Journal of Chromatography, 249 (1982) 369–372
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,257

Note

Separation of cinchona alkaloids by high-performance liquid chromatography

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(Received August 2nd, 1982)

Analysis of the cinchona alkaloid quinidine has received considerable attention because of the wide therapeutic use of this compound and certain derivatives as anti-arrhythmic agents[1]. Methods are available for its assay in solid dosage form[2] as well as in plasma and urine[3–5]. Most modern techniques use some form of high-performance liquid chromatography although gas chromatographic methods have been reported with packed[6–8] and glass wall coated open tubular columns[9].

As part of a programme to develop plant cell cultures for the production of specialised food ingredients, a separation of the four major alkaloids quinine, quinidine, cinchonine and cinchonidine was required in extracts of cinchona and other plant tissues. Two restraints were placed upon the development of a suitable method: it had to run isocratically to allow the maximum throughput of samples and fractions had to be collected for mass spectral examination. This latter condition precluded the use of non-volatile organic reagents such as those used in ion-pairing techniques. Extensive examination of an apparently suitable technique[2] failed to produce an adequate separation even though three different columns (Waters μBondapak C_{18}, Perkin-Elmer Spherisorb C_{18} and RP-8) were tested.

This paper describes the use of a bonded cyanopropyl column in the reversed-phase mode with a quaternary solvent system optimized for maximum resolution to separate the alkaloids both as a standard mixture and in a plant tissue extract.

EXPERIMENTAL

Methanol and acetonitrile were of HPLC grade (Fisons Scientific), tetrahydrofuran and reagents were of Analar grade (BDH). The mobile phase consisted of selected proportions of orthophosphoric acid (0.0068 *M*, buffered to pH 7.0 with 1 *M* sodium hydroxide), acetonitrile, methanol and tetrahydrofuran. Because of the lack of quaternary solvent delivery on the pumping system, the different mobile phases were mixed separately in batches of 200 ml. All solutions were filtered through a 0.48-μm membrane and degassed by sonication under vacuum before use. The alkaloids were obtained from a commercial supplier and used without further purification. A stock solution of each alkaloid was made in water (800 μg ml^{-1}) and a mixture containing 200 μg ml^{-1} of each component was used for the optimization procedure. The liquid chromatograph comprised a Model 3B pumping system, Model LC100 oven at 50°, a Rheodyne 7125 valve injector with a 20-μl loop and a Model LC75 variable wavelength detector at 231 nm linked to a Sigma 15 data console (all Perkin-

0021-9673/82/0000–0000/$02.75

TABLE I

MOBILE PHASE COMPOSITION DURING OPTIMIZATION OF CINCHONA ALKALOID SEPARATION

*Mixture**	*Composition (%, v/v)*			
	Buffer	*Acetonitrile*	*Methanol*	*Tetrahydrofuran*
a	40	60	–	–
b	57	–	–	43
c	48	–	52	–
d	52.5	–	26	21.5
e	48.5	30	–	21.5
f	44	30	26	–
g	48.4	20	17.3	14.3
h	50	17	28.7	3.3

* Mixtures a–g (inclusive) were used to optimize the separation, mixture h was the final mixture.

Elmer). The column was 25 cm × 0.46 cm I.D. slurry packed [in sodium acetate–methanol, 0.2 g l^{-1} (ref. 10)] in this laboratory with 5-μm Spherisorb CN, a bonded cyanopropyl spherical silica (Phase Separations). The mobile phase flow-rate was 1.5 ml min^{-1}.

Optimization of the mobile phase composition to achieve base line resolution of the four alkaloids was carried out by the overlapping resolution method[11] using seven calculated mixtures in varying proportions of buffer, acetonitrile, methanol and tetrahydrofuran at equivalent solvent strengths calculated on the assumption that the buffer had the same polarity as water. Resolution maps were sketched and the optimum overlap estimated visually to establish the optimum phase composition. The polarity of the mixture was then adjusted by a slight increase in the buffer concentration to increase the solute capacity factor, k, and thereby improve the separation of the alkaloids from polar components in plant tissue extracts. The selected mobile phase was used to analyse a simple extract of *Cinchona succiruba*, prepared by homogenization of the frozen leaf in phosphate buffer at pH 7.5 containing 0.1 % (w/v) of the detergent Tween 20.

RESULTS AND DISCUSSION

Optimization of the separation of the alkaloids was quite straightforward. The compositions of the seven trial mobile phases (a–g) and the final optimized mixture (h) are listed in Table I. Corresponding chromatograms and the optimized separation are shown in Fig. 1. Almost complete baseline resolution of the four compounds has been achieved although there is some variation in the retention times (Table II). This could be due to inaccuracies in the preparation of the mobile phase mixtures which could be overcome by the manufacture of larger batch volumes or the use of a liquid chromatograph with quaternary solvent delivery. This separation has been found to be quite reproducible from column to column provided that the column is conditioned before use by the passage of at least 400 ml of the optimized mobile phase. The retention times of the alkaloids were sufficiently long to enable them to be clearly separated from other components of the leaf extract (Fig. 2) and enabled peaks of interest to be collected for further examination and identification. The use of the fluorescence detector was examined but neither cinchonine nor cinchonidine fluoresced under the conditions of the separation.

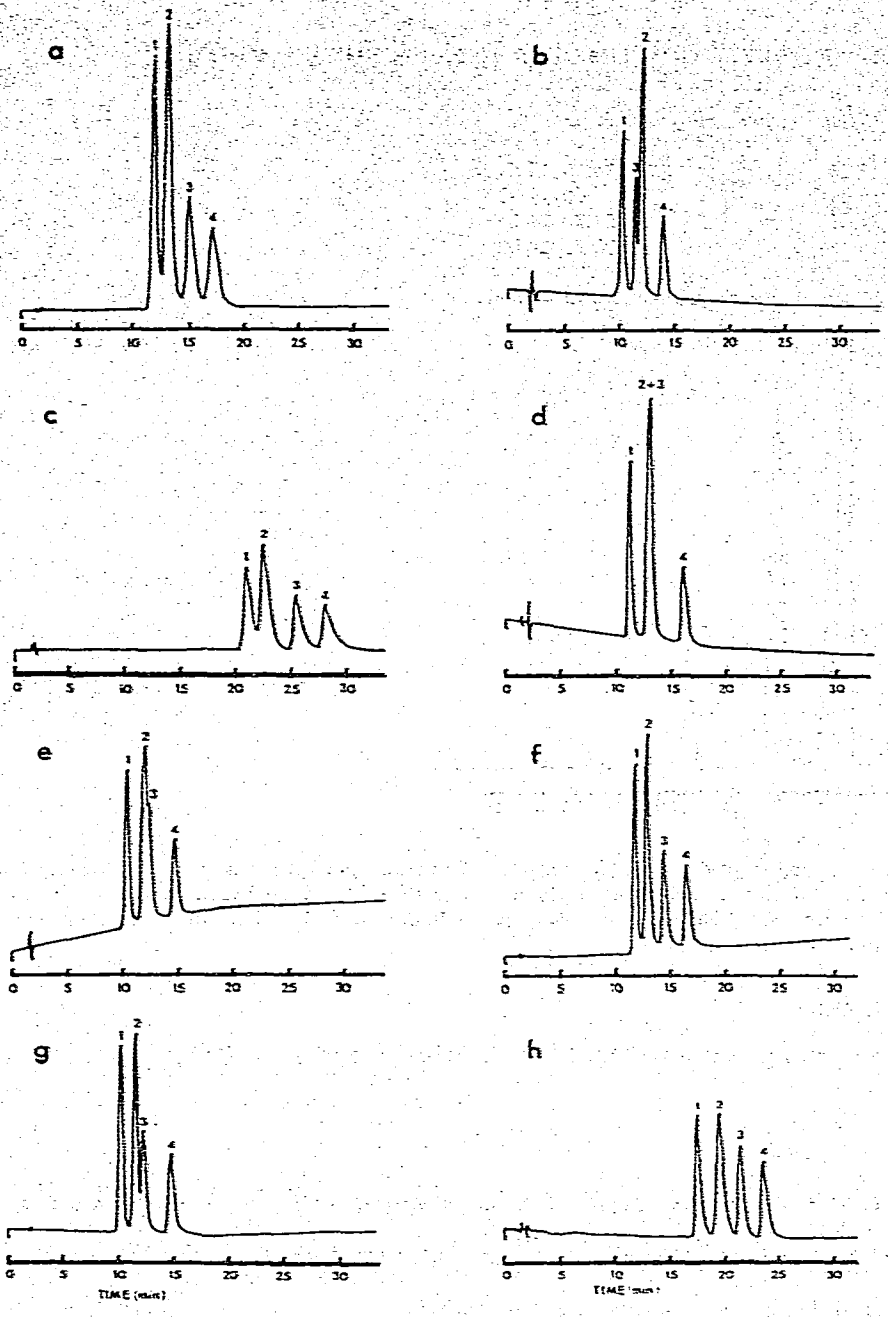

Fig. 1. Development of the optimized separation of the four cinchona alkaloids. Column, 5 μm Spherisorb CN at 50°C; mobile phase, varying proportions of buffer, acetonitrile, methanol and tetrahydrofuran (see Table I, mixtures a–h); flow-rate, 1.5 ml min^{-1}; UV detector at 231 nm. Peak identities: 1 = quinine; 2 = quinidine; 3 = cinchonidine and 4 = cinchonine.

TABLE II

REPRODUCIBILITY OF RETENTION TIME FOR THE CINCHONA ALKALOIDS

Compound	*Retention time (min)**	*Range*
Quinine	17.26	16.60–17.89
Quinidine	19.29	18.60–19.93
Cinchonidine	21.63	21.20–21.85
Cinchonine	23.76	23.25–24.02

* Mean value from three separate batches of solvent.

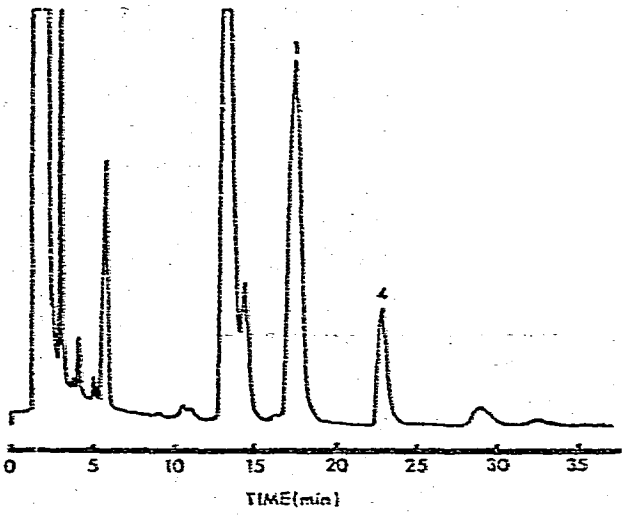

Fig. 2. Chromatogram of extract of *Cinchona succiruba* leaf. Column, 5 μm Spherisorb CN at 50°C; mobile phase, 0.0068 *M* orthophosphoric acid (adjusted to pH 7.0 with 1 *M* sodium hydroxide)–acetonitrile–methanol–tetrahydrofuran (50:17:28.7:3.3, v/v); flow-rate, 1.5 ml min^{-1}; UV detector at 231 nm. Peak identities: 1 = quinine; 4 = cinchonine.

ACKNOWLEDGEMENT

The authors are grateful to Drs M. J. C. Rhodes and R. J. Robins for helpful discussions.

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