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ISOLATION, SEPARATION AND DETECTION VIA HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF ACIDIC AND NEUTRAL ACETYLATED REARRANGEMENT PRODUCTS OF OPIUM ALKALOIDS

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SUMMARY

The isolation, separation and detection of acidic and neutral acetylated rearrangement products of opium alkaloids, which are major sources of heroin processing impurities, are described. Preparative reversed-phase high-performance liquid chromatography with the aid of a micro-computer has been utilized for the isolation of products obtained from the action of acetic anhydride on thebaine and noscapine alkaloids. Results obtained show that a high-speed C_{18} analytical column (3 μm particle size, 3.0 cm × 4.6 mm I.D.) containing a monomeric bonded phase can be used to develop separations for a polymeric bonded-phase preparative column (10 μm particle size, 25 cm × 22 mm I.D.).

An optimized reversed-phase separation was developed for the acetylated thebaine and noscapine compounds and for acetylated rearrangement products from three other opium alkaloids: morphine, codeine and norlaudanosine.

Ultraviolet, fluorescence and electrochemical detection were compared for these compounds. A detection system optimized in terms of sensitivity and selectivity was developed utilizing two variable-wavelength UV detectors, programmable fluorescence detector, and an electrochemical detector in series.

INTRODUCTION

Chromatograms of trace impurities found in heroin can be important for intelligence purposes and can form the basis for comparative analysis of exhibits. Impurities in heroin can be basic, acidic or neutral. Acidic and neutral acetylated rearrangement products, which arise from the acetylation of opium alkaloids present in crude morphine, are generally found at levels below 0.5%[1].

Capillary gas chromatographic methodology using flame ionization detection, nitrogen–phosphorus detection, electron-impact mass spectrometry and electron-capture detection has been reported for the detection of underivatized and derivatized extracts of acidic and neutral acetylated rearrangement products arising from heroin[2–4]. Reversed-phase high-performance liquid chromatography (HPLC) with UV detection has been employed for the determination of the neutral compounds ace-

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tylthebaol and 3,6-dimethoxy-4,5-epoxyphenanthrene, which arise from the acetylation of thebaine[5]. These impurities plus thebaol have been determined by means of normal-phase HPLC with fluorescence detection[6].

This paper reports analytical and preparative separations prerequisite for the identification of acidic and neutral acetylated rearrangement products arising from the reaction of the opium alkaloids noscapine and thebaine with acetic anhydride[3,7]. Many of these impurities have been identified in heroin exhibits[2–6]. In addition, an optimized high-speed reversed-phase chromatographic system is described that separates 13 compounds isolated from thebaine-noscapine reaction mixtures plus 7 compounds arising from the acetylation of the opium alkaloids morphine, codeine, thebaine and norlaudanosine. Ultraviolet, fluorescence and electrochemical detectors are used in series for the sensitive and selective determination of the above compounds.

EXPERIMENTAL

Materials

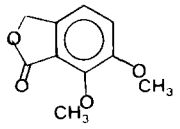
Acetonitrile, methanol and tetrahydrofuran were from Burdick & Jackson (distilled in glass). Other chemicals were reagent grade. The acetylated products of thebaine and noscapine were prepared as previously reported[3,7] except for a noscapine reaction mixture prepared as follows: 10 g of noscapine, 150 ml of acetic anhydride and 5 g of sodium acetate were refluxed for 18 h. After excess acetic anhydride was removed via rotary evaporation, the residue was dissolved in 200 ml of diethyl ether–methylene chloride (60:40) plus 100 ml chloroform. The resulting solution was extracted sequentially against 1 *M* sodium carbonate, 2 *M* sulfuric acid, and 1 *M* sodium carbonate, then evaporated to dryness. Because a shorter reaction time was employed, this extract differs from the one previously reported[7] in that it contains a higher percentage of noscapine and a lower percentage of the compounds of interest.

Standard compounds used in the analytical HPLC studies were synthesized in our laboratory, several with the aid of the preparative HPLC method described in this report. Proton magnetic resonance, mass spectroscopy, elemental analysis, and melting point were used to confirm the identity of standard compounds. Structures of the various compounds discussed are presented in Table I.

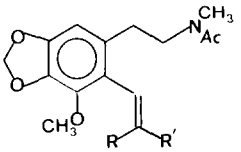
For preparative chromatography, the mobile phases consisted of premixed combinations of organic solvents and phosphate buffer or water. The phosphate buffer consisted of 870 parts water, 30 parts 2 *M* sodium hydroxide and 10 parts phosphoric acid. The mobile phase was adjusted to pH 2.2 with 2 *M* sodium hydroxide. All extracts from reaction mixtures were dissolved in mobile phase prior to chromatography. For analytical HPLC, the mobile phases for the Waters liquid chromatograph consisted of premixed mobile phases as described above except 7 ml hexylamine was added to 870 ml of phosphate buffer, and the final pH was adjusted to 2.2 with 2 *M* sodium hydroxide. For the Series 4 liquid chromatograph the mobile phases were mixed internally from solvent reservoirs containing: methanol, acetonitrile, tetrahydrofuran and either water or an amine–phosphate buffer.

TABLE I

SOME ACIDIC AND NEUTRAL ACETYLATED REARRANGEMENT PRODUCTS OF OPIUM ALKALOIDS



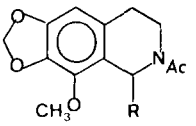
1.



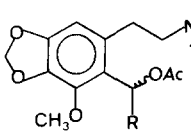
2. R=H, R'=COOH

6. "E", R-R'=1.

7. "Z", R-R'=1.

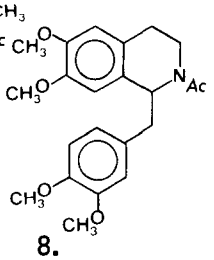


3. R=1.

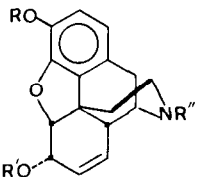


4 erythro, R=1.

5 threo, R=1.



8.



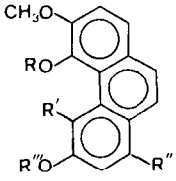
9 R,R'=H, R"=Ac

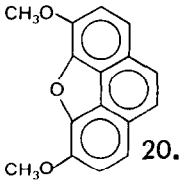
10 R=H, R'R"=Ac

11 R,R'R"=Ac

12 R=CH_3, R'=H, R"=Ac

13 R=CH_3 R'R"=Ac





20.

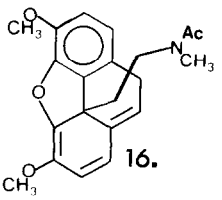
14 R=Ac, R'=$CH_2CH_2NAcCH_3$, R"=H, R'"=CH_3

15 R=Ac, R'=H, R"=$CH_2CH_2NAcCH_3$ R'"=CH_3

17 R=Ac, R'R"=H, R'"=Ac

18 R=H, R'R"=H, R'"=CH_3

19 R=Ac, R'R"=H, R'"=CH_3



16.

Compound	*Name*
1	Meconin
2	3-{2-[2-(N-methylacetamido)-ethyl]}-4,5-methylenedioxy-6-methoxyphenylacrylic acid
3	N-Acetylnornarcotine
4	*erythro*-1-Acetyloxy-N-acetylanhydro-1,9-dihydronornarceine
5	*threo*-1-Acetyloxy-N-acetylanhydro-1,9-dihydronornarceine
6	*cis*-N-Acetylanhydronornarceine
7	*trans*-N-Acetylanhydronornarceine
8	N-Acetylnorlaudanoisine
9	N-Acetylnormorphine
10	N,6-Diacetylnormorphine
11	N,3,6-Triacetylnormorphine
12	N-Acetylnorcodeine
13	N,6-Diacetylnorcodeine
14	3,6-Dimethoxy-4-acetyloxy-5-[2-(N-methylacetamido)]-ethylphenanthrene
15	3,6-Dimethoxy-4-acetyloxy-8-[2-(N-methylacetamido)]-ethylphenanthrene
16	$\Delta^{5,7,9(14)}$-N-Acetyldesthebaine
17	3-Methoxy-4,6-diacetyloxyphenanthrene
18	Thebaol
19	Acetylthebaol
20	3,6-Dimethoxy-4,5-epoxyphenanthrene

TABLE II

COMPARISON OF PHYSICAL PARAMETERS OF ANALYTICAL COLUMNS

Data obtained from Perkin-Elmer promotional literature.

Column	*HS-5 C_{18}*	*3 × 3 C_{18}*	*HS-5 C_{18}*
Column dimensions			
I.D. (mm)	4.6	4.6	4.6
Length (cm)	12.5	3.3	10.0
Typical column efficiency (Theoretical plates)	10,000	5000	14,800
Bonded surface	Polymeric	Monomeric	Monomeric
Carbon loading (%)	10	12	12
End capping	Yes	Yes	Yes
Average pore diameter (Å)	60	80	80
Particle shape	Irregular	Spherical	Spherical

Instruments

Two liquid chromatographs were used for preparative HPLC separations. One consisted of a Model 6000A pump (Waters Assoc., Milford, MA, U.S.A.); a Model 7125 injector fitted with a 10-ml loop (Rheodyne, Berkley, CA, U.S.A.); a Zorbax ODS column (25 cm × 21.2 mm) (DuPont, Wilmington, DE, U.S.A.); a Model 401 differential refractometer (Waters Assoc.); a Model FC100 fraction collector (Gilson, Middletown, WI, U.S.A.); and an Omniscribe dual-pen recorder (Houston, Austin, TX, U.S.A.). The other instrument consisted of a Model 8800 four-solvent gradient system with oven, fitted with a preparative head (DuPont); a fixed-loop injector fitted with a 40-ml loop (Valco, Houston, TX, U.S.A.); a Zorbax ODS column, as described, or a preparative C_{18} column (25 cm × 22.0 mm) (Perkin-Elmer, Norwalk, CT, U.S.A.); a Model LC85 variable-wavelength UV detector containing a 8.0-μl flow cell (Perkin-Elmer); and other components as described.

The analytical separations also employed two liquid chromatographs. The first consisted of a Model 6000A pump; a Model 660 solvent programmer (Waters Assoc.); a Model U6K injector (Waters Assoc.); or a Model 7125 injector fitted with a 5-μl loop (Rheodyne); a Zorbax ODS column (15 cm × 4.6 mm I.D.) (DuPont) or a HS-5 C_{18} column (12.5 cm × 4.6 mm I.D.) (Perkin-Elmer) or a 3 × 3 C_{18} column (3.0 cm × 4.6 mm I.D.) (Perkin-Elmer). (Physical parameters of the HS-5 C_{18} and 3 × 3 C_{18} columns are presented in Table II.) Model 440 fixed-wavelength detector containing a 12.5-μl flow cell (Waters Assoc.); a Model LC85 variable-wavelength UV detector containing a 2.5-μl flow cell; a Model LS-4 fluorescence spectrometer fitted with a 4-μl flow cell (Perkin-Elmer); a Model LC-4B amperometric detector fitted with a TL-5 glassy carbon electrode cell and an Ag/AgCl reference electrode, with the top half of the cell serving as the auxiliary electrode (Perkin-Elmer); a Sigma 15 data system (Perkin-Elmer) and a Model 56 dual-pen recorder (Perkin-Elmer). The second instrument consisted of a Series 4 liquid chromatograph; an ISS-100 autosampler fitted with a 6-μl loop (Perkin-Elmer); columns as described; two Model LC85B detectors fitted with 1.5-μl flow cells (Perkin-Elmer); a Model LS-4 fluorescence spectrometer, fitted with a 4-μl flow cell, and a Model LC-4B amperometric detector, as described; a Model 3600 data station, equipped with Chromatographics 2 software (Perkin-Elmer); and a Model 660 printer (Perkin-Elmer).

Computer programs

A computer program supplied by Perkin-Elmer, written in BASIC[8], was used with modification to estimate parameters needed to scale up for preparative work. The equation in the program for N, the number of theoretical plates required for a resolution of 1, was changed to:

$$(4k' + 4)^2/(k'\alpha - k')^2 \quad (1)$$

In addition, the molar capacity number (M_c) of the Zorbax ODS preparative column was assumed to be equal to that of the Perkin-Elmer C_{18} preparative column. The program used the following equations to calculate the maximum injection volume, VL, and the maximum sample loading, CL in order to obtain a resolution of 1 for the worst-resolved pair:

$$\mathrm{VL} = \mathrm{VO}\,(\alpha - 1)\,k' \quad (2)$$

$$\mathrm{CL} = (k' + 1)\,M_c\mathrm{MW}/Nk' \quad (3)$$

where VO is the void volume of the preparative column, α is the selectivity factor, k' is the capacity factor of the first solute, and MW is the gram molecular weight of the first solute. The values of α and k' are determined from data obtained during analytical separations.

The percent recovery for the preparative separations is determined using the following formula:

$$\text{percent recovery} = H_f V_f \times 100/H_s V_s \quad (4)$$

where H_f is the peak height of the monitored fraction, V_f is the volume of monitored fraction, H_s is the peak height due to the sample obtained during analytical chromatography and V_s is the volume injected for preparative analysis. The actual calculations are carried out on the Model 3600 data station using Chromatographics 2 software with the following substitutions:

amount from calibration = 1
dilution factor = volume of monitored fraction × 100
sample volume = volume of preparative injection
standard volume = 1.00

RESULTS AND DISCUSSION

Preparative HPLC was utilized for the isolation of acidic and neutral rearrangement products obtained from acetylating thebaine and noscapine. The analytical and preparative column utilized were of matched surface properties. A computer program written in BASIC was used as an aid in predicting the maximum injection volume and maximum column load for a required separation.

The analytical and preparative separations as well as relevant data such as chromatographic conditions, predicted variables and preparative recoveries are presented in Tables III and IV and Figs. 1–3.

As Figs. 1 and 2 indicate, the separations obtained for the analytical and pre-

TABLE III

PREDICTED AND EXPERIMENTAL DATA FOR PREPARATIVE SEPARATION OF ACETYLATED REARRANGEMENT PRODUCTS OF THEBAINE

Separation	*Flow-rate (ml/min)*	*Column*	*VL (ml)*	*CL*** *(mg)*	*α*§*	*Amount injected*		*Peak no.*	*Recovery (percent)*	*Yield (mg)*
						wt. (mg)	*vol. (ml)*			
Analytical*	2.0	Zorbax ODS 4.6 mm I.D.	–	–	1.5	–	–	–	–	–
Preparative**	25	Zorbax ODS 21.2 mm I.D.	125	266		1152	40	1 2 4	77 75 84	99 28 127

* UV detection at 214 nm.

** Refractive index detection.

*** Value for VL and CL are those predicted from BASIC program as described in Experimental section. CL is maximum sample load for peak 1.

§ Selectivity factor for peaks 1 and 2.

TABLE IV

PREDICTED AND EXPERIMENTAL DATA FOR PREPARATIVE SEPARATION OF ACETYLATED REARRANGEMENT PRODUCTS OF NOSCAPINE

The eluent used for all separations was acetonitrile–methanol–phosphate buffer (25.2:13.4:61.4).

Separation	*Flow-rate (ml/min)*	*Column*	*VL*§ *(ml)*	*CL*§ *(mg)*	α§§	*Amount injected*		*Peak no.*	*Recovery (percent)*	*Yield (mg)*
						wt. (mg)	*vol. (ml)*			
Analytical*	2.0	HS-5 C_{18}	–	–	1.07	–	–	–	–	–
Preparative**	20	Prep C_{18}	115	8.4	–	300	40	5	74	13
								6	71	6
								7	69	9
								8	78	11
Preparative***	20	Prep C_{18}	115	8.4	–	1000	40	5	70	126
								6	51	46
								7	53	63
								8	72	58

* UV detection at 214 nm.
** UV detection at 340 nm.
*** Refractive index detection.
§ Values VL and CL are predicted. Value for CL calculated for peak 7.
§§ Selectivity factor determined for peaks 7 and 8.

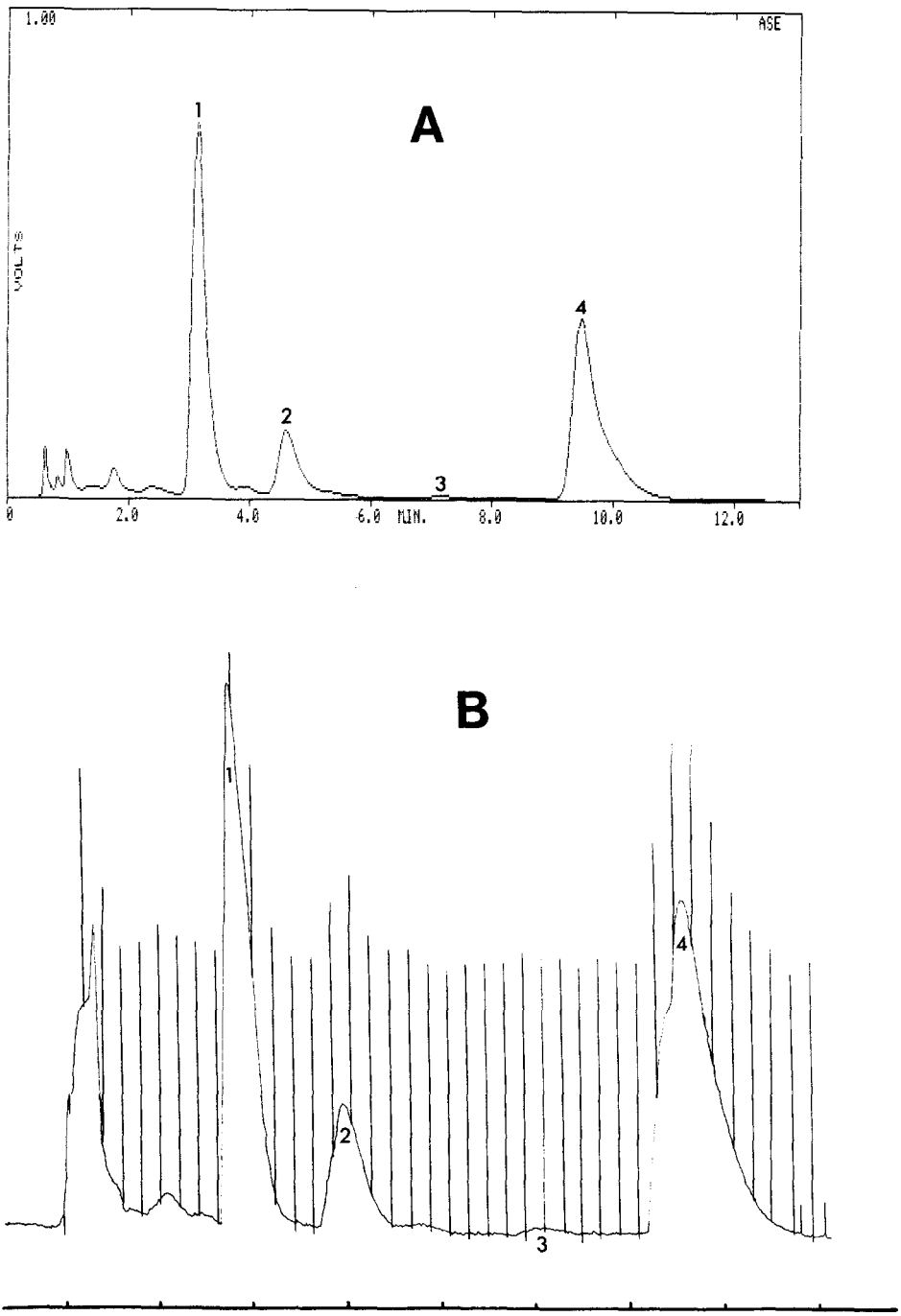


Fig. 1. Analytical (A) and preparative (B) separation of acetylated rearrangement products of thebaine with acetonitrile–water (48:52) as eluent. Peaks: 1 = compound 14; 2 = compound 15; 3 = compound 17; 4 = compound 19.

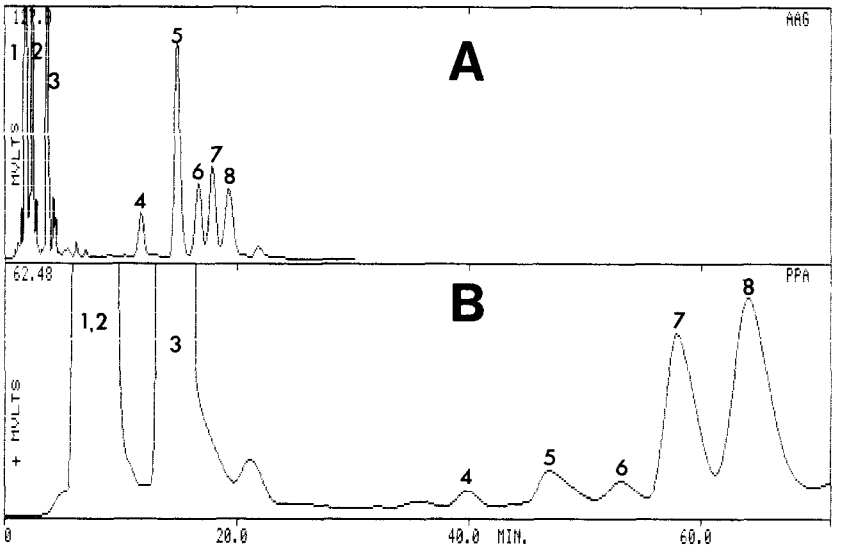


Fig. 2. Analytical (A) and preparative (B) separation of acetylated rearrangement products of noscapine with acetonitrile–methanol–phosphate buffer (25.2:13.4:61.4) as eluent. Peaks: 1 = unreacted noscapine; 2 = compound 1; 3 = compound 2; 4 = compound 3; 5 = compound 4; 6 = compound 5; 7 = compound 6; 8 = compound 7.

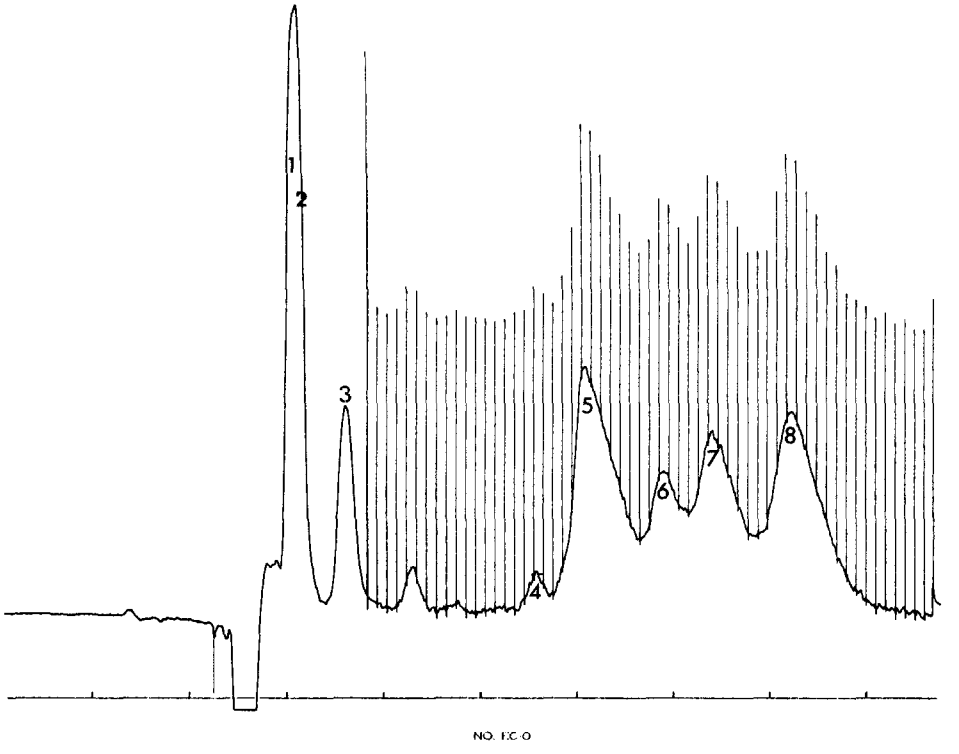


Fig. 3. Preparative separation and refractive index detection of acetylated rearrangement products of noscapine with the same eluent as described in Fig. 2; Peaks: 1 = unreacted noscapine; 2 = compound 1; 3 = compound 2; 4 = compound 3; 5 = compound 4; 6 = compound 5; 7 = compound 6; 8 = compound 7.

parative separations were very similar. For products obtained from the thebaine reaction mixture, the computer predicted that for the least-resolved pair of compounds, 14 and 15 (see Table I), which have a high value of α of 1.5, only 85 theoretical plates would be required for a resolution of 1. Since our Zorbax ODS column was rated by the manufacturer at 5000 theoretical plates minimum, the maximum predicted load of 266 mg for compound 14 should have been easily obtainable. However, because of solubility limitations, only half of this load could actually be placed on the column.

Compounds 16 and 20 were also isolated using the identical conditions described in Fig. 1.

The original preparative separation of acetylated rearrangement products of thebaine used a flow-rate of 10.0 ml/min and an injection volume of 10 ml[7]. The separation reported in Fig. 1 accomplished a ten-fold increase in sample throughput per unit time.

For the acetylated rearrangement products of noscapine (6 and 7 in Table I), which have a relatively low α value of 1.07, the program predicted that 2682 plates would be required for a resolution of 1.0. Since the Prep C_{18} column used for this separation was rated at 10,000 plates by the manufacturer, a predicted maximum load of at least 8.4 mg would appear to be feasible. The computer program had good predictive capability, since a load of 13 mg of compound 6 gave a satisfactory separation.

As Figs. 1 and 2 and Tables III and IV show, the computer predictions were generally satisfactory and good separations were obtained. For the separation of the acetylated rearrangement products of noscapine, an approximate ten-fold increase in mass resulted only in a small loss in recovery. As indicated in Fig. 3, this loss was due to decreased resolution between the peaks of interest.

As Fig. 1 indicates, considerable tailing was observed for compound 14. This was not a serious liability, since the isolation of compounds 14 and 15 was of primary interest and compound 19 was recovered in high yield. Compounds 2–7 and 14–16 were isolated and their identities were confirmed by combinations of proton magnetic resonance spectroscopy, mass spectrometry, elemental analysis and melting point; while compounds 1 and 17–20 were confirmed by retention time and 214–230 absorbance ratios.

Analytical HPLC

A four-solvent optimization scheme based on an optimization triangle[9] was used to develop both the analytical and preparative separation of the acetylated rearrangement products. The scheme employed three binary solvents of equal solvent strength, based on compound 15 as the reference compound. The organic modifiers consisted of acetonitrile, methanol, and tetrahydrofuran. Ternary and quaternary solvent mixtures used in the seven experiments defining the optimization triangle consisted of equal ratios of the binary mixtures. A visual evaluation of the experiments was used to define an approximate optimum solvent composition within the optimization triangle. This composition was refined through successive approximations of composition by interpolation. Further refinement in the mobile phase was obtained by increasing the ratio of aqueous to organic components. Chromatographic data for the binary solvent mixtures and the optimum separation of acidic and

neutral rearrangement products are presented in Tables V and VI, respectively. The optimum separation of the noscapine-related compounds was presented previously (see Fig. 2). If no amine modifier is present in the mobile phase given above, separations identical to the optimum resulted, except that values of k' decreased by about 20%.

TABLE V

EFFECT OF ORGANIC MODIFIER AND C_{18} STATIONARY PHASE ON k' AND α

Buffer = phosphate buffer with hexylamine as described in the Experimental section.

Compound number (see Table I)	*Mobile phase*											
	Buffer–acetonitrile (60:40)				*Buffer–methanol (40.5:59.5)*				*Buffer–tetrahydrofuran (78.8:21.2)*			
	Column											
	3 × 3		*HS-5*		*3 × 3*		*HS-5*		*3 × 3*		*HS-5*	
	k'	α	k'	α	k'	α	k'	α	k'	α	k'	α
2	0.65	1.69	0.95	1.40	1.09	2.53*	1.78	2.78*	2.04	2.49*	4.00	2.60*
1	1.10	2.96	1.33	3.08	0.43	5.86	0.64	5.38	0.82	5.49	1.54	5.44
3	3.26	1.09	4.10	1.12	2.52	1.12	3.44	1.11	4.50	1.05	8.37	1.03
4	3.55	1.11	4.60	1.13	2.83	1.09	3.83	1.08	4.73	1.03	8.65	–
5	3.95	1.09	5.22	1.11	3.09	1.20	4.15	1.18	4.86	1.61	9.36	1.58
6	4.30	1.06	5.79	1.05	3.70	1.14	4.90	1.10	7.82	1.12	14.8	1.11
7	4.55	–	6.09	–	4.22	–	5.37	–	8.77	–	16.50	–
9	0.10	4.00	–	–	0.09	4.33	–	–	0.36	1.25	–	–
12	0.40	1.25	–	–	0.39	1.30	–	–	0.45	2.22	–	–
10	0.50	2.80	–	–	0.30	2.90	–	–	1.00	1.59	–	–
11	1.40	1.18	2.24	1.17	0.87	1.45	–	–	1.59	1.31	–	–
13	1.65	1.15	2.62	–	1.26	1.52	–	–	2.09	1.31	–	–
8	1.90	1.60	–	–	1.91	1.48	–	–	1.59	–	–	–
14	3.75	1.71	5.26	1.66	2.83	2.21	–	–	3.27	2.03	–	–
15**	6.40	1.19	8.71	–	6.26	1.37	–	–	6.64	1.70	–	–
16	7.60	2.32	–	–	8.56	1.02*	–	–	11.3	2.98	2.98	–
17	17.6	1.37	–	–	8.39	1.53	–	–	33.7	1.63	–	–
19	24.1	1.05*	22.6	1.10*	12.8	1.07	–	–	54.8	2.29	–	–
18	22.9	1.67	20.6	–	13.7	3.69	–	–	125.6	1.82	–	–
20	61.1		–		50.6		–	–	229		–	

* Alpha values derived from reciprocal k' values.

** Reference compound.

The 3 × 3 C_{18} and HS-5 columns were used for analytical and preparative method development, respectively. Identical mobile phases were employed to compare the retention characteristics of compounds of interest on both columns. Physical data for these columns and the HS-3 C_{18} column are compared in Table II. The HS-3 C_{18} column has been shown to contain a significantly greater number of non-bonded silanol sites than the HS-5 C_{18} column[10]. These sites could prove deleterious, since unwanted absorption could occur, involving the silanol groups on the

TABLE VI

RETENTION DATA AND COMPARISON OF DETECTION MODES FOR HIGH-SPEED SEPARATION OF ACIDIC AND NEUTRAL ACETYLATED REARRANGEMENT PRODUCTS OF OPIUM ALKALOIDS

Stationary phase: two 3 × 3 C_{18} columns in series. Eluent: 22.4% acetonitrile, 18.8% methanol, 1% tetrahydrofuran, 57.8% phosphate–hexylamine buffer. MDQ = minimum detectable quantity.

Compound (see Table I)	*RRT*	*Ultraviolet*		*Electrochemical*		*Fluorescence*		
		UV_{max} (nm)	*MDQ*	*Ep[§] (V)*	*MDQ*	*$\lambda_{ex(max)}$ (nm)*	*$\lambda_{em(max)}$ (nm)*	*MDQ*
9	0.08	210	480 pg (S/N = 2)	0.85	480 pg (S/N = 4)	230[§§§]	340	240 pg (S/N = 5)
10	0.10	**	–	0.85	–	230[§§§]	328	–
12	0.10	210	500 pg (S/N = 5)	1.45	–	230[§§§]	340	100 pg (S/N = 4)
1	0.11	**	2.5 ng (S/N = 7)	[§§]	–	230	390	10 pg (S/N = 3)
2	0.17	**	–	1.20	–	Weak fluor.		–
11	0.21	**	500 pg (S/N = 4)	1.35	–	Weak fluor.		–
13	0.24	210	500 pg (S/N = 4)	1.40	–	230[§§§]	340	500 pg (S/N = 5)
8	0.30	208	–	1.35	–	230[§§§]	312	–
3	0.42	208	2.4 ng (S/N = 2)	1.15	–	Weak fluor.		–
14	0.48	267	1.2 ng (S/N = 2)*	1.15	–	258	403	19 pg (S/N = 3)
4	0.52	211	2.4 ng (S/N = 4)	1.30	–	Weak fluor.		–
5	0.57	**	–	1.30	–	Weak fluor.		–
6	0.61	**	–	1.15	–	Weak fluor.		–
7	0.66	**	6.0 ng (S/N = 3)	1.15	–	Weak fluor.		–
15	1.00***	260	3.0 ng (S/N = 3)	1.20	–	258	383	6.0 pg (S/N = 2)[†]
16	1.12	235	–	0.85	–	Weak fluor.		–
17	1.65	254	–	1.35	–	255	372	–
18	2.25	257	3.0 ng (S/N = 3)*	0.45	600 pg (S/N = 3)	257	380	12 pg (S/N = 4)[†]
19	2.30	256	3.0 ng (S/N = 3)*	1.25	–	256	380	6.0 pg (S/N = 2)[†]
20	6.36	–	–	–	–	–	–	–

* MDQ measured at 260 nm.

** Absorbance at 210 nm exceeds absorbance at longer wavelengths.

*** Retention time 7.18 min.

§ Oxidation potential 0.50 V greater than point at which electrode response becomes independent of potential.

§§ Electrode response still increasing at 1.50 V.

§§§ Fluorescence from excitation at 230 nm is greater than that from longer wavelengths.

† MDQ measured at λ_{ex} = 257 nm; λ_{em} = 383 nm.

stationary phase and polar moieties on the compounds of interest. Since a 3 × 3 C_{18} column has packing material identical to a HS-3 C_{18} column, an amine modifier was added to block unwanted silanol sites. To compare retention data from Table II for the HS-5 C_{18} and the 3 × 3 C_{18} column, the following regression equations were calculated, using data from the nine compounds chromatographed with acetonitrile as the organic modifier:

$$ya = 0.95xb + 0.08 \qquad r = 0.985, n = 9 \tag{5}$$

where *ya* represents a value of α for the 3 × 3 C_{18} column and *xb* the value of α for the HS-5 C_{18} column. This correlation compares favorably with the one obtained for the same compounds on two different 3 × 3 C_{18} stationary phases:

$$ya = 1.00xa + 0.05 \qquad r = 0.983, n = 9 \tag{6}$$

where *xa* is an α value obtained from experiments with an alternate 3 × 3 C_{18} column. For noscapine-related compounds, chromatographed on all three binary mobile phases, the following relation exists between the 3 × 3 C_{18} column and the HS-5 C_{18} column:

$$ya = 1.04xb - 0.05 \qquad r = 0.995, n = 18 \tag{7}$$

The good agreement in selectivity factors between these columns has two implications. First, since the optimization of α values is of primary importance in developing a preparative separation, the 3 × 3 column could provide a faster alternative to the HS-5 C_{18} column. The 3 × 3 column is not only faster by virtue of its shorter length, but for a given mobile phase, lower k' values were generally obtained with this column than with the HS-5 C_{18} column. The 3 × 3 column has been used successfully for the development of preparative separations of acetylated rearrangement products of thebaine other than those reported here[11]. Second, using two 3 × 3 columns in series gives faster separations with lower limits of detection than those obtained with a single HS-5 C_{18} column without any sacrifice in separation efficiency. Two 3 × 3 columns in series were used for the optimized separation described in Table VI.

The agreement in α values between these columns is probably due to the presence of hexylamine in the mobile phase. The amine modifier acted by tying up nonbonded silanol sites and made the stationary phases more hydrophobic and therefore more like the stationary phase of the HS-5 C_{18} column.

Effect of structure on retention

Significant changes in polar group selectivity have been observed for solutes in binary mobile phases, containing acetonitrile, methanol, or tetrahydrofuran (THF) at approximately equal solvent strength[9,12,13]. As Table V shows, these effects are in evidence for the acidic and neutral rearrangement products from the acetylation of opium alkaloids. For example, compounds containing phenolic substituents are significantly retarded in the mobile phase containing THF. This effect has been previously reported for phenol[13]. Compounds 6 and 7 are also selectively retarded in the mobile phase containing THF. On the other hand, compound 11 with two

O-acetyl groups and a N-acetyl group is retained less strongly in the system containing methanol.

Hydrophobic effects are very much in evidence for the separations described in Table V. Horváth *et al.*[14] show that for reversed-phase chromatography, the driving force in retention is the decrease in non-polar surface area of a solute exposed to the solvent. This decrease can be expressed by ΔA, the contact area, which is shown to be proportional to the non-polar surface area of a molecule[14]. In all mobile phases examined, the k' of compound 15 was greater than that of compound 14, a positional isomer. This occurred because compound 15 is less compact and therefore has a greater contact area. The same effect explains why the k' value of compound 7 was greater than that of compound 6, its *cis* isomer. In addition, for the pairs of compounds 12–13 and 17–18, the replacement of a moderately polar acetyl group by a methoxy group resulted in an increase in k'. The substitution of the O-acetyl group at C-4 of compound 19 demonstrates that the epoxide bridging C-4 and C-5 as in compound 20 is also able to increase retention time significantly.

For all three binary mobile phases studied, the k' values for compound 5 are greater than those for the *erythro* isomer, compound 4.

Detection

The sensitivity and selectivity of ultraviolet, fluorescence and electrochemical detectors for the compounds discussed in this paper are reported in Table VI, where optimized detection settings for all three detectors, as well as minimum detection limits for selected compounds, are presented. Detection at the low picogram level was obtained via fluorescence for phenanthrene-like compounds and for meconin. High sensitivity as well as excellent selectivity were obtained via electrochemical detection for compounds such as thebaol, N-acetylnormorphine, and N,O^6-diacetylnormorphine, all of which contain phenolic moieties. It has been shown for morphine, which is structurally related to the first two compounds, that the catechol-type oxygen between C-4 and C-5 is responsible for the observed amperometric response[15].

In order to utilize fully the advantages of multi-detection modes for the sensitive and selective detection of our compounds, two variable-wavelength UV detectors were employed in series with a programmable fluorescence detector and an electrochemical detector. Compromise wavelengths of 210 and 260 nm and a compromise oxidation potential of +0.85V were employed for the UV and electrochemical detectors, respectively. Five different pairs of excitation and emission wavelengths were employed with the fluorescence detector. Fig. 4 illustrates an optimized high-speed separation for selected compounds employing multi-detection modes. A further advantage of using detectors in series is the ability to generate response ratio data. The ratio of the peak height or peak area responses of individual detectors may be used to check peak identity or peak purity. This approach was used by Christiansen and Rasmussen, who employed ultraviolet, fluorescence and electrochemical detection for the screening of hallucinogenic mushrooms[16].

CONCLUSIONS

Preparative HPLC was used to isolate trace impurities found in illicit heroin.

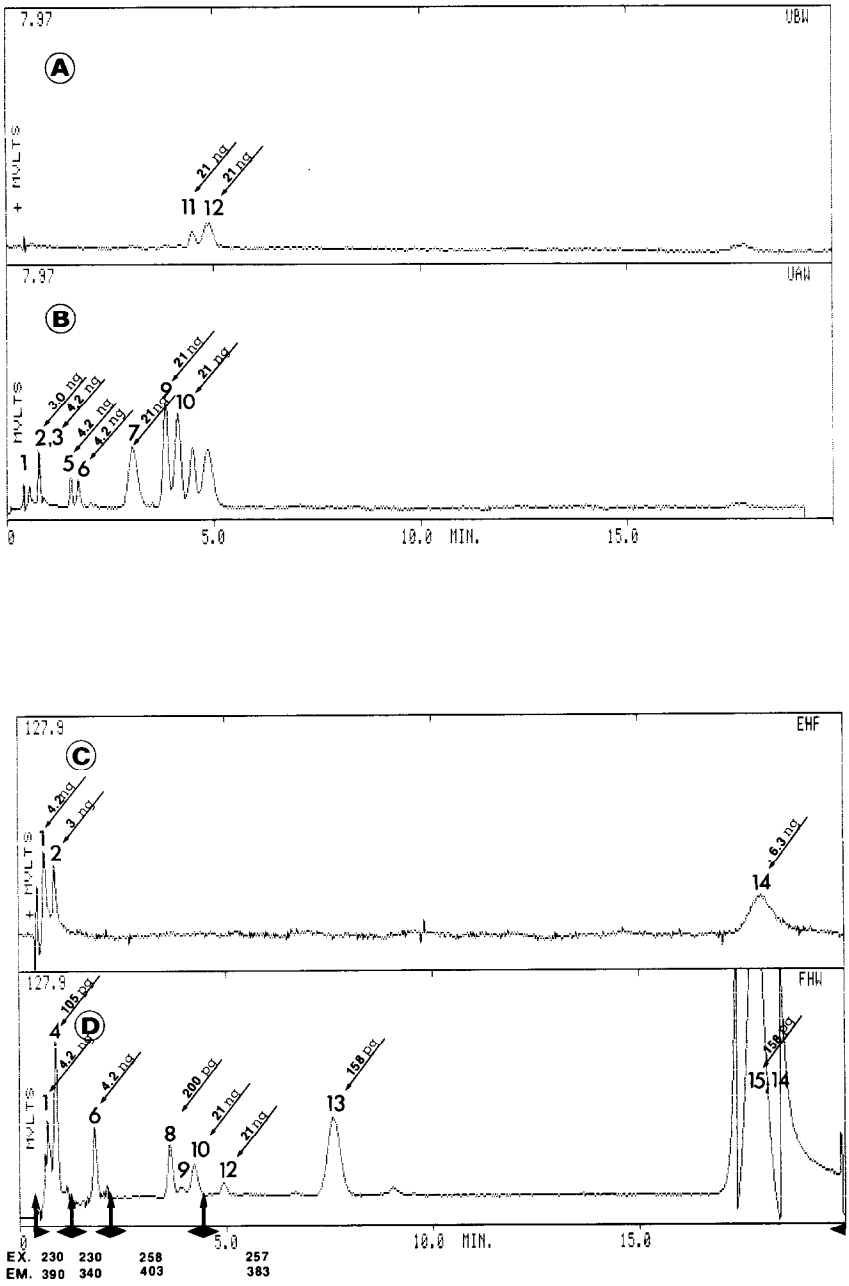


Fig. 4. Chromatogram of standard mixture containing (1) compound 9; (2) compound 10; (3) compound 12; (4) compound 1; (5) compound 11; (6) compound 13; (7) compound 3; (8) compound 14; (9) compound 4; (10) compound 5; (11) compound 6; (12) compound 7; (13) compound 15; (14) compound 18; (15) compound 19. Detection, (A) UV 260 nm; (B) UV 210 nm; (C) electrochemical 0.85 V; (D) fluorescence, programmable excitation and emission wavelengths; mobile phase, 22.4% acetonitrile, 18.8% methanol, 1% THF, 57.8% buffer (amine + phosphate); flow-rate, 2.0 ml/min; column, two 3 × 3 C_{18} in series.

Results indicated that the fast 3 × 3 C_{18} column can be used to develop preparative separations for the Prep C_{18} column, even though the bonded stationary phases are different.

Neutral and acidic acetylated rearrangement products of opium alkaloids were separated by an optimized liquid chromatographic procedure and were detected by employing multi-detection modes. Ultraviolet, fluorescence, and electrochemical detectors in series produced highly sensitive and selective detections of the compounds studied. The methodology is being expanded to include the analysis of uncut and adulterated heroin samples.

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