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EFFECTS OF SYSTEM PEAKS IN ION-PAIR REVERSED-PHASE LIQUID CHROMATOGRAPHY FOR NOSCAPINE AND METABOLITES

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SUMMARY

System peaks were generated in an ion-pair reversed-phase system by co-injection of an alkylsulphate with the analytes. The acidic mobile phase contained acetonitrile and an aliphatic tertiary amine as a co-ion. The retention time of the system peak was regulated by the concentration and hydrophobicity of the co-ion and the alkylsulphate. The peak performance of the analytes was affected by co-elution with a system peak. Both peak distortions and improvements appeared, and the principles for the latter could be applied in a dual-column system involving trace enrichment and column switching.

INTRODUCTION

Ion-pair-reversed phase liquid chromatography is widely used for the separation of ionizable compounds. The ionic reagent is normally a component of the mobile phase. In order to achieve special effects with respect to retention and/or efficiency, an ionic compound may be injected before[1] or at the same time as the analytes[2,3] or at pulsed intervals during the separation[4,5]. Injection of a solution with a composition deviating from the mobile phase gives rise to disturbances in the established equilibria in the column. These disturbances are registered as peaks or zones with a refractive index (RI) detector using a common UV-transparent mobile phase, or they can be monitored with a UV detector, if the mobile phase contains at least one UV-absorbing compound that participates in the disturbed equilibria. Each component in the injected solution, which deviates from the mobile phase, gives rise to two kinds of peaks. One is the ordinary peak corresponding to the injected compound and the other originates from the disturbances of the equilibria described above, and is due to changes in the concentrations of mobile phase components. The retention time of the latter kind of peaks is characteristic of the mobile phase component from which the peak or zone originates[6–10]. It is called the system peak (or system zone), as it is characteristic of the actual chromatographic system.

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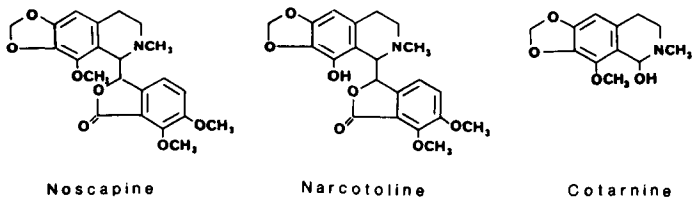

Fig. 1. Structures of noscapine, narcotoline and cotarnine.

Co-elution of an analyte with the zone may affect the peak shape in different ways, giving either distorted or improved peak performance. In a system with an acidic mobile phase containing a tertiary aliphatic amine it was possible to achieve an extremely compressed analyte peak for a cationic benzamide which co-eluted with the back of a system peak, created by the co-injection of an organic alkylsulphonate[2].

The aim of this work was to study the conditions for the peak compression of noscapine, narcotoline and cotarnine (Fig. 1). The analytes were injected on to the analytical column in a solution containing octyl- or decylsulphate in high concentrations. Correlations between the peak performance of the analytes and the retention of the system peak were studied using an RI and a UV detector coupled in series.

The conditions established for peak compression of noscapine were applied in a dual-column system. The analytes were enriched as ion pairs on a small polar precolumn, with a mobile phase containing alkylsulphate and a low content of acetonitrile, followed by back-flushing to the analytical column with a mobile phase including a tertiary aliphatic amine and a higher content of acetonitrile.

EXPERIMENTAL

Chemicals

The sodium salts of octyl- and decylsulphate were obtained from Eastman Kodak (Rochester, NY, U.S.A.). N,N-Dimethyl-N-octylamine (DMOA), N,N-dimethyl-N-hexylamine (DMHA) and N,N-dimethyl-N-dodecylamine (DMDDA) were obtained from ICN Pharmaceuticals (Plainview, NY, U.S.A.). Noscapine was obtained from Macfarlan Smith (Edinburg, U.K.), cotarnine chloride dihydrate from EGA-Chemie (Albuch, F.R.G.) and narcotoline from Diosynth-Apeldoorn (Oss, The Netherlands). All other chemicals were of analytical-reagent or HPLC grade.

LC apparatus

The chromatographic system consisted of an LKB 2150 pump (LKB, Bromma, Sweden), a Waters M 45 pump (Waters Assoc., Milford, MA, U.S.A.), a Kontron Tracer MCS 670 valve-switching unit with a Tracer timer 210 (Kontron, Zürich, Switzerland) and a Waters WISP 710 B automatic injector. The UV absorbance was monitored at 310 or 210 nm with a Spectroflow 783 (Kratos Analytical, Ramsey, NJ, U.S.A.) and the refractive index was monitored with a Waters Model R 401 refractometer. The recorder was a dual-channel Servogor 220 (Gortz, Vienna, Australia) and the integrator was a Shimadzu C-R3A (Shimadzu, Kyoto, Japan).

Chromatographic system

The analytical column (100 × 4.6 mm or 100 × 4.0 mm I.D.) was packed with Nucleosil C_{18} (3 μm) from Machery, Nagel & Co. (Düren, F.R.G.). In the dual-column system the precolumn (20 × 3.8 mm I.D.) was packed with Nucleosil C_4 (5 μm). The columns were packed with dichloromethane as the slurry medium and methanol as the eluent. The mobile phase consisted of various concentrations of an aliphatic tertiary amine in different percentages of acetonitrile in phosphate buffer (pH 2) (μ = 0.05).

The analytes were dissolved in phosphate buffer (pH 2) (μ = 0.05) containing octyl- or decylsulphate (0, 0.01–0.10 *M*) and 200 μl were injected on to the analytical column. In the dual-column system the analytes were dissolved in phosphate buffer (pH 2) (μ = 0.05) and 500 μl were injected on to the precolumn.

Calculation of plate height

The plate number was calculated from the retention time and the peak width at half-height. This is not correct when the analyte elutes in a gradient; however, in this study such calculations were made only for comparisons of the peak performances.

Mass spectrometry

The mass spectrometer was a Shimadzu QP 1000 equipped with a direct inlet probe. The probe temperature was programmed from room temperature to 250°C at 80°C/min. Electron-impact ionization was performed at 70 eV. The mass was scanned from *m/z* 38 to 400 with a cycle time of 4 s. The presence of sulphate ions was monitored using the reconstructed ion chromatogram (RIC) of the ions of *m/z* 48 and 64.

Fractions of mobile phase from the LC system collected between 2–12 min (2-ml portions), 22–24 min, 2.5–3 h (5-ml portions) and 4–4.08 h (5-ml portions). The time for fraction collection was decided by monitoring the RI detector (2–12 min) and the UV detector at 210 nm (12 min–4.5 h).

An aliquot of the fractions (1 or 2 ml) was mixed with 0.5 ml of phosphate buffer (pH 2, μ = 0.05), containing 2.5 m*M* DMOA, before extraction with dichloromethane. After centrifugation, 2 μl of the organic phase were evaporated at the direct probe.

THEORY

It has been found in ion-pair chromatography that solid phases of the hydrophobized silica gel type seem in most instances to contain at least two different adsorption sites with different total adsorption capacities and affinities[11,12]. The retentions of both analytes and mobile phase components (system peak) are determined by the partial derivative of the adsorption isotherm at the actual mobile phase composition. However, as the analyte concentration from the start of elution is zero, whereas those of the mobile phase components are non-zero, different retention equations will result[9,13]. When the analyte concentrations are high and influence the retention and peak shape, no exact theory is yet available. Empirically it has been found that when high analyte concentrations are injected the retention volume will be influenced as if the denominator contained an additional term including the analyte concentration,

according to eqns. 1 and 2. These equations are derived from simplified models relating the capacity ratios directly to distribution. They can be useful for qualitative discussions about retention.

Cationic analyte, HA^+:

$$V_{N,HA} = \frac{W_s K_0 K_{HAX}[X^-]_m}{1 + K_{QX}[Q^+]_m[X^-]_m + K_{HAX}[HA^+]_m[X^-]_m} + \\ + \frac{W_s K_0^* K_{HAX}^*[X^-]_m}{1 + K_{QX}^*[Q^+]_m[X^-]_m + K_{HAX}^*[HA^+]_m[X^-]_m} \quad (1)$$

Anionic analyte, Z^-:

$$V_{N,Z} = \frac{W_s K_0 K_{QZ}[Q^+]_m}{1 + K_{QX}[Q^+]_m[X^-]_m + K_{QZ}[Q^+]_m[Z^-]_m} + \\ + \frac{W_s K_0^* K_{QZ}^*[Q^+]_m}{1 + K_{QX}^*[Q^+]_m[X^-]_m + K_{QZ}^*[Q^+]_m[Z^-]_m} \quad (2)$$

System peak:

$$V_{N,Q} = W_s \cdot \frac{\partial C_{Q,s}}{\partial C_{Q,m}} = \frac{W_s K_0 K_{QX}[X^-]_m}{(1 + K_{QX}[Q^+]_m[X^-]_m)^2} + \frac{W_s K_0^* K_{QX}^*[X^-]_m}{(1 + K_{QX}^*[Q^+]_m[X^-]_m)^2} \quad (3)$$

where V_N = net retention volume, W_s = weight of solid phase, K_0, K_0^* = total adsorption capacity of the two sites, K, K^* = equilibrium constants, Q^+ = mobile phase component (tertiary aliphatic amine) and X^- = buffer component (dihydrogenphosphate); the subscript m denotes mobile phase.

The system peaks will be positive or negative depending on the disturbance of the adsorption equilibria[6–9].

RESULTS AND DISCUSSION

Origin of system peaks

The injection of a solution deviating in composition from the mobile phase may give rise to system peaks. One such peak is obtained for each component in the mobile phase, which participates in an interaction common with a compound in the injected solution. The retention times of the system peaks are characteristic of the mobile phase component from which respective peak originates. System peaks are only detected when retarded from the front disturbances and when at least one of the mobile phase components involved in the disturbed equilibria gives a detector response. As RI detection provides an almost general response, most system peaks present will be registered, whereas an UV-absorbing mobile phase component is necessary for UV

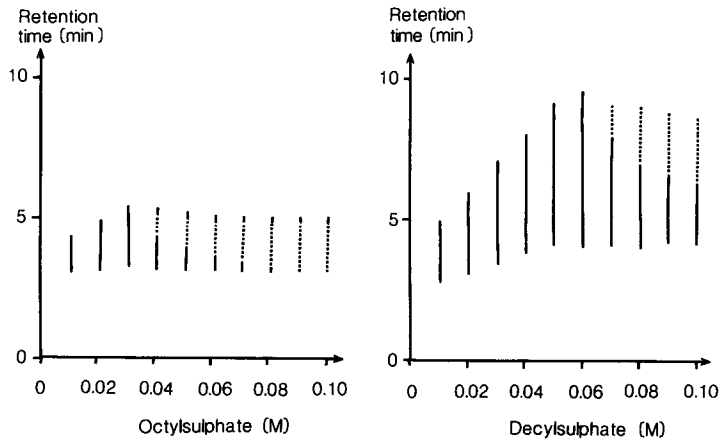

Fig. 2. Width and retention times of the system peak (DMOA) (solid lines) and octyl- or decylsulphate peaks (broken lines). Mobile phase, 2.5 m*M* DMOA in 25% (v/v) of acetonitrile in phosphate buffer (pH 2).

detection. The direction of the system peak, *i.e.*, positive or negative in relation to the baseline, is determined by the charge and relative retention of the solutes relative to the component from which the system peak originates[6–9].

In this study, system peaks were generated by the injection of octyl- or decylsulphate (0.01–0.1 *M*) into an acidic mobile phase with acetonitrile (25%, v/v) and containing an aliphatic tertiary amine, *i.e.*, DMHA, DMOA or DMDDA. The widths and retention times of the system peaks (DMOA) monitored by a RI detector after the injection of various concentrations of octyl- or decylsulphate are shown in Fig. 2. Injection of a low concentration of alkylsulphate gave only one peak (peak 1). When the retention time of this peak reached a nearly constant value a second peak (peak 2) appeared with the opposite response direction. The retention time of the second peak decreased with increasing injected concentrations of alkylsulphate, whereas the area of peak 1 increased until the retention had attained a constant level with an increasing injected alkylsulphate concentration. At the level of constant retention time the peak area was difficult to evaluate owing to a disturbance from peak 2. The area of peak 2 increased continuously with increasing concentration of alkylsulphate.

The equations given under Theoretical were used for qualitative interpretations in this paper. An alkylsulphate peak with a longer retention time than that of the induced system peak will contain an excess of tertiary amine in the alkylsulphate peak, leaving a deficiency of this mobile phase component to elute in the system peak. Injection of increasing amounts of alkylsulphate will decrease the amount of tertiary amine in the system peak. The retention of the system peak will increase with a decreasing concentration of the tertiary amine in the peak (see eqn. 3) and the area will also increase. A constant retention time and a constant area may be obtained when the system peak contains a very low concentration of cation (Q^+). This retention behaviour is in accordance with peak 1 on the RI trace, which means that this system peak contains a deficiency of DMOA. The large amount of alkylsulphate

TABLE I

RETENTION TIMES OF SYSTEM PEAKS AND ANALYTES WITH DIFFERENT TERTIARY ALIPHATIC AMINES IN THE MOBILE PHASE

The analytes were injected with 0.1 *M* of the respective counter ion.

Amine	*Concentration (mM)*	*Counter ion*	*Retention time (min)*			
			System-peak	*Cotarnine*	*Narcotoline*	*Noscapine*
DMHA	2.5	OS	4	4.2	4.5	13
DMOA	2.5	OS	5	4.5	3.7	5
DMOA	2.5	DS	8	8.6	8.4	9.3
DMOA	0.5	OS	9	5.5	6.8	9
DMDDA	0.5	OS	23	2.5	2.7	6.5

injected may also lead to this compound having a more direct influence on the retention time of the mobile phase component (Q^+) by retaining it as an ion pair during the period of co-elution of the two zones, that is, at the start of elution and during elution for different times depending on the alkylsulphate concentration in each injection.

The retention behaviour of the second peak (alkylsulphate) can be understood qualitatively in light of eqn. 2, which shows that increasing analyte concentrations will decrease the retention. This peak appears first when the system peak has reached a constant retention. It was further observed that very broad peaks with long retention times of about 1.5 and 2.3 h for octylsulphate and decylsulphate, respectively, appeared. The presence of two or three peaks from a homogeneous sample has been reported previously[14–16]. One interpretation of this phenomenon involved slow interconversion between two forms of a pure compound[14] and another was based on a dual retention mechanism, *i.e.*, both ion-pair and dynamic ion exchange[16].

In this work the two peaks appeared only when a high concentration of alkylsulphate was injected. It is assuming that at a certain critical concentration of each anion the adsorption capacity of the solid phase is exceeded and only a fraction of this compound is retained with the hydrophobic cation in the injection zone. Then the remaining fraction of the molecules will elute faster, initially only being retained by buffer cations (Q^+ in eqn. 2 then corresponds to Na^+), and in fact being identical with the peak which is detected according to Fig. 2. For the less hydrophobic anion OS the visible critical concentration was about 0.04 *M* with 2.5 m*M* DMOA in the mobile phase (*cf.*, Fig. 2) and <0.01 *M* with 0.5 m*M* DMOA. For the stronger adsorbed DS the critical concentration was 0.07 *M* with 2.5 m*M* DMOA in the mobile phase (*cf.*, Fig. 2).

The hypothesis was supported by mass spectrometric identification of DS both in the early (2–10 min) and the late (2.5–3.0 h) fractions. This is discussed further below.

The stronger adsorption of decylsulphate compared with octylsulphate will deplete the DMOA zone more efficiently. Accordingly, the concentration of DMOA in the deficiency zone will be lower after decylsulphate resulting in a longer retention time (eqn. 3). However, ion-pairing effects during co-elution between the two kinds of

peaks will also be stronger with decylsulphate. The peak width of the system peak was broader with decylsulphate than octylsulphate, although the peak height was about the same. However, the peak height decreased with decreasing concentration of amine in the mobile phase.

The retention of the system peak increases with increasing lipophilicity of the tertiary aliphatic amine (Table I). Further, the retention of the system peaks increases with decreasing concentration of the amine in the mobile phase, qualitatively in accordance with eqn. 3.

Mass spectral identification of alkylsulphate in the LC peaks

The mass spectral (MS) properties of *n*-alkylsulphates with sodium and amines as counter ions have been studied previously[17]. In both instances the characteristic fragments from the sulphate were SO^+, SO_2^+ and SO_3^+ (m/z 48, 64 and 80).

Batch extractions of DS with dichloromethane gave considerably higher recoveries as measured from MS signals from the evaporated dichloromethane when the extraction was performed with a buffer containing DMOA compared with a buffer without the amine. This was probably due to the better extraction properties of DS as an ion pair with DMOA in combination with a higher volatility of the DS–DMOA ion pair compared with the sodium salt of DS.

MS analysis of the collected fractions confirmed that DS was present both in an early peak (2–8 min) and in a very late and broad peak (2.5–3.0 h). No DS was found in a fraction collected between 20 and 22 min or in a very late fraction (4.0–4.1 h). These studies were performed on a solid phase from a different batch then that for the results shown in Figs. 2–4. The retention times for the system and alkylsulphate peaks differ slightly between the two batches, as well as the critical concentration of alkylsulphate exceeding the adsorption capacity. However, the retention behaviour was similar for the two different batches of solid phase.

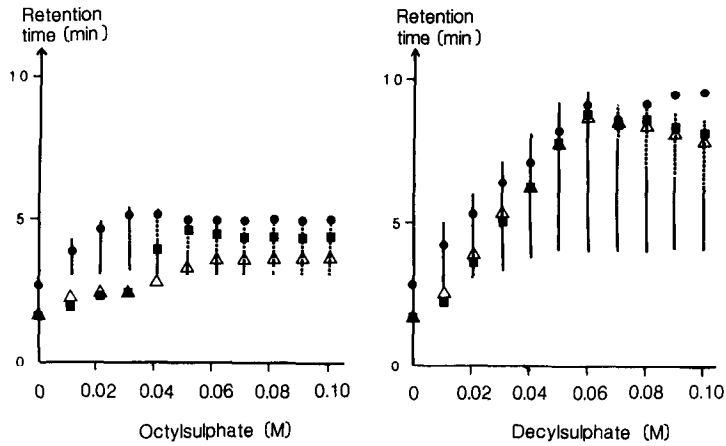

Fig. 3. Retention times of (●) noscapine, (△) narcotoline and (■) cotarnine co-injected with octyl- or decylsulphate. Chromatographic conditions as in Fig. 2.

Retention times of the analytes as influenced by the co-injected organic anion

Noscapine, narcotoline and cotarnine were injected together with octyl- or decylsulphate in phosphate buffer. With increasing concentration of the organic anion the retention times of the analytes increased until an upper level was reached (octylsulphate); a more complex retention behaviour was obtained with decylsulphate (Fig. 3). The retention times were measured from the middle of the peak when there was no sharp top to the peak. In most instances the analytes elute within the system peak and the special conditions prevailing there with different gradients of the mobile phase components will influence the retention times. A detailed discussion of the retention behaviour of the analytes is therefore difficult. At octylsulphate levels >0.06 *M* the retention does not change with increasing anion concentrations. This may indicate that the relative influences of DMOA and OS compensate each other in the combined system and octylsulphate peaks at these high levels. However, with decylsulphate the retention of noscapine, the most retarded analyte, increases with increasing decylsulphate concentration (an ion-pairing effect), whereas the opposite behaviour was observed for the polar metabolites. The noscapine peak elutes under these circumstances outside the combined system and DS peaks and was affected by strong ion pairing in the injection zone. The selectivities between the analytes were generally lower with co-injection of decylsulphate than with octylsulphate.

Retention times of the analytes as influenced by the aliphatic tertiary amine in the mobile phase

Addition of an aliphatic amine to the mobile phase has been found to improve the peak shape of cationic analytes owing to competition for adsorption sites[12]. With increasing lipophilicity of the competing amine the retention of the analytes will be reduced more effectively (Table I). Further, an increasing concentration of the amine will have a similar effect owing to increasing competition.

Influences of system and alkylsulphate peaks on the analyte peak performance

In trace analysis, involving no preconcentration step, the injection of a large volume may be necessary to achieve the required detection limits. In order to retain the chromatographic efficiency a low eluting strenght of the injected solution is essential, as the analytes will then be enriched on the top of the column before elution starts with the bulk mobile phase. Combining this principle with co-injection of an ion-pairing agent a highly compressed analyte peak has been obtained in favourable instances, when the analyte co-elutes with a system zone of suitable composition[2].

As discussed earlier, both alkylsulphate peaks contain an excess of the tertiary amine, leaving a deficiency of this mobile phase component to elute in the system peak. However, when the adsorption capacity of the solid phase is exceeded the excess of the alkylsulphate will elute in an additional peak that in some instances also has an influence on the analyte peaks.

Decylsulphate

Some typical chromatograms with different concentrations of decylsulphate as the anionic system peak inducer are illustrated in Fig. 4. The RI trace shows the system peak (DMOA-deficient zone) and with increasing anion concentration the area of the peak with the excess of decylsulphate also increases. It is first visible as a

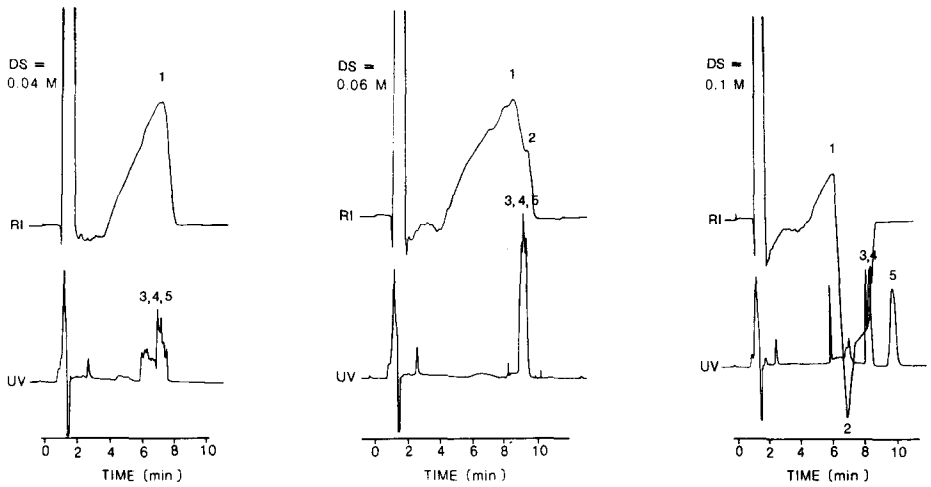

Fig. 4. Simultaneous recording of UV (310 nm) and RI traces. The analytes were dissolved in phosphate buffer (pH 2) containing decylsulphate at the different concentrations indicated. 1 = DMOA deficiency; 2 = decylsulphate; 3 = narcotoline; 4 = cotarnine; 5 = noscapine. Chromatographic conditions as in Fig. 2.

disturbance of the system peak at a concentration of the anion of 0.06 *M*. One fraction of the decylsulphate elutes, as mentioned before, very late after about 2.3 h in a very broad peak outside this part of the chromatogram.

Analyte peaks are seriously disturbed at the lowest concentration of the anion (0.04 *M*) in this example. The compounds elute in the middle of the amine-deficient zone and each analyte seems to give a split peak. At the next higher concentration (0.06 *M*) the three analytes are clearly visible, and all three peaks seem to have a very high performance with a high degree of peak compression. However, the selectivity is low and the peaks are not separated. Here all three components elute at the back part of the system zone, which further is disturbed by a small decylsulphate peak.

The last example (0.10 *M*) shows a complete separation of noscapine from the two metabolites. Noscapine elutes here after both the system zone and the excess alkylsulphate peak, and its chromatographic performance seems to be unaffected by the presence of these zones, its efficiency corresponding to values obtained in a conventional system (reduced plate height, $h = 10$). The metabolites are not baseline separated and elute at the back of the system and anion peaks. They are both still compressed, especially narcotoline, which elutes first and gives an efficiency corresponding to "h" < 1. Obviously, as remarked under Experimental as the peaks in systems of this kind are affected by different special effects, such as gradients of mobile phase components, it is not possible to compare the apparent "efficiency" data obtained in this study with normal isocratic systems. The peak compression effects are, however, difficult to illustrate by other means and have therefore been expressed by this parameter in this paper.

At a concentration of the anion of 0.06 *M* the analytes largely elute under the conditions described in ref. 2, *i.e.*, with a decreasing DMOA deficiency zone. Although the alkylsulphate peak is also present, with a slight excess of DMOA, causing

a disturbance of the DMOA gradient, in this instance it probably has only a marginal effect on the analyte peak shapes. At a high concentration of the anion (0.10 *M*), however, the conditions are different. The most hydrophobic analyte, noscapine, is so strongly retained by ion pairing with the decylsulphate on injection that it elutes later than the system and alkylsulphate zones. The two metabolites elute here at the back of the decylsulphate excess peak.

Two effects may operate in this position. First, alkylsulphate, which is absent from the mobile phase, is available here as a decreasing gradient, offering possibilities for ion pairing with the cationic analytes. This effect is then strongest at the earliest eluting parts of the analyte peaks, giving a peak compression effect, which is evident from the peak shape in Fig. 4c. Second, as the conditions for the DMOA concentrations in this area of the chromatogram are complex it is not possible to give a detailed interpretation of the results. Obviously peak compression effects dominate, giving high apparent efficiencies for the two metabolites. Narcotoline, which elutes first, where the decylsulphate concentration is highest, gives the best apparent efficiency.

The split-peak behaviour at a low concentration of decylsulphate (0.04 *M*, Fig. 4a) may be explained as follows. The ion-pairing effect in the injection zone is lower, which means that the analytes will start the elution earlier than with larger amounts of the organic anion in the injected solution. During elution the system peak may catch up the analyte peaks, which will be exposed to both increasing and decreasing gradients of DMOA. The ultimate result will be split peaks, as different parts of a peak simultaneously experience forces of different strengths of competing properties.

Octylsulphate

The comparatively more polar anion octylsulphate is also more strongly retained than the DMOA system peak. As already mentioned, it elutes after about 1.5 h, but the excess of alkylsulphate will interfere with the system peak at a lower

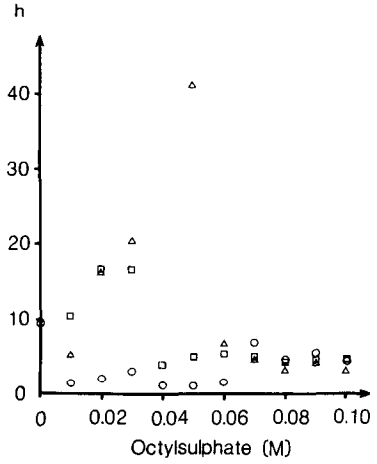

Fig. 5. Effects on the apparent peak efficiency, *h*, of the concentration of octylsulphate co-injected with the analytes: (○) noscapine; (△) narcotoline; (□) cotarnine. Chromatographic conditions as in Fig. 2.

concentration of injected anion compared with decylsulphate (Fig. 3). As indicated in Fig. 3, this peak is first visible at 0.04 *M* of injected anion, when it elutes at the back of the system peak. With increasing concentration the peak will gradually acquire decreasing retention times (*cf.*, eqn. 2) and finally, at concentrations ≥ 0.09 *M* it is the dominant part of the mixed peak and elutes first. Obviously this behaviour is more complicated than for decylsulphate and will make the interpretation of analyte peak performances more difficult, and here only the most evident effects are discussed.

Calculation of the dependence of the apparent efficiencies of the analytes on the injected octylsulphate concentration illustrates some of the effects that may be obtained in systems of this kind (Fig. 5). Noscapine elutes within the DMOA system peak in all systems used (see Fig. 3) and in many instances a peak compression effect was obtained, corresponding maximally to an apparent reduced plate height of 1. The more polar metabolites have lower retention times than the DMOA system peak with small amounts of the co-injected anion. However, with increasing anion concentration and resulting increasing retention times of the metabolites, in addition to a broader system zone of DMOA, they will elute within the zone, cotarnine at OS > 0.03 *M* and narcotoline at > 0.04 *M*. Their apparent efficiencies are initially low but with increasing amounts of injected octylsulphate attain a higher and fairly constant value, cotarnine at OS > 0.03 *M* and narcotoline at OS > 0.05 *M*. The reason for the low efficiencies at low anion concentrations may be insufficient trace enrichment effects on the top of the column. DMOA is more strongly retained than the metabolites in the system and consequently competes efficiently with the metabolites for ion pairing with the OS anion. The remaining octylsulphate will not be sufficient for an effective enrichment and the analytes are already spread out over a significant part of the column at the start; the volume injected was 200 µl. With increasing concentration of the anion an enrichment effect is gradually obtained. Further, the efficiency obtained seems to be higher even than a reference value obtained for noscapine when no organic alkylsulphate was injected. At these concentrations of the anion the metabolites also elute within the alkylsulphate peak, and will consequently experience an additional retaining factor, in addition to the deficiency of the DMOA, namely ion pairing with the octylsulphate.

Application of the peak compression principle in a dual-column system

The levels of noscapine in biological fluids after administration of the drug to humans are low, probably owing to an extensive first-pass metabolism. The compound has no useful physico-chemical properties that can be utilized for detection apart from UV absorbance in the low wavelength range. An application of the peak compression effect may be useful for bioanalytical purposes and some limited experiments in that direction were performed.

For simple sample preparation a direct injection technique is desirable. In such applications a precolumn is necessary to protect the analytical column from the biological fluid. Often the precolumn is placed in a loop of an injection valve, making it possible to inject the analytes from one direction and then to back-flush the compounds on to the analytical column. Hence the precolumn must first retain the analytes from the injected sample, and then release them in a small volume of the mobile phase for further transport to the analytical column. By a careful choice of the polarity of the precolumn and the mobile phase, *e.g.*, the ion-pairing agent and the content

TABLE II

BREAKTHROUGH VOLUMES OF COTARNINE ON ENRICHMENT COLUMNS (20 × 3.8 mm I.D.)

Column	*Counter ion*		*Content of acetonitrile (%)*	V_b *(ml)*
	Type	*Concentration (mM)*		
Nucleosil CN (5 μm)	Decyl sulphate	1	5	1.2
Nucleosil C_4 (5 μm)	Decyl sulphate	1	5	16.8
	Decyl sulphate	1	10	3.9
	Octyl sulphate	1	10	<2.1
	Octyl sulphate	10	10	9.0
Nucleosil C_8 (5 μm)	Octyl sulphate	1	10	<4.1
	Decyl sulphate	1	10	18.7
Spherisorb Ph (5 μm)	Decyl sulphate	1	5	14.6*
	Octyl sulphate	10	5	21.5**

* V_b for narcotoline was 11.7 ml.
** V_b for narcotoline was 9.2 ml.

of organic modifier, the analytes can be enriched on the top of the precolumn. After reversal of the flow the analytes can be desorbed by applying a mobile phase containing a competing compound and a larger content of organic modifier than the initial mobile phase.

The breakthrough volumes and retention volumes were measured on a number of solid phases for the least retarded compound, cotarnine (Table II). As expected, decylsulphate gave a higher breakthrough volume than octylsulphate. Further, the breakthrough volumes increased with increasing lipophilicity of the solid phase. On Spherisorb phenyl support a reversal of the retention order occurred, narcotoline eluting before cotarnine.

The necessary compromise between enrichment ability and desorption effects with the different mobile phases indicated that Nucleosil C_4 might be a suitable solid phase, with decylsulphate (1 m*M*) in phosphate buffer (pH 2) containing of 5% acetonitrile for the enrichment conditions. The precolumn was utilized in the dual-column system shown in Fig. 6, where the analytical column was Nucleosil C_{18}. The precolumn will be saturated with decylsulphate and the analytes were retained effectively by ion-pair formation. After switching the valve the analytes were desorbed by the mobile phase for the analytical column, containing 2.5 m*M* DMOA in 20% of acetonitrile in phosphate buffer (pH 2). Simultaneously with desorption of the analytes, a fraction of decylsulphate from the first mobile phase will be transferred to the analytical column. A strong equilibrium disturbance will be obtained, creating a DMOA-deficient system zone eluting on the column.

Under the applied conditions noscapine co-eluted with the back of the DMOA system peak, resulting in a peak compression effect (see Fig. 7). The metabolites elute before the system zone, cotarnine even within the front. Injection of only 0.03 nmol of noscapine gave a very high apparent efficiency, corresponding to an apparent reduced plate height, "*h*", of 1.8. However, when a ten times larger amount of the compound

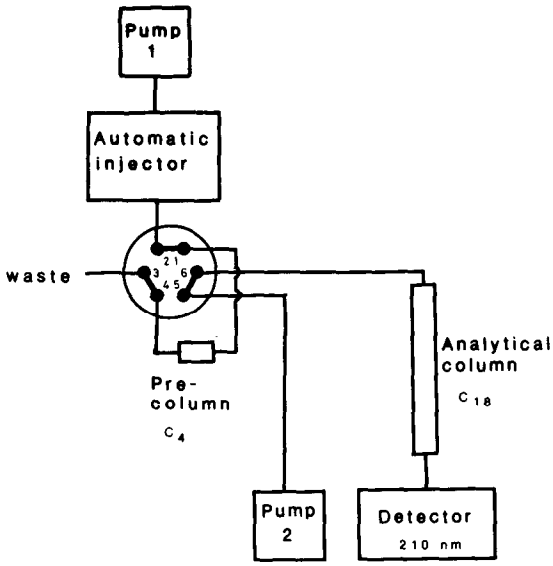

Fig. 6. Scheme of the dual-column system. Chromatographic conditions: precolumn, Nucleosil C_4, 5 μm (20 × 3.8 mm I.D.); analytical column, Nucleosil C_{18}, 3 μm (100 × 4.0 mm I.D.); precolumn mobile phase, 1 m*M* decylsulphate in 5% (v/v) of acetonitrile in phosphate buffer (pH 2); analytical column mobile phase, 2.5 m*M* DMOA in 20% (v/v) of acetonitrile in phosphate buffer (pH 2).

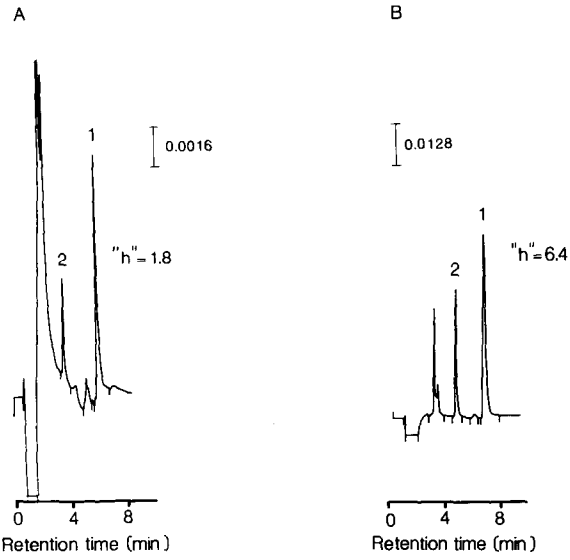

Fig. 7. Chromatogram obtained by injection of 500 μl of phosphate buffer (pH 2) containing the analytes. Enrichment on the precolumn was performed for 4 min, before back-flushing. UV detection at 210 nm. 1 = Noscapine; 2 = narcotoline. Amounts injected: (A) noscapine 31 and narcotoline 23 pmol; (B) noscapine 310 and narcotoline 230 pmol.

was injected the efficiency dropped to "h" = 6.4. One reason is probably small changes in retention time, giving co-elution with different parts of the DMOA gradient, which considerably affect the apparent efficiency. An alternative reason is that with such a large amount the concentration of DMOA in the system zone is affected, resulting in a disturbance of its gradient[18]. Another drawback with this system was a late-eluting (1 h) and broad decylsulphate peak, visible when UV monitoring at 211 nm was used.

CONCLUSIONS

The injection of a solution deviating in composition from the mobile phase creates system peaks in chromatographic systems. The presence of an organic anion in the injected sample was used to generate system peaks containing a deficiency of a tertiary aliphatic amine, a component of an acidic mobile phase with acetonitrile as the organic modifier. The peak shapes of analytes co-eluting with the system peak were affected by the special conditions prevailing there, *i.e.*, gradients of DMOA at the back and front in addition to low levels in the middle of the peak. The analyte peak shapes vary from severely distorted, such as peak splitting, to greatly improved (peak compression), depending on the conditions during elution and in the system peak. The analyte peak shapes could be regulated by the character and concentration of the co-injected organic anion. Apparent reduced plate heights < 1 were obtained in optimal instances for some of the analytes (noscapine and two of its metabolites, narcotoline and cotarnine).

The principle was applied in a dual-column system where the analytes were injected on to a pre-column (C_4) equilibrated with an acidic mobile phase including an organic anion and a low content of acetonitrile. Back-flushing with a mobile phase containing a tertiary amine and a higher concentration of acetonitrile transferred the analytes to the separation column (C_{18}), where a system peak with a deficiency of the tertiary amine was created by the simultaneously transferred fraction of the organic anion from the first mobile phase. Under these conditions noscapine eluting in this system peak experienced a peak compression effect corresponding to an apparent reduced efficiency of 1.8.

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REFERENCES

1 V. V. Berry and R. E. Shansky, *J. Chromatogr.*, 284 (1984) 303.
2 L. B. Nilsson and D. Westerlund, *Anal. Chem.*, 57 (1985) 1835.
3 M. Krejci, K. Slais and A. Kunath, *Chromatographia*, 22 (1986) 311.
4 J. J. Stranahan, S. N. Deming and B. Sachok, *J. Chromatogr.*, 202 (1980) 233.
5 V. V. Berry, *J. Chromatogr.*, 321 (1985) 33.
6 L. Hackzell and G. Schill, *Chromatographia*, 15 (1982) 437.
7 M. Denkert, L. Hackzell, G. Schill and E. Sjögren, *J. Chromatogr.*, 218 (1981) 31.
8 G. Schill and J. Crommen, *Trends Anal. Chem.*, 6 (1987) 111.

9 J. Crommen, L. Hackzell, G. Schill and D. Westerlund, *Chromatographia*, 24 (1987) 252.
10 S. Levin and E. Grushka, *Anal. Chem.*, 59 (1987) 1157.
11 A. Tilly-Melin, Y. Askemark, K.-G. Wahlund and G. Schill, *Anal. Chem.*, 51 (1979) 976.
12 A. Sokolowski and K.-G. Wahlund, *J. Chromatogr.*, 189 (1980) 299.
13 A. Sokolowski, T. Fornstedt and D. Westerlund, *J. Liq. Chromatogr.*, 10 (1987) 1629.
14 R. A. Keller and J. C. Giddings, *J. Chromatogr.*, 3 (1960) 205.
15 G. K. C. Low, A. M. Duffield and P. R. Haddad, *Chromatographia*, 15 (1982) 289.
16 G. K. C. Low, P. R. Haddad and A. M. Duffield, *J. Chromatogr.*, 336 (1984) 15.
17 D. P. Kirby, P. Vouros, B. L. Karger, B. Hidy and B. Petersen, *J. Chromatogr.*, 203 (1981) 139.
18 A. Sokolowski, T. Fornstedt and D. Westerlund, *J. Liq. Chromatogr.*, in press.