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Note

High-performance liquid chromatography of some tropane alkaloids

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Recently, we described the separation of alkaloids by means of high-performance liquid chromatography (HPLC) on small-particle silica columns[1–3] and surveyed the HPLC of alkaloids[4]. In this paper, we describe the separation of atropine, scopolamine and apoatropine.

The analysis of tropane alkaloids has been described by Stutz and Sass[5], who used Sil-X adsorbent as the stationary phase and ammonia solution–tetrahydrofuran (1:100) as the mobile phase and obtained detection limits of 50 μg with RI detection and 1 μg with UV detection at 254 nm. Honigberg *et al.*[6] analyzed scopolamine and atropine in antispasmodic mixtures, using reversed-phase chromatography on octadecyltrichlorosilane or phenyldichlorosilane on pellicular beads as the stationary phase and mixtures of methanol and water buffered with ammonium dihydrogen orthophosphate (1 %) or ammonium carbonate (0.5 %) as the mobile phase. However, the results were not satisfactory because neither scopolamine nor atropine could be detected at therapeutic dose levels. Several of the solvent systems used gave rise to tailing and/or multiple peaks. These results are in accordance with those obtained by Twitchett *et al.*[7], who found that the octadecyltrichlorosilane stationary phase gave satisfactory results only with acidic and neutral solutes, having a very poor separation efficiency for most basic compounds.

EXPERIMENTAL

A Packard Model 8200 liquid chromatograph (Packard Becker, Delft, The Netherlands) equipped with a Model 1130 UV absorbance detector for the wavelengths 254 and 280 nm was used.

Two types of column were used. A stainless-steel column of 30 cm length and 4.6 mm I.D. was filled with Partisil 5 μm (Chrompack Nederland, Middelburg, The Netherlands) by the balanced-density slurry technique, and a stainless-steel column of 127 cm length and 2 mm I.D. was filled with 5.5 g of Pellosil HC (Reeve-Angel, Clifton, N.J., U.S.A.) by the tap-and-fill technique. The Partisil column was operated at a pressure of 40–65 kg/cm^2, flow-rate 2.00–2.29 ml/min and temperature 25°, with 1 % of diethylamine in diethyl ether–methanol (proportions as in Table I) as the solvent system. The Pellosil column was operated at a pressure of 70 kg/cm^2, flow-rate 1.33 ml/min and temperature 20°, with 1 % of diethylamine in diethyl ether–methanol (95:5) as solvent system. The solvents used were diethylamine (puriss, p.a., freshly

distilled, Cat. No. 31730, Fluka, Buchs, Switzerland), diethyl ether (p.a., Cat. No. 921, E. Merck, Darmstadt, G.F.R.) and methanol (Baker Analyzed Reagent, Cat. No. 8045, J. T. Baker Chemicals, Deventer, The Netherlands). The sample size was 15–150 μg in 5 μl of methanol.

RESULTS AND DISCUSSION

The number of possible mobile phases with low absorption at 254 nm is limited. On Pellosil HC, different mobile phases were tested and diethyl ether containing 1% of diethylamine gave the best results. When chloroform–methanol mixtures were used, the large difference between the k' values of scopolamine and atropine prevented useful separations from being achieved under isocratic conditions. Because of the small UV absorption of the tropane alkaloids ($E_{1cm}^{1\%}$ of the UV maxima of atropine in methanol is 6 at 252 nm, 7 at 258 nm and 6 at 262 nm), rather large amounts of the alkaloids have to be injected for analysis. On the pellicular adsorbent, not only was tailing observed, but also the retention times were found to vary, depending on the concentrations of the alkaloids. These phenomena may be explained by the unfavourable ratio of the sample size to the capacity of the pellicular bead stationary phase. Therefore, a completely porous small-particle silica column with a much higher capacity was applied.

A 5-μm Partisil column was tested with methanol, ammonia solution and ammonium nitrate solution mixtures as the mobile phase, as described by Jane[8]. Atropine and scopolamine were separated, whereas atropine and apoatropine could be separated only partially ($R_s = 0.2$). The peak shape of atropine was not symmetrical and seemed to be formed from two poorly resolved peaks. This may be caused by a partial separation of atropine in *l*- and *d*-hyoscyamine. In order to achieve a separation of atropine and apoatropine, solvent systems of diethyl ether plus methanol in different proportions, containing 1% of diethylamine, were tested. It can be seen from the results in Table I that the solvent system diethyl ether–methanol (9:1) plus

TABLE I

HPLC SEPARATION OF SOME TROPANE ALKALOIDS ON A PARTISIL COLUMN

5-μm Partisil column, 30 cm long, 4.6 mm I.D. Solvent system: diethyl ether–methanol in various proportions, containing 1% of diethylamine.

Diethyl ether:methanol ratio in solvent	*Pressure (kg/cm²)*	*Flow-rate (ml/min)*	*Time per analysis (min)*	*Solvent per analysis (ml)*	$k' = \frac{tR_1 - tR_0}{tR_0}$ *(ref. 9)*			*Resolution:* $R_s = \frac{2(tR_2 - tR_1)}{w_1 + w_2}$ *(ref. 9)*	
					Atropine	*Apoatropine*	*Scopolamine*	*Atropine–apoatropine*	*Apoatropine–scopolamine*
7:3	65	2.00	4.5	9	1.21	0.98	0.24	0.6	2.8
8:2	60	2.17	5	10.35	1.50	1.09	0.27	0.9	2.9
9:1	55	2.29	6.5	14.90	2.35	1.54	0.46	1.5	3.2
95:5	45	2.14	9	19.26	3.64	2.08	0.70	2.2	3.5

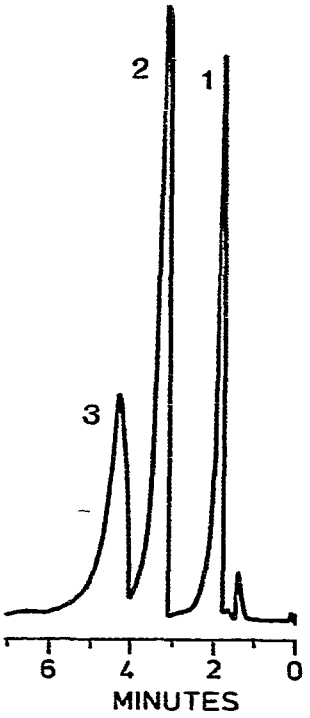

Fig. 1. Separation under the conditions given in Table I. Solvent: diethyl ether–methanol (9:1) + 1% of diethylamine. Peaks: 1 = scopolamine; 2 = apoatropine; 3 = atropine.

1% of diethylamine gave the best results, and a small resolution of the atropine peak into two peaks was also observed (Fig. 1). On the 5-μm Partisil column, no changes in the retention behaviour were noticed for various concentrations of the tropane alkaloids in the range used (15–150 μg).

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