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Note

High-performance liquid chromatography of *Cinchona* alkaloids

I. Normal-phase high-performance liquid chromatography

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In a previous paper[1] we reported on the thin-layer chromatographic (TLC) analysis of *Cinchona* alkaloids. In this paper high-performance liquid chromatographic (HPLC) analysis of these alkaloids by means of normal-phase systems is described.

A numbers of papers have been published on the HPLC analysis of quinidine and its metabolites in biological materials. However, only a few investigations have dealt with naturally occurring mixtures of *Cinchona* alkaloids and none of these was able to separate the eight major quinoline alkaloids (quinine, quinidine, cinchonine, cinchonidine and their dihydro derivatives), permitting their simultaneous determination in extracts from tissue cultures of *Cinchona*. The best results could presumably be obtained by means of the methods developed by Pound and Sears[2], Johnston *et al.*[3] and Bauer and Untz[4].

EXPERIMENTAL

Chromatographic procedures

A Waters Model 6000A pump, a Waters Model U6K injector and a Schoeffel Model SF770 variable-wavelength detector operating at 325 nm were employed. LiChrosorb Si 60 (5 μm) was used as the stationary phase, packed in 200 × 4.6 mm I.D. stainless-steel columns. The following mobile phases were used at a flow-rate of 1 ml/min: S1, toluene–ethyl acetate–diethylamine (7:2:1); S2, methanol–25% ammonia (100:1); S3, chloroform–isopropanol–diethylamine (96:1:2); S4, chloroform–diethylamine (98:2); S5, chloroform–ethanolamine (100:0.5); S6, diisopropyl ether–methanol–triethylamine (70:5:5).

Chemicals

The solvents were obtained from J. T. Baker (Deventer, The Netherlands) and were of Baker analyzed reagent quality. Diethylamine was freshly distilled prior to its use. The test compounds were kindly provided by ACF Chemiefarma (Maarssen, The Netherlands), except for isocinchophyllamine, which was isolated from plant material.

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RESULTS AND DISCUSSION

The methods reported by Pound and Sears[2], Johnston *et al.*[3], Bauer und Untz[4] and several others, based on our experience with the TLC analysis of *Cinchona* alkaloids[1], were tested.

First a silica gel column and the mobile phase toluene–ethyl acetate–diethylamine (7:2:1) (S1), which gave the best TLC separation[1], were tested. For detection a wavelength of 325 nm was chosen. Fairly good results were obtained (Fig. 1). The four parent alkaloids (quinine, quinidine, cinchonine and cinchonidine) were separated completely (peaks 6, 5, 1 and 3, respectively), but the separation from their dihydro derivatives (peaks 2, 4 and 7) was poor. Changes in the proportions in the solvent system did not improve the separation. Using the triethylamine[5] instead of diethylamine in the mobile phase (less aggressive for silica gel) did not give satisfactory results.

The solvent methanol–25% ammonia (100:1) (S2), which separated the vinyl alkaloids from the dihydro alkaloids on silica gel TLC plates, gave similar results in HPLC, but the separation of the parent alkaloids was poor.

At this stage of our investigations a publication by Bauer and Untz[4] appeared, describing the separation of a series of *Cinchona* alkaloids using chloroform–isopropanol–diethylamine (940:57:1), containing 2.65 ml/l of water, as the mobile

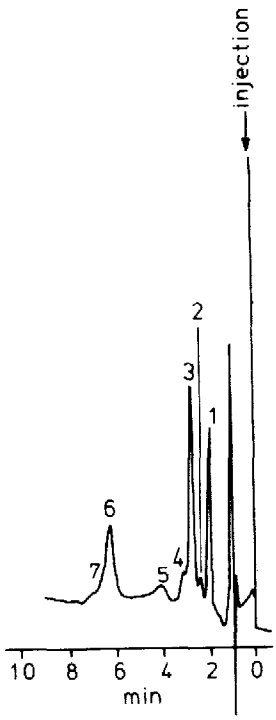

Fig. 1. Separation of *Cinchona* bark alkaloid extract. Column: 200 × 4.6 mm I.D. filled with LiChrosorb Si 60 (5 μm). Mobile phase: toluene ethyl acetate–diethylamine (7:2:1) (S1); flow-rate, 3 ml/min. Detection: 325 nm. Peaks: 1 = cinchonine; 2 = dihydrocinchonine; 3 = cinchonidine; 4 = dihydrocinchonidine; 5 = quinidine; 6 = quinine; 7 = dihydroquinine.

phase. The exact water content was crucial for the separation, and had to be determined by Karl Fischer titration. The laborious preparation of the mobile phase and the fact that we did not obtain a complete separation of the eight quinoline alkaloids led us to try several modifications. The best results were obtained with chloroform–isopropanol–diethylamine (94:1:2) (S3), and chloroform–diethylamine (98:2) (S4) was also suitable. However, as diethylamine is known to dissolve silica gel[5] and coloured solutions are formed readily in combination with chloroform, other bases were tested. With triethylamine the retention times of the vinyl alkaloids decreased considerably compared with the diethylamine-containing solvent; the retention times of the dihydro alkaloids were not affected as much. By means of chloroform–triethylamine (94:1) useful retention times were obtained, but the peak width was increased and the alkaloids showed tailing compared with diethylamine-containing systems. Replacement of diethylamine by ethanolamine[6,7] shortened the retention times drastically. By reducing the ethanolamine concentration to 0.5% reasonable separations were obtained, but further reduction in the ethanolamine concentration resulted in tailing.

The advantages of the solvent system chloroform–ethanolamine (100:0.5) (S5) were its stability (no coloration was observed) and very little baseline drift. Changes in retention times due to evaporation of the base, observed when ammonia and diethylamine were used, did not occur with ethanolamine.

A solvent system that had given good results for other types of alkaloids, *viz.*, diisopropyl ether–methanol–triethylamine (70:5:5) (S6), also gave reasonable separations of the quinoline alkaloids, but not complete separation of all eight parent alkaloids.

Table I gives the results obtained by means of some of the normal-phase systems described above. They can all be applied to the analysis of a limited number of quinoline alkaloids but none of them is capable of separating all four parent alkaloids and their dihydro derivatives, necessitating the use of two solvents for the HPLC analysis of extracts of tissue cultures.

TABLE I

HPLC SEPARATION OF SOME *CINCHONA* ALKALOIDS ON SILICA GEL

Conditions and solvent systems as described in the text.

Alkaloid	*k'*			
	S1	*S2*	*S5*	*S6*
Quinine (Q)	6.7	1.3	1.5	10.6
Dihydro-Q	7.7	2.1	1.7	10.4
Quinidine (Qd)	4.1	0.9	0.8	5.5
Dihydro-Qd	4.5	1.9	1.1	7.1
Cinchonine (C)	1.4	1.8	1.0	5.3
Dihydro-C	1.9	2.5	1.1	7.7
Cinchonidine (Cd)	2.6	1.7	1.3	6.6
Dihydro-Cd	2.8	2.0	2.5	8.1
Quinidinone	0.4	0.2	1.0	
Isocinchophyllamine		0.6	0.5	5.2

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