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## Note

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### **Ion-exchange and straight-phase partition high-performance liquid chromatography of the opiates on silica**

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Liquid chromatographic systems containing acidic amine salt buffers and using silica columns have recently been reported<sup>1–3</sup>. These systems were originally designed for simultaneous separations of primary, secondary and tertiary amines as well as quaternary ammonium salts. Recently one of these systems has been used for the determination of morphine in opium<sup>4</sup>. All the systems published can be described as straight-phase partition chromatography.

It is known that silica gel may also act as a cation exchanger in water-rich systems<sup>5</sup>.

The opiates have previously been separated by high-performance liquid chromatography (HPLC) using straight-phase adsorption<sup>6–9</sup>, straight-phase partition<sup>4,10,11</sup>, reversed-phase<sup>11–16</sup> and ion-exchange chromatography<sup>17</sup>. In this study the influence on the retention of some opium alkaloids of a gradual change in the polarity of the mobile phase from a straight-phase system containing 1% water to an ion-exchange system containing 90% water is investigated using a silica column.

## EXPERIMENTAL

### *Apparatus*

A liquid chromatograph comprised of a Waters 6000 pump, a Rheodyne 7120 loop-injector, a Waters 440 UV-absorbance detector (254 nm) and an Omniscribe 5117-5 recorder was used.

### *Chemicals*

All chemicals used were of analytical reagent grade, and the amines were distilled before use.

### *Chromatography*

The chromatographic column (150 × 4.65 mm I.D.) was packed with Li-Chrosorb SI 60 5- $\mu$ m particles as previously described<sup>1</sup>. The efficiency of the column was 6000 plates for thebaine (capacity ratio,  $k' = 2.0$ ) measured with acetonitrile–water–glacial acetic acid–diethylamine (10:90:0.5:0.5) as the mobile phase.

A column identical to the chromatographic column was installed between the pump and the loop-injector in order to saturate the mobile phase with silica.

## RESULTS AND DISCUSSION

The basis for this investigation was a straight-phase partition chromatographic system with silica as the solid phase and tetrahydrofuran-methanol-glacial acetic acid-diethylamine-water (80:20:0.5:0.5:1.0) as the mobile phase.

The polarity of the mobile phase was gradually increased by substituting methanol for tetrahydrofuran (Fig. 1). This resulted in a decreased  $k'$  value for normorphine, but increased  $k'$  values for the other opiates tested. The  $k'$  value for morphine increased less than the values for codeine and thebaine.

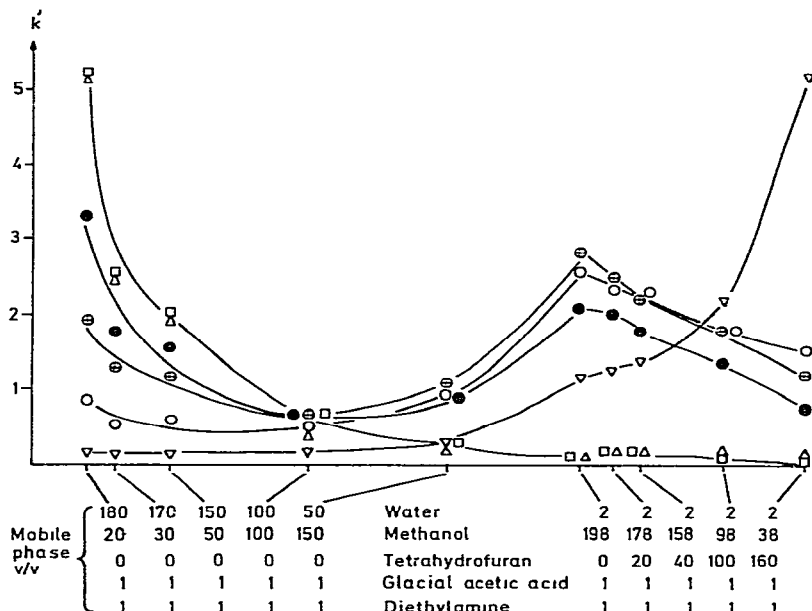


Fig. 1. Plots of the  $k'$  values of opiates obtained on unmodified silica versus the polarity of the mobile phase. Opiates:  $\oplus$ , codeine;  $\circ$ , morphine;  $\nabla$ , normorphine;  $\square$ , noscapine;  $\triangle$ , papaverine;  $\bullet$ , thebaine.

This behaviour may be due to solvation of the solutes and the diethylammonium acetate. Solvation of the solutes results in decreased  $k'$  values, the more polar substances being affected the most. Therefore a drastic change in retention is seen for normorphine. On the other hand, at the higher methanol concentration, less diethylammonium acetate will be adsorbed to the silica, which gives the solutes more access to the silica surface. This results in an increase in the retention of the less solvated semipolar compounds, thebaine and codeine.

When the polarity of the mobile phase was further increased by substituting water for methanol, the  $k'$  values for all the opiates decreased as a consequence of the deactivation of the silica. However, when the water content increased beyond *ca.* 50%, the  $k'$  values again began to increase, and the order of elution became the exact opposite of what was seen with the straight phase system at 80% tetrahydrofuran.

The retention of the opiates using mobile phases with from 90 to *ca.* 50% water may be ascribed to an ion-exchange effect, while the retention mechanism when using

mobile phases containing from *ca.* 50 down to 1% water seems more complex, probably being a mixture of several mechanisms.

The influence of the pH of the mobile phase on the retention of the opiates in the cation-exchange system was investigated in the pH range 4–6 (Table I). As expected, an increase in retention is seen with increasing pH due to the increase in ionization of the silica gel.

TABLE I

*k'* VALUES FOR OPIATES OBTAINED ON SILICA

Eluent: methanol–0.05 *M* diethylamine buffer (15:85); the pH of the buffer was adjusted with acetic acid. Opiates: NM = normorphine; M = morphine; C = codeine; T = thebaine; N = noscapine; P = papaverine.

<i>pH</i>	<i>k'</i>					
	<i>NM</i>	<i>M</i>	<i>C</i>	<i>T</i>	<i>N</i>	<i>P</i>
4.0	0.0	0.2	0.6	1.0	1.6	1.8
5.0	0.1	0.4	0.7	1.4	2.3	2.4
5.5	0.2	0.8	1.3	2.1	3.2	3.3
6.0	0.4	1.6	1.9	3.2	4.5	4.6

Increasing the polarity of the amine resulted in increased *k'* values (Table II) probably due to the decreased affinity (increased solvation) of the more polar ammonium ions for the ion-exchange sites on the silica. The competition between the amines and the opium alkaloids for the ion-exchange sites is therefore in favour of the alkaloids. The pH values given in Table II were measured in the eluent containing methanol. Besides the increase in retention, the most polar amines gave rise to some peak asymmetry.

TABLE II

*k'* VALUES FOR OPIATES OBTAINED ON SILICA IN THE PRESENCE OF DIFFERENT AMINES

Eluent: water containing 15% methanol, 0.088 *M* acetic acid and 0.05 *M* amine; the pH value is given in brackets.

<i>Amine</i>	<i>k'</i>					
	<i>NM</i>	<i>M</i>	<i>C</i>	<i>T</i>	<i>N</i>	<i>P</i>
Triethylamine (5.2)	0	0.4	0.7	1.2	1.7	1.7
Dibutylamine (5.2)	0.1	0.6	0.9	1.3	1.8	1.8
Diisopropylamine (5.1)	0.1	0.6	0.9	1.3	2.0	2.0
Diethylamine (5.1)	0.1	0.6	1.2	1.8	2.4	2.3
<i>n</i> -Butylamine (5.2)	0.2	0.8	1.7	2.2	3.2	3.1
Isopropylamine (5.1)	0.2	0.9	1.8	2.7	3.6	3.6
Diethanolamine (5.0)	0.2	0.9	2.0	2.9	4.3	4.3
Ethanolamine (5.0)	0.4	1.2	2.6	3.7	5.3	5.3

When another acid was substituted for acetic acid (Table III), it was seen that stronger acids gave smaller  $k'$  values. This is probably due to the change in pH since the adsorption of cations to silica decreases with decreasing pH.

TABLE III

$k'$  VALUES FOR OPIATES OBTAINED ON SILICA IN THE PRESENCE OF DIFFERENT ACIDS

Eluent: water containing 15% methanol, 0.088 *M* acid and 0.05 *M* diethylamine; the pH value is given in brackets.

Acid	$k'$					
	NM	M	C	T	N	P
Perchloric (1.0)	0	0.2	0.7	1.0	1.3	1.5
Methanesulphonic (1.2)	0	0.4	0.8	1.2	1.7	1.9
Formic (3.6)	0	0.4	1.0	1.5	2.0	2.2
Acetic (5.1)	0.1	0.6	1.2	1.8	2.4	2.3
Propionic (5.5)	0.2	0.7	1.4	2.1	3.1	2.7

A change in the nature of the organic solvent in the mobile phase led to decreased  $k'$  values with decreasing polarity (Table IV). This behaviour is similar to that observed in other ion-exchange experiments<sup>18,19</sup>, but the changes in  $k'$  values ongoing from isopropanol to acetonitrile to tetrahydrofuran were very small on the silica. A change in selectivity was seen when using tetrahydrofuran.

TABLE IV

$k'$  VALUES FOR OPIATES OBTAINED ON SILICA IN THE PRESENCE OF DIFFERENT ORGANIC SOLVENTS

Eluent: water containing 10% organic solvent, 0.088 *M* glacial acetic acid and 0.05 *M* diethylamine.

Solvent	$k'$					
	NM	M	C	T	N	P
Methanol	0.2	0.9	2.0	3.2	5.3	5.2
Isopropanol	0.2	0.7	1.4	1.9	2.7	2.8
Acetonitrile	0.2	0.7	1.3	1.9	2.7	2.8
Tetrahydrofuran	0.1	0.6	0.9	1.7	2.1	2.5

The overall conclusion is that it is possible to separate the opium alkaloids in the straight-phase partition as well as in the ion-exchange mode on unmodified silica. The ion-exchange system behaves very much like a reversed-phase system and has the same advantages. This gives the analyst more possibilities for selection of the appropriate chromatographic system without changing the column.

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