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Note

Reversed-phase ion-pair chromatography of alkaloids on dodecylsulphonic acid and cetrimide (hexadecyltrimethylammonium)-impregnated C_{18} columns

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The analysis of alkaloids by high-performance liquid chromatography (HPLC) has always been hampered by the basic properties of the alkaloids and the weakly acidic properties of the silanol groups present in silica gel. Even in chemically bonded stationary phases uncovered silanol groups may interact with the bases, resulting in chemisorption, usually recognized by tailing of peaks[1–3]. The addition of basic modifiers such as ammonia or amines to the mobile phase[4,5] often improves the column performance and the peak shapes of basic compounds. Dimethylformamide has also been reported as a useful additive for improving the peak shapes of basic compounds in reversed-phase HPLC[6]. As chemically bonded phases and silica gel deteriorate rapidly under basic conditions owing to dissolution of the silica[5], either guard columns can be used to protect them, or the mobile phase can be saturated with silica[7] to prevent dissolution of the stationary phase.

The problems of column degradation mentioned can be circumvented if the analyses are performed under acidic conditions. Under such conditions the alkaloids are protonated and require a more polar eluent in normal-phase HPLC than do non-protonated alkaloids, whereas the opposite holds true for reversed-phase HPLC. Here, ion-pair chromatography has proved to be a very useful technique. Various theories have been proposed to explain the mechanism of the ion-pair chromatography of both acidic and basic compounds. Owing to the different concepts involved, various names have been used to describe ion-pair chromatography, including ion-pair chromatography[8], soap chromatography[9], surfactant chromatography[10] and dynamic ion-exchange chromatography[11].

The application of ion-pair chromatography to the analysis of alkaloids has been reported by numerous workers. In our investigations of the separation of various alkaloids by using reversed-phase ion-pair chromatography as reported by Lurie and Demchuk[12–14] we observed poor peak shapes for some of the strongly basic alkaloids. Therefore, some of the methods described in the literature for improving peak shapes in reversed-phase HPLC were investigated.

Kubiak and Munson[15] reported the addition of 0.001 *M* ammonium or sodium nitrate to the mobile phase in reversed-phase ion-pair chromatography, claiming that the nitrate ions would occupy the uncoated sites on the solid support and thus reduce

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tailing. Bonora *et al.*[16] added a non-polar solvent such as tetrahydrofuran to the mobile phase to improve the peak shape for *Cinchona* alkaloids in reversed-phase chromatography.

Several workers have reported improved peak shapes on addition of long-chain amines to the mobile phase[17–24]. Low concentrations of tetramethylammonium in the mobile phase have also been reported to improve peak shapes in reversed-phase ion-pair HPLC[25,26]. The beneficial effect of the addition of amines to the mobile phase was explained by the masking of the free silanol groups by the amines.

Several workers have compared the effects of different amines on column performance[19,22–24]. Gill *et al.*[24] found that an increase in the chain length of the amine additive results in a significant improvement in the peak shapes of basic compounds. The introduction of hydroxyl groups in the amine considerably reduced the benificial effect of the amine additive. The geometry of the amine was also an important factor. Of the isomers triethylamine and hexylamine, the latter gave better results than the former, indicating that the primary amine interacts more strongly with the active silanol groups of the octadecyl stationary phase. Introduction of one or two further methyl groups on the nitrogen in hexylamine did not affect the interaction of the amine with the silanol groups. Bij *et al.*[22] found that long-chain amines even at very low concentrations (1 m*M*) were much more efficient in masking silanol groups than, for example, the more bulky triethylamine. They described a method for measuring the silanol masking effect and found hexadecyltrimethylammonium bromide (cetrimide) to be the most active compound.

In our experiments on the reversed-phase ion-pair chromatography of alkaloids we found it of interest to investigate the influence of the addition of the quaternary long-chain amine cetrimide on the separation and peak shapes of alkaloids, and considerable changes in these parameters were observed on addition of low concentrations of cetrimide to the mobile phase.

Because in reversed-phase ion-pair chromatography the columns have to be equilibrated with the mobile phase, in order to obtain saturation of the column with the pairing ion and as a similar equilibration can be expected to occur with the amine additive, the reversed-phase column was first loaded with dodecylsulphonic acid and then with cetrimide. Using such a loaded column a considerable improvement in column performance was observed for alkaloids, whereas for neutral compounds there was no change in the number of theoretical plates of the column (Table I). Changing the sequence of the loading procedure, *i.e.*, the amine before the sulphonic acid, did not lead to satisfactory column performance. Subsequent alterations to the mobile phase led to the conclusion that with the impregnated columns the amines could be omitted from the mobile phase, with still a good column performance. Various counter ions could also be used in the mobile phase, and studies of this aspect resulted in the use of a mobile phase consisting of 0.02 M methanesulphonic acid in 0–20% methanol in water at pH 3–4. The addition of 1–5% of dioxane or tetrahydrofuran to the mobile phase further improved the peak shape.

Preliminary tests with some types of reversed-phase materials other than the initially used LiChrosorb RP-18 showed that in some instances the characteristics of the column were so altered that the alkaloids were no longer retained. Separations on impregnated LiChrosorb RP-18 columns with the above-mentioned solvent systems proved to be very useful for the analysis of various types of alkaloids. Highly

TABLE I

NUMBER OF THEORETICAL PLATES OF TREATED AND UNTREATED COLUMNS AS CALCULATED FOR QUININE AND ANTHRACENE

Column	*Calculated number of theoretical plates*	
	*Quinine**	*Anthracene***
Untreated	5	5844
Impregnated with dodecylsulphonic acid	31	6294
Impregnated with dodecylsulphonic acid and then with cetrimide	3136	–
Impregnated with cetrimide	313	7000
Impregnated with cetrimide and then with dodecylsulphonic acid	324	

* Mobile phase: 0.02 *M* methanesulphonic acid in water–dioxane–sulphuric acid (98.5:1.5:0.5) (pH = 3.5 with 10 *M* sodium hydroxide).

** Mobile phase methanol–water (7:3).

selective separations could be obtained of closely related alkaloids and examples are shown in Figs. 1 and 2. The columns could be used for several weeks without a decrease in efficiency.

The selectivity of the separations can be changed by altering the ratio of water to organic solvents or the pH of the mobile phase; *e.g.*, the separation of morphine and codeine (Fig. 1) can be improved by decreasing the concentration of methanol to 1.5%.

This type of separation system has been applied to the analysis of *Cinchona* and *Tabernaemontana* alkaloids.

EXPERIMENTAL

A Waters Model 6000A pump, a Waters Model U6K injector and a Waters Model 440 UV detector operating at 280 nm were employed for the HPLC analyses. LiChrosorb RP-18, 5 μm, was used, as the stationary phase, packed in 250 or 300 × 4.6 mm I.D. stainless-steel columns. The flow-rates employed were 1.0–1.2 ml/min.

Column loading procedure

The column was packed using a slurry of the stationary phase in methanol. Subsequently the column was washed with 100 ml of 0.02 *M* methanesulphonic acid in water–dioxane–sulphuric acid (98.5:1:0.5) (pH = 3.5 with 10 *M* sodium hydroxide). The dodecylsulphonic acid was loaded on the column by washing it with 100 ml of 0.01 *M* dodecylsulphonic acid in methanol–water (1:1), followed by recycling this mobile phase overnight at a flow-rate of 0.5 ml/min. The column was washed with the above-mentioned mobile phase until a stable baseline was obtained, then loaded with cetrimide by pumping through 100 ml of 0.02 *M* aqueous cetrimide, followed by recycling this solution overnight at a flow-rate of 0.5 ml/min. Finally, the column was washed with the mobile phase until a stable baseline was obtained, and the column was then ready for the analyses. For the mobile phases used, see Figs. 1 and 2.

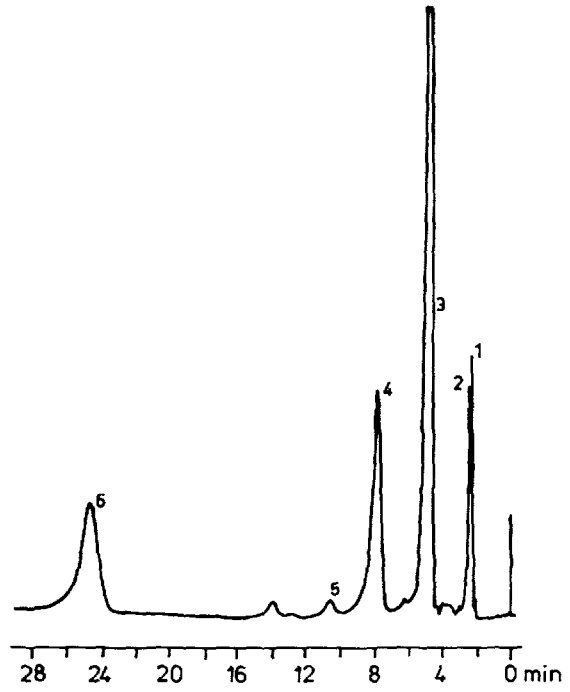

Fig. 1. Separation of some opium alkaloids. Column, LiChrosorb RP-18, 5 μm, dodecylsulphonic acid and cetrimide impregnated (250 × 4.6 mm I.D.); mobile phase, 0.02 *M* methanesulphonic acid in water–methanol–dioxane–sulphuric acid (85:15:1:0.5) (pH = 3.5 with 10 *M* sodium hydroxide); flow-rate, 1.2 ml/min; detection, UV (280 nm). Peaks: 1 = morphine; 2 = codeine; 3 = thebaine; 4 = papaverine; 5 = noscapine; 6 = narceine.

Fig. 2. Separation of scopolamine and atropine. Column, as in Fig. 1; mobile phase, 0.02 *M* methanesulphonic acid in water–methanol–dioxane–sulphuric acid (75:23.5:1:0.5) (pH = 3.5 with 10 *M* sodium hydroxide); flow-rate, 1.2 ml/min; detection, UV (254 nm). Peaks: 1 = scopolamine; 2 = atropine.

Chemicals

The solvents were obtained from J. T. Baker (Deventer, The Netherlands) and were of Baker analysed reagent quality. Water was freshly distilled before use. The stationary phase used was LiChrosorb RP-18, 5 μm (E. Merck, Darmstadt, F.R.G.).

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