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APPLICATION OF HIGH-SPEED LIQUID CHROMATOGRAPHY TO THE ANALYSIS OF MORPHINE, HEROIN, 6-(O-ACETYL)MORPHINE AND METHADONE

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SUMMARY

The chromatographic behaviour of morphine, heroin, 6-(O-acetyl)morphine and methadone has been investigated using high-speed ion-exchange chromatography with UV detection. Conditions for the rapid quantitative determination of these compounds are outlined. Morphine, heroin and 6-(O-acetyl)morphine were analysed on Zipax SCX (strong cation exchanger), and methadone was analysed on Zipax SAX (strong anion exchanger). Eluted components were identified by their mass spectra and UV absorption spectra.

INTRODUCTION

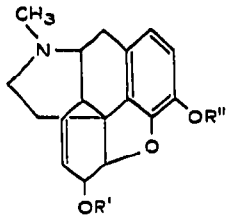
The development of rapid sensitive quantitative methods for the analysis of narcotics has attracted much interest in analytical toxicology and in forensic science. Gas chromatography (GC) has, for example, been applied to the determination of drugs in body fluids[1,2], morphine in opium[3], and heroin in illicit street preparations[4], but requires extraction by organic solvents followed by derivatization prior to GC itself, and for morphine the formation of a derivative before extraction[5]. Such preparative procedures can introduce considerable scope for error.

With the development of high-speed liquid chromatography (HSLC)[6,7], ion-exchange chromatography, previously a very slow technique, can now be carried out in a manner similar to GC[8], and has already been applied to the analysis of drugs such as diphenylhydantoin, barbiturates[9], phenacetin and its metabolites[10].

The aim of the present work was to apply high-speed ion-exchange chromatography to the detection, separation and quantitative determination of morphine, heroin, 6-(O-acetyl)morphine and methadone (formulae I and II).

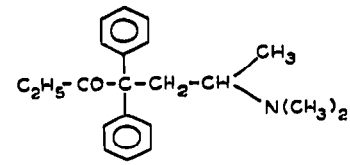
In order to determine optimal conditions for their separation the effect of pH, ionic strength and the presence of organic components in the eluent on the column capacity ratio, k', and the plate height, H, have been determined. The capacity ratio, k', which is determined entirely by thermodynamic parameters[11], is obtained from the elution chromatogram using eqn. 1

$$k' = (t_R - t_0)/t_0 \qquad (1)$$



(I)

Morphine: R′=R″=H
6-(O-Acetyl)morphine: R′=$COCH_3$, R″=H
Heroin: R′=R″=$COCH_3$



(II)

Methadone

where t_R and t_0 are the elution times of a retained and unretained solute. k' is related to the R_F value of a solute by eqn. 2

$$R_F = 1/(1+k') \tag{2}$$

The plate height, H, is a measure of the dispersive capacity of the column and is determined largely by kinetic factors[11]. It is obtained from the elution chromatogram using eqn. 3

$$H = (L/16)(w_t/t_R)^2 \tag{3}$$

where L is the column length, w_t is the peak width at the base obtained by extrapolation of tangents to the points of inflexion of the peak to the base line, and t_R the retention time.

EXPERIMENTAL

Equipment

The high-speed liquid chromatograph was constructed in the laboratory. Degassed eluent passed through a 10-μ Nupro filter to a high-pressure Model DMP1515 piston pump (Orlita, Giessen, G.F.R.) and then past a Bourdon pressure gauge which served as a pulse damper to the column inlet. Columns were of heavy walled 2-mm-bore glass or stainless-steel tubing, 80 to 100 cm in length, fitted with heads for injection by syringe through a septum. The packing was retained by a 5-μ frit (stainless steel or PTFE). Columns were operated at room temperature. Eluted samples were detected by a variable wavelength Type CE212 UV monitor (Cecil Instruments, Cambridge, Great Britain) fitted with an 8-μl flow cell (Du Pont Co., Wilmington, Del., U.S.A.).

Columns were packed by the rotate, bounce and tap method[12] with Zipax SCX (strong cation-exchange) or Zipax SAX (strong anion-exchange) pellicular resins. Before use these materials were sieved to give a 37- to 44-μ cut.

Samples were identified after elution by their mass spectra using an AEI MS902 mass spectrometer (AEI Scientific Apparatus Ltd., Harlow, Great Britain), or by their UV spectra using a Model 402 spectrophotometer (Perkin-Elmer, Beaconsfield, Great Britain).

Materials

Morphine, thebaine, codeine, cryptopine, narcotine alkaloids, morphine sulphate and papaverine hydrochloride were gifted by Macfarlane Smith Ltd. (Edinburgh, Great Britain). Methadone hydrochloride B.P. and heroin hydrochloride B.P. were pharmaceutical preparations.

Preparation of solutions

Alkaloid salts were dissolved in water or methanol. Alkaloid bases were dissolved in methanol or weak sulphuric acid. Solutions of heroin were prepared immediately before they were required to avoid hydrolysis to 6-(O-acetyl)morphine[13]. Alcoholic solutions were more stable in this respect than aqueous solutions. A solution of 6-(O-acetyl)morphine was obtained by dissolving heroin hydrochloride in 0.02 *M* $NaOH/H_3BO_3$ buffer and heating briefly to 100°. The solution so obtained also contained a small proportion of morphine.

RESULTS

1. General elution patterns

Morphine, 6-(O-acetyl)morphine and heroin were eluted in order from Zipax SCX using $NaOH/H_3BO_3$ buffer solutions with pH values from 9.2 to 10.0 and ionic strengths from 0.02 to 0.2 *M*. In general, elution was accelerated by increase of pH and ionic strength and by the addition of organic components to the mobile phase. Morphine could be eluted in a few minutes by buffers of ionic strength between 0.02 and 0.2 *M*; 6-(O-acetyl)morphine could be eluted close to morphine with a buffer of ionic strength 0.2 *M* at pH around 9.8, but heroin could be eluted in a reasonable time only when 5–15% of acetonitrile was added to the mobile phase.

On Zipax SAX, morphine, heroin and 6-(O-acetyl)morphine were not retained with an acid buffer. They were slightly retained with an alkaline buffer, but not sufficiently for adequate resolution. Methadone, on the other hand, was reasonably strongly retained with a $NaOH/H_3BO_3$ buffer of ionic strength between 0.1 and 0.2 *M*, and pH around 8.7, but the peaks were broad and the plate height poor.

2. Effect of pH and ionic strength on k' and H

Elution from Zipax SCX. The capacity ratio, *k'*, was measured as a function of pH and ionic strength for morphine and 6-(O-acetyl)morphine eluted by aqueous $NaOH/H_3BO_3$ buffers, and for heroin eluted by buffers containing acetonitrile. The results are summarised in Figs. 1 and 2. Log *k'* fell linearly with pH and *k'* was inversely related to ionic strength. The pH dependence is given by eqns. 4–6.

Morphine:	$\log k' = 14.4 - 1.5 \times pH$	(4)
6-(O-Acetyl)morphine:	$\log k' = 10.4 - 1.0 \times pH$	(5)
Heroin:	$\log k' = 6.4 - 0.6 \times pH$	(6)

The plate height, *H*, showed a different response to changes in pH for the three alkaloids: for morphine *H* decreased, for 6-(O-acetyl)morphine *H* increased (Fig. 3), while for heroin *H* was unaffected by increase of pH. Changes in ionic strength had

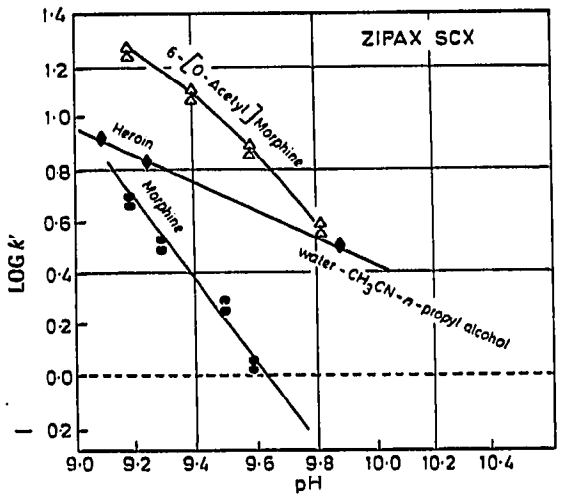


Fig. 1. Effect of pH on k' for morphine alkaloids (morphine, 6-(O-acetyl)morphine and heroin). Packing: 1 m column, Zipax SCX 37–44 μ. Eluent: for morphine and 6-(O-acetyl)morphine, 0.1 M NaOH with H_3BO_3 added to pH required; for heroin, 0.2 M NaOH with H_3BO_3 added to pH required + 0.1 M KNO_3 in water–acetonitrile–n-propanol (85:14:1, v/v).

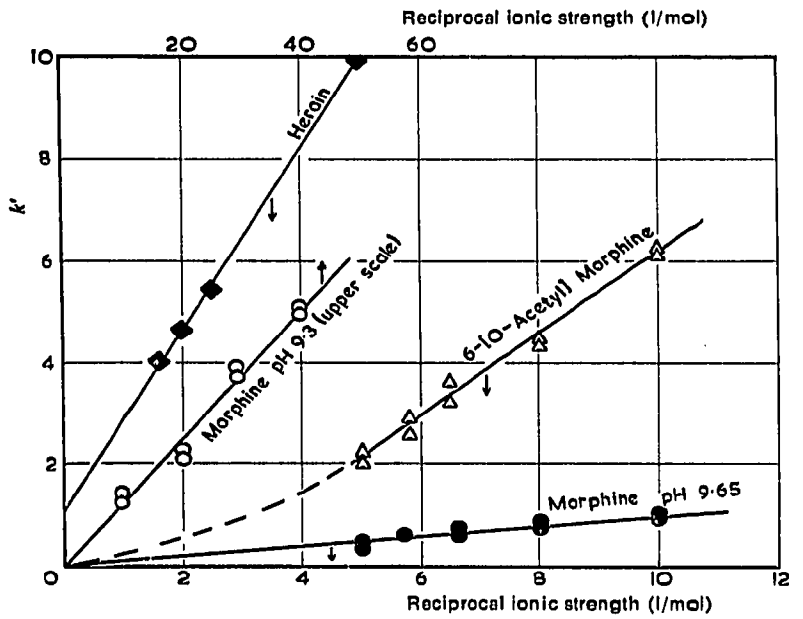


Fig. 2. Dependence of k' on reciprocal of ionic strength for morphine alkaloids. Eluent: for morphine, pH 9.35, NaOH + H_3BO_3, concentration adjusted to give required ionic strength (lower scale); for morphine and 6-(O-acetyl)morphine, pH 9.65, 0.1 M NaOH with H_3BO_3 added to required pH, and KNO_3 added to give required ionic strength (lower scale); for heroin, 0.2 M NaOH with H_3BO_3 added to pH 10.2 and KNO_3 added to give required ionic strength (upper scale) in water–acetonitrile–n-propanol (94.5:4.5:1). Column as for Fig. 1.

no significant effect on *H*. The values of *H* for unretained solutes were generally small (in the region of 0.3 mm), but those for retained solutes were very much higher (1 to 3 mm). In this respect Zipax ion exchangers contrast with Zipax when used in liquid–liquid partition chromatography, where the plate heights for retained and unretained solutes differ by factors of not more than two[14,15].

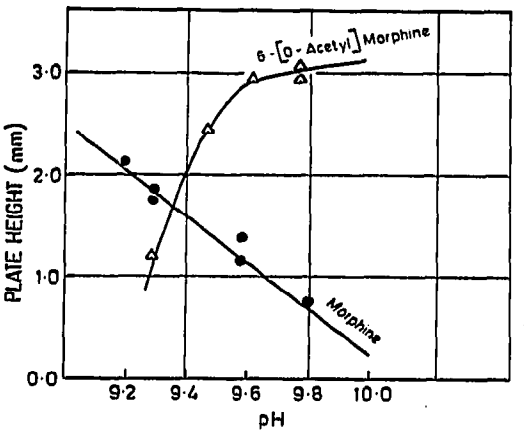


Fig. 3. Effect of pH on plate height, *H*. Conditions as for Fig. 1.

Elution from Zipax SAX. Fig. 4 shows the dependence of k' for methadone, morphine and 6-(O-acetyl)morphine on pH and ionic strength. The small values of k' for the latter two components and the small differences between them emphasize the difficulty of separating them by anion-exchange chromatography. By contrast methadone was well retained by Zipax SAX at pH between 8.4 and 9.6, with log k' increasing roughly linearly with pH, the gradient being about 0.3 (*cf.* eqns. 4–6). At low pH the rate of elution of methadone was roughly halved by increase in the ionic strength from 0.1 to 0.15 *M*. While Zipax SAX cannot be used for the separation of morphine and its acetylated derivatives from each other, it can be used for their group separation from methadone.

3. Addition of organic components to eluent

Addition of organic components to the mobile phase was beneficial only with Zipax SCX resin. The results obtained with methanol, *n*-propanol, and acetonitrile are summarised in Table I. 1–2% of propanol added to aqueous buffers reduced both k' and *H* more effectively than acetonitrile or methanol in equivalent concentration but could not be used in concentrations exceeding 3% without damaging the resin. By contrast acetonitrile could be added in concentrations as high as 15% without ill effects. For this reason concentrated solutions of acetonitrile were preferred when it was desired to increase the rate of elution of heroin.

4. Optimum conditions for the quantitation of morphine alkaloids and methadone

As shown by the results presented in paragraphs 2 and 3, the analysis of morphine requires different chromatographic conditions from those for the analysis of morphine in the presence of heroin and 6-(O-acetyl)morphine. The conditions selected

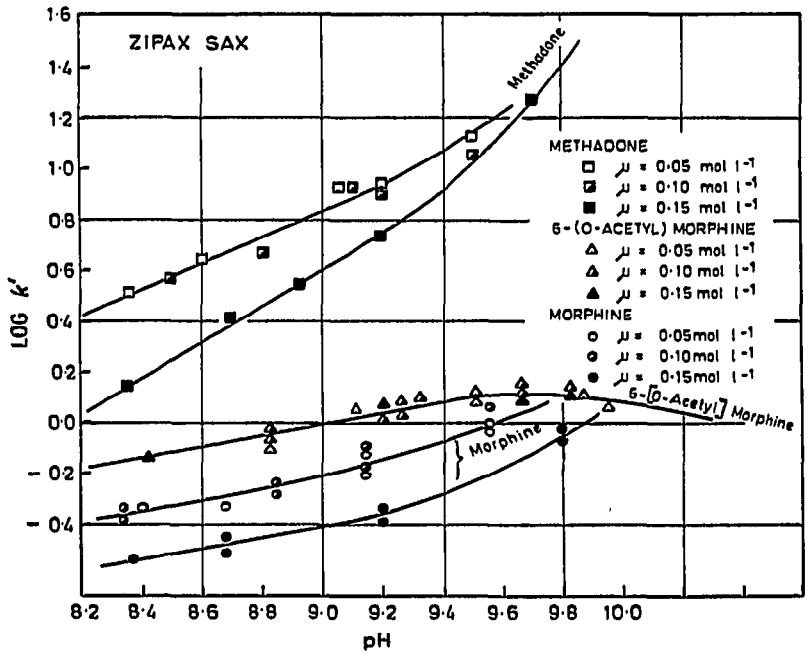


Fig. 4. Effect of pH and ionic strength for elution from Zipax SAX. Eluent: NaOH with H_3BO_3 added to give required pH and ionic strength.

TABLE I

EFFECT OF ADDITION OF ORGANIC COMPONENTS TO ELUENT ON k' AND H FOR ZIPAX SCX

The changes in k' and H for heroin are those occurring when the content of acetonitrile is increased from 4.5% v/v to the values given. k'^- and H^- are the values of k' and H with no organic additive present (morphine and 6-(O-acetyl)morphine) and with 4.5% acetonitrile present (heroin).

Compound	*Organic additive (% v/v)*	k'/k'^-	H/H^-	*No. of observations*
Morphine	Methanol 5%	0.68–0.70	0.78–0.82	5
	Methanol 7%	0.50–0.55	0.69–0.80	3
	n-Propanol 1–2%	0.29–0.44	0.52–0.68	7
	Acetonitrile 1–2%	0.68–0.74	0.82–0.91	4
6-(O-Acetyl)morphine	*n*-Propanol 1–2%	0.40–0.52	0.45–0.54	6
	Acetonitrile 1%	0.40–0.45	0.95	3
Heroin	Acetonitrile 5.5%	0.75	0.78	2
	Acetonitrile 10%	0.18	0.90	2
	Acetonitrile 15%	0.12	0.88	2

for the analysis of morphine also depend upon how much unretained material is present in the specimen. When the proportion is large, elution of morphine must be retarded and a pH of between 9.3 and 9.6 is recommended with an ionic strength

below 0.1 *M*. When the proportion is small, elution can be accelerated to obtain the highest sensitivity of detection by using a buffer of pH around 9.8 and an ionic strength around 0.15 *M*. When accurate quantitation is required pyridine or γ-picoline can be used as internal standard, as shown in Fig. 5A.

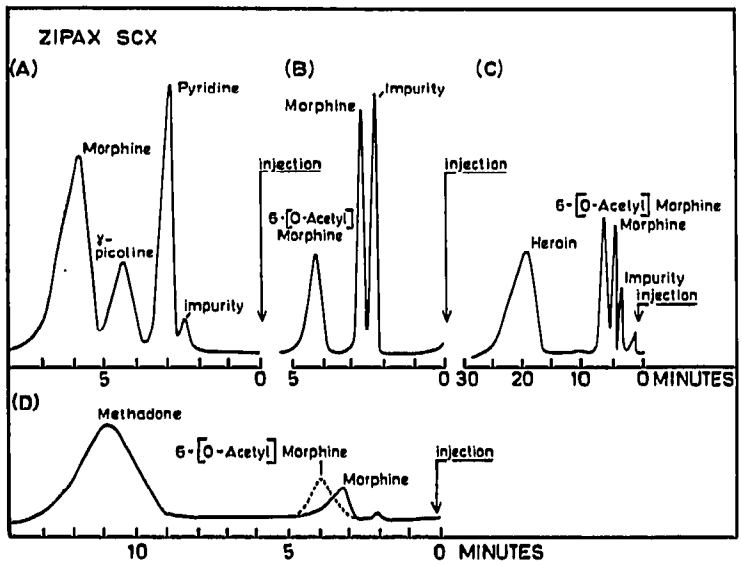


Fig. 5. High-speed liquid chromatograms on 1-m columns containing 37–44 μ Zipax SCX (A, B, C) or SAX (D). Detector: variable-wavelength UV photometer. Eluents: NaOH + H_3BO_3 buffers. Details of elution conditions are given below.

	Molarity of NaOH/pH	*W–A–P**	*Pressure (p.s.i.g.)*	*Flow-rate (cm/sec)*	*Detection wavelength (nm)*	*Detector sensitivity (AUFS)***
A	0.08/9.5	100:0:0	800	0.62	250	0.1
B	0.15/9.8	98:2:0	1100	0.82	230	0.3
C	0.04/9.3	86:12:2	1600	0.44	280	0.1
D	0.15/8.8	100:0:0	500	0.72	280	0.1

* W–A–P = ratio water–acetonitrile–*n*-propanol by volume.
** AUFS = Absorbance units full-scale deflection.

Heroin may be determined directly using a buffer containing 8–12% of acetonitrile, but under these conditions morphine and 6-(O-acetyl)morphine are not retained unless a buffer of very low ionic strength and pH is used. Conditions under which heroin can be quantitated together with 6-(O-acetyl)morphine are shown in Fig. 5C. Alternatively heroin may be completely hydrolyzed to 6-(O-acetyl)morphine by heating to 100° in an alkaline aqueous buffer for a few minutes. The optimum conditions are then a buffer of pH 9.9 and an ionic strength of 0.2 *M* with no addition of organic component; a typical chromatogram is shown in Fig. 5B.

Methadone may be quantitated using Zipax SAX. The optimum eluent is then a buffer of pH 8.6 and ionic strength 0.2 *M* (Fig. 5D). With buffers of lower pH, elution is faster but the peaks are badly tailed.

TABLE II

MASS SPECTRA FOR MORPHINE ALKALOIDS AND METHADONE

A=Alkaloid salt; B=alkaloid base extracted by chloroform from buffered solution of salt; C=alkaloid base extracted by chloroform from eluent obtained from chromatographic column.

Compound		*Mass Nos. of fragments*																			
		370	*369*	*328*	*327*	*310*	*286*	*285*	*284*	*268*	*215*	*204*	*174*	*162*	*124*	*94*	*81*	*70*	*55*	*43*	*42*
		Percentage of fragment ions relative to base intensity																			
Heroin	A	19	95	19	100	33	–	1	–	26	14	21	–	–	9	5	9	9	–	23	–
$C_{21}H_{23}NO_5$	B	25	100	19	95	37	–	5	–	25	13	19	–	–	8	1	25	10	–	37	–
Mol. wt.=369	C	26	100	21	94	35	–	6	–	25	15	21	–	–	9	6	26	18	–	42	–
6-(O-Acetyl)morphine	A	data not obtained																			
$C_{19}H_{21}NO_4$	B			20	10	–	–	15	–	45	18	9	–	–	9	4	6	4	–	13	–
Mol. wt.=327	C			19	100	–	–	5	–	46	19	8	–	–	11	5	11	11	–	26	–
Morphine	A						17	100	12	12	17	–	12	23	17	–	–	14	1	–	17
$C_{17}H_{19}NO_3$	B						19	100	14	10	17	–	17	19	12	–	–	10	10	–	35
Mol. wt.=285	C						19	100	14	12	20	–	8	19	16	–	–	24	12	–	15

		Mass Nos. of fragments									
		309	*294*	*265*	*223*	*208*	*165*	*115*	*91*	*85*	*72*
		Percentage of fragment ions relative to base intensity									
Methadone	A	3	4	1	2	1	2	1	2	2	100
$C_{21}H_{27}NO$	B	2	2	1	1	1	3	1	3	2	100
Mol. wt.=309	C	2	3	1	4	1	2	2	3	4	100

5. Identification of eluted solutes by mass spectrometry

The mass spectra of the bases morphine, 6-(O-acetyl)morphine, heroin and methadone, and of the salts morphine sulphate, heroin hydrochloride and methadone hydrochloride were determined using an AEI MS902 mass spectrometer. The organic bases were extracted with chloroform from solutions of their salts in the same buffers as were used for elution in chromatography. Solutions for mass spectrometric identification were first concentrated by evaporation of solvent in a stream of nitrogen and then taken up on a sintered mass spectrometer probe where the last traces of solvent were evaporated in the air before insertion of the probe into the mass spectrometer source. Bases in eluted bands were also extracted with chloroform in the same way. The data are presented in Table II, and, apart from small discrepancies in the proportions of minor peaks, the mass spectra of the salts, and of the bases extracted either from salt solutions or from the chromatographic eluents are identical. The identities of the chromatographic bands are thereby established without doubt.

For the morphine alkaloids the base peaks (*i.e.* the most abundant ion peaks) arise from the parent ion. The peaks at the parent mass + 1 arise from alkaloid molecules containing one atom of ^{13}C (1.1% of all C atoms are ^{13}C). The major fragments from heroin at mass numbers 327, 310 and 268 arise by loss of $COCH_2$, $OCOCH_3$, $COCH_2 + OCOCH_3$, respectively (see formula I), and those with mass numbers 215 and 204 arise from ring cleavage followed by loss of peripheral groups. The fragments arising from morphine and 6-(O-acetyl)morphine are essentially the same as those from heroin after allowing for the differences in the number of acetyl groups initially present. The parent ion of methadone (II) is formed to the extent of only 3%, and the base peak at mass number 72 arises from the fragment $CH_3CHN(CH_3)_2$. Ions of mass numbers 294, 265 and 223 arise from the loss of CH_3, $N(CH_3)_2$, and $CH_2–CH(CH_3)–N(CH_3)_2$, respectively.

6. Identification by UV spectroscopy

The spectra of bases eluted from the column were obtained and compared with those of pure samples using a Perkin-Elmer Model 402 spectrophotometer. In addition the sensitivity of response of the chromatograph to the different alkaloids was determined at various wavelengths. Fig. 6 shows the excellent agreement obtained for the peak identified from its elution time as morphine. The UV absorption maximum occurs for morphine at 215 nm but, because of the increasing noise from the detector and the onset of absorbance by the buffer itself, the highest sensitivity relative to noise was attained at around 230 nm, being nearly three times higher than at the wavelength 254 nm commonly used in single-wavelength UV photometers for HSLC.

Heroin, as already noted, undergoes rapid hydrolysis to 6-(O-acetyl)morphine[13] and it was important to check whether or not hydrolysis occurred on the chromatographic column. Fig. 7 shows the changes which occur in the UV spectrum of heroin on standing in a buffered solution at room temperature. Rapid hydrolysis occurs initially and is complete in about 4 h. Heroin shows an absorption maximum at about 275 nm and a minimum at about 250 nm, whereas 6-(O-acetyl)morphine shows a shallow minimum at 275 nm and a maximum at 295 nm. Measurements of the sensitivity of detection as a function of wavelength for the peaks identified from their elution times as heroin and 6-(O-acetyl)morphine are shown in the lower part of Fig. 7. The changes in sensitivity with wavelength confirm that the eluted bands were

in fact unhydrolysed heroin and 6-(O-acetyl)morphine, respectively. Hydrolysis does not therefore occur during the process of chromatography. As with morphine, maximum absorption occurs at wavelengths just above 200 nm, but the optimum wavelength for quantitation is around 230 nm.

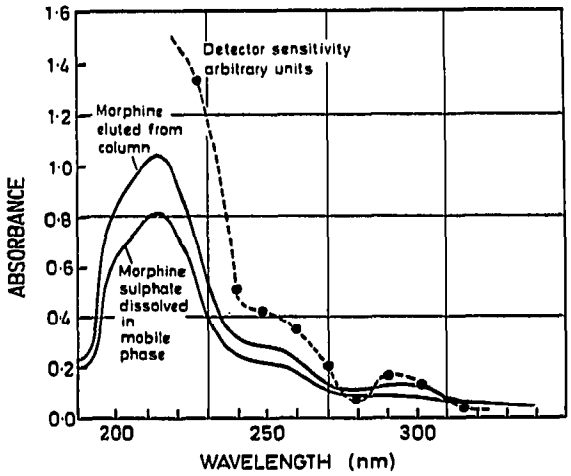


Fig. 6. UV absorption of morphine sulphate at two concentrations, and of morphine eluted from Zipax SCX (———), and detector sensitivity (●---●) as functions of wavelength. Elution conditions as for Fig. 5A.

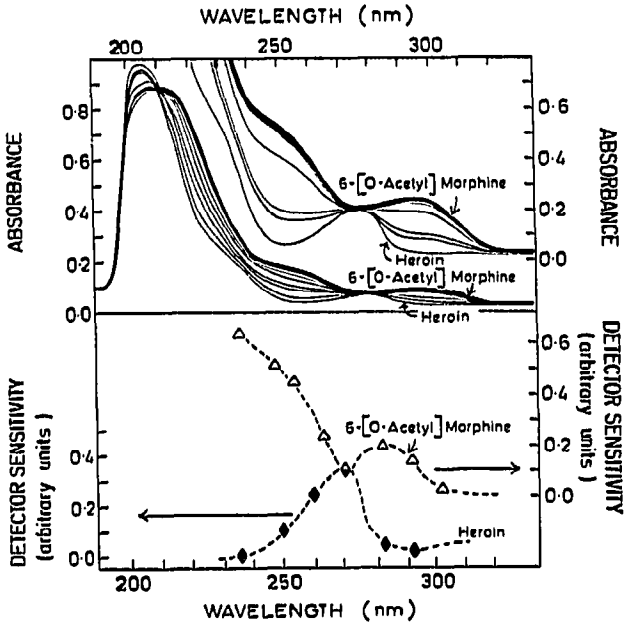


Fig. 7. Changes in the UV spectrum of heroin dissolved in mobile phase at two concentrations (upper) on standing over a 5-h period, illustrating hydrolysis reactions. Detector sensitivity (lower) for heroin (◆---◆) and 6-(O-acetyl)morphine (△---△). Chromatographic conditions as for Fig. 5B but with pH 9.9.

Methadone was likewise identified by comparison of the absorption spectrum of eluate thought to contain methadone and that of authentic methadone.

7. Quantitative analysis

Fig. 8 shows the linear correlation between the quantity injected and the peak area (for morphine, 6-(O-acetyl)morphine and heroin) and the peak height (for methadone). The detection limit for accurate quantitation depends upon the degree of retention; the more a compound is retained the higher the detection limit. For morphine the most reliable results were obtained with samples in the region of 1–5 μg, when k' was around unity, and from 60–300 ng, when k' was around 0.5.

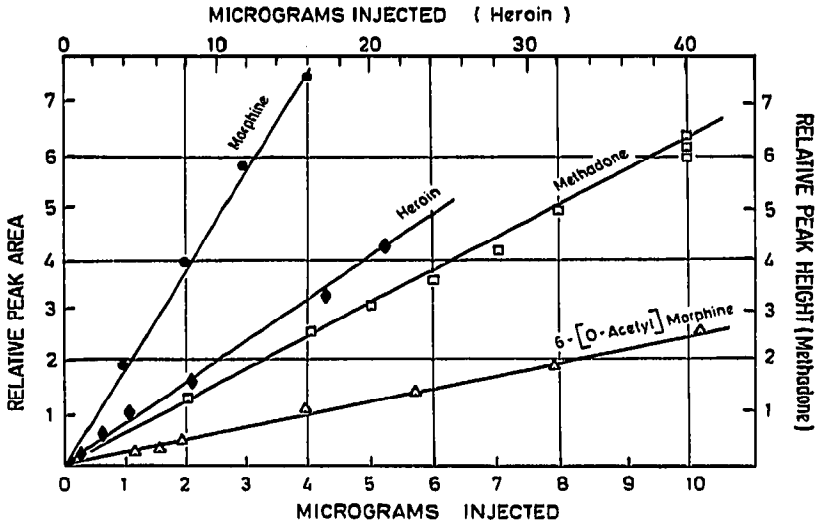


Fig. 8. Correlation between peak area and mass injected for morphine alkaloids and between peak height and mass injected for methadone. Elution conditions as for Fig. 5A (morphine), Fig. 5B (6-(O-acetyl)morphine), Fig. 1 with pH 10.2 (heroin), and Fig. 5D (methadone).

The use of γ-picoline as internal standard for morphine gave increased quantitative accuracy, and using 250-nm radiation the calibration factor F in eqn. 7 was between 1.00 and 1.15, depending upon the precise chromatographic conditions used.

$$\frac{\text{Weight of morphine}}{\text{Weight of } \gamma\text{-picoline}} = F \times \frac{\text{Peak area of morphine}}{\text{Peak area of } \gamma\text{-picoline}} \qquad (7)$$

8. Interfering alkaloids

Alkaloids likely to be present in natural sources of morphine are papaverine, thebaine, narcotine, cryptopine, and codeine. Using a buffer of pH 9.6 and ionic strength 0.1 M, all five alkaloids were shown to be strongly retained by Zipax SCX. They therefore do not interfere with the analysis of morphine or 6-(O-acetyl)morphine. Codeine, the first of these alkaloids to be eluted, has k' in the range 2 to 3 using a buffer of pH 11 to 12 and an ionic strength of 0.2 M.

DISCUSSION

Retention of solutes in ion-exchange chromatography depends strongly upon

the composition of the eluent; it can be altered by changes in ionic strength, nature of the counter ion, pH, presence of complexing agents and the addition of organic components[16,17]. These effects can be understood qualitatively at least in terms of various equilibria, in particular the ion-exchange equilibrium (reaction (i) below), the acid or base dissociation equilibrium (reaction (ii) below), complex formation and the sorption–desorption equilibria for unionised molecules. The kinetics of ion exchange which govern the plate height depend strongly upon the charge on the species being equilibrated, upon the molecular or ionic size and upon the degree of crosslinking, or more precisely the size of reticulation within the resin.

In the present work it has been shown that increase of ionic strength and pH, and the addition of organic solutes strongly decrease retention: the plate heights are much higher than those observed in liquid–liquid partition chromatography and also depend upon the eluent composition. These features are generally explicable as follows.

The ion-exchange equilibrium for an exchange of a base ion BH^+ with a strong cation-exchange resin in the sodium form can be represented by reaction (i) in which barred symbols refer to ions in the resin phase.

$$\overline{Na^+} + BH^+ \rightleftharpoons Na^+ + \overline{BH^+} \qquad \text{(i)}$$

This leads to eqn. 8 for the column capacity ratio of BH^+, the capacity ratio being the ratio of the quantity of BH^+ in the stationary phase (resin) to the quantity in the mobile phase[18,19].

$$k'_{BH^+} = \frac{v_r[\overline{BH^+}]}{v_m[BH^+]} = K_{IE}\frac{v_r}{v_m}\frac{\alpha_{\overline{Na^+}}}{\alpha_{Na^+}} \qquad (8)$$

In eqn. 8 v_r and v_m are the volumes of the resin and eluent in the column, respectively; K_{IE} is the equilibrium constant for the ion-exchange reaction (i); and $\alpha_{\overline{Na^+}}$ and α_{Na^+} are the sodium ion activities in the resin and eluent.

Since $\alpha_{\overline{Na^+}}$ is more or less unaffected by α_{Na^+} because of the Donnan effect, k'_{BH^+} to a first approximation is inversely proportional to $[Na^+]$, that is

$$k'_{BH^+} = C_{IE}/[Na^+] \qquad (9)$$

where C_{IE} is a constant governed by the equilibrium constant for ion exchange, the relative volume of the resin and eluent, and the structure of the resin.

When the mechanism of retention is pure ion exchange, k' should be inversely proportional to ionic strength. This is roughly true for the morphine alkaloids, as shown in Fig. 2. The intercept for heroin (considered later) may be explained by adsorption of unionised species. The curvature for 6-(O-acetyl)morphine is not simply explained.

The retention of a weak base (or acid) by ion exchange will be strongly influenced by pH if the base is weakly ionised (reaction ii)

$$B + H_2O \rightleftharpoons BH^+ + OH^- \qquad \text{(ii)}$$

for the fraction of the base in the BH^+ form which is sorbed by ion exchange is then given by eqn. 10

$$\frac{[\overline{BH^+}]}{[B]+[BH^+]} \approx \frac{[\overline{BH^+}]}{[B]} = \frac{K_{IE}K_b\alpha_{\overline{Na^+}}}{K_w[Na^+]\,[H^+]} \tag{10}$$

K_b being the base ionisation constant and K_w the ionic product of water.

For molecules of high molecular weight the undissociated base B may also be sorbed by the resin according to reaction (iii)

$$B \rightleftharpoons \bar{B} \tag{iii}$$

thus

$$\frac{[\bar{B}]}{[B]} = K_s \tag{11}$$

The capacity ratio for partition of the base as a whole is then given by eqn. 12.

$$k'_B = \frac{v_s}{v_m}\frac{\{[\bar{B}]+[\overline{BH^+}]\}}{\{[B]+[BH^+]\}} = \frac{v_s}{v_m}\frac{[\bar{B}]+[\overline{BH^+}]}{[B]} = \frac{v_s}{v_m}\left\{K_s + \frac{K_{IE}K_b\alpha_{\overline{Na^+}}}{K_w[Na^+]\,[H^+]}\right\} \tag{12}$$

We therefore expect in general that a plot of k'_{base} against $1/[Na^+]$ should be a straight line with an intercept corresponding to K_s. The curve in Fig. 2 for heroin shows such an intercept giving $K_s=1.0$. Morphine shows no intercept and is therefore unsorbed in the molecular form. 6-(O-Acetyl)morphine shows a non-linear dependence, which is not readily explained on the above theory.

When adsorption is unimportant log k' should fall linearly with pH with unit gradient. Fig. 1 and eqns. 4–6 indeed show a more or less linear dependence but with gradients of 1.5, 1.0 and 0.6, respectively, for morphine, 6-(O-acetyl)morphine and heroin. The low gradient for heroin is in accord with the fact that adsorption of the unionised base contributes somewhat to retention. The high gradient for morphine is not readily explained.

The order of elution, morphine, 6-(O-acetyl)morphine, heroin is in order of decreasing acidity but at pH$\approx$9.4 the phenolic groups of the alkaloids will be essentially unionised and therefore unlikely to influence retention by any ion-exchange effect. It is most probable that with progressive removal of hydroxyl groups the unionised bases become less soluble in water and interact more strongly with the resin structure (see the adsorption of heroin) and at the same time K_{IE} increases due to greater interaction of the ionised base with the structure. Thus both K_{IE} and K_s are increased by replacement of the hydroxyl group by $-OCOCH_3$.

The addition of organic components to the eluent can in principle either increase or decrease retention. In general organic solutes such as isopropanol and acetonitrile will be preferentially sorbed by the resin phase[16] and therefore ions will tend to be rejected by the resin although undissociated base will tend to be more strongly sorbed. In the present instance, where ion exchange appears to be the predominant retentive mechanism, sorption of organic components by the resin is expected to reduce retention in agreement with our results. However, increase of sorption of unionised molecules may be expected as found for heroin in the presence of acetonitrile.

The retention of the alkaloids on Zipax SAX is relatively little affected by pH or ionic strength and the mechanism here is almost certainly adsorption.

The kinetics of the ion-exchange process using Zipax ion exchangers may be considered by examining the plate heights for the alkaloids. At a velocity of around 1 cm/sec the plate heights observed were 1–3 mm. For 6-(O-acetyl)morphine H rose from 1.2 to 3.0 mm as the pH changed from 9.2 to 9.8 and k' changed from about 18 to 4. For morphine H fell from 2 mm to 0.7 mm when k' fell from about 4 to unity.

These values compare with values obtained by Kirkland[8] of about 1.5 mm for 2-aminobenzimidazole ($k'=2.9$) and are of the same order. They are, however, very much higher than the values of around 0.25 mm obtainable in liquid–liquid partition chromatography and with unretained solutes on the ion exchangers (see the early peaks in the chromatograms of Fig. 7). There are two likely reasons for this difference in performance. Firstly, large molecules are to a considerable extent excluded from parts of the resin structure because of the smallness of the pores in the organic polymer[16]. Secondly, counter-ions within any ion-exchange resin, even if small, are much less mobile than in a solution as established by Hamilton[20] for amino acid cations and elsewhere for inorganic ions[16]. Both these effects combine to reduce the rate of mass transfer of large ions such as those of the alkaloids and lead to inefficient chromatography and large values of H.

In general, H is expected from chromatographic theory[19] to pass through a maximum at k' near or just above unity. The fall in H for 6-(O-acetyl)morphine as k' increases from 4 to 18 appears to agree with this prediction but the rise for morphine as k' rises from 1 to 4 cannot be readily explained, for, over this range, H might be expected to remain more or less constant. The explanation of how H depends upon eluent compositions and upon k' is still unclear.

Organic components added to the eluent reduce H and this may be due to the reduction in the dielectric constant causing ion pair formation which will allow higher mobility of the bases within the resin.

REFERENCES

1 M. W. Anders and G. J. Mannering, *Anal. Chem.*, 34 (1962) 730.
2 G. R. Wilkinson and E. L. Way, *Biochem. Pharmacol.*, 18 (1969) 1435.
3 E. Brochman-Hanssen and A. B. Swensden, *J. Pharm. Sci.*, 52 (1963) 1134.
4 P. DeZaan and J. Fasanello, *J. Chromatogr. Sci.*, 10 (1972) 333.
5 R. Hakim and J. M. Fujimoto, *J. Pharm. Sci.*, 59 (1970) 1783.
6 J. J. Kirkland (Editor), *Modern Practice of Liquid Chromatography*, Wiley, New York, 1971.
7 J. N. Done, G. J. Kennedy and J. H. Knox, *Nature (London)*, 237 (1972) 77.
8 J. J. Kirkland, *J. Chromatogr. Sci.*, 7 (1969) 361.
9 M. W. Anders and J. P. Latorre, *Anal. Chem.*, 42 (1970) 1430.
10 M. W. Anders and J. P. Latorre, *J. Chromatogr.*, 55 (1971) 409.
11 J. H. Knox, *Ann. Rev. Phys. Chem.*, 24 (1973) 1.
12 G. J. Kennedy and J. H. Knox, *J. Chromatogr. Sci.*, 10 (1972) 549.
13 E. G. Clark (Editor), *Isolation and Identification of Drugs in Pharmaceuticals, Body Fluids and Post-mortem Material*, Pharmaceutical Press, London, 1969, p. 292.
14 J. N. Done and J. H. Knox, *J. Chromatogr. Sci.*, 10 (1972) 606.
15 J. J. Kirkland, *J. Chromatogr. Sci.*, 10 (1972) 129.
16 F. Helferich, *Ion Exchange*, McGraw-Hill, New York, p. 1062; *Advan. Chromatogr.*, 1 (1965) 3.
17 O. Samuelson, *Ion-Exchange Methods in Analytical Chemistry*, Wiley, New York, 1963.
18 A. J. P. Martin and R. L. M. Synge, *Biochem. J.*, 35 (1941) 1358.
19 J. C. Giddings, *Dynamics of Chromatography*, Part 1, Marcel Dekker, New York, 1965.
20 P. B. Hamilton, D. C. Bogue and R. A. Anderson, *Anal. Chem.*, 32 (1960) 1782.