% (*R*)-atropine in (*S*)-atropine could be detected. It may be added that when checking the prepared stock solution of (*S*)-atropine no (*R*)-form could be detected.

TABLE III

DETERMINATION OF TOTAL ATROPINE

For chromatographic conditions see Experimental section. For samples see Table II.

(*R*)-Atropine added (%)	Total concentration of atropine × 10^5 (M)			Number of injections
	Added	Found	R.S.D. (%)	
0.98*	2.41	2.45	0.8	5
1.11	2.13	2.14	0.9	15
2.00	2.36	2.40	1.7	6
2.83*	2.51	2.52	0.7	7
3.33	2.13	2.16	0.9	8
5.53	2.14	2.15	2.8	7
5.55*	2.13	2.17	3.2	8
9.99*	2.36	2.36	0.4	7
10.9	2.17	2.23	1.8	9

Analysis of (S)-atropine in tablets

The racemization in commercially available depot tablets when stored under different conditions for six years was monitored. It can be concluded that the preparations are very optically stable during the storage conditions used. Compared with storage at 25°C, storage at 30°C and 30% relative humidity or at 37°C caused only a minor degree of racemization, though increasing significantly ($P = 0.01$) with increasing temperature (Table IV). The original ratio of the two enantiomers is unknown. An example from the analysis of tablets stored at 37°C is shown in Fig. 2.

TABLE IV

ENANTIOMERIC PURITY OF (*S*)-ATROPINE DEPOT TABLETS

For chromatographic conditions see Experimental section. For sample preparation see *Assay procedure*. The data were obtained on intra-assay basis.

Storage temperature (°C)	Amount of atropine per tablet		(*R*)-Atropine		n
	mg	R.S.D. (%)	(%)	R.S.D. (%)	
25	0.201	2.5	0.46	15	8
25	0.201	1.5	0.51	5.9	5
25	0.187	0.5	0.44	11	4
30	0.202	1.0	1.08	14	9
30	0.198	2.5	0.99	14	9
30	0.183	1.6	0.95	23	6
37	0.197	1.0	1.52	8.6	11
37	0.190	1.6	1.40	17	7
37	0.190	1.6	1.61	17	6

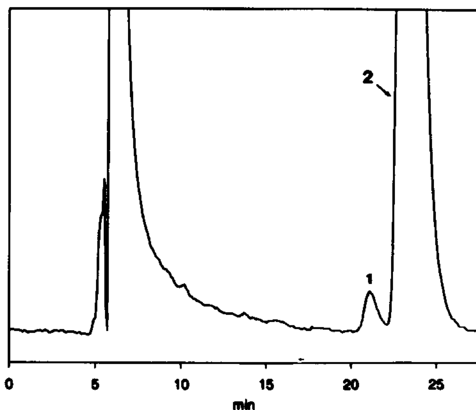

Fig. 2. Enantiomeric impurity in (*S*)-atropine depot tablets stored at 37°C. For chromatographic conditions see Experimental section. Peaks: 1 = (*R*)-atropine, 1.5% of total concentration; 2 = (*S*)-atropine.

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