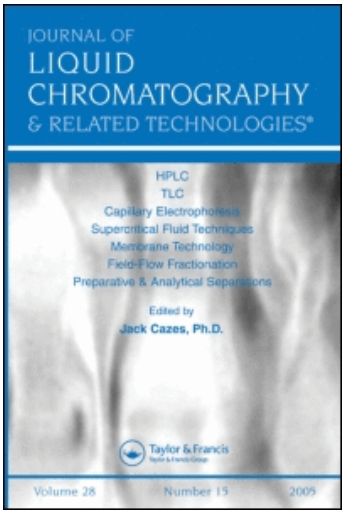

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OPTIMIZED AND VALIDATED HPLC METHODS FOR COMPENDIAL QUALITY ASSESSMENT. IV. NON-CHIRAL AND CHIRAL PURITY TESTS FOR SOLANACEOUS (TROPANE) ALKALOIDS

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OPTIMIZED AND VALIDATED HPLC METHODS FOR COMPENDIAL QUALITY ASSESSMENT. IV. NON-CHIRAL AND CHIRAL PURITY TESTS FOR SOLANACEOUS (TROPANE) ALKALOIDS

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ABSTRACT

The main alkaloids of certain plants of the Solanacea family, such as atropine and scopolamine, together with the synthetic homatropine and methylhomatropine, are routinely used medicinal agents and have their monographs in the modern pharmacopeias. In these monographs, non selective chemical reactions and/or TLC serve for the detection of "foreign alkaloids, related substances, etc." impurities.

In the present paper selective HPLC methods for the compendial purity control of these alkaloids are published. The methods allow the detection of 0.05 μg (= 0.1 %) and the quantitation of 0.05 - 5.0 μg of tropic acid or mandelic acid as hydrolytic degradation product impurities (chromatographic system : C_{18} / methanol - phosphate buffer pH 5 - 5.5). The limit of detection is 0.025 μg. Also, HPLC for the separation of tropane alkaloid enantiomers (chromatographic system : Chiralcel OD / n-hexane - isopropanol - methanol - triethylamine) is presented. Procedure for the enantiomeric purity control of S - (–) - hyoscyamine, the detection of R - (+) - distomer is suggested.

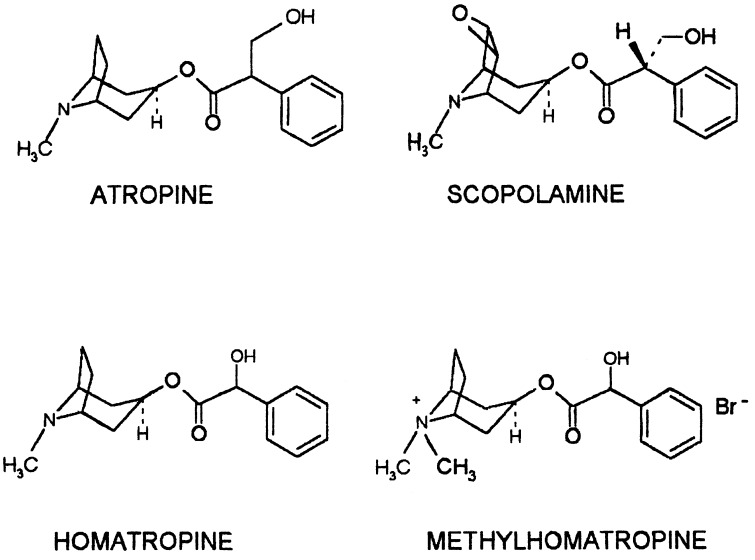

Figure 1. Chemical structures of the model compounds.

INTRODUCTION

The plants of the Fam. Solanacea, their different parts (leaf, root, etc.) also the products (tinctures, extracts) prepared by them, are used through centuries in the medication. Together with numerous other active agents, the tropic acid ester hyoscyamine (atropine) and Scopolamine (structures see in Fig. 1) are valuable tools of therapy even nowadays. Hyoscyamine as competitive acetylcholine inhibitor is used as strong spasmolytics and widely effective antidote (intoxication by morphine, pilocarpine, ammanita muscarea, phosphate insecticides, etc.). Scopolamine, out of its parasympatholytic effect, has a strong central inhibitory activity. Both compounds, alike to the synthetic, mandelic acid ester homatropine and methylhomatropine (Fig.1) have their own monograph in several pharmacopeias. These tropanol ester alkaloids either alone or in combination with other compounds (sedatohypnotics, tranquilizers, etc.) are daily used tools of the therapy. Due to the very strong biological activity, their unit dose falls within the 0.1 mg region, therefore their detection and quantitation from dosage forms needs the use of sensitive, mostly chromatographic methods. For such tasks HPLC proved especially suitable in case of plant extracts[1-6] and multi-component galenics.[7-11]. HPLC - methods were also published for the determination of Solanaceous alkaloids in biological samples[8,12-14] and feeds.[15]

For the purity control of the tropane alkaloids only non specific tests may be found in the pharmacopeial monographs. So, the Hungarian[16] and the European Pharmacopeia[17] prescribes thin layer chromatographic control of "foreign alkaloids and degradation products" limiting in 0.5 - 1 % the strength of foreign spots.

Considering the quality of the spraying reagents (iodo-bismuthate, iodo-platinate) the method seems suitable for the detection of such basic hydrolytic products as tropanol, N-methyltropanol, scopoline, as potential impurities. The USP[18] prescribes "other alkaloid" detection in atropine sulfate and scopolamine bromide with chemical reactions, while the "atropine and other solanaceous alkaloid content" of methylhomatropine is detected in an alkaline chloroformic extract by mercuric chloride reagent. Although the Solanaceous alkaloids are chiral compounds, similar to several other pharmacons, they are monographed as racemates in the pharmacopeias. An exception occurs for scopolamine, as its more effective S - (–) - enantiomer (the eutomer) is used. USP 23 registers the racemate of tropanoltropate (atropine) and the eutomer S - (–) hyoscyamine also has a monograph. As by the previous parts of this series, the suitability of HPLC for compendial purity tests of methylxanthines[19] and opium - alkaloids[20] was shown. In this work RPHPLC methods for the specific and sensitive detection of tropic acid, mandelic acid as potential (hydrolytic degradation product) impurities of tropane alkaloids, are published. Chiral-HPLC for the separation of the enantiomers and the enantiomeric purity control of S - (–) - hyoscyamine is also included.

EXPERIMENTAL

Materials

Atropine sulfate, Scopolamine bromide, Homatropine bromide, and Methylhomatropine bromide met the requirements of the Hungarian Pharmacopeia.[16] From their methanolic solution 10 μL aliquots were injected. Tropic acid(Fluka); Mandelic acid, D - (–) (Fluka); Hyoscyamine sulfate , S - (–) (Merck); Tetrabutylammonium hydrogen sulfate, 97 % (Aldrich); Triethylamine, Methanol, n- Hexane, 2-Propanol, all were "for HPLC" quality, Chemolab (Budapest); water, deionized, double distilled. Buffer solutions were prepared by mixing at pHs 4.0,5.0,5.5 the proper volumes of 0.067 M aqueous solutions of potassium dihydrogenphosphate and sodium hydrogenphosphate (KH_2PO_4, $Na_2HPO_4.2H_2O$, analytical grade, Reanal, Budapest). The pH of the solutions was tested by potentiometry with an accuracy ± 0.02 unit.

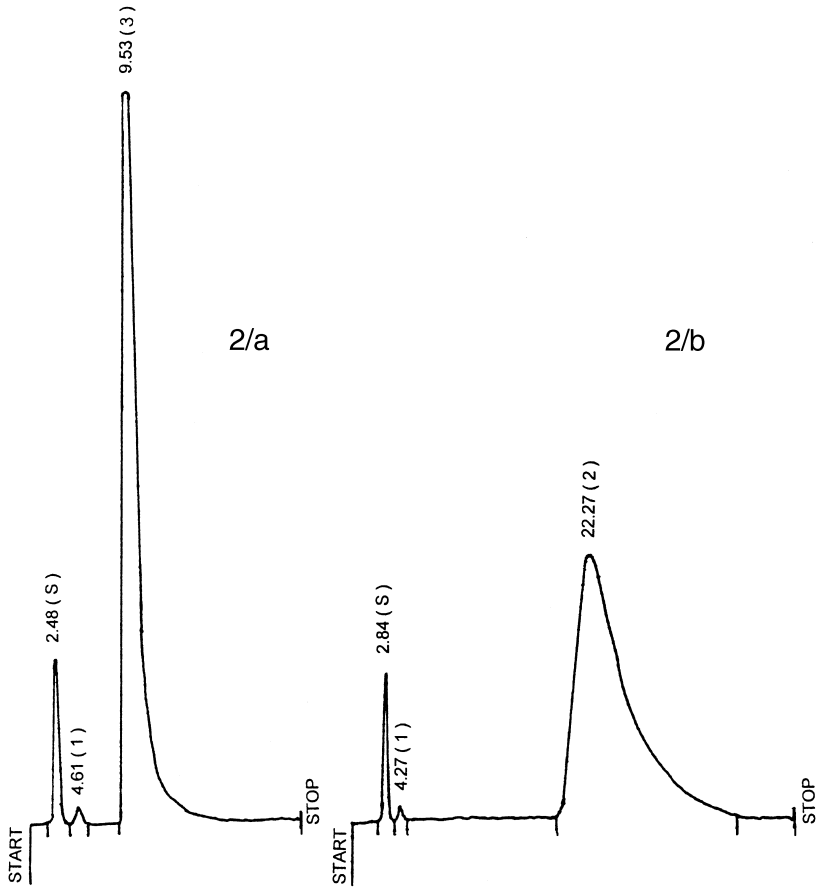

Figure 2. Detection of 0.1 % of Tropic acid (1) in Atropine (2) and Scopolamine (3). Injected : 0.05 µg (1) 50.0 µg (2 and 3). Mobile phase : Methanol - Phosphate buffer (pH 5.0) 25 : 75. Solvent peak : (S).

Chromatography

The HPLC apparatus comprised an ISCO pump Model 2350 (U.S.A.) combined with a Valco - injector unit (10 µL loop). An ISCO variable wavelength absorbance detector (230 - 800 nm) was used. The equipment units subsequent to the pump, was thermostatted at 25 ± 0.1^{o} (Column heater and chiller, Model 7955, Jones Chromatography LTD., Wales). The chromatograms were recorded; the data handling was effected by a Hewlett - Packard integrator Model 3396 Ser. II.

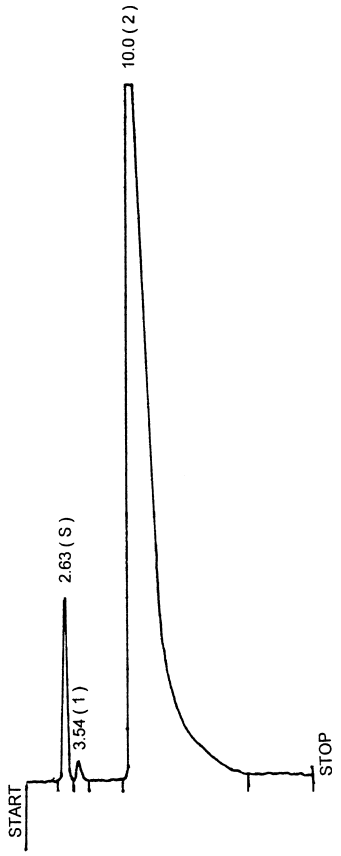

Figure 3. Detection of 0.1 % of mandelic acid (1) in homatropine (2). injected; 0.05 μg (1) 50.0 μg (2). Mobile phase : methanol - phosphate buffer (pH 5.5) 25 : 75.

For the non-chiral experiments the C_{18} - sorbent, Hypersil-5 ODS (Shandon) particle size 5 μm, was packed in a stainless column (250 x 4.0 mm I.D., BST, Budapest). As mobile phase, sonically degassed and filtered mixtures of methanol and aqueous phosphate buffer (pH = 4.0, 5.0 and 5.5) occasionally containing 0.01 M of tetrabutylammonium bromide, was applied. The chiral analysis has been performed on Chiralcel OD (Daicel) column 250 x 4.0 mm I.D. As mobile phase degassed and filtered mixtures 80:10 :10 and 85 : 5 : 10 of n-hexane - 2-propanol - methanol containing 12.5 - 25 μLof tri - ethylamine, were used. The column void time was signaled by the solvent peak of methanol.

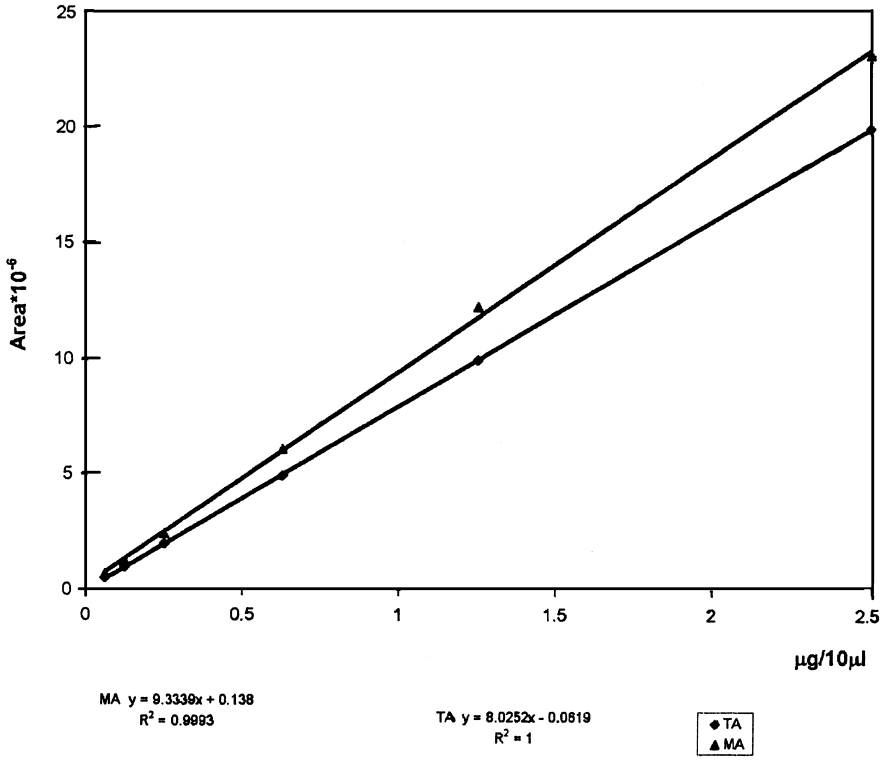

Figure 4. Standard calibration plot of Mandelic acid (MA) and Tropic acid (TA).

Following testing the C_{18} columns were brought to the initial state by washing with a 75 : 25 mixture of water - methanol (60 ml) and then with methanol (60 ml). Chiralcel OD column was reconditioned after a washing with a 50 : 50 and subsequently 90 : 10 mixture of n-hexane - 2-propanol (60 mL each).

Chromatographic System Optimization

In case of non-chiral analysis, the methanol content (40 - 70 v/v %) and the pH (4.0 - 5.5) of the eluent, convenient for a reasonable retention time (5 - 15 min.) and optimum selectivity was established; the temperature dependence of separation (15^o - 30^o) was also examined. For testing of enantiomeric purity the optimum ratio of isopropanol and methanol (3:1 through 1:3) was to be established.

Table 1

pH Dependent Retention Times (min.) for the Alkaloids and the Hydrolytic Product Acids

Compound	pH		
	4.0	5.0	5.5
Methylhomatropine	10.84	11.98	12.42
Homatropine	8.93	10.86	12.71
Atropine	20.30	24.35	27.66
Scopolamine	8.36	11.11	11.90
Tropic Acid	7.71	4.61	4.12
Mandelic Acid	9.93	3.37	3.31

Mobile phase: methanol-phosphate buffer 25 : 75.

Table 2

Mass Dependence of the Retention

Compound	Retention (min.)			
Atropine*	50 μg :	20.57	10μg :	24.35
Scopolamine		10.67		11.11
Tropic Acid	0.05 μg :	4.79	10 μg:	4.61
Homatropine**	50 μg :	10.63	10 μg :	12.71
Methylhomatropine		10.32		12.42
Mandelic Acid	0.05 μg :	3.31	1.0 μg :	3.31

* Mobile phase: methanol-phosphate buffer (pH 5.0) 25.75.
** (pH 5.5) 25.75.

Validation

Each data of retention was calculated as an average of min. 5 parallel runs, the difference between the extreme retention values might not exceed 3 %. The same was taken valid for the deviation of t_0 values. As limit of detection a signal - to - noise ratio 3 : 1 was accepted. The eluent flow rate was 1.0 mL / min. The effluent was monitored at 220 nm.

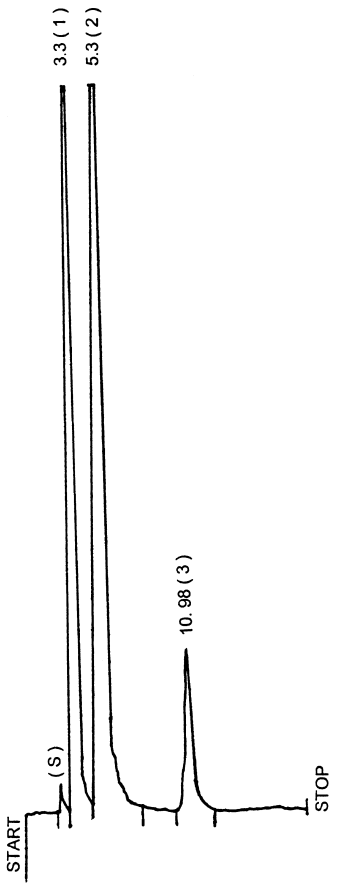

Figure 5. Separation of tropic acid (3) atropine (2) and scopolamine (1) by the addition of tetrabutylammonium bromide (TBA) to the eluent. Injected: 1.0 μg (3) 25 μg (2) and (1). Mobile phase: methanol-phosphate buffer (pH 5.0) 25 : 75 + 0.01 M TBA.

Detection of Tropic Acid Impurity in Atropine or Scopolamine Salts

0.050 g of the tested substance is dissolved in 10.0 mL of methanol. 10 μL (= 50 μg) of the solution is injected and chromatographed in the chromatographic system as shown by Figure 2. The appearance of a definitely observable peak of tropic acid indicates the presence of impurity equal or more than 0.05 % (0.025 μg). Eluent flow rate 1.0 mL/min.

Table 3

Separation of Tropane Alkaloid Enantiomers

Compound	k'_1	k'_2	α
Atropine*	1.87	2.98	1.59
Homatropine*	2.16	5.52	2.55
Methylhomatropine**	5.47	7.03	1.29

Mobile phase: n-Hexane- 2-propanol- methanol + Triethylamine

*	85	5	10	12.5 μL
**	80	10	10	25.0 μL

Detection of Mandelic Acid Impurity in Homatropine and Methyl-Homatropine Bromide

The method, described for the purity control of atropine, is used (see Figure 3).

Detection of R-(+)-Hyoscyamine Impurity of S-(-)-Hyoscyamine

0.050 g of hyoscyamine bromide or sulfate (H) is dissolved in 10.0 mL of methanol. 10 μL (= 50.0 μg H) of the solution is injected and chromatographed in the chromatographic system shown by Table 3 and Fig. 6. Any definitely observable peak of R - (+) - hyoscyamine indicates an amount equal or more than 1.0 μg (= 2 %).

RESULTS AND DISCUSSION

Characteristic, accompanying impurities of tropanol-ester alkaloids are the hydrolytic products of tropanol, and the corresponding acids. With regard to the UV activities, it seemed reasonable to aim the detection of the acid (tropic-, mandelic acid) impurity content as a quality indicator for the Solanaceous alkaloids. The data of Table 1 show, that in the reversed phase (C_{18}) system the pH increase effects a significant retention increase for the tertiary alkaloids, while the retention of the quarter amine methylhomatropine increases very moderately. At the same time, the retention of the acids, due to the increased ionization, decreases. Consequently, pH 5.0 and 5.5 together with 25 % methanol content were found as optimum for the purity test of alkaloids.

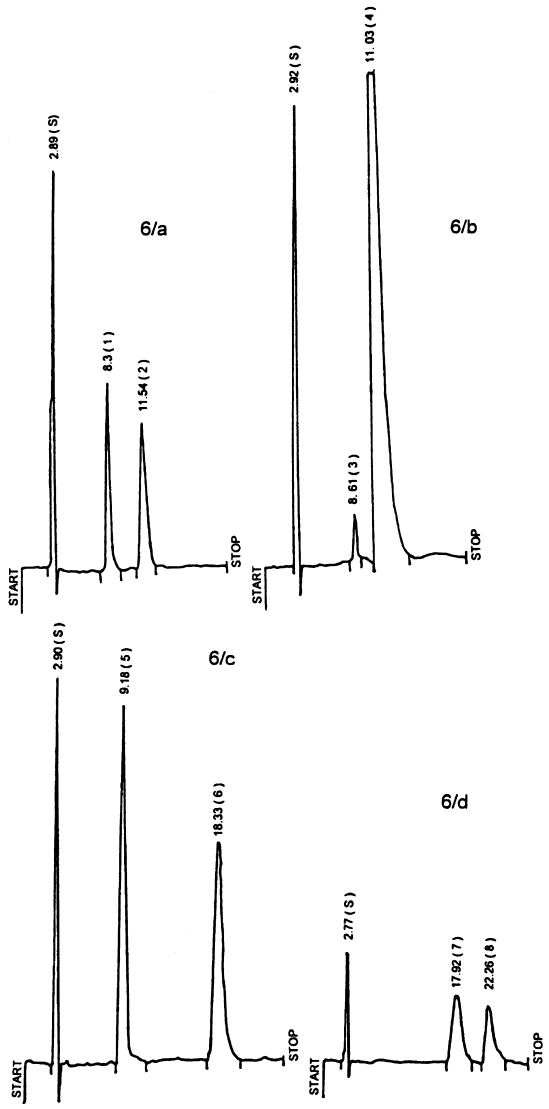

Figure 6. Separation of Solanaceous alkaloid enantiomers. (1) and (2) Hyoscyamine enantiomers (injected: atropine). (3) R-(+)-Hyoscyamine, 1.0 μg. (4) S-(-)-Hyoscyamine, 50.0 μg. (5) and (6) Homatropine enantiomers. (7) and (8) Methylhomatropine enantiomers.

The retention times are shown in Table 1. Of course, with a marked increase of the amount injected, the retention of the analyte decreases. Since, for the modeling of an impurity test, the amount of the tested substance and that of its assumed impurity must be chosen as high and low as it is possible and occasionally the difference of retentions decreases; but, in our case, it remains great enough for a sensitive purity testing. The mass dependence of retention is shown by Table 2.

Also the tropic or mandelic acid impurities of tropane alkaloids may be quantitated in the range 0.05-5.0 μg. Diagram and statistics are shown by Figure 4. The limit of detection lies for both acids at 0.025 μg.

Figure 5 demonstrates how the addition of tetrabutylammonium bromide (TBA) alters the peak sequence basically: as TBA, via ion pair formation, greatly increases the retention time of tropic acid, reversibly, it inhibits by competition the adsorption of atropine and scopolamine. Consequently, this way the peak of tropic acid (mandelic acid) impurity appears later than that of the correspondent ester-alkaloids.

As earlier experiences indicated, the Chiralcel OD column is highly suitable for the separation of certain tropane enantiomers.[21] However, methods suitable for the compendial chiral purity testing of tropane alkaloids, could not be found in the literature. We observed, in accordance to the establishment of Tang[21] that partial substitution of 2-propanol by methanol in the mobile phase dramatically increased the separation power of the used chromatographic system.

It is our own experience, that opposite to certain opinions[21] in the presence of 2-propanol : n-hexane can dissolve methanol much over 5 % and the increase of methanol concentration up to 10 % enhanced the enantiomer separation enormously (see Table 3, homatropine).

On the other hand, the addition to the eluent trace amount of triethylamine[21] highly improves the peak quality and also increases the chiral discrimination power of the chromatographic system. So, the mobile phase containing n-hexane and both of the two alcohol - modifiers, proved suitable for the purity control of S-(-)-hyoscyamine, allowing the detection of 2 % of the distomer R-(+)-enantiomer (Fig. 6).

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