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A High Performance Liquid Chromatographic Method for the Analysis of Illicit Heroin

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ABSTRACT: A method using high performance liquid chromatography with an ammonium acetate-buffered acetonitrile/water mobile phase has been developed for the analysis for heroin. The method gives good resolution of the opiates found in illicit heroin.

KEY WORDS: toxicology, heroin, chromatographic analysis, high performance liquid chromatography

Heroin is one of the more commonly encountered illicit drugs and hence must often be identified and quantified in samples involved in illegal drug proceedings. Such samples may contain a wide range of heroin concentrations, a number of related compounds such as morphine, codeine, acetylcodeine, and monoacetylmorphine, other drugs, and a number of diluents. A suitable method of analysis must be able to distinguish heroin from other constituents and allow a simple quantification of the compounds of interest.

Until recently gas chromatography has been the most widely used method for the quantification of heroin in illicit drug samples. It has the advantages of speed, high precision, and efficiency but suffers from some disadvantages. The analyses are performed at high temperatures, which may cause decomposition of heroin, and some compounds of interest such as morphine cannot be satisfactorily assayed by gas chromatography without the use of special techniques such as derivatization [*1*] and the preparation of special liquid-coated solid supports [*2*]. No single really satisfactory method has yet been developed [*3*].

High performance liquid chromatography (HPLC) offers an alternative approach for the analysis of heroin samples and combines the merits of gas chromatography with the added advantages of low temperature operation and ease of collection of components. It is inherently suitable for the analysis of nonvolatile, highly polar, and temperature-sensitive compounds such as are found in illicit heroin samples, and these may be chromatographed without derivatization.

Several satisfactory methods that use HPLC to analyze heroin samples involve normal-phase silica columns with polar eluting solvents [*4-7*]. These give good resolution of morphine, codeine, monoacetylmorphine, acetylcodeine, and caffeine. However, these silica columns are susceptible to contamination from other polar materials (such as sugars, which are a common diluent in heroin samples) and deactivation from traces of water in the solvent [*8*].

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Although it is claimed that the use of a polar eluting solvent minimizes this deterioration so that several months of routine analyses may be performed without noticeable loss in column performance [*4*], column life is very dependent on both the level and nature of the polar impurities in the samples injected.

An alternative approach involves the analysis of heroin samples on cation exchange columns [*9–11*]. Although satisfactory separations of the various morphinan derivatives in illicit heroin have been performed on ion exchange columns, the resolution of these compounds is critically dependent on the pH, ionic strength, and organic content of the eluting solvent. After a few months in use these columns have a markedly reduced ion exchange capacity [*9*].

Reversed-phase columns have less stringent solvent and sample requirements. Several reports have appeared on the analysis of morphinan derivatives using this type of column and an ammonium carbonate-buffered water/acetonitrile mobile phase [*12–14*]. However, with this type of system it is difficult to resolve codeine from heroin, the peaks of the morphine derivatives often tail badly, and the pH of the ammonium carbonate buffer (about pH 8.3) is slightly higher than the maximum pH of 8 recommended by most column manufacturers. An alternative to working at this high pH is to employ an ion-pairing agent. Two recent papers report the separation of morphinan alkaloids on a C18 reversed-phase column with water/methanol and water/acetonitrile eluant containing *n*-heptane sulfonic acid [*15,16*]. Reference *15* does not give retention data for acetylcodeine and monoacetylmorphine, while Ref *16* reports poor separation of codeine and monoacetylmorphine and of heroin and acetylcodeine. Ion-pairing methods do not seem to be suitable for the analysis of illicit heroin samples.

This paper reports a more satisfactory method for the analysis of illicit heroin samples using a reversed-phase column and an ammonium acetate-buffered mobile phase. The resolution of the common morphinan derivatives is as good as has been reported on normal-phase silica columns [*7*] while the ammonium acetate buffer produces a pH of approximately 7 in the mobile phase, which is well within the range recommended by column manufacturers. An added advantage is that ammonium acetate is relatively soluble in methanol, acetonitrile, and water.

Experimental Procedure

High Pressure Liquid Chromatograph

All components were obtained from Waters Associates and included their Model 6000A pump, Model U6K injector, Model 440 ultraviolet detector fitted with a 280-nm converter, and a 30-cm micro-Bondapack C18 column.

Mobile Phase

The chromatograph was operated without varying the solvent concentration and with the mobile phase composed of 65% acetonitrile and 35% aqueous buffer containing 0.75 g of ammonium acetate per 100 ml. All solvents were filtered through a 0.45-μm filter (Millipore FHUP 04700). The acetonitrile was purified according to the method of Walter and Ramaley [*17*].

Preparation of Standards

Pure standards of morphine were obtained from May and Baker, codeine phosphate from MacFarlane Smith, and heroin from Chemistry Division, Department of Scientific and Industrial Research, Auckland, New Zealand.

Monoacetylmorphine was prepared by the method of Wright [*18*].

Acetylcodeine was prepared by the following procedure: 1.5 g of codeine phosphate was

dissolved in water, buffered to pH 9 with sodium carbonate, and extracted twice with chloroform/isopropanol (4:1). The combined extracts were filtered through Whatman #1 phase separating paper and evaporated to dryness under reduced pressure. Approximately 1.2 g of codeine base was recovered. It was dissolved in acetic acid/pyridine (25 ml, 1:1), placed in a water bath (60°C, 3 h), and then left at room temperature overnight. The acetic anhydride/pyridine was removed under reduced pressure on a rotary evaporator at 50°C, and the residue was dissolved in dilute sulfuric acid (0.1*N*), buffered to pH 9 with solid sodium carbonate, and extracted twice with chloroform/isopropanol (4:1). The combined extracts were filtered through Whatman #1 phase separating paper and evaporated to dryness on a rotary evaporator at 40°C, and the residue was recrystallized three times from methanol.

Samples of the pure drugs were accurately weighed and dissolved in methanol to give concentrations of 1 to 2 mg/ml. The solutions were filtered through a 0.45-μm Teflon® filter (Millipore FHLP 01300) and then stored in a deep freeze in small, tightly stoppered glass containers.

Preparation of Samples

An accurately weighed portion of the sample for analysis was dissolved and then made to volume in methanol. It is desirable that the final solution contains from 1 to 2 mg/ml of heroin or the other opiates of interest. The solutions were filtered and stored in a refrigerator until analysis.

Determination

The flow of the mobile phase was set at 1.5 ml/min and the detector at a sensitivity of 0.1 absorbance, full scale, using a 280-nm light source. Ten-microlitre volumes of standards and samples were injected in triplicate and the amount of each component present was determined by a comparison of peak heights, which were reproducible to within 1% (coefficient of variation, 0.008).

In the range of about 5 to 40 μg of component injected, heroin, codeine, acetylcodeine, and monoacetylmorphine produced peak heights directly proportional to the amount injected. Morphine gave a nonlinear response and a calibration curve should be constructed for this compound, especially if the amount present must be accurately determined.

Internal Standard

An internal standard can be used with the method if required. Both papaverine and thebaine are suitable as both give linear responses of peak height to weight of component injected. They should be added at 0.15 mg/ml to the methanol used to dissolve the standards and samples. If papaverine is chosen as the internal standard, the composition of the mobile phase should be checked to confirm that the papaverine is well resolved from both the monoacetylmorphine and morphine (see Fig. 1). If the papaverine peak is too close to the monoacetylmorphine peak, then the addition of about 5% of acetonitrile (relative to reservoir eluant volume) should center the papaverine peak (Fig. 1); if the papaverine is too close to the morphine peak, then the addition of 3% of buffer solution should correct the problem. Generally, one addition of either of these quantities is sufficient. The resultant effects on the relative retention volumes are shown in Table 1.

Thebaine does not pose this problem, but its retention volume is significantly greater than those of the other alkaloids and is consequently less desirable.

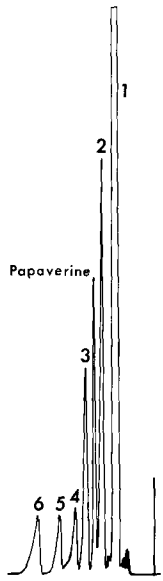

FIG. 1— *Chromatogram of mixed opiates, caffeine, and papaverine internal standard;* (1) *caffeine,* (2) *morphine,* (3) *monoacetylmorphine,* (4) *codeine,* (5) *heroin, and* (6) *acetylcodeine. Chromatographic conditions are given in the text.*

TABLE 1—*Variations of the retention volumes relative to heroin of morphine, papaverine, monoacetylmorphine, codeine, and acetylcodeine as the mobile phase composition is varied.*[a]

Compound	Acetonitrile Content		
	67%	65%	63%
Morphine	0.58	0.55	0.54
Papaverine	0.61	0.63	0.65
Monoacetylmorphine	0.74	0.72	0.71
Codeine	0.87	0.84	0.78
Acetylcodeine	1.26	1.24	1.19

[a]Chromatography on a 30-cm micro-Bondapak C18 column with mobile phase consisting of 65% acetonitrile/35% of 0.75% aqueous ammonium acetate.

Discussion

This method of heroin analysis is quick, involves minimum sample preparation, and uses a simple binary solvent mixture for the mobile phase. The resolution of the various morphinan derivatives commonly associated with heroin is shown in Fig. 2 and their retention times relative to heroin are given in Table 2. The peaks of the morphinan derivatives are better resolved than in any other reported method on a reversed-phase column.

In this method, the relationship between the amount of morphine injected and the peak height was not linear. Thus 1, 2.5, and 5 μg of morphine (each in 10 μl of methanol) produced peaks with heights in the ratio 1:2.3:3.8, respectively. However, as the peaks became larger, they also became broader (at half peak height), so that peak areas were essentially linear. A similar behavior was found with the ammonium carbonate-buffered water/

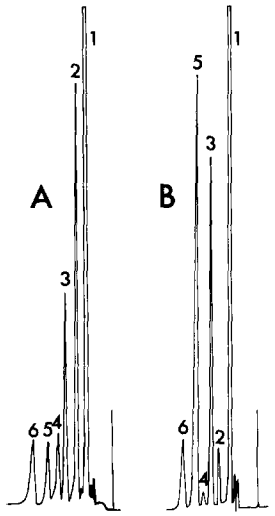

FIG. 2— *Chromatograms of* (A) *mixed opiates and caffeine and* (B) *Chinese heroin sample;* (1) *caffeine,* (2) *morphine,* (3) *monoacetylmorphine,* (4) *codeine,* (5) *heroin, and* (6) *acetylcodeine. Chromatographic conditions are given in the text.*

TABLE 2—*Retention volumes (in millilitres)*[a] *of various compounds commonly found in illicit heroin, morphine, and opium.*

Compound	Retention Volume, ml
Caffeine	3.8
Morphine	5.0
Papaverine	5.7
Monoacetylmorphine	6.5
Codeine	7.5
Narcotine	8.4
Heroin	9.0
Acetylcodeine	11.1
Thebaine	13.5
Strychnine	22.2

[a]65% acetonitrile/35% of 0.75% aqueous ammonium acetate on a 30-cm micro-Bondapak C18 column.

acetonitrile system [*13,19*]. Wu and Wittick [*14*] observed that codeine was the only alkaloid in opium that gave a linear response when chromatographed on a micro-Bondapak C18 column with a phosphate-buffered water/acetonitrile mobile phase. Similar results have been reported with other drugs on C18 reversed-phase columns [*20–22*] and were attributed to free silanol sites on the column packing phase resulting from an incomplete reaction with C18 functional groups. This leads to a mixed retention mechanism [*23*] with the relatively polar compounds such as morphine being held back and eluted as broad peaks. At pH > 7 these groups can also have a cation exchange effect. Residual silanol groups have been shown to give changes in retention time, order of elution, and efficiency of separation relative to columns containing packings that have been exhaustively treated with silane [*20*]. A similar effect has been observed in gas chromatographic systems [*24*]. The proposed HPLC system has not been investigated on a column exhaustively treated with silane.

Both papaverine and thebaine are suitable as internal standards because they give linear

responses of peak heights to amounts injected and, although these are naturally present in opium [25], no compounds of similar retention volumes have been found in any of the heroin samples we have examined. However, no matter what internal standard is used, it is still necessary to ensure that no such components are present in the samples analyzed. An ever-increasing number of chemicals are being added to illicit heroin, for instance dyes to mask dilution [26]. In this respect, the problems found by the Pharmaceutical Society of London should be considered [3]. In a collaborative survey of gas chromatographic methods for the analysis of alkaloids in opium, the Society observed that no one internal standard could be found that was suitable for the analysis of all the samples by the different laboratories.

In the present method, the ammonium acetate is used at a concentration (0.75% w/w water) higher than that used in other reversed-phase methods. This reduced peak tailing but had little effect on the relative retention times of the alkaloids. The peak tailing became pronounced if the concentration of ammonium acetate in the buffer was below 0.4%. Because of the high solubility of the ammonium acetate in water, acetonitrile, and methanol, buffer precipitation, often associated with high buffer concentrations, is not a problem, and the column may be flushed directly with methanol without the need for an intermediate aqueous solvent.

This method is quick and reliable but should be used with other confirming techniques, as is standard practice in this type of work. Some of these confirming techniques have been compared by Clark [27] and by Manura et al [28]. These workers did not consider HPLC but they did outline the considerations required in interpreting results obtained from gas chromatography, and similar conditions should apply to HPLC.

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