Effects of system peaks in a coupled column system for noscapine and its metabolites*

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Abstract: System peaks created in a coupled column system, after transfer of the precolumn mobile phase to the analytical column have been investigated. Co-elution of an analyte with a system peak may affect the chromatographic performance of the analyte. The possibility of using the peak compression effect, in order to increase the detectability of the compounds noscapine, narcotoline and cotarnine, respectively, is studied. Three different mobile phases for the analytical column have been tested, the first contained a co-ion, the second a counter-ion and the last both a co-ion and a counter-ion. A compressed noscapine peak is obtained not only when it is eluted in a positive gradient of the co-ion in the first system, but also in a positive gradient of the counter-ion in the second system. Slightly compressed narcotoline and cotarnine peaks are obtained in the third system as long as they eluted before the deficiency peak of the counter-ion.

Keywords: *Liquid chromatography; noscapine; coupled columns; system peaks.*

Introduction

Over recent years coupled column systems have frequently been used in liquid chromatography. The technique has often been used to increase the selectivity in trace analysis of complex samples, or to enrich a large volume of a diluted sample. Furthermore, the technique enables determination of compounds with different polarities, e.g. a drug and its metabolites without using gradient elution [1, 2].

It is well known that injection of a solution with a composition deviating from that of the mobile phase causes disturbances in the established equilibria in the column. This phenomenon will certainly occur in coupled column systems when the precolumn mobile phase is transferred to the analytical column. Each deviating mobile phase component gives rise to at least one migrating zone on the analytical column [3–7]. The zones can be monitored with an RI-detector provided they are retarded from the front disturbances. Co-elution of the analytes with a system zone may give rise to compressed, split or broadened peaks [8–10].

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This paper presents an investigation of the peak performance of noscapine and metabolites, accompanying the formation of system peaks on the analytical column after the introduction of the precolumn mobile phase. Two different precolumn mobile phases have been used, one was based on a low content of acetonitrile in an acidic buffer (pH 2), the second containing in addition a co-ion, a tertiary aliphatic amine. On the analytical column three different mobile phases were tested: the first contained the same co-ion as for the precolumn, the second a counter-ion (an alkylsulphate) and the third the same co-ion together with the counter-ion. The retention of the system peaks is regulated by varying the concentrations of mobile phase components. The stability (in retention times) of the different systems is investigated by multiple sample injections.

Experimental

Chemicals

Noscapine was obtained from Macfarlan Smith Ltd (Edinburgh, UK), cotarnine chloride dihydrate from EGA-Chemie (Albuch, FRG) and narcotoline from Diosynth Apeldoorn (OSS, The Netherlands). The sodium salts of octylsulphate (OS), decylsulphate (DS), dodecylsulphate (DDS) and tetradecylsulphate (TDS) were received from Eastman Kodak Co (Rochester, NY, USA). *N,N*-dimethyloctyl-*N*-amine (DMOA) was obtained from ICN Pharmaceuticals (Plainview, NY, USA).

Acetonitrile (HPLC-quality) and buffer substances (analytical-reagent grade) were from Merck (Darmstadt, FRG).

Apparatus

The liquid chromatographic system consisted of a Kontron Tracer MCS, 670 valve switching unit, with a Tracer timer 210 (Kontron AG, Zürich, Switzerland). The pumps were LKB Model 2150 (LKB, Bromma, Sweden) or Waters M-6000A (Waters Assoc., Milford, MA, USA). The samples were introduced by means of a Waters WISP 710 B automatic injector or a Rheodyne Model 7120 injection valve, equipped with a 50 μl loop.

Noscapine was detected at 210 or 310 nm, narcotoline and cotarnine at 310 nm with a Spectroflow 783 (Kratos Analytical, NJ, USA) or an LDC/Milton Roy SM 4000 detector (Laboratory Data Control, Riviera Beach, FL, USA). The system zones were monitored with a Model R 401 refractometer (Waters Assoc.) coupled in series with the UV-detector. The system and analyte peaks were registered with a dual channel Servogor 220 recorder (Gortz, Wien, Austria). The output signal from the UV-detector was also connected to a Shimadzu C-R3A-integrator (Shimadzu, Kyoto, Japan).

Chromatographic system

The precolumn was a Brownlee Spheri-5 CN (30 × 4.6 mm i.d., 5 μm) (Brownlee Labs, Santa Clara, CA, USA) or a home-packed Nucleosil CN 5 μm (35 × 4.6 mm i.d.) (Machery-Nagel, Düren, FRG). The precolumn mobile phase consisted of acetonitrile and phosphate buffer, pH 2 (μ = 0.05) 10:90, v/v or acetonitrile and 1 mM DMOA in phosphate buffer, pH 2 (μ = 0.05) 94–99:6–1, v/v. The analytical column was a home-packed Nucleosil C_{18}, 3 μm (100 × 4.6 mm i.d.) or a Spherisorb ODS-2, 3 μm (100 × 4.6 mm i.d.) (Phase Separations, Queensferry, UK). The home-made columns were packed with methylene chloride as the slurry medium and methanol as the eluent. The mobile phase for the analytical column consisted of 1–7.5 mM of an alkylsulphate and 0.1 mM of DMOA in acetonitrile and a phosphate

buffer, pH 2 (μ = 0.05), or 0.1–5 mM of DMOA in acetonitrile and a phosphate buffer of pH 2 (μ = 0.05), or 1–9 mM of DDS in acetonitrile and a phosphate buffer, pH 2 (μ = 0.05). Throughout a flow rate of 1.0 ml min^{-1} was used. The asymmetry factor (As$_{10}$) was measured at 10% of the total peak height by dividing the distance after the peak centre by the distance before the peak centre.

Column switching

Noscapine and its metabolites were separated into two separate peaks on the precolumn. Each peak was transferred to an analytical column for further separation.

During the elution from the analytical column, the precolumn was cleaned by 1.6 ml of a mixture of acetonitrile in a phosphate buffer, pH 2 (μ = 0.05) (50:50, v/v). The mixture was pumped into the loop in the first valve using the pump in the Kontron unit. An acetonitrile gradient was created by valve switching.

Sample preparation

Acetonitrile (0.125 ml) and perchloric acid (0.250 ml) were added to 1.00 ml plasma or phosphate buffer, pH 7.4 (μ = 0.2) and mixed carefully. The plasma samples were then centrifuged at 5000 rpm for 10 min. Next 0.380 ml of a 1 M trisodiumcitrate and 5 M sodium hydroxide solution (1 + 4) were added to 0.80 ml of the supernatant or the buffer mixture, giving a pH of about 3. An aliquot of 500 µl was then injected into the precolumn.

Results and Discussion

Initially a dual column system was tested. This was based on the enrichment of noscapine and metabolites on a precolumn followed by backflush to the analytical column. The precolumn mobile phase was acidic and contained an alkyl sulphate for trace enrichment of the analytes as ion-pairs. The analytes were then transferred by backflush to the analytical column with a mobile phase containing DMOA, which competed with the analytes for adsorption sites [6]. This system could, however, not be used in routine work due to a large late-eluting peak due to the alkylsulphate [9].

In order to avoid this complication due to the late-eluting peak, coupled column systems based on the heart-cut technique were investigated. One advantage with this principle was that strongly retained components were not transferred to the analytical column. In addition, a more efficient sample clean-up was achieved, since only the fraction containing the analytes was transferred. Noscapine was separated from its metabolites on a polar precolumn (CN) (Fig. 1) and each fraction was transferred to the respective analytical column (C$_{18}$) for the final separation.

Precolumn

The selection of a suitable precolumn is essential for a coupled column system to work properly. The first requirement of the precolumn is that it has such a character that a mobile phase with a lower eluting strength than that used for the following column can be used. Secondly, the precolumn must show an adequate chromatographic efficiency and symmetry, especially when it is used for group-separation, as in this study. If these requirements are met an efficient enrichment of the analytes will be obtained on the top of the next column.
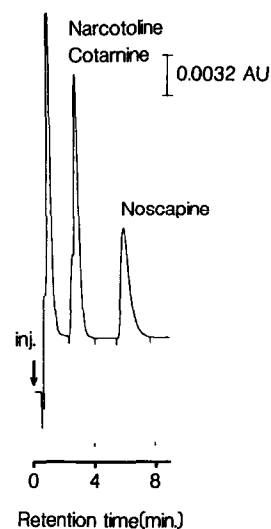
Figure 1
Chromatogram from the precolumn.
Chromatographic conditions: column, Brownlee CN,
5 μm (30 × 4.6 mm); mobile phase, 10% (v/v) of
acetonitrile in phosphate buffer, pH 2 (μ = 0.05);
detection wavelength, 310 nm. Injected volume:
500 μl containing cotarnine (482 ng), narcotoline
(473 ng) and noscapine (602 ng).

Table 1
Retention times of cotarnine, narcotoline and noscapine on different precolumns. The plate numbers (*N*) were calculated for noscapine. 500 μl was injected

| Column | Mobile phase* | Retention time (min) | | | *N* |
		Narcoline	Cotarnine	Noscapine	
Sulphopropyl 5 μm (50 × 4.6)	1% acetonitrile	1.6	2.4	2.3	60
Nucleosil CN 5 μm (35 × 4.6)	5% acetonitrile	2.0	1.4	3.7	110
Nucleosil CN 5 μm (35 × 4.6)	5% acetonitrile 0.5 mM DMOA	1.8	1.2	3.0	450
Nucleosil CN 5 μm (35 × 4.6)	1% acetonitrile 1.0 mM DMOA	3.0	2.4	6.2	610
Brownlee CN 5 μm (30 × 4.6)	4% acetonitrile 1.0 mM DMOA	2.7	2.3	7.1	540
Brownlee CN 5 μm (30 × 4.6)	10% acetonitrile	2.8	2.9	6.1	640
Spherisorb C_1 3 μm (20 × 3.8)	3.5% acetonitrile 1.0 mM DMOA	2.2	4.1	8.1	290
Nucleosil C_4 5 μm (35 × 4.6)	5% acetonitrile 1.0 mM DMOA	5.3	2.0	28	440
Spherisorb PH 5 μm (20 × 3.8)	10% acetonitrile	1.9	4.3	5.6	240

*Additions to phosphate buffer pH 2 (μ = 0.05).

Several stationary and mobile phases were tested regarding efficiency and retention of noscapine and its metabolites (Table 1). The precolumn had to be capable of group-separation of the metabolites from noscapine. This was achieved for the nitrile-, the C_1- and C_4-phases, however, the two alkyl-bonded phases gave either too high selectivity or too strong retention. This would involve the transfer of a large volume to the following column, or the use of too strong a mobile phase for the precolumn, respectively. The chromatographic performance on the analytical column would then deteriorate as a consequence.

The retention and efficiency of the nitrile columns were finally optimised by adjusting the concentrations of acetonitrile and DMOA in the acidic mobile phase. Two different precolumns were used: Nucleosil CN with a mobile phase containing 1 mM of DMOA in 1% (v/v) acetonitrile in acidic buffer or Brownlee CN with 10% (v/v) acetonitrile in acidic buffer (efficiency and symmetry adequate without addition of DMOA). The precolumn was purified between each injection by switching in a loop containing 1.6 ml of acetonitrile phosphate buffer, pH 2 ($\mu = 0.05$) (50:50, v/v).

System peaks

The compositions of the mobile phases required for the precolumn and the next columns were different; generally for the latter a higher content of the organic modifier was necessary. In some cases, it contained a counter-ion as well as a variable amount of co-ion (DMOA). When a fraction was transferred from the precolumn, system peaks were produced, which were monitored by RI-detection. A systematic study was performed on the influence of the system peaks on co-eluting analytes for the different coupled column systems.

Mobile phase with a co-ion

The chromatographic system used is given in Fig. 2. An additional injection valve was directly connected to the analytical column in order to make it possible to test band broadening effects in the system. The precolumn mobile phase contained a co-ion, a tertiary aliphatic amine (DMOA). In order to reduce the influence of system peaks, the mobile phases for the precolumn and the analytical column were made as similar as possible.
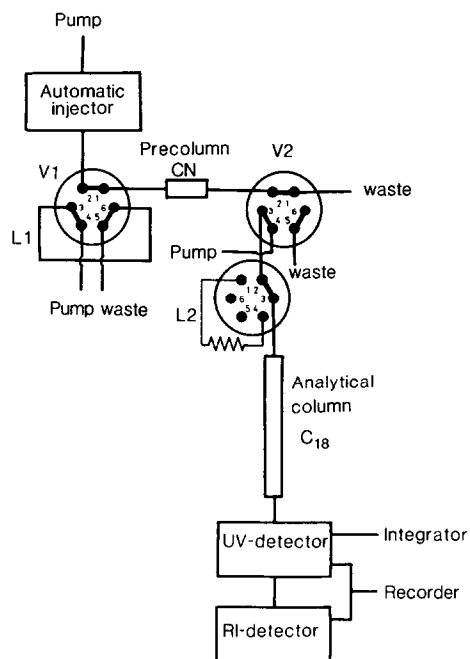
Figure 2
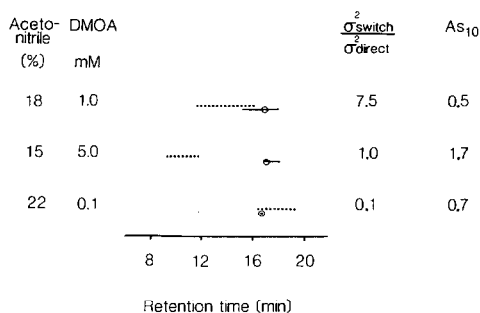Scheme of the chromatographic system. Precolumn: Brownlee CN, 5 μm (30 × 4.6 mm) or Nucleosil CN, 5 μm (35 × 4.6 mm); analytical column, Nucleosil C_{18}, 3 μm (100 × 4.6 mm); detection wavelength, 211 nm or 310 nm. $L_1 = 1.6$ ml, $L_2 = 50$ μl.

Figure 3

Influence of system peak on the peak variance ratio ($\sigma^2_{switch}/\sigma^2_{direct}$) of noscapine with varying concentrations of DMOA. ——, noscapine peak width; O, peak maximum; ----, DMOA peak width. Chromatographic system was as in Fig. 2. The precolumn mobile phase contained 1 mM of DMOA in 1% (v/v) acetonitrile in a phosphate buffer, pH 2.

Aceto-nitrile (%)	DMOA mM		$\dfrac{\sigma^2_{switch}}{\sigma^2_{direct}}$	As_{10}
18	1.0		7.5	0.5
15	5.0		1.0	1.7
22	0.1		0.1	0.7

Retention time (min)

The apparent efficiency of the noscapine peak depends on its retention relative the DMOA system peak (Fig. 3). The retentions of both the noscapine and system peaks were dependent on the concentrations of DMOA and acetonitrile. However, the system peak was affected to a greater extent by varying the DMOA concentration. This is a consequence of a Langmuir-type of adsorption onto the solid phase. The content of acetonitrile was adjusted to keep the noscapine retention at approximately the same value. Three principally different results were obtained; the noscapine peak eluted at the back, well separated from, or in front of the system peak. The ratios of the variances of the noscapine peaks ($\sigma^2_{switch}/\sigma^2_{direct}$) demonstrate that severe band broadening and leading were obtained with 1 mM of DMOA. The reason is the competitive effect of DMOA which causes the front of the noscapine peak to elute faster than the other parts. When the two peaks were well separated the noscapine peak performance was unaffected. A small concentration of DMOA (0.1 mM) gave a longer retention of the system peak and the noscapine peak eluted within the front of DMOA. An increasingly steep gradient of DMOA competes effectively with the back part of the noscapine peak and results in a strong peak compression effect producing a 10-fold reduction of the variance. However, it was not possible to use this effect in practice, since disturbances appeared in the chromatograms even when recording at 310 nm (Fig. 4).

When the system and noscapine peaks were well separated (DMOA 5 mM), the plasma blanks were clean in the region of the noscapine peak even when registered at 211 nm (Fig. 5). In this chromatogram noscapine appears to be surrounded by two unidentified tentative metabolites. However, the stability of this system was not acceptable because, after each injection, the retention time for noscapine decreased by an almost constant reduction. The mobile phases from the precolumn and the analytical column differed in their content of acetonitrile (1 and 15%, respectively) and DMOA (1 and 5 mM, respectively), which gave a difference in the adsorption of DMOA. The introduction of the precolumn mobile phase onto the analytical column caused a stronger adsorption of the amine and repeated injections probably caused an accumulation of DMOA resulting in the decreasing retention times of noscapine. The initial retention times could be restored by desorption of DMOA from the analytical column with 50% acetonitrile in a phosphate buffer, pH 2.

In order to circumvent this problem a precolumn mobile phase with no addition of DMOA but a higher content of acetonitrile (10%) was tried. However, the system was still found to be unstable. A probable explanation is that during the transfer of the precolumn mobile phase, the analytical column at the top, equilibrates with the
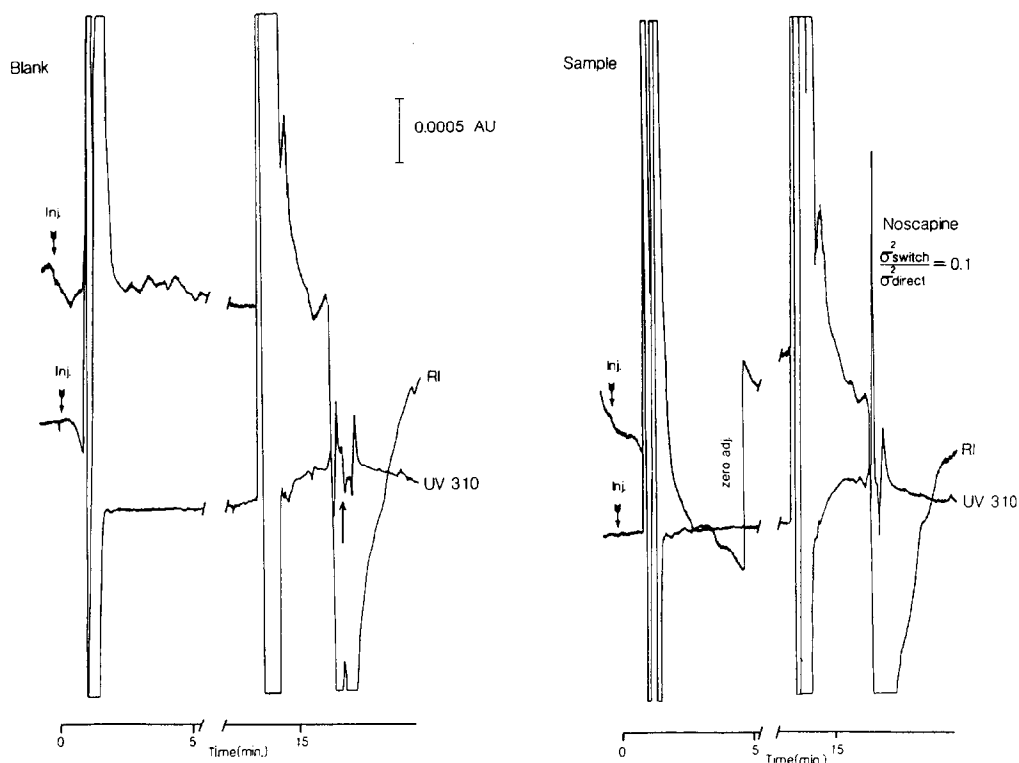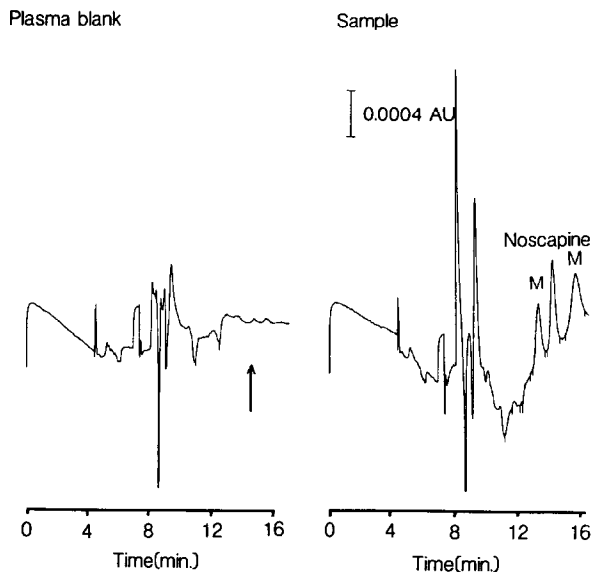
Figure 4

Chromatograms from UV- and RI-traces, after transfer of 3.00 ml precolumn mobile phase (blank) containing the noscapine fraction (sample). Chromatographic system was as described in Fig. 2. The precolumn mobile phase contained 1 mM of DMOA in 1% (v/v) acetonitrile in a phosphate buffer of pH 2. The analytical column mobile phase contained 0.1 mM of DMOA in 22% (v/v) of acetonitrile in phosphate buffer, pH 2 (μ = 0.05). Detection wavelength: 310 nm. Injected solution: 500 μl of deproteinized pH adjusted plasma (pH 3), to which 50 ng ml^{-1} of noscapine was added.

precolumn mobile phase with its low content of acetonitrile (10%) and no DMOA. When changing to the analytical column mobile phase containing DMOA (5 mM), more DMOA was initially adsorbed after each injection. The adsorbed excess was not completely released by the analytical column mobile phase due to the low content of acetonitrile (15%).

Mobile phase with a co-ion and counter-ion

The co-ion in the precolumn mobile phase was DMOA. In addition to this an alkylsulphate was present in the mobile phase for the analytical column. The transfer of a fraction from the precolumn to the analytical column gave rise to two peaks with opposite directions on the RI-recording (Fig. 6). The content of DMOA in the precolumn phase was 10 times higher than that of the second mobile phase, an excess of DMOA was thus transferred. Its retention increased with an increase in the concentration of the organic anion, DS (Fig. 7). The retention of the system peak (a deficiency of the alkylsulphate) decreased with an increasing anion concentration, as expected theoretically [11]. However, when the DS concentration reached 7.5 mM the system peak changed response direction indicating the elution of excess reagent. The

Plasma blank   Sample

I 0.0004 AU

Noscapine
M

M

0   4   8   12   16   0   4   8   12   16
        Time(min.)   Time(min.)

Figure 5
Plasma blank and sample chromatogram. The noscapine concentration corresponds to 13 ng ml^{-1} plasma.
Chromatographic system was as described in Fig. 2 with an additional enrichment column (C_{18}) used in the
foreflush mode before the analytical column. The precolumn mobile phase contained 1 mM of DMOA in 6%
(v/v) of acetonitrile in phosphate buffer, pH 2. The analytical column mobile phase contained 5 mM of DMOA
in 13% (v/v) acetonitrile in a phosphate buffer, pH 2. Detection wavelength: 211 nm. M = Unidentified
metabolite.

conditions at the top of the analytical column are complex following the introduction of
the precolumn mobile phase. Nevertheless the response change may be explained. The
precolumn mobile phase had a much lower acetonitrile content and during mixing with
the mobile phase from the analytical column the adsorption of its components to the
solid phase may have increased. At a certain critical concentration of DS this increased
adsorption causes an excess of anion in the injection zone that appears as a positive
system peak. At the lower DS concentrations, on the other hand, the ion-pairing effect
of DMOA consumed the excess yielding a net deficiency of the anion in the injection
zone. In comparison, when the precolumn mobile phase did not contain DMOA (10%
acetonitrile in a buffer, pH 2), an excess of DS eluted as the system peak at all tested
concentrations of DS. This indicated that the adsorption of the anion was stronger from
the precolumn mobile phase compared to the analytical column mobile phase in spite of
the absence of an ion-pairing effect by DMOA. The retentions of the cationic analytes
increased with increasing anion concentrations according to conventional ion-pair theory
[12]. When the analyte peaks were retained more than the system peak, which contained
a deficiency of the anion the analytes peak variances increased twice as much as those
obtained by direct injections (Fig. 8B, 5 mM of DS). The probable reason is that the
analytes and the system peak co-elute during an early part of the elution and, in the
system zone which contains a deficiency of DS, the analytes are retained less than during
elution in the bulk mobile phase. The result is that the peaks spread out. A further
support for this explanation is that when the system peak contained an excess of DS, the
peak variances of the analytes were unaffected (Fig. 8A).
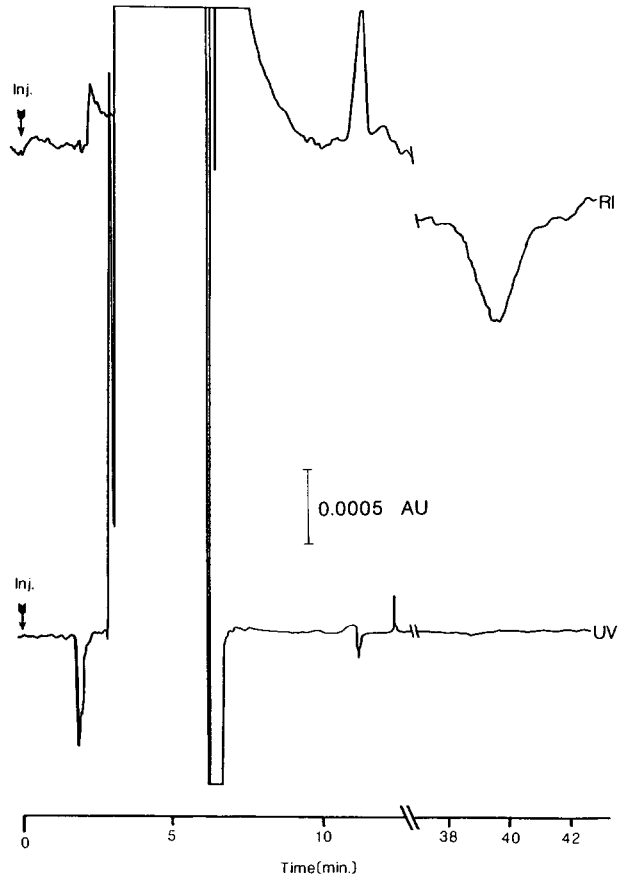
Figure 6
RI- and UV-traces after transfer of 3.00 ml of precolumn mobile phase. Chromatographic system was as described in Fig. 2. Precolumn mobile phase: 1 mM of DMOA in 1% (v/v) acetonitrile in phosphate buffer, pH 2. Analytical column mobile phase: 2 mM DS, 0.1 mM DMOA in 30% acetonitrile in phosphate buffer, pH 2. UV-detection: 310 nm.
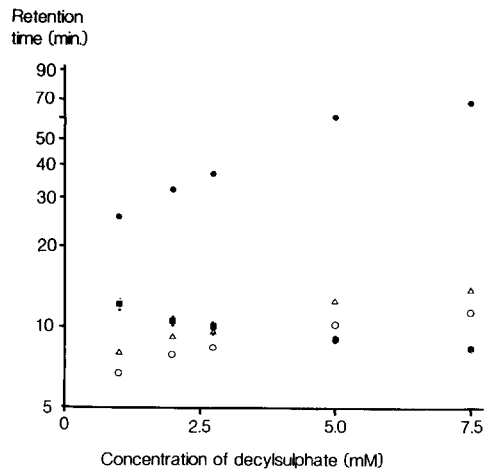
Figure 7
Regulation of retention time by decylsulphate. Chromatographic conditions were as described in Fig. 2. Analytes: ○, cotarnine; △, narcotoline; System peaks: ●, DMOA; ■ decylsulphate peak width.

The retentions of cotarnine, narcotoline and DMOA as ion-pairs with different counter-ions were studied (Table 2). The retention times were adjusted to suitable values by altering the acetonitrile content. In all cases the sytem peak eluted after the analytes, which, according to the discussion above, implies that no extra band broadening effects were obtained. The selectivity decreased, however, with increasing hydrophobicity of the counter-ion.

In bioanalytical applications the use of DS as the counter-ion, however, resulted in interference of endogenous compounds with analyte retentions. This problem could be solved by changing to the more hydrophobic anion DDS (Fig. 9). The stability of this kind of system was high. Using a mobile phase containing 2 mM of DDS and 0.1 mM DMOA in 35% of acetonitrile in a phosphate buffer of pH 2, more than 100 injections of 500 µl deproteinated plasma were made without any change in the retention times of the analytes. Despite the fact that a fraction of 1.5 ml of the precolumn mobile phase was transferred into the analytical column with every injection.

Mobile phase with a counter-ion

The chromatographic system employed for this investigation (Fig. 10) possessed in addition to the previous one (Fig. 2), an enrichment column (C_{18}) used in the backflush mode. The system was used for the analysis of noscapine. The time-table of the switching events is given as Table 3. After injection into the precolumn, endogenous compounds and the noscapine metabolites were allowed to drain off for about 4 min and then the enrichment column was equilibrated with the mobile phase for the analytical column.

Figure 8
The influence on peak variance ratios ($\sigma^2_{switch}/\sigma^2_{direct}$) by the DS concentration. Chromatographic system as in Fig. 2. Analytes: $\triangle$, cotarnine; $\bigcirc$, narcotoline. Analytical column mobile phase: varying concentration of DS, 0.1 M DMOA in 30% acetonitrile in a phosphate buffer, pH 2. Precolumn mobile phase: A, 10% (v/v) acetonitrile in acidic phosphate buffer; B, 1 mM of DMOA in 1% acetonitrile in acidic phosphate buffer.
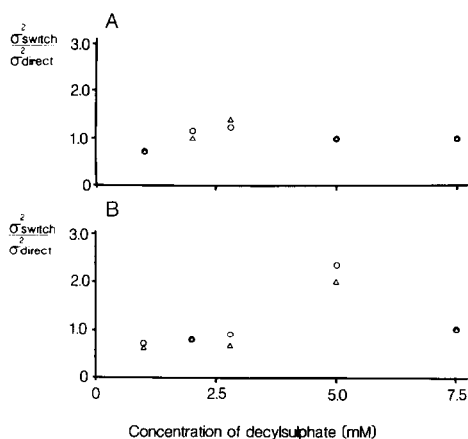
Table 2
Retention times of cotarnine, narcotoline, DMOA and the system peak with different alkylsulphates

Type	Counter ion Concentration (mM)	Acetonitrile (%)	System peak	Retention time (min) DMOA	Cotarnine	Narcotoline	α^*
OS	1.0	22	14.6	47	8.7	11.3	1.30
DS	1.0	30	12.3	26	8.1	9.1	1.12
DDS	1.0	33	13.6	35	8.6	9.5	1.10
TDS	0.5	35	—†	—†	7.9	8.5	1.07

*Retention time of narcotoline relative to that of cotarnine.
†Not visible.

Figure 9
Plasma chromatograms without and with addition of 25 ng ml^{-1} of cotarine and narcotoline, respectively. Chromatographic system were as described in Fig. 2. Precolumn mobile phase: 10% (v/v) acetonitrile in acidic phosphate buffer. Analytical column mobile phase: 2 mM DDS, 0.1 mM DMOA in 35% acetonitrile in phosphate buffer pH 2. Detection wavelength: 310 nm.

Figure 10
Scheme of the chromatographic system. Precolumn: Brownlee CN (30 × 4.6 mm); enrichment column, Spherisorb ODS 2 (20 × 3.8 mm); analytical column, Spherisorb ODS 2 (100 × 4.6 mm). Detection wavelength: 310 nm; $L_1 = 1.6$ ml; $L_2 = 50$ µl.

The fraction from the precolumn was then introduced into the enrichment column for 2.5 min, after which the analyte was transferred to the analytical column by backflush. The enrichment and analytical columns were run on-line for 1.3 min and then both the precolumn and the enrichment column were washed with 50% acetonitrile in the acidic buffer.

Table 3
Switching events for the chromatographic system in Fig. 10

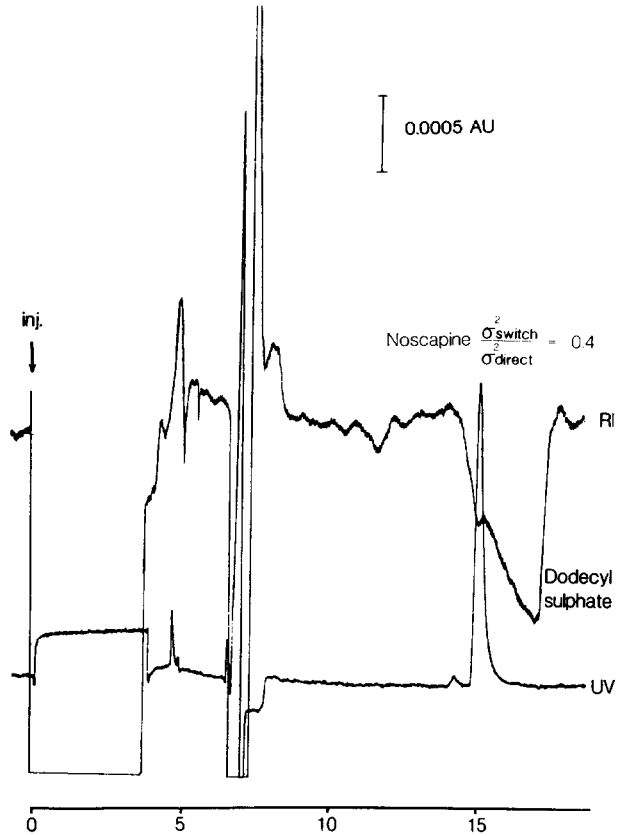| Time	Valve	Event
0		Plasma sample is injected
0.1	V_2 off	The first fraction from the precolumn is drained off. The enrichment and the analytical column are connected in series
0.2	V_3 off	The enrichment and the analytical column are disconnected
0.9A*	V_2 on	The precolumn and the enrichment column are connected in series and noscapine is enriched
0.9A* + 0.1	V_3 on	The analytical column is equilibrated with its mobile phase
B†	V_2 off	The enrichment column and the analytical column are connected
B† + 1.0	pump on	The loop is filled with 50% (v/v) of acetonitrile in phosphate buffer, pH 2
B† + 1.6	pump off	
B† + 1.3	V_2 on	The enrichment column and the analytical column are disconnected. The enrichment column is equilibrated with precolumn mobile phase
B† + 2.1	V_1 on	The precolumn and the enrichment column are purified with 50% (v/v) of acetonitrile in acidic buffer
B† + 3.6	V_1 off	Regeneration of the precolumn and the enrichment column is completed

 * A The time of the start of the noscapine peak.
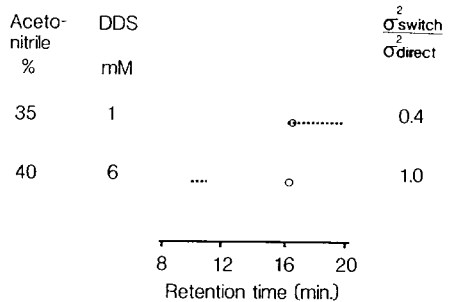 † B The time of the end of the noscapine peak.

When the mobile phase for the analytical column contained 1 mM DDS in 35% acetonitrile in a buffer, pH 2, the noscapine peak co-eluted with the front of the system peak (Fig. 11). The latter contained an excess of DDS and consequently the analyte was exposed to a rather steep positive gradient of the anion. In this situation noscapine is retained more strongly than in the bulk mobile phase resulting in a peak compression effect (Figs 11 and 12). The response was linear as measured both from, peak height and peak area between 10 ng ml^{-1} and >1000 ng ml^{-1}. However, when using a new batch of the solid phase the adsorption capacity of the enrichment column with regard to DDS was lower. This resulted in a smaller system peak causing a diminished steepness of the gradient and a longer retention time. The co-eluted noscapine peak was now distorted, since the end of the peak was eluted in a decreasing gradient (Fig. 13). By decreasing the DDS concentration in the mobile phase the retention of DDS increased and of noscapine decreased. Co-elution of the whole noscapine peak with the positive gradient of DDS was obtained at 0.8 mM of DDS. But in this latter case the steepness of the gradient was lower causing a less compressed peak. However, co-elution of the analyte with an excess of DDS could not be detected at 211 nM because the co-eluting DDS caused UV-absorbance.

An alternative system was therefore tested where the analyte was fully separated from the system peak. This mobile phase was composed of 6 mM DDS in 40% acetonitrile in the acidic buffer. As expected, no peak compression effect was obtained in this case (Fig. 12).

0.0005 AU

inj.

Noscapine $\dfrac{\sigma^2_{switch}}{\sigma^2_{direct}} = 0.4$

RI

Dodecyl sulphate

UV

0   5   10   15

Figure 11
Chromatogram from RI- and UV-traces after transfer of 2.5 ml of precolumn mobile phase containing the noscapine fraction. Chromatographic system was as described in Fig. 10. Precolumn mobile phase: 10% (v/v) acetonitrile in phosphate buffer, pH 2; analytical column mobile phase, 1 mM DDS in 35% (v/v) acetonitrile in phosphate buffer pH 2; detection wavelength: 310 nm.

Figure 12
Influence of the peak variance ratio ($\sigma^2_{switch}/\sigma^2_{direct}$) and retention time of noscapine and the retention time of the system peak (DDS) with varying decylsulphate concentrations. ---, DDS peak width; O, noscapine retention. Chromatographic system was as in Fig. 10. The precolumn mobile phase contained 10% (v/v) acetonitrile in a phosphate buffer, pH 2.

Aceto-nitrile %	DDS mM			$\dfrac{\sigma^2_{switch}}{\sigma^2_{direct}}$
35	1		o··········	0.4
40	6	....	o	1.0

8   12   16   20
Retention time (min.)

The system peak, which contains an excess of the anion, originates from the equilibrium conditions of the enrichment step. Before injection, the enrichment column was equilibrated with the precolumn mobile phase, which contained a low content of acetonitrile. During the first 4 min after injection the enrichment column was equilibrated with the mobile phase containing DDS and initially DDS was strongly

Figure 13
Chromatogram from RI- and UV-traces after transfer
of 2.5 ml of precolumn mobile phase containing the
noscapine fraction. Chromatographic system was as
described in Fig. 10. Precolumn mobile phase: 10%
(v/v) acetonitrile in a phosphate buffer, pH 2;
analytical column mobile phase, 1 mM DDS in 35%
(v/v) acetonitrile in phosphate buffer of pH 2;
detection wavelength: 310 nm.
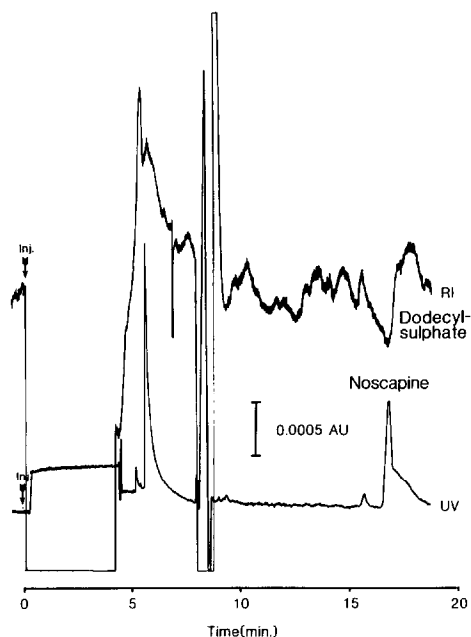
adsorbed. When the noscapine fraction from the precolumn was transferred to the enrichment column the latter equilibrated at least partly with the precolumn mobile phase. During the initial phase of the backflush step, once again DDS was strongly adsorbed onto the solid phase. The adsorbed excess was finally released during the subsequent elution with the analytical mobile phase which contained a higher amount of acetonitrile (35%). This gave rise to the observed system peak.

This explanation is supported by the fact that the system peak was absent when the enrichment and analytical columns were run on-line after enrichment of the noscapine fraction. This involves no equilibration with precolumn mobile phase and therefore no increased adsorption of DDS.

An application of the peak compression effect (1 mM DDS, 35% acetonitrile, Fig. 12) to the analysis of plasma gave a chromatogram containing noscapine as well as a small peak of an unidentified metabolite (Fig. 14). The peaks were, however not resolved ($Rs = 0.6$).

Increasing the concentrations of DDS (3 mM) and acetonitrile (40%) resulted in higher resolution (Fig. 15, $Rs = 0.9$). If the counter-ion concentrations are raised the system and analyte peaks separate (Fig. 12). In turn this eliminates the peak compression effects of co-eluting peaks. This means that although the apparent efficiency of analyte peaks decreases, the selectivity increases so much that the resolution is still improved. This indicates a possible drawback of peak compression effects. When two or more structurally related compounds co-elute in the same gradient of a system peak the selectivity may be negatively affected [9]. A further increase of the counter-ion concentration (9 mM DDS and 40% acetonitrile), however, lowered the resolution ($Rs = 0.6$) again. This is often observed in practice. The reason is probably that there is a difference in the relative affinity of the different binding sites for analytes available on silica based solid phases [11–13].
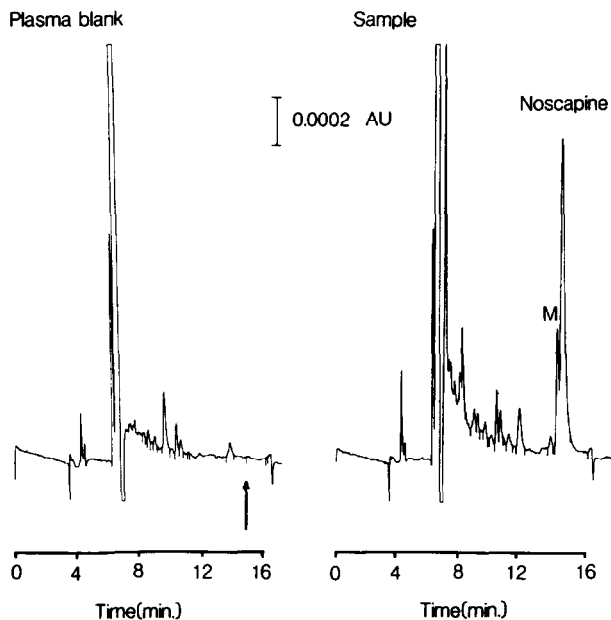
Figure 14
Plasma blank and sample chromatogram. The noscapine concentration corresponds to 256 ng ml^{-1} plasma.
Chromatographic conditions were as described in Fig. 11. M = unidentified metabolite.
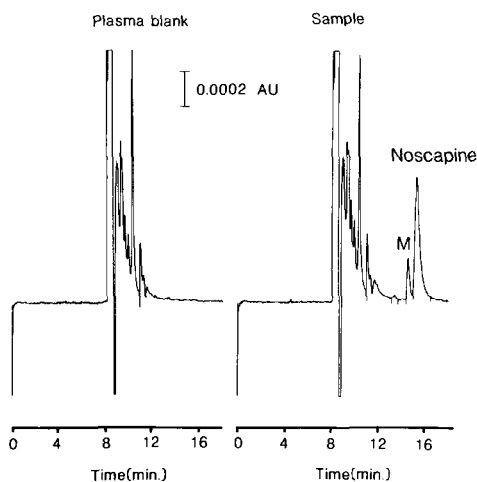
Figure 15
Plasma blank and sample chromatogram. The
noscapine concentration corresponds to 206 ng ml^{-1}
plasma. Chromatographic system was as described in
Fig. 10. Precolumn mobile phase: 10% (v/v)
acetonitrile in a phosphate buffer, pH 2; analytical
column mobile phase, 3 mM DDS in 40% (v/v)
acetonitrile in a phosphate buffer of pH 2; detection
wavelength: 310 nm. M = unidentified metabolite.

Conclusions

The chromatographic performance of noscapine, narcotoline and cotarnine was
affected during co-elution with a system peak created in a coupled column system. The
retention and character of the system peak were regulated by the composition of the
mobile phases in the precolumn and the analytical columns, respectively. System peaks
containing an excess of a co-ion, or an excess or a deficiency of a counter-ion were
produced after transfer of the precolumn mobile phase.

A compressed noscapine peak was created when the analyte co-eluted with a positive gradient of a co-ion or a counter-ion, while a broad and leading peak was produced when the analyte co-eluted with a negative gradient of a co-ion or a counter-ion. An unaffected noscapine peak was obtained when it was well separated from the system peak. Slightly compressed narcotoline and cotarnine peaks were produced when they eluted before an excess or a deficiency counter-ion system peak. Elution after a deficiency counter-ion system peak gave broad narcotoline and cotarine peaks. In the case of elution after an excess counter-ion system peak the analytes were unaffected. Since small differences in retention time may give drastic effects on the peak performance, robust and reproducible solid phases are necessary.

A high detection selectivity for the analyte is necessary when co-elution with a system peak occur in order to avoid detection interferences. A drawback with system peaks in bioanalytical applications may be lower selectivity against structurally related compounds, such as metabolites.

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