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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF HEROIN SAMPLES ON ALUMINA BY ION EXCHANGE IN MIXED AQUEOUS–ORGANIC MOBILE PHASES

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SUMMARY

Alkaloids can be separated as cations on ion-exchange material. In contrast with silica-based ion-exchangers, bare alumina can be packed into columns with high permeability and efficiency yielding symmetrical peaks. By using mixed aqueous–organic mobile phases, the selectivity of the system can be enhanced and manipulated in order to optimize separations. Chromatograms of illicit heroin and opium samples illustrate the applicability of the technique.

INTRODUCTION

Illicit heroin samples contain a number of other compounds. They can be divided in two groups. First those introduced by a poor preparative technique and secondly the adulterants added after preparation.

The major impurities acetylcodeine and 6-monoacetylmorphine are introduced by an acetylation of codeine respectively hydrolysis during the preparation[1]. Codeine is originally present in the raw morphine base. Major adulterants with physiological effects include caffeine, strychnine and procaine. The compounds most commonly found in illicit heroin are listed in Table I. The typical concentrations given depend on the geographical origin of the mixture.

Opium is the basic source for the preparation of heroins. It is the latex obtained by incision of the unripe seed capsule of *Papaver somniferum* L., dried or partly dried by heat or spontaneous evaporation. Opium contains 25–30 alkaloids[2] of which morphine, codeine, narcotine and papaverine are the most important. These are also included in Table I.

In order to lay criminal charges, precise identification and quantitation of the compounds of the seizure are necessary. A comparison supported by chemical analysis also permits the association of different samples and hence investigation in the distribution chains. This aspect is very interesting and several analytical methods have been explored.

Recently, Huizer[3] described a technique using fluorescent compounds present

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TABLE I

COMPOUNDS COMMONLY FOUND IN ILLICIT HEROIN AND OPIUM SAMPLES

Concentrations are given as percentages.

Compound	*Heroin of Middle East*	*Heroin of Far East*	*Opium*
Heroin	70	30	–
6-Monoacetylmorphine	5	10	–
Acetylcodeine	5	7	–
Codeine	Low	Low	+
Morphine	Low	Low	+
Papaverine	2	Low	+
Narcotine	1–5	Low	+
Strychnine	–	0.5–1	–
Caffeine	–	50	–
Procaine	Low	–	–
Thebaine	–	–	+

in low concentrations in the heroin sample. The pattern obtained on the basis of the relative concentrations of these secondary compounds remains intact despite the addition of adulterants, and permits the recognition of the origin of the seizure.

Separation, identification and quantitation of the compounds in heroin samples have received more attention. The separation of narcotics is mostly achieved by chromatography[4,5] but spectroscopic techniques are used for identification and quantitation. Mass spectrometry[6], nuclear magnetic resonance spectrometry[7] and infrared spectroscopy[8] provide reliable identification and ultraviolet spectroscopy is often used for quantitation after separation by high-performance liquid chromatography (HPLC).

Gas chromatography is most commonly used for rapid quantitative analysis[9], but is not optimal for the determination of polar compounds such as morphine. Therefore, recently published work has concentrated on the use of HPLC. Liquid–solid chromatography (LSC)[10] is still very popular, but the low solubility of heroin and various adulterants in the relatively non-polar solvents employed, forms a major limitation. Reversed-phase[11] and ion-pairing techniques[12] are more promising. However, interactions with residual silanol groups give rise to tailing elution peaks and the number of parameters to take into consideration renders routine analysis problematic. The number of compounds to be separated simultaneously demands that optimal conditions are transferable from one column to another or from one laboratory to another. This aspect renders applicability of these techniques limited.

To the best of our knowledge ion-exchange HPLC has not been reported for the separation of heroin samples, despite the fact that alkaloids are easily ionized. This may be attributed to the low selectivity when pure aqueous solvents are used.

We have shown previously that the use of mixed aqueous–organic mobile phases significantly enhances the selectivity of ion-exchange chromatography on alumina[13]. The use of an inorganic exchanger means that swelling effects can be neglected so that the specific action of the solvent on the solute can be exploited. Aluminium oxide is amphoteric and can be used as anion exchanger in an acidic solution or as

cation exchanger in a basic solution[14]. The transition between these two mechanisms takes place at a pH value where the net charge of the surface is zero (zero point of charge, ZPC). We have shown[15] that this ZPC moves along the pH scale depending on the nature of the buffer used. With a citrate buffer the ZPC is as low as pH 3.5. When the pH of the mobile phase incorporating a citrate buffer exceeds 3.5, alumina acts as a cation exchanger. On the other hand, basic compounds such as alkaloids will be positively charged, when the pH is below their pK_a values. Consequently, the pH chosen will be between ZPC = 3.5 and the pK_a of the solutes.

The separations presented in this paper illustrate the possibilities of ion-exchange chromatography on alumina for routine analysis of heroin samples.

EXPERIMENTAL

The chromatograph includes an M 6000 pump, a UV detector Model 440 (both from Waters Assoc.) and a Rheodyne 7125 injector with a 20-μl loop. The columns were enclosed by a water bath and the temperature was controlled by a Tamson (Holland) thermostat. Columns of standard dimensions (25 cm × 4.6 mm I.D.) were filled with Spherisorb A5Y from Chrompack. Columns were packed in our laboratory by the balanced density technique using a slurry in 15% glycerol in water and pure methanol as packing solvents; they yielded plate numbers of *ca.* 4000 at 20°C. At the same temperature commercial columns supplied by Chrompack yielded a plate number of 8000. To prevent damage to the column, we used a precolumn (7.5 cm × 2.1 mm I.D.) filled with pellicular alumina, also from Chrompack.

Commercial columns have been tested by the supplier with 2.5% isopropanol in isooctane. Remaining traces of these solvents were washed from the column with 100 ml of pure methanol. After changeover to pure aqueous solvent, the columns were washed with 1 *M* sodium chloride solution to displace chemisorbed compounds. The column is equilibrated to a certain pH by eluting 100 ml of concentrated (greater than 0.1 *M*) aqueous buffer solution of appropriate pH and finally 20 ml of the diluted buffer solution (either water or a water–organic mixture). The same procedure is repeated when the pH is changed to another value.

The organic solvents (methanol, acetonitrile, tetrahydrofuran) were obtained from Rathburn Chemicals, U.K. Distilled water was further purified by ion-exchange and carbon filters. The buffers used were of the highest quality commercially available. The buffers in mixed water–organic solvent were prepared by adding to a concentrated aqueous buffer an appropriate amount of water and organic modifier.

All drugs and illicit samples were obtained from Dr. Huizer of the Forensic Science Laboratory, Rijswijk (The Netherlands). Except for natural opium, which requires vigorous ultrasonic stirring, all samples dissolve readily in water or mixed water–organic solvents. After filtration to remove particulate material as a precaution, samples were injected with a 50-μl syringe into a 20-μl sample loop. Quantitative analysis was based on peak areas determined with a C-E1B integrator from Shimadzu.

RESULTS AND DISCUSSION

Except for caffeine, all the substances included in Table I can be ionized. The

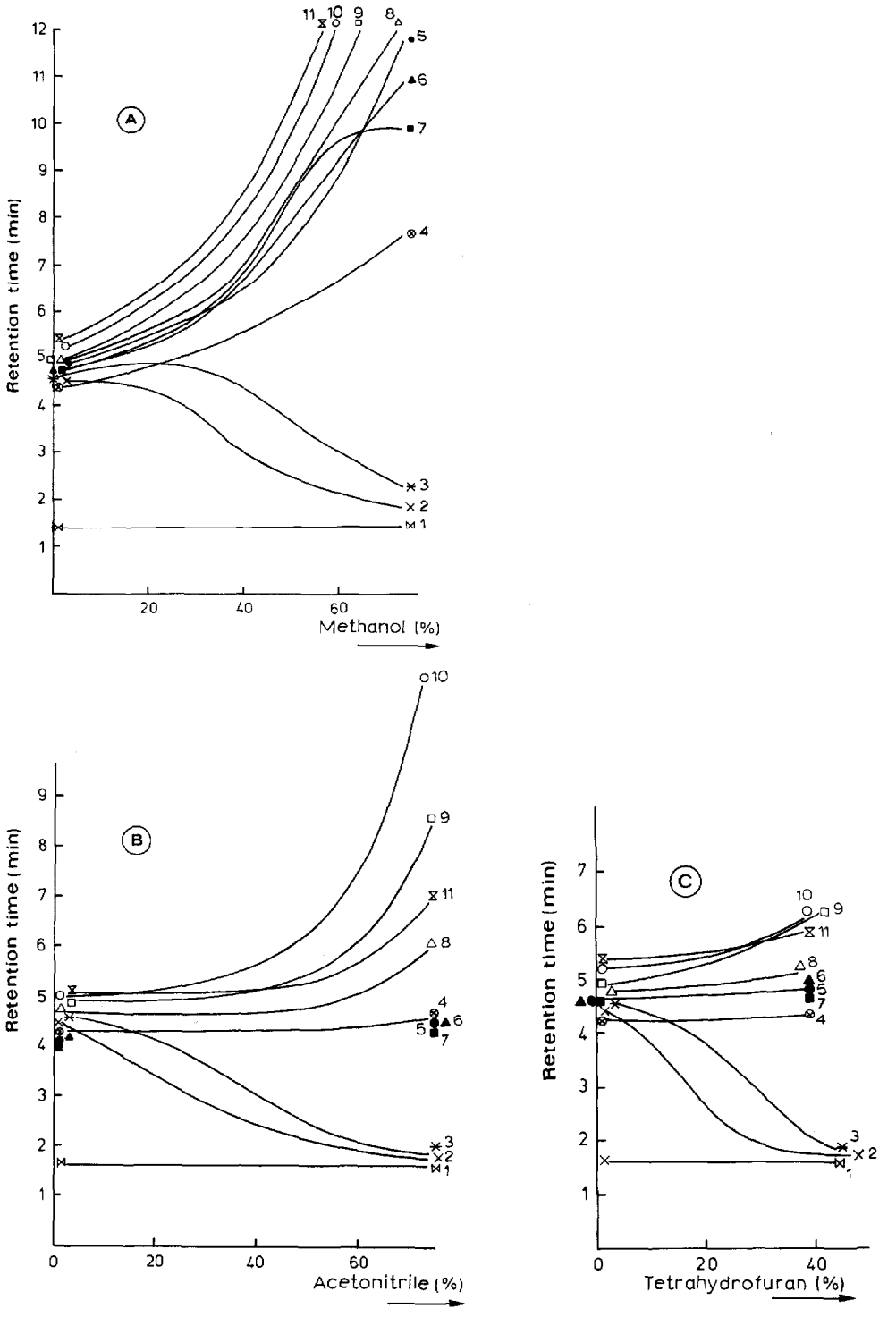

Fig. 1. Retention behaviour of heroin and opium compounds with increasing concentration of (A) methanol, (B) acetonitrile and (C) tetrahydrofuran. Conditions: citric acid and tetramethylammonium hydroxide (0.01 *M*), pH 5. Solutes: caffeine (1), papaverine (2), narcotine (3), procaine (4), thebaine (5), acetylcodeine (6), heroin (7), 6-monoacetylmorphine (8), codeine (9), morphine (10) and strychnine (11).

pK_a values vary between 6 and 9. In a citrate buffer of pH 5 they are positively charged and retained on alumina, which under these conditions acts as a cation exchanger[12]. For the basic component of the buffer we chose tetramethylammonium hydroxide (TMA), which is well solvated in organic solvents. Since the solutes compete with the TMA cations for active sites on the alumina surface, their retention can be controlled by the concentration of TMA.

Fig. 1 shows that a solution of 0.01 *M* TMA yields acceptable retention for all solutes, except, of course, for caffeine which is not retained. In pure aqueous solvents the cations elute too close together for adequate separation, but the addition of organic modifier significantly changes their retention as a result of two opposing mechanisms.

TMA is readily soluble in acetonitrile and particularly methanol, and the enhanced solvation in these solvents reduces its competition for active sites on alumina. Indeed, for the majority of compounds in Fig. 1, the retention increases steadily with increasing percentage of methanol or, to a lesser extent, acetonitrile. Although the variation is largely similar for all these solutes, some specific effects can be observed, *e.g.* the crossings of the retention curves of heroin and acetylcodeine in Fig. 1A and of strychine and codeine in Fig. 1B.

Although the increase in retention is accompanied by an increase in relative retention, this increase in selectivity is generally insufficient to separate all components. This is particularly true for structurally very similar compounds, such as thebaine, acetylcodeine and heroin. However, it is also clear from Fig. 1 that the addition of organic solvents dramatically increases the selectivity between the cations considered so far on the one hand and papaverine and narcotine on the other hand. Indeed, the latter two solutes are seen to decrease rather than increase their retention as the percentage of organic solvent increases.

The reason is that the pK_a values of narcotine and papaverine (5.5–6.5) are close to the pK of 5 chosen for the aqueous buffer in Fig. 1. As discussed in a previous publication[10], the addition of organic solvents simultaneously effects an increase of the apparent pH and a decrease of the effective pK_a of the solute. The two effects co-operate to reduce the solute ionization and, hence, their cation-exchange retention. The sigmoidal shape of the retention curves observed for papaverine and narcotine resembles the effect of a pH variation in pure aqueous solvent. Indeed, the addition of up to 75% methanol, 60% acetonitrile or 40% tetrahydrofuran is similar to a variation by two pH units. Obviously, however, the addition of organic solvent allows a much finer control. For components other than narcotine or papaverine with pK_a values further away from the starting pH value of 5, the apparent pH variation is insufficient to influence their retention. It is only for heroin (pK_a = 7.6) that the effect is just noticeable for methanol contents exceeding 60% (Fig. 1A). Clearly, the selection of a higher starting pH would produce similar effects for these solutes. Simultaneously, however, solutes with low pK_a values, such as papaverine, would then be non-ionised and, hence, non-retained. These predictions will be confirmed below.

It is clear that the choice of the buffer pH and the concentration and nature of the organic solvent provides us with great flexibility to adapt the separation conditions to the composition of a particular sample. Although it would be difficult to formulate conditions for a complete separation of all components presented in Table

I and Fig. 1, the following two examples demonstrate that it is possible to perform less demanding separations.

The first aim is the separation of the major alkaloids in opium (Table I) and the quantitative determination of the most important one, morphine. Fig. 1 shows that separation should be possible over a wide range of methanol (20–70%) or acetonitrile (30–60%) contents. Indeed, Fig. 2a shows that good selectivity between all solutes is obtained in acetonitrile–water (40:60) at pH 5. In comparison with Fig. 1 the overall retention is somewhat larger, because a lower concentration (0.003 *M*) is selected for TMA.

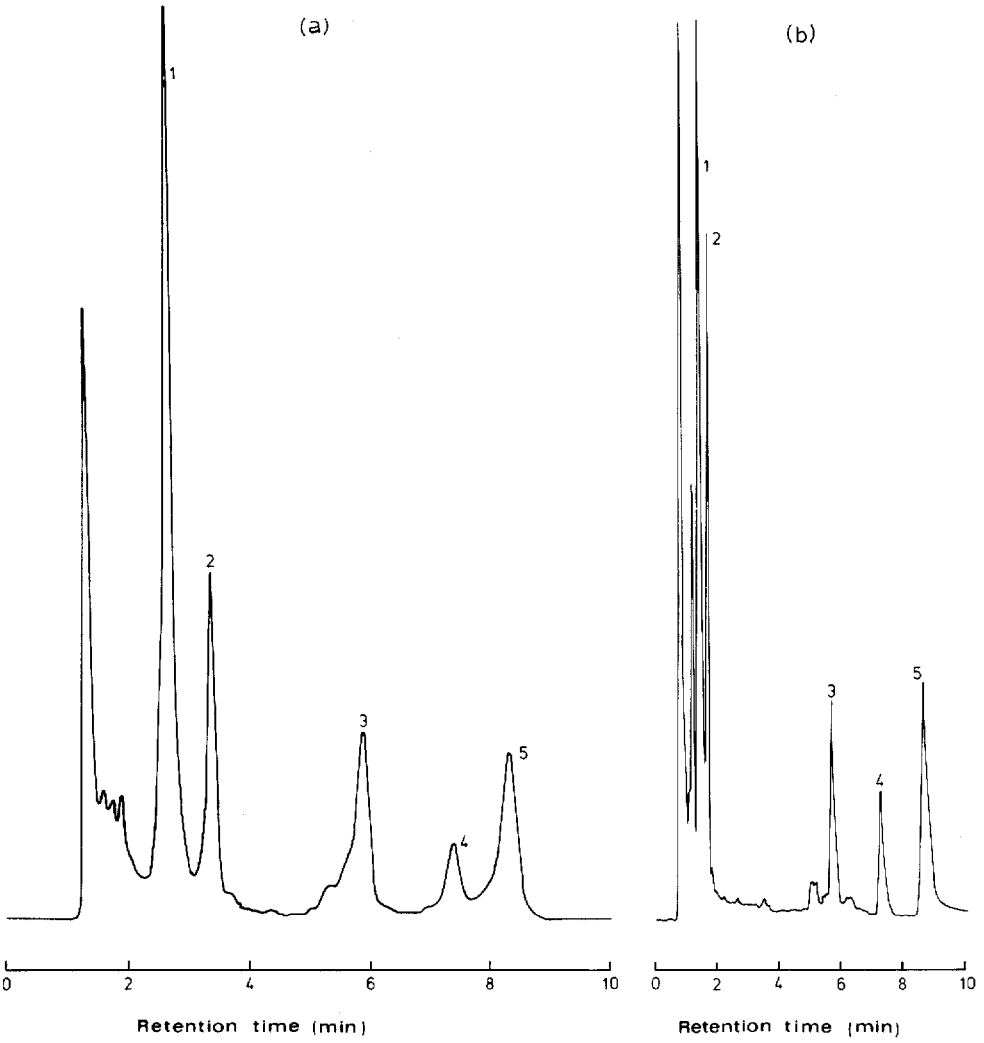

Fig. 2. Chromatograms of an opium sample. Peaks: 1 = papaverine; 2 = narcotine; 3 = thebaine; 4 = codeine; 5 = morphine. Conditions: citric acid and tetramethylammonium hydroxide, pH 5, in 40% acetonitrile. (a) TMA = 0.003 *M*. Column, home packed with Spherisorb A5Y. temperature, 20°C. (b) TMA = 0.01 *M*. Commercial column filled with Spherisorb A5Y. temperature, 60°C.

If the main interest is a precise quantitative determination of morphine, a larger plate number may be desirable than the 4000 plates realized at 20°C with our own column (Fig. 2a). One possibility is an increase of the column temperature, which accelerates the ion-exchange kinetics. We have observed a 30% increase in plate number when the column temperature is raised from 20°C to 60°C. The temperature increase does not influence the retention of completely ionized solutes, such as morphine, but it decreases the retention times of partially ionized solutes, such as narcotine and papaverine.

Fig. 2b shows that the combined effects of increased temperature and a change-over to a more efficient commercial column lead to a plate number of *ca.* 10,000. Whereas narcotine and papaverine are now less well separated, the sharp and completely isolated peak obtained for morphine allows an easy quantification. From the linear calibration curve in Fig. 3 (correlation coefficient of 0.997 without internal standard) the morphine content of the sample in Fig. 2b is derived as 10.0 ± 0.4%.

As a second example we consider the separation of all components in illicit heroin samples (Table I). Their pK_a values are all *ca.* 8. To increase the selectivity it is, therefore, necessary to increase the pH from 5.0 to 6.5. Fig. 4 demonstrates that their retention behaviour with increasing amounts of organic solvent conforms to our expectations outlined above. Papaverine and narcotine, which are not present in heroin samples, would now be unretained, but the components of interest reflect the opposing influences of the decreasing degree of ionization and the increasing solvation of TMA.

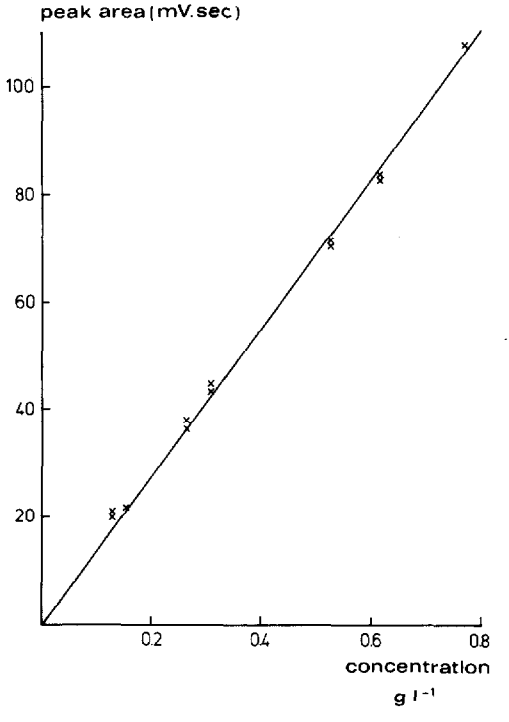

Fig. 3. Calibration curve for the quantitation of morphine.

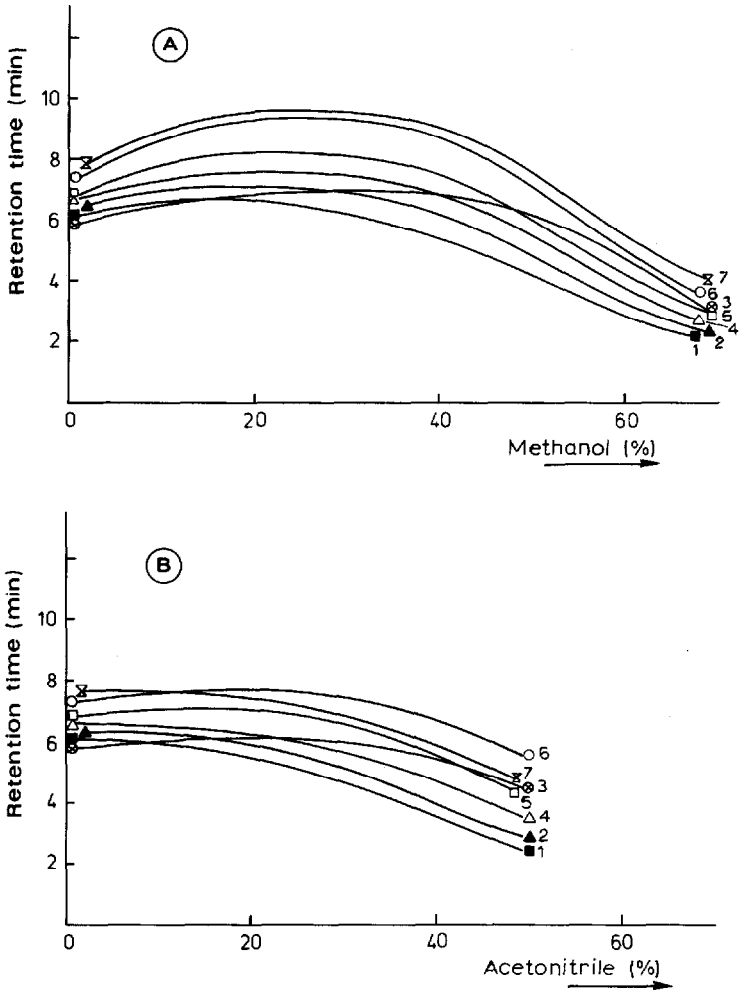

Fig. 4. Retention behaviour of heroin compounds with increasing concentration of (A) methanol, (B) acetonitrile. Conditions: citric acid and tetramethylammonium hydroxide (0.01 *M*), pH 6.5. Solutes: heroin (1), acetylcodeine (2), procaine (3), 6-monoacetylmorphine (4), codeine (5), morphine (6) and strychnine (7).

In Fig. 5 the horizontal displacement between the retention curves arises from small differences between the pK_a values of the solutes. Variations in the vertical direction are attributed to specific solvation effects, which depend on the nature of the solute and of the organic solvent. Nevertheless, there is no binary composition of either methanol–water or acetonitrile–water that permits a separation of all components with resolution of at least one. However, the shifts observed for strychnine and procaine between 25% methanol and 25% acetonitrile clearly point to a ternary mobile phase composition (Fig. 5). Using the optimization procedure described by Drouen *et al.*[16] we arrived at an optimum ternary composition of methanol–acetonitrile–water (12.5:12.5:75).

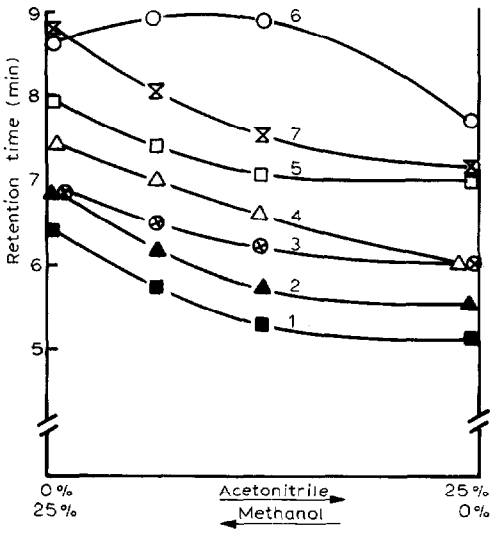

Fig. 5. Retention behaviour of the heroin compounds listed in Fig. 4 in acetonitrile–methanol–water. For other conditions see Fig. 4.

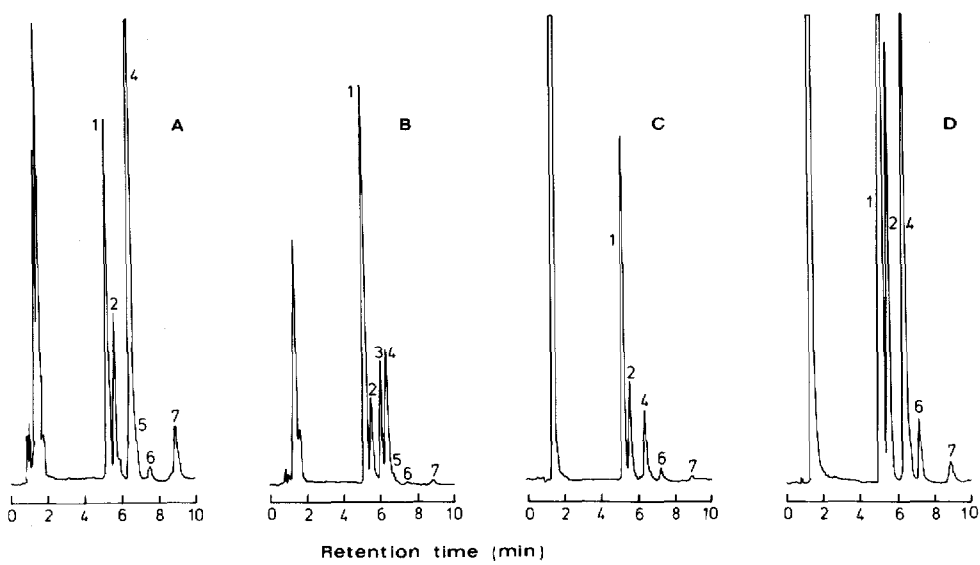

Fig. 6. Chromatograms of illicit heroin samples originating from the Middle East (A, B) and the Far East (C, D). Conditions: citric acid and tetramethylammonium hydroxide (0.01 *M*) pH 6.5, in methanol–acetonitrile–water (12.5:12.5:75). Peaks: 1 = heroin; 2 = acetylcodeine; 3 = procaine; 4 = 6-monoacetylmorphine; 5 = codeine; 6 = strychnine; 7 = morphine. The identity of 6 in A and B is uncertain.

The chromatograms presented show excellent separations of samples originating from the Middle East (Fig. 6A and B) and the Far East (Fig. 6C and D).

CONCLUSION

The present study provides a second example of the significant increase in selectivity that can be realized in ion-exchange chromatography by the use of mixed aqueous–organic mobile phases[13]. Very similar cationic species, such as morphians, can be separated on the basis of minor differences in structure and pK_a value. These compounds, which are difficult to separate on bonded-silica stationary phases, are much more amenable to the simple cation-exchange system developed in this study. Indeed, the availability of efficient alumina columns of good stability provides the means for a straightforward and precise quantitative determination of basic drugs. Sample preparation is extremely simple, and intercolumn reproducibility of separation conditions is excellent (*cf.* Fig. 2). This is in contrast to the ion-pairing techniques required for reserved-phase separations on bonded silica.

On the other hand, the compatibility of the mobile phases used in reversed-phase liquid chromatography and in the proposed ion-exchange system could be exploited in coupled column techniques for those samples where one retention mechanism provides insufficient separation.

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