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High Performance Liquid Chromatographic Analysis of Heroin by Reverse Phase Ion-Pair Chromatography

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ABSTRACT: A method using high performance liquid chromatography (HPLC) with an acetonitrile, water, phosphoric acid, and methane sulfonic acid mobile phase and a microparticulate octadecylsilane bonded column has been developed for the rapid, sensitive, selective, and accurate analysis of heroin. Retention data relative to heroin and 220:254 nm absorbance ratios are presented for the various adulterants in heroin exhibits.

KEYWORDS: criminalistics, heroin, chromatographic analysis, high performance liquid chromatography, adulterants, street samples, absorbance ratios

This paper presents a rapid, highly sensitive, specific, and accurate high performance liquid chromatographic (HPLC) method for the quantitation of heroin and identification of adulterants and synthetic by-products in illicit heroin exhibits. It is applicable to complex mixtures containing heroin, even at low levels.

Various HPLC systems have been reported for heroin analysis, including ion exchange [*1,2*], adsorption [*3,4*], reverse phase [*5*], and reverse phase ion-pair chromatography [*6,7*]. Ion exchange requires careful control of such factors as pH and salt concentration. In addition, ion-exchange columns do not appear to be stable for more than a few months [*8*].

Although good separations have been obtained using adsorption chromatography, the presence of water in the eluant will deactivate the column. Therefore, careful control of the amount of water in the mobile phase is required. Because a basic mobile phase was employed, acidic adulterants would exhibit appreciable tailing. Of more concern is the fact that heroin and various adulterants have limited solubility in the relatively nonpolar solvents that have been employed to dissolve samples.

An interesting system employing the use of a silica column with a polar eluant instead of the usual nonpolar mobile phase has been reported for the analysis of heroin [*9*]. In this system acetylmorphine and acetylcodeine, present in nearly all heroin exhibits, were not sepa-

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rated. Also, many of the adulterants had short retention times that were indistinguishable from each other. Finally, since the mobile phase is basic, its utility for the analysis of acidic components is limited.

Love and Pannel [5] published a reverse phase chromatographic method for the analysis of heroin samples that used an acetonitrile, water, ammonium acetate buffer mobile phase at pH 7 with a Microbondapak-C18 column. Good separation was obtained for heroin, acetylcodeine, and acetylmorphine. Retention data were presented only for the adulterants caffeine and strychnine. We found that the chromatographic system presented by Love and Pannel did not resolve heroin from the adulterant quinine, which is present in many of the heroin exhibits analyzed by our laboratory. Acidic adulterants were poorly retained and exhibited poor peak shape. The system lacked specificity for many adulterants. In addition, tailing was observed for heroin and basic drugs in general. The analysis of bases by reverse phase chromatography using microparticulate nonpolar bonded phase columns via an ion suppression mechanism is not recommended [*10*]. Under these conditions, bases exhibit poor chromatographic performance, probably because mixed mechanisms such as adsorption, ion exchange, partition, and ion pairing occur. Better column efficiencies and peak shapes are obtained for bases in general when a reverse phase ion-pair chromatographic system is employed at an acidic pH. Under these conditions, the predominant mechanism will only be ion pairing.

Reverse phase ion-pair chromatography, a technique in which a lipophilic counter ion capable of forming an ion pair with an oppositely charged species is employed with a nonpolar bonded phase column, appears to be well suited for the analysis of illicit heroin exhibits. If an acidic mobile phase is employed, most bases would be ionized and would be available to pair with a negative counter ion, such as an alkylsulfonate. Retention would occur as a result of some interaction of this ion pair with the stationary phase. Via an ion suppression mechanism acidic drugs would interact as the free acid with the nonpolar bonded phase column. Thus, reverse phase ion-pair chromatography would be viable for the simultaneous chromatography of both acidic and basic drugs.

Soni and Dugar [*6*] have reported a reverse phase ion-pair chromatographic system in which retention times for heroin and acetylcodeine differed by more than 5 min. In an earlier work, Lurie [*11*] had found that heroin coeluted with acetylcodeine when similar conditions were used. Recent studies by Lurie and Demchuk [*12,13*] substantiate Lurie's earlier findings.[3] Lurie has recently developed a system in which the resolution between heroin and acetylcodeine was approximately 1 [*7*].

Baker et al [*14*] have employed a reverse phase system that uses relative retention time and 254:280 nm absorbance ratios to identify 78 drugs. Based on Beer's law, the ratio of the absorbances at any two wavelengths is proportional to the ratio of the extinction coefficients [*15*]. Because extinction coefficients are intrinsic characteristics, an absorbance ratio coupled with a retention time provides highly specific identification. Using this system, Baker found that 95% of the drugs included in the study could be uniquely identified. In contrast, only 9% could be uniquely identified by relative retention time alone.

The present work describes a reverse phase ion-pair chromatographic method for the analysis of heroin exhibits. Retention data on two different column substrates is presented relative to heroin for 46 adulterants or synthetic by-products that have been found in heroin samples. Quantitation of the heroin, as well as qualitative identification of the various components, is done by peak area with a variable ultraviolet (UV) detector at 220 nm in series with a fixed UV detector at 254 nm. The quantitative results are compared with those obtained by using an established gas chromatographic procedure.

[3]I. S. Lurie, personal communication, 1979.

Experimental Procedure

High Performance Liquid Chromatography

The chromatographic system consisted of a Model 6000-A pump (Waters Associates, Milford, MA), a Model 710A auto injector (Waters), a Model 770 variable UV detector at 220 nm (Schoeffel Instruments, Westwood, NJ), a Model 440 fixed UV detector at 254 nm (Waters), and a System IVB integrator (Spectra Physics, Santa Clara, CA). Column I was a 3.9-mm by 30-cm stainless steel column, prepacked with Microbondapak-C18 (Waters). Column II was a stainless steel column, 4.6 by 25 cm, prepacked with Partisil 10 ODS-3 (Whatman, Clifton, NJ).

The mobile phase comprised 12% acetonitrile, 87% water, 1% phosphoric acid, and 0.02*M* methanesulfonic acid adjusted to pH 2.2 with 2*N* sodium hydroxide. This eluant was prepared by filtering and degassing through a 0.50-μm filter (Millipore Corp., Bedford, MA) a solution consisting of 3480 mL water, 40 mL phosphoric acid, 120 mL 2*N* sodium hydroxide, and 7.6 g methanesulfonic acid. This solution was degassed for 5 min prior to the addition of 480 mL of filtered and degassed acetonitrile. The final solution was then degassed for 2 min. A flow rate of 3.0 mL/min was used.

The materials used were methanesulfonic acid (Eastman Chemicals, Rochester, NY) and acetonitrile, Omnisolv, distilled in glass (MCB, Cincinnati, OH); all other chemicals were reagent grade. The drug standards were of U.S. Pharmacopeia/National Formulary quality.

Relative retention time and absorbance ratios were determined by using 10-μL injections of methanolic solutions containing 0.25 mg/mL each of heroin and another drug standard. Samples were prepared by dissolving 100 mg in 5 mL of methanol containing 0.5 mg/mL amylocaine hydrochloride as an internal standard. Injection volume was 10 μL. For samples containing greater than 10% heroin, a twentyfold dilution was made for the heroin quantitation.

Gas-Liquid Chromatography

A Hewlett-Packard gas liquid chromatograph, Model 5840A, was used. It was equippec with a flame ionization detector, a printer-plotter data system, and a Model 7671-A auto sampler (Hewlett Packard, Avondale, PA). A 1.8-m (6-ft) by 4 mm inside diameter glass column packed with 10% OV-1 on Chromosorb WHP 100-120 mesh was used for analysis. The column temperature was 250°C, detector and injection port temperatures were 300°C, and the nitrogen carrier gas flow rate was at 60 mL/min.

The sample was prepared by dissolving the equivalent of 3.5 mg of heroin in 1 mL of methanol, adding 5 mL of chloroform containing 0.4 mg/mL of docosane internal standard, and diluting to 10 mL with chloroform. The standard contained 0.2 mg/mL of docosane internal standard and 0.35 mg/mL of heroin in chloroform.

Results and Discussion

A major requirement in developing a method of analyzing illicit heroin exhibits is separating heroin and acetylcodeine. Lurie [7] separated these two compounds using a Microbondapak-C18 column with a water (79%), methanol, acetic acid, and methanesulfonic acid system. Increasing the percentage of water provides excellent separation, but it also increases the time of analysis. For example, the retention time of papaverine, a common impurity, would exceed 2 h at a flow rate of 2.0 mL/min. By substituting acetonitrile for methanol, higher flow rates can be used because of the lower viscosity and hence lower back pressure. In addition, a smaller relative retention time was obtained for papaverine versus heroin. Thus, at a flow rate of 3.0 mL/min, the retention time of papaverine was approximately 1 h.

Because of the absorbance of acetic acid at 220 nm, phosphoric acid was used in its place. The use of 220 nm was based on the high sensitivity of detection for heroin over that obtained for the commonly employed wavelengths of 254 and 280 nm. At a flow rate of 3.0 mL/min, the peak area response from a 10-μL injection of a 0.5-mg/mL solution of heroin at 254 nm is approximately 65 000 counts. In comparison, the peak area at 280 nm is approximately 4 times greater, while at 220 nm the response is approximately 24 times greater.

The use of absorbance ratios in conjunction with relative retention times is a powerful technique for the identification of a wide variety of compounds. In addition, they can be used to verify that the response for a given peak is due to only one component. Since the adulterants and by-products commonly encountered in heroin exhibits have good UV responses at 220 nm, this wavelength is more suitable for determining ratios than the 280 nm reported by Baker et al [*14*]. Phenethylamines and barbiturates, for example, have negligible absorbance at 280 nm.

Relative retention times and the 220:254 nm absorbance ratios of various adulterants and other impurities are presented in Table 1 for the Microbondapak-C18 and Partisil 10 ODS-3 columns. Although the Microbondapak-C18 column gave retention times similar to those of the Partisil column, the latter column was preferred because of the higher efficiencies and better peak symmetries of the various compounds.

The values for relative retention times and absorbance ratios for the Partisil 10 ODS-3 column were remeasured after six weeks. The average long-term reproducibility of the relative retention times was 3.5% while the average variability of the absorbance ratios was 3.4%, using peak area. In comparison, Baker et al [*14*] obtained long-term average reproducibilities of 3.2% for relative retention times and 21% for absorbance ratios, using peak height. After prolonged use, retention times decreased. By successively washing the column with 15 mL of 50:50 water/methanol, methanol, chloroform, dimethylsulfoxide, and methanol, retention times close to the original may be restored.

Absorption of heroin at 220 nm was found to be linear between 0.062 and 4.0 mg/mL. At 254 nm, linearity was limited to the range from 0.5 to 4.0 mg/mL. The smaller range of linearity at 254 nm is probably due to the larger error of peak area measurements at low concentrations.

The accuracy and precision of the HPLC method reported for the quantitation of heroin was checked by analyzing five separate weighings of synthetic samples consisting of 49% heroin, 25% quinine, and 25.3% mannitol. In addition, the chromatographic precision was measured by injecting one sample four times. Using the 220-nm UV detector, the percentage of error for the average of five separate weighings was 0 with a 1.7% relative standard deviation. The chromatographic precision for the variable UV detector at 220 nm was 0.8%, while that of the 254 nm UV detector was 2.8%. Because of its greater precision, accuracy, and sensitivity, the use of the variable wavelength UV detector at 220 nm is preferred for heroin quantitation.

Eleven heroin exhibits previously analyzed by a combination of other techniques, including gas chromatography (GC), thin-layer chromatography, infrared analysis, and gas chromatography/mass spectrometry, were assayed by HPLC. A typical chromatogram is depicted in Fig. 1. As Table 2 indicates, good agreement was obtained between the GC and HPLC quantitative determinations. In addition, good agreement was obtained between the adulterants and by-products determined by HPLC and other methods. In certain instances, relative retention values differing by approximately 10% from those obtained in Table 1 were observed for sample peaks. These discrepancies occurred at low retention values where small differences in mobile phase composition or temperature can lead to moderate variations in relative retention values. A closer match between sample and standard relative retention times could be obtained by running a standard the same day as the sample.

Larger discrepancies for absorbance ratio values were obtained in certain instances when two compounds were present under a peak. This occurred primarily in those cases when syn-

TABLE 1—*Relative retention data and 220:254 absorbance ratios for heroin and its adulterants and by-products.*

Drug	Microbondapak-C18 RRT (Heroin)	Partisil 10-ODS-3 RRT (Heroin)	Partisil 10-ODS-3 220:254
L-Ascorbic acid	...	0.05	...[a]
Isonicotinamide	0.05	0.05	...[a]
Morphine	0.09	0.08	12.1
Aminopyrine	0.15	0.12	0.9
Procaine	0.12	0.12	4.1
Ephedrine	0.14	0.12	3.5
Acetaminophen	0.11	0.12	0.6
Theophylline	0.12	0.13	1.4
Methapyrilene	0.15	0.13	2.0
Tripelennamine	0.16	0.13	2.4
Codeine	0.17	0.16	8.9
Pyrilamine	0.18	0.17	3.2
Quinidine	0.19	0.19	0.7
Barbital	0.23	0.21	62.0
Quinine	...	0.22	0.7
Acetylmorphine	0.23	0.22	14.7
Caffeine	0.19	0.23	2.2
Phentermine	0.28	0.25	5.1
Lidocaine	0.30	0.30	16.6
Quinine (second peak)	...	0.30	0.7
Acetylprocaine	0.28	0.30	0.6
Prilocaine	...	0.31	3.5
Salicylamide	...	0.31	6.0
Antipyrine	0.36	0.36	1.4
Hyoscyamine	0.41	0.39	21.7
Strychnine	0.38	0.41	0.56
Benzocaine	0.50	0.58	4.9
Aspirin	0.60	0.58	10.7
Sodium salicylate	...	0.75	7.3
Tropacocaine	0.71	0.76	7.7
Phenobarbital	0.81	0.85	11.0
Benzoyltropeine	0.82	0.87	8.1
Acetylcodeine	0.85	0.88	11.2
Thebaine	0.88	0.92	4.6
Phenacetin	0.81	0.95	0.5
Meperidine	...	1.00	25.5
Heroin	1.00	1.00 (19 min)	24.0
Cocaine	1.05	1.11	6.0
Amylocaine	...	1.37	5.9
Phencyclidine	2.12	2.15	8.5
Noscapine	2.30	2.36	10.8
Tetracaine	2.28	2.38	10.3
Papaverine	2.80	3.17	0.4
Tartaric acid	...	...[b]	...
Diphenhydramine	...	...[c]	...
Methadone	...	...[c]	...
Phenylbutazone	...	...[c]	...

[a] Elutes near solvent front.
[b] Exhibits no UV at 254 nm.
[c] Retention time greater than 1 h.

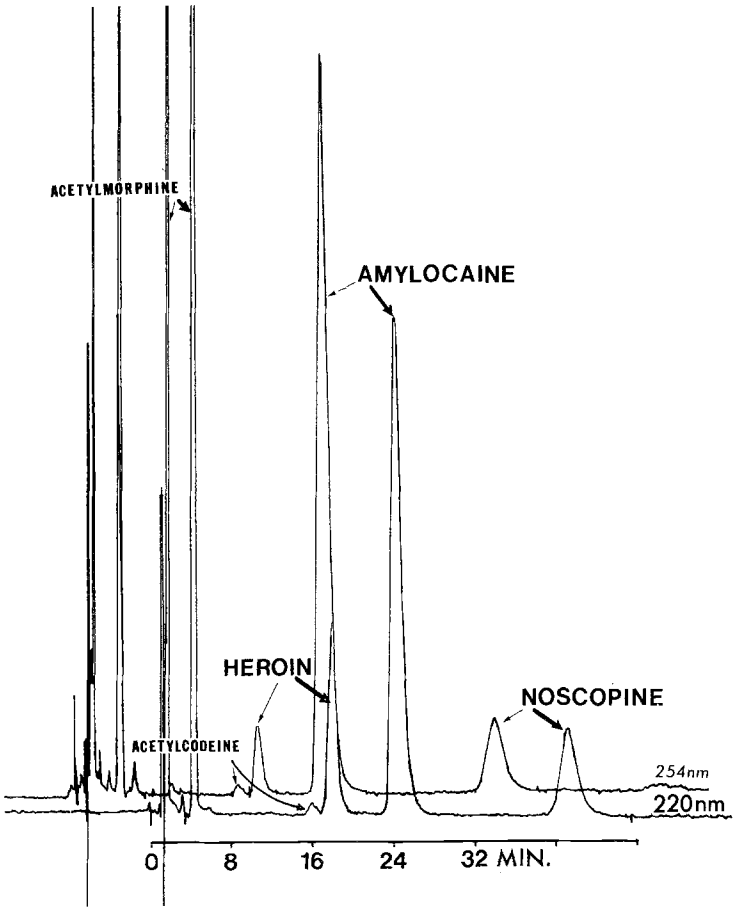

FIG. 1—*Chromatogram of a heroin exhibit containing acetylmorphine, acetylcodeine, heroin, amylocaine (internal standard), and noscapine. Conditions are described in text.*

thetic by-products such as acetylcodeine and acetylmorphine coeluted with adulterant peaks. For example, benzoyltropeine is not resolved from acetylcodeine, and caffeine and quinine are not separated from acetylmorphine. Because the amount of adulterant is usually much greater than that of the by-products, the effects of the interferences are generally small and predictable.

For heroin samples greater than 10%, an undiluted sample is assayed to identify peaks that would be too small to be detected. In this instance, relative retention times versus amylocaine instead of heroin were employed, since the retention time of the heroin peak varies with concentration at heroin concentrations greater than 2.0 mg/mL. Several peaks were obtained in these concentrated samples that could not be identified by relative retention times and absorbance ratios.

An advantage of the ion-pairing system is the ability to increase the retention of certain compounds selectively by increasing the size of the counter ion employed. For example, in the system used in this paper, if the chain length of the alkylsulfonic counter ion is increased, only the retention times of moderate to strong bases would be increased. Thus, acetylmorphine could be separated from caffeine by the use of a higher chain length alkylsulfonate counter ion.

TABLE 2—*Comparison of quantitation of illicit heroin samples by HPLC versus GC.*

Sample	Adulterants and Synthetic By-Products	%Heroin by HPLC (220 nm)	% Heroin by GC
1	acetaminophen codeine noscapine	4.1	3.9
2	quinine phenobarbital	2.8	3.0
3	benzoyltropeine noscapine	4.6	4.5
4	quinine benzoyltropeine noscapine	2.1	1.9
5	aspirin salicylic acid benzoyltropeine noscapine	4.8	5.3
6	benzoyltropeine quinine noscapine papaverine	4.1	4.1
7	procaine quinine noscapine papaverine	14.9	16.9
8	noscapine	26.2	25.4
9	tripelennamine	13.2	11.4
10	caffeine	82.0	79.8
11	pyrilamine quinine noscapine	2.7	2.9

In addition, other variables, such as counter ion concentration, solute charge, and the organic/water ratio in the mobile phase, could be altered to change separations [*12,13*].

Conclusion

A reverse phase ion-pair liquid chromatographic system has been developed for the analysis of illicit heroin exhibits. The system offers the following advantages:

1. High accuracy, precision, sensitivity, and linearity of response is obtained for the quantitative determination of heroin.
2. High specificity is realized by the use of relative retention times and 220:254 nm adsorbance ratios for the identification of adulterants and synthetic by-products in heroin exhibits.
3. Detection sensitivity for adulterants and by-products is obtained to a level of at least 1% of sample.
4. Good chromatographic performance is obtained for acidic, neutral, and basic compounds.
5. Basic compounds in general can be separated from acidic and neutral compounds by changing the size or concentration of the counter ion.
6. Peaks present in the chromatogram can easily be collected for identification by spectrographic techniques.
7. The method easily lends itself to automation.

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