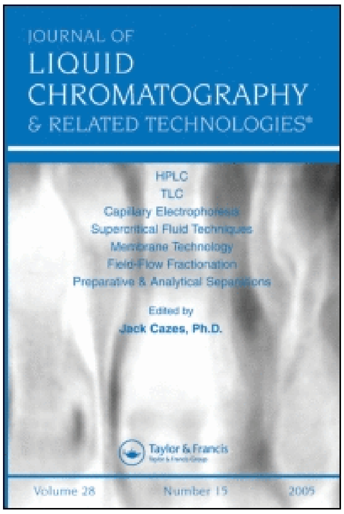

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The Quantitation of Heroin and Selected Basic Impurities Via Reversed Phase HPLC. I. The Analysis of Unadulterated Heroin Samples

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THE QUANTITATION OF HEROIN AND SELECTED BASIC IMPURITIES VIA REVERSED PHASE HPLC. I. THE ANALYSIS OF UNADULTERATED HEROIN SAMPLES

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ABSTRACT

Methodology was developed employing reversed phase liquid chromatography for the simultaneous determination of heroin, O^3-monoacetylmorphine, O^6-monoacetylmorphine, acetylcodeine, noscapine and papaverine in unadulterated illicit powders. An HS-5 C18 column was used with a gradient system using methanol and a hexylamine phosphate buffer at pH 2.2. This method, suitable for automated analysis, used a multi-mode detection scheme via the use of a photodiode array detector. In order to arrive at the optimum chromatographic conditions in terms of selectivity and stability, a study was performed on the effect of various mobile phase parameters on log k' for heroin, various impurities, and common adulterants. The mobile phase parameters included amine concentration, organic modifier type, and eluent pH.

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INTRODUCTION

The determination of heroin and its impurities can be important for intelligence purposes and can form the basis for comparative analysis of exhibits. Acidic, neutral, or basic impurities which arise during heroin manufacture are a result of acetylation of opium alkaloids, or hydrolysis of these by-products. Quantitative procedures have been reported for the determination of heroin and basic impurities in illicit heroin samples that employ either GLC or HPLC methodology. Packed column and capillary column GLC have been employed for the quantitation of heroin and certain basic impurities such as O^6-monoacetylmorphine, O^3-monoacetylmorphine, acetylcodeine, morphine, codeine, and papaverine using both FID[1-5] and ECD[6,7] detection. Normal phase HPLC with conventional UV detection has been utilized by Huizer[8-10] and White, et. al.[11], for the quantitation of heroin and some of the above impurities, plus noscapine. An alleged reversed phase procedure has been reported by Baker and Gough [12]. This separation appears to be proceeding by a different mechanism since the more lipophilic compounds are eluting first. Fell, et. al., used HPLC with photodiode array UV detection for the determination of heroin and selected basic impurities[13-15] utilizing the chromatographic system developed by Baker and Gough[12]. None of these methods were found to be satisfactory for our needs because of either incomplete resolution of compounds of interest, probable interferences from adulterants or other heroin impurities, or lack of ease of sample preparation. Common to these methods is the elution of noscapine near the void volume where it is subject to interference from non-retained compounds.

This paper reports a reversed phase procedure for the simultaneous quantitation of heroin, O^3-monoacetylmorphine,

O^6-monoacetylmorphine, acetylcodeine, noscapine, and papaverine in illicitly manufactured heroin samples using photodiode array UV detection. In order to obtain optimum chromatographic conditions for heroin and other compounds of interest, a study of the effect of mobile phase parameters such as amine concentration, organic modifier type and eluent pH on k' was conducted. A previous report on the analysis of heroin demonstrated the value of hexylamine in reducing k' values and providing good peakshape[16]. In this paper, only the quantitations in unadulterated samples are included.

EXPERIMENTAL

Materials

Methanol, tetrahydrofuran, and acetonitrile were acquired from Burdick and Jackson (Muskegon, MI, USA). Reagent grade hexylamine and piperonal were acquired from Eastman Chemicals (Rochester, NY, USA). Propiophenone standard was obtained from Pierce Chemical (Rockford, IL, USA). Other chemicals were reagent grade. Drug standards of USP/NF quality were employed.

The mobile phases were mixed internally from solvent reservoirs containing pure organic component or an amine-phosphate buffer. The phosphate buffer consisted of 870 parts water, 30 parts 2M sodium hydroxide, and 10 parts phosphoric acid. Hexylamine was added to this buffer after it was filtered and degassed, due to possible volatility losses. The final pH was adjusted to pH 2.2 with 2M sodium hydroxide or phosphoric acid, if necessary.

Equipment

Two systems were employed for the chromatographic separations. The first system used in chromatographic optimization studies consisted of a Series 4 liquid chromatograph (Perkin-Elmer, Norwalk, CT, USA); an ISS-100 autosampler fitted with either a 6ul or 50ul loop (Perkin-Elmer); an HS-5 C18 column (12.5cm x 4.6mm I.D.) (Perkin-Elmer); two Model LC85B detectors fitted with 1.5ul flow cells (Perkin-Elmer); a Model 3600 data station, equipped with Chromatographics 2 software (Perkin-Elmer), and a Model 660 printer (Perkin-Elmer).

The second system used for heroin sample analysis consisted of a Series 4 liquid chromatograph; an ISS-100 autosampler fitted with a 50ul loop; an HS-5 C18 column; a Model 1040a photodiode array HPLC detection system (Hewlett-Packard, Waldbronn, FRG); two Model LCI-100 laboratory computing intergrators (Perkin-Elmer) interfaced to a Model 7500 data station equipped with Chromatographics 3 software (Perkin-Elmer), and a Model PR-210 printer (Perkin-Elmer).

Quantitative Determination of Heroin and Selected Basic Impurities

Standard Preparation

The following were accurately weighed into a 100ml volumetric flask: 1.0mg each of O^3-monoacetylmorphine sulfamate, noscapine base, and papaverine base; 1.5mg of O^6-monoacetylmorphine base, 3.0mg of acetylcodeine base and 90mg of heroin base. Prior to dilution to volume with injection solvent, propiophenone internal standard was added in injection solvent to give a final concentration of 0.08mg/ml. Inject 50ul onto the liquid chromatograph.

Sample Preparation

An amount of heroin was weighed into a volumetric flask which resulted in an approximate heroin base concentration of 0.90mg/ml after dilution to volume. Prior to dilution to volume with injection solvent, propiophenone was added in injection solvent to give a final concentration of 0.08mg/ml. Subsequent to 50ul injections into the liquid chromatograph, heroin and selected basic impurities were determined via peak height or peak area using single point calibration. For automated determinations the standard mixture was injected after every four sample injections.

Chromatographic Conditions

Gradient elution was employed followed by an isocratic step. The initial solvent consisted of 5% methanol and 95% phosphate buffer (0.023M hexylamine, pH 2.2); while the final solvent consisted of 30% methanol, 70% phosphate buffer (0.023M hexylamine, pH 2.2). A linear gradient for 20 minutes was employed with the final solvent held for 25 minutes. Solvent flow rate was 1.5ml/min. Analogue signal A on the photodiode array detector was kept at 218nm for 12 minutes then switched to 228nm, and subsequently switched to 240nm at 30 minutes. Analogue signal B was run at 228nm and switched to 240nm at 12 minutes.

Injection Solvent

The injection solvent consisted of 10 parts acetonitrile, 89 parts water and 1 part acetic acid and was adjusted to pH 3.7 with 2N sodium hydroxide.

RESULTS AND DISCUSSION

Mobile Phase Optimization

The goal in our study was to develop a chromatographic system suitable for the quantitation of heroin and selected basic impurities in both uncut and adulterated heroin samples. A previously reported system used an HS-5 C18 column and a mobile phase containing 12% acetonitrile and 88% phosphate buffer (0.072M hexylamine) that gave an excellent separation of 0^6-monoacetyl morphine, acetylcodeine, heroin, papaverine, and noscapine in that order[16]. However, the following problems were observed with this system:

(a) Although noscapine and papaverine peaks were well resolved when new columns were used, their resolution decreased with time and they eventually merged after several weeks of column usage.

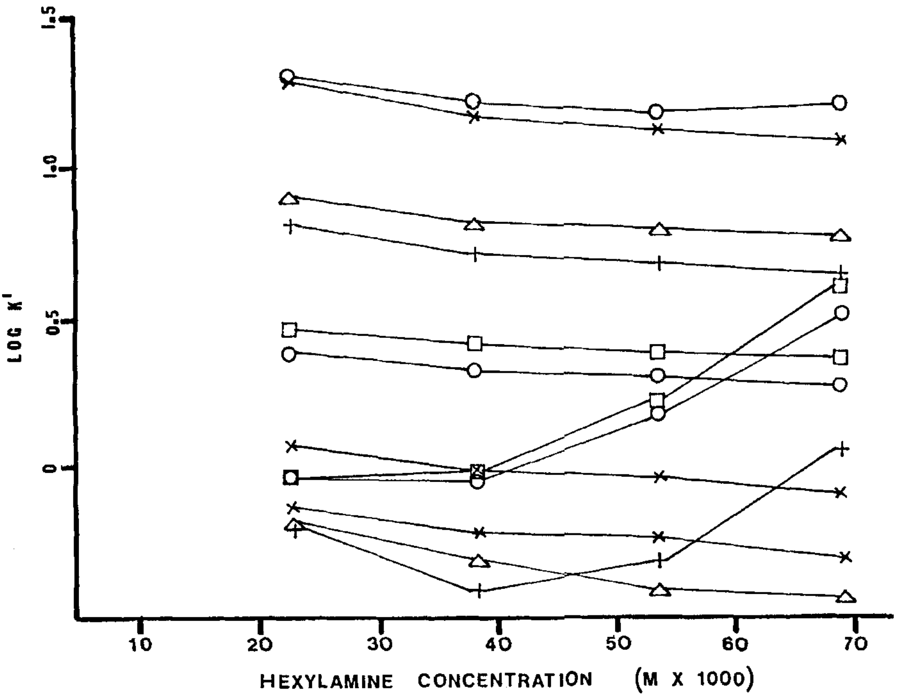
(b) 0^6-monoacetylmorphine co-eluted with the adulterant acetylprocaine.

(c) The adulterants quinine, methapyrilene, and aminopyrine exhibited large day-to-day variations in retention time relative to heroin.

With respect to Problem (a), it was observed that with continuous column usage papaverine had a more dramatic retention time shift than noscapine. It was postulated that a modified mobile phase could shift the elution order and thus resolve this problem, as well as Problem (b). Problem (c) appeared to be a result of an unstable chromatographic system in which k' varies significantly with small changes in concentration of one or more components of the mobile phase.

A study was performed to measure the effect of amine concentration on retention for the compounds of interest. As indicated in Figure 1, the log k' of quinine, methapyrilene, and aminopyrine first decreased then increased greatly with increasing hexylamine concentration. This was in contrast to the behavior of the other moderate to strong bases examined which exhibited the expected behavior, where the log k' values decrease with increasing amine concentration[17,18]. In addition, none of the systems indicated in Figure 1 appeared to be viable for the quantitation of O^6-monoacetylmorphine in the presence of acetylprocaine and no change in elution order was observed for noscapine and papaverine.

Since the pH of the eluents used in Figure 1 changed with amine concentration, it was desired to ascertain whether the retention behavior of the three adulterants, quinine, methapyrilene, and aminopyrine were pH related. Therefore the variation of log k' versus amine concentration was studied at pH 2.2 and 3.0. As Figures 2 and 3 illustrate, the log k' of the three adulterants listed increased significantly with mobile phase pH and exhibited normal behavior with respect to the effect of amine concentration. The change in retention times for quinine, methapyrilene, and aminopyrine with pH were probably due to changes in ionization state of the compounds to a more lipophilic species. The diprotic amines, quinine and methapyrilene, have reported second pKa values of 4.2 and 3.7 respectively; while aminopyrine, a monoprotic amine, has a reported pKa of 5.0. The reported values were determined in aqueous solution and the addition of an organic modifier effects simultaneously an increase of the apparent pH of the eluent and a decrease of the

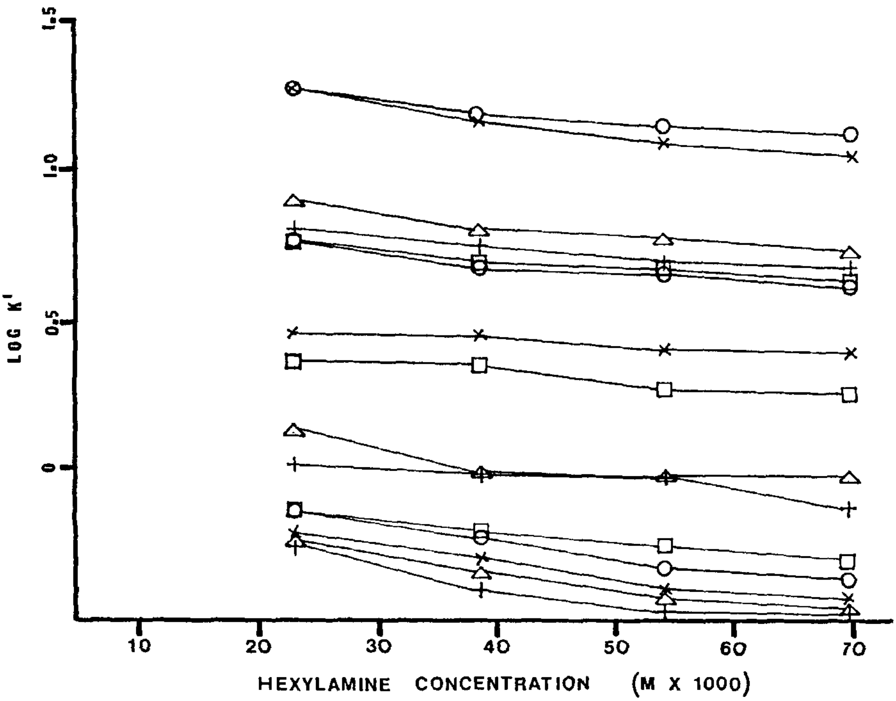


1. Plot of log k' versus hexylamine concentration for heroin, selected basic impurities, and various adulterants. Conditions: 12% acetonitrile and 87% phosphate buffer (see experimental section) with various concentrations of hexylamine.

The symbols are listed from the left, top to bottom, and represent the following: o-noscapine, x-papaverine, Δ-heroin, +-acetylcodeine, □-cocaine, o-strychnine, x-acetylprocaine and O^6-monoacetylmorphine, □-quinine, o-methapyrilene, x-codeine, Δ-procaine, +-aminopyrine.

effective pKa of the solute[19]. It would appear that in order to obtain a more stable chromatographic system, a buffer pH of 2.2 would be required. At this pH, each of the compounds of interest would be expected to exist as single species.

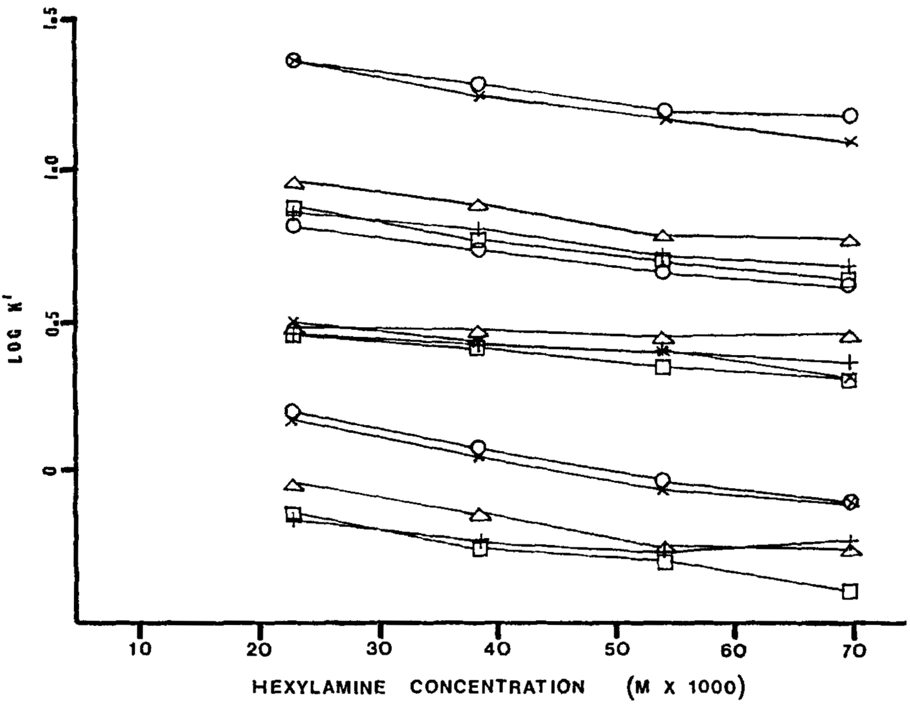
In Figures 1-3, the increase in selectivity for papaverine versus noscapine with an increase in hexylamine concentration is



2. Plot of log k' versus hexylamine concentration for heroin, selected basic impurities, and various adulterants. Conditions: same as Figure 1 except final pH adjusted to 2.2 with phosphoric acid if necessary.

The symbols are listed from the left, top to bottom, and represent the following: o-noscapine, x-papaverine, △-heroin, +-acetylcodeine, □-cocaine, o-benzoyltropeine, x-caffeine, □-strychnine, △-acetylprocaine, +-0^6-monoacetylmorphine, □-codeine, o-quinine, x-methapyrilene, △-aminopyrine, +-procaine.

of particular interest because of Problem (a). The change in selectivity can be explained by the two site mechanism of retention on the solid phase[17,20]. One kind of site, thought to be unbonded silanol groups, would be involved in silanophilic interactions while the other kind of site, thought to be the bonded alkyl chain, would be involved in solvophobic interactions. The reduction in k' versus amine concentration for both



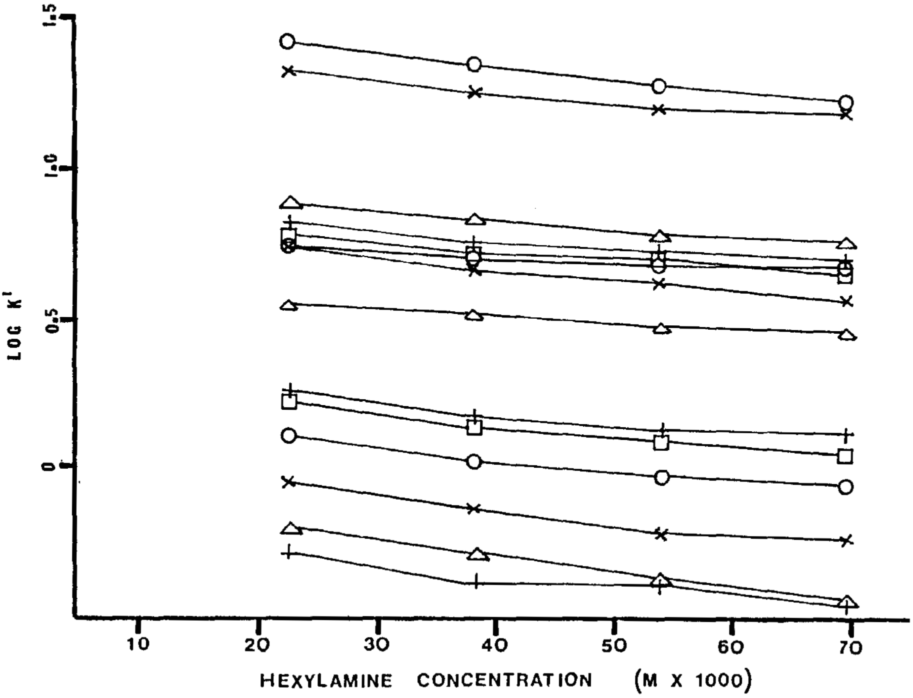
3. Plot of log k' versus hexylamine concentration for heroin, selected basic impurities, and various adulterants. Conditions: same as Figure 1 except final pH adjusted to 3.0 with phosphoric acid or 2N sodium hydroxide if necessary.

The symbols are listed from the left, top to bottom, and represent the following: o-noscapine, x-papaverine, Δ-heroin, □-cocaine, +-acetylcodeine, o-benzoyltropeine, x-methapyrilene, Δ-caffeine, +-quinine, □-strychnine, o-acetylprocaine, x-O^6-monoacetylmorphine, Δ-codeine, □-procaine, +-aminopyrine.

solutes can be explained by an increased competition between the solute and the amine for the available silanophilic sites. Papaverine, being less bulky and having a lower hydrophobic contact area than noscapine, should more easily undergo silanophilic interactions with the partially shielded unbonded silanol sites. Therefore, the effect of the amine modifier will be greater on papaverine than noscapine. This explanation is consistent with a previous report that competing amines with shorter hydrophobic side chains will be less strongly attracted to the stationary phase carbon chain and will tend to better undergo silanophilic interactions[21].

The loss in resolution between noscapine and papaverine with column usage could result from loss of C18 groups from the stationary phase with time and the resultant formation of silanol sites. Since papaverine undergoes silanophilic interactions more easily than noscapine, its affinity for the stationary phase would increase at a greater rate, gradually increasing its retention time relative to noscapine.

Since varying the amine concentration in a mobile phase containing 12% acetonitrile did not result in satisfactory separations for many of the compounds of interest, different organic modifiers at the same solvent strength necessary to elute heroin were investigated. The effect of hexylamine concentration on log k' for heroin and selected compounds in 21% methanol and 3% tetrahydrofuran is presented in Figures 4 and 5, respectively. Certain compounds were omitted from these figures because of their very low retention. It is of interest to note, especially for noscapine versus papaverine and O^6-monoacetylmorphine versus acetylprocaine and quinine, the large selectivity differences that resulted from changing the organic modifier. The elution order for noscapine and papaverine was reversed when an eluent containing methanol or tetrahydrofuran was used. With eluents containing methanol, better selectivity was observed between O^6-monoacetylmorphine and acetylprocaine and quinine eluted after O^6-monoacetylmorphine. This elution order for O^6-monoacetylmorphine and quinine was desirable since large quantities of the latter compound and its later eluting impurity dihydroquinine were less likely to interfere with the former compound. Figure 4 also shows that significantly improved separations were

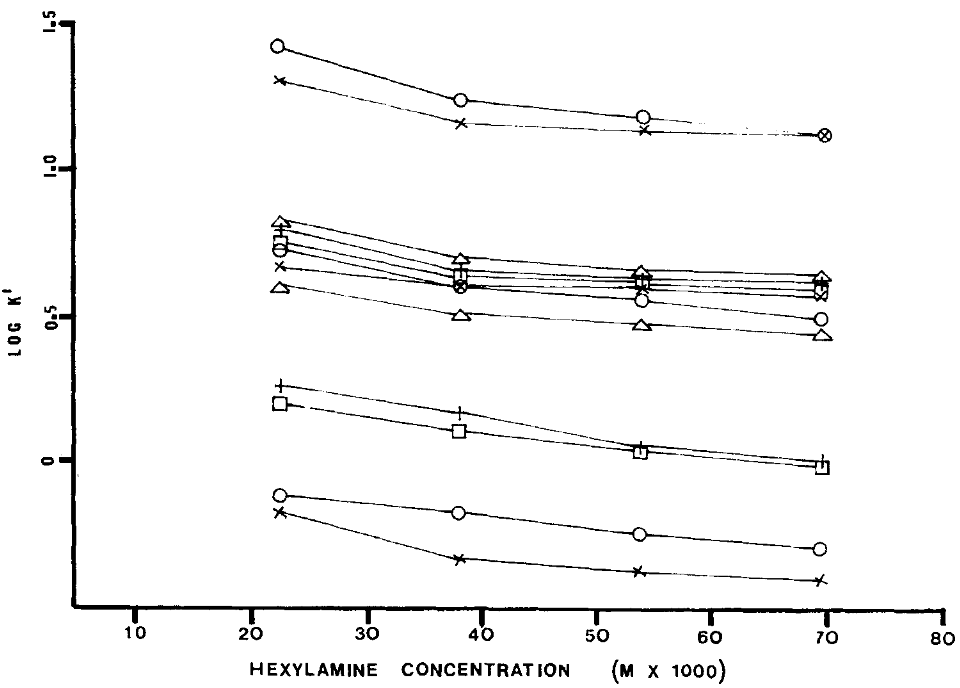


4. Plot of log k' versus hexylamine concentration for heroin, selected basic impurities, and various adulterants. Conditions: 21% methanol and 79% phosphate buffer (see text) with various concentrations of hexylamine added; final pH adjusted to 2.2 with phosphoric acid if necessary.

The symbols are listed from the left, top to bottom, and represent the following: o-papaverine, x-noscapine, Δ-heroin, +-acetylcodeine, □-benzoyltropeine, o-caffeine, x-cocaine, Δ-strychnine, +-quinine, □-acetylprocaine, o-O^6-monoacetylmorphine, x-codeine, Δ-methapyrilene, +-aminopyrine.

obtained using a mobile phase containing 21% methanol and 0.023M hexylamine. However, even under these chromatographic conditions, samples containing high levels of acetylprocaine would still interfere with O^6-monoacetylmorphine. In addition, O^3-monoacetylmorphine would not be separated from O^6-monoacetylmorphine.

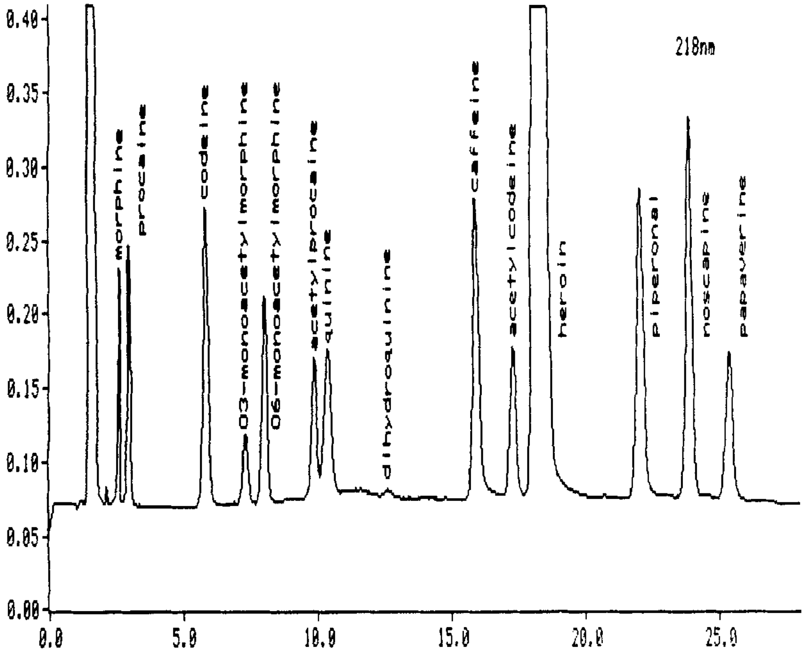
In order to improve the separation further, a lower solvent strength was necessary for earlier eluting compounds, and



5. Plot of log k' versus hexylamine concentration for heroin, selected basic impurities, and various adulterants. Conditions: 3% tetrahydrofuran, 97% phosphate buffer (see text) with various concentrations of hexylamine added; final pH adjusted to 2.2 with phosphoric acid if necessary.

The symbols are listed from the left, top to bottom, and represent the following: o-papaverine. x-noscapine, △-heroin, +-acetylcodeine, □-benzoyltropeine, o-cocaine, x-caffeine, △-strychnine, +-acetyprocaine, □-O^6-monoacetylmorphine, o-codeine, x-quinine.

therefore gradient elution was desirable. The initial chromatographic conditions consisted of 5% methanol, 95% phosphate buffer (.023M hexylamine, pH 2.2); while the final chromatographic conditions consisted of 30% methanol, 70% phosphate buffer (.023M hexylamine, pH 2.2). A linear gradient was used for 20 minutes with the final solvent held for 8 minutes. An optimum separation employing gradient elution for heroin, selected basic impurities, a suitable internal standard, piperonal, and the most common adulterants is presented in Figure 6.



6. Chromatogram of gradient separation of standard mixture of heroin, selected basic impurities, and the most common adulterants. Conditions: initial solvent - 5% methanol, 95% phosphate buffer (0.023M hexylamine, pH 2.2); final solvent - 30% methanol, 70% phosphate buffer (0.023M hexylamine, pH 2.2), 20' linear gradient: hold 8'; flow 1.5ml/min.

The short term stability of the chromatographic system was good, with relative retention times of the compounds of interest versus the internal standard varying by no more than approximately 1.0% during a 10 hour run. However, as expected, the resolution between noscapine and papaverine increased with column lifetime. Conversely, the resolution between heroin and piperonal decreased with column lifetime. These changes in the first few weeks of column usage were initially large but eventually leveled off. After several months of column usage, the internal standard co-eluted with meconin. This neutral impurity derived from noscapine can be found in significant quantities in certain heroin samples, especially those originating in Mexico. In addition, other impurities found in heroin originating in Mexico could co-elute with the internal standard piperonal. Therefore propiophenone, which elutes after any possible sample interferences, was chosen as an internal standard. The elution of this compound was accomplished by extending the hold time of the final solvent to 25 minutes. Although propiophenone behaved similarly to piperonal, in that its retention time decreased with column lifetime, after several months of use this internal standard was not subject to co-elution with heroin impurities. The retention time of propiophenone using a new column was approximately 44 minutes. After a relatively short period of time, the retention time of the internal standard was under 38 minutes and its rate of decrease of retention time with column lifetime was significantly reduced.

The changes in relative retention times for the internal standards and certain compounds is thought to be as described earlier for noscapine and papaverine. As a result of the cleavage of C18 groups from the stationary phase, unbonded silanol sites are formed. For example the internal standard piperonal undergoes silanophilic interactions less than heroin and meconin and therefore would be retarded on the stationary phase to a smaller degree. It would seem that the use of a precolumn, located between the pump and injector, containing the same stationary phase as the analytical column would inhibit loss of C18 groups. In fact the use of this type of saturation column or the employment of a silica precolumn had no effect on the resolution changes which occurred. A possible explanation for this phenomena was that the mobile phase was not fully saturated with column breakdown products. Since the largest variation in relative retention values occurs at initial column use, the column should be initially conditioned overnight at a flow rate of 0.5ml/min. This led to a more stable chromatographic system and to the desired separations.

QUANTITATIVE ANALYSIS

Injection Solvent

Since methodology suitable for automation was desired, the hydrolysis of heroin to O^6-monoacetylmorphine on standing in the injection solvent was of major concern. For aqueous solutions, this breakdown has been shown to proceed via first order kinetics and to depend on solvent properties such as pH, ionic strength, non-aqueous components, and temperature[22-24]. A study of the effect of various injection solvents, pH, and temperature on the formation of O^6-monoacetylmorphine from heroin on standing was conducted and is presented in Table 1. In agreement with previous studies[22-24], heroin hydrolysis was found to decrease significantly with a decrease in ionic strength or in temperature and to be highly pH dependent. Adjustments in injection solvent composition were explored. From Table 1 it appeared that for the analysis of both heroin base and heroin hydrochloride at room temperature, an injection solvent of 20% acetonitrile, 79.8% water, and 0.2% acetic acid (pH 3.7) would be optimum. However, this injection solvent would have inadequate buffering capacity to handle some cut samples. In addition, it was found that earlier eluting compounds such as procaine exhibited peak distortion because the injection solvent was too strong. An injection solvent consisting of 10% acetonitrile, 89% water, and 1% acetic acid (pH 3.7) was therefore chosen to minimize this effect. Also as indicated in Table 1, the heroin salt form or the presence of other bases had only a small effect on the rate

Table 1 - Study of Effect of Injection Solvent, pH and Temperature on Formation of O^6-monoacetylmorphine Base from Heroin on Standing.

Column - HS-5 C-18; Mobile Phase - 12% acetonitrile, 88% buffer (0.05M hexylamine, pH 2.2). Flow 2.0ml/min. Or Mobile Phase 5% methanol, 95% buffer (0.023M hexylamine, pH 2.2) - 30% methanol, 70% buffer (0.23M hexylamine, pH 2.2); 20' linear gradient: hold 8'; flow 1.5ml/min.

Sample	Solvent	pH(a)	Temp.	O^6 Formed (%)	Time of Standing (Hrs.)
Heroin HCl(b)	methanol	3.6	amb.	0.01(c)	21.0
Heroin base(b)	methanol	7.9	amb.	4.7	21.5
Heroin base(b)	acetonitrile	8.8	amb.	0.01(c)	21.5
Heroin base(b)	tetrahydrofuran	6.8	amb.	0.01(c)	6.8
Heroin base(b)	(d)	4.7	amb.	0.30	18.0
Heroin base(b)	(d)	4.3	amb.	0.23	19.0
Heroin base(e)	(f)	4.7	amb.	0.19	23.0
Heroin base(e)	(f)	4.4	amb.	0.14	23.0
Heroin base(e)	(f)	4.1	amb.	0.17	29.5
Heroin base(e)	(f)	3.7	amb.	0.13	29.5
Heroin base(e)	(d)	3.7	amb.	0.18	23.5
Heroin base + basic impur.(g)	(d)	3.7	amb.	0.19	24.0
Heroin base(e)	(f)	4.7	9 C	0.07	27.5
Heroin base(e)	(f)	3.7	9 C	0.02	27.0
Heroin base(e)	(f)	4.7	-10 C	0.00	22.0
Heroin base(e)	(f)	3.7	-10 C	0.00	21.0
Heroin HCl(e)	(f)	4.7	amb.	0.19(h)	28.0
Heroin base(e)	(i)	4.7	amb.	0.11	28.0
Heroin base(e)	(j)	4.6	amb.	0.59	19.0
Heroin base(e)	(j)	3.7	amb.	0.42	18.5
Heroin base(e)	(k)	3.8	amb.	0.35	19.5
Heroin base(e)	(l)	3.8	amb.	0.17	20.0
Heroin base(e)	(l)	4.7	amb.	0.54	21.0

(a)adjusted with 2M sodium hydroxide
(b)3.0 mg/ml
(c)heroin peak undergoes severe peak distortion
(d)10% acetonitrile, 89% water, 1% acetic acid
(e)0.90 mg/ml
(f)20% acetonitrile, 79% water, 1% acetic acid
(g)0.90 mg/ml heroin base, 0.10 mg/ml acetylcodeine base, 0.25 mg/ml noscapine and 0.12mg/ml papaverine
(h)calculated as hydrochloride
(i)20% acetonitrile, 79.8% water, 0.2% acetic acid
(j)99% water, 1% acetic acid
(k)20% methanol, 79% water, 1% acetic acid
(l)50% methanol, 49% water, 1% acetic acid

of hydrolysis of heroin in a buffered system. Since we found no hydrolysis of heroin at freezing temperature, samples could be frozen for re-analysis. The use of a cooling unit on the autosampler would be beneficial in reducing the amount of heroin hydrolysis.

Detection System

A photodiode array detector was used for sample analysis. This detection scheme, which simultaneously measures absorbance as functions of wavelength and time has been used previously to monitor peak purity and confirm peak identity in heroin exhibits[13-15]. For peak purity, Fell, et. al.[13,14], obtained a continuous plot of the 254:280nm absorbance ratio. Also in order to check peak identity, spectra and derivatives of spectra captured at peak apexes were utilized by Fell, et. al.[13,14],. In addition, UV detection at 280nm was used for the quantitation of heroin and basic impurities; while the internal standard was measured at 254nm.

Our approach was to use a software program "EVALUP" provided by the vendor to monitor peak purity. This non-interactive program first obtains a UV spectrum on the upslope of a peak which becomes the reference spectrum. This spectrum is then subtracted from the acquired spectrum on the apex of the peak which becomes the new reference spectrum. The new reference spectrum is then subtracted from spectra acquired on the downslope and end of the peak with the resultant spectrum inverted. All three resultant spectra are normalized and compared by direct overlay indicating peak purity. This approach would offer no advantage over absorbance ratio techniques utilizing a limited number of wavelengths when the interfering UV spectrum is similar to that of the compound of interest. However, two compounds can have similar absorbance ratios at one point in their UV spectra, but differ significantly at other points. The UV spectra generated were also used to confirm peak identity.

Both analogue signals on the photodiode array detector were time programmed so that the wavelengths were switched 12 minutes after the beginning of the run. For Signal A, detection was first at 218nm, then at 228nm; at 30 minutes this signal was switched to 240nm. Signal B was switched from 228nm to 240nm. For quantitative analysis the wavelength of choice for O^3-monoacetylmorphine and O^6-monoacetylmorphine was 218nm, while 228nm was chosen for the other compounds of interest. Although 218nm was more sensitive overall for the compounds of interest, at 228nm greater selectivity of detection was obtained for acetylcodeine in the presence of heroin. Additionally at 228nm, noscapine and papaverine were not subjected to baseline disturbances. In addition more selective detection was obtained for noscapine at 228nm in samples where papaverine-like impurities were present. A similar situation would hold for papaverine at 240nm in the presence of any noscapine-like impurities, if needed.

Linearity, Precision, and Sample Analysis

As indicated in Table 2, linear responses over specified concentration ranges were obtained for the compounds of interest at the wavelengths of choice with correlation coefficients of 0.999 or greater using peak height or peak area. In addition, computed regression lines passed essentially through the origin. It was apparent from the linearity studies that at higher concentration levels peak area was preferred over peak height for

Table 2 - Linearity and Chromatographic Reproducibility Via Photodiode Detection. Chromatographic conditions as described in experimental section.

Compound	Conc. Range (mg/ml)	Correlation Coefficient	Conc. (mg/ml)	CV[#] (%)	Wave-length (nm)	Area or Height[x]
O^3-monoacetylmorphine	0.0014-0.0250	1.000	0.0030	1.2	218	height
O^6-monoacetylmorphine	0.0037-0.2250	0.999	0.0275	0.6	218	height
Acetylcodeine	0.0040-0.1980	1.000	0.0158	1.0	228	height
Acetylcodeine	0.0040-0.4960	1.000	0.0158	2.3	228	area
Noscapine	0.0005-0.204	1.000	0.0010	2.8	228	height
Noscapine	0.0005-0.510	1.000	0.0010	8.0	228	area
Papaverine	0.0005-0.206	0.999	0.0010	2.2	228	height
Papaverine	0.0005-0.5160	1.000	0.0010	2.4	228	area
Heroin	0.1125-1.80	1.000	0.900	0.8	228	area

all free bases except O^3-monoacetylmorphine, which is sulfamate.
[#] coefficient of variation based on five injections.
[x] parameter used for computation.

computational purposes. At concentration levels above approximately 0.2mg/ml, nonlinear isotherms existed and detector saturation was observed which affect peak height measurements much more than peak area measurements. As indicated in Table 2, good chromatographic precision was obtained for the compounds of interest even when they were present at relatively low levels. Conversely, at lower solute concentrations significantly greater precision could be obtained for calculations based on peak height than for peak area.

Because of the high concentration of heroin present in the material analyzed, peak area should be used to determine this component. For the other components peak height should be utilized, provided that the solute concentration is within the linear range. The use of peak height has the further advantage of allowing more accurate quantitation in the event that minor components elute near the basic impurities of interest. The "EVALUP" program was used to monitor peak purity at the various solute concentrations used in our linearity studies. This methodology was successful for O^3-monoacetylmorphine and acetylcodeine down to a concentration level of 0.009mg/ml; while for O^6-monoacetylmorphine, noscapine, and papaverine, half this concentration level was monitored successfully. The absorbance peak height or peak area ratio from the analogue signal could be used in those instances when "EVALUP" was not successful. At higher solute concentration levels, where peak height linearity no longer holds, normalized UV spectra overlay could not be obtained because of detector overload.

Unadulterated samples seized in Southeast Asia, the Middle East, Europe and Mexico were analyzed. For many of these exhibits a comparison was made between the quantitative results obtained for O^6-monoacetylmorphine, acetylcodeine, and heroin via HPLC and the original results obtained when these samples were first examined via GLC procedures[5,25]. While in most instances good agreement was obtained for the quantitation of acetylcodeine and heroin, the agreement for O^6-monoacetylmorphine was much poorer. A comparison of results for more recently submitted samples is given in Table 3. Values for O^3-monoacetylmorphine via HPLC is also presented since O^3-monoacetylmorphine levels can be significant. This can contribute to the lack of agreement because the GLC method does not resolve the monoacetylmorphines. Other factors which can contribute to these discrepancies include

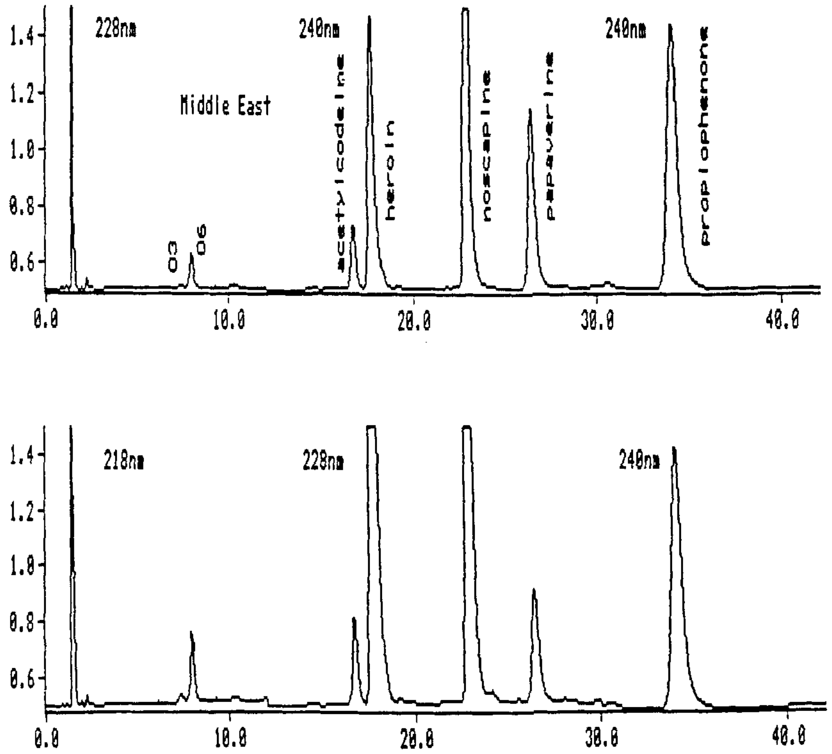
Table 3 - Comparison of Quantitative Determination$^+$ of O^6-monoacetylmorphine, Acetylcodeine, and Heroin via HPLC and GLC. HPLC chromatographic conditions are described in Experimental Section.

Sample No.	%O3	%O6		%Acetylcodeine		%Heroin		Heroin
	HPLC	HPLC	GLC	HPLC	GLC	HPLC	GLC	Type
1)	0.5	5.9	7.3	1.5	1.5	87.4	88.8	HCl
2)	0.1	0.7	0.9	4.3	4.4	63.8	61.8	HCl
3)	0.4	9.1	11.2	1.3	0.9	86.8	81.1	HCl
4)	0.2	9.7	10.0	4.4	3.9	74.7	75.2	HCl
5)	0.2	6.2	7.1	3.5	3.8	60.1	63.7	HCl
6)	0.1	1.2	1.0	4.0	3.7	60.3	62.7	base
7)	0.3	5.9	7.0	2.8	2.6	60.7	57.7	HCl
8)	0.2	1.1	1.8	4.5	5.0	69.8	72.7	HCl
9)	0.4	5.1	6.5	5.1	4.9	70.3	67.8	HCl
10)	0.9	0.7	1.1	4.1	4.0	54.0	52.7	base

$^+$based on single determination via GLC or HPLC

transacetylation between heroin and some of the constituents of illicitly prepared heroin to produce O^6-monoacetylmorphine[26], production of O^6-monoacetylmorphine from heroin in the heated injection port[27], hydrolysis of heroin on storage, and the instability of O^3-monoacetylmorphine over time[12].

During an unattended run, 10 samples could be assayed before heroin hydrolysis provided any significant errors. For samples containing 0.6% O^6-monoacetylmorphine, which was in practice the lowest amount encountered, a positive error of no more than 10% would be obtained. This error in O^6-monoacetylmorphine determination was minimized by using a sample concentration of heroin approximately equal to that of the mixed standard. The hydrolysis of heroin in the sample was partially compensated for by hydrolysis of heroin in the standard. By using a normalized heroin content, consistent lower limits of quantitation were



7. Chromatographic separation using multi-wavelength detection of a heroin sample seized in the Middle East. For chromatographic conditions, see experimental section.

obtained for O^6-monoacetylmorphine and other compounds of interest. With a heroin concentration of 0.9mg/ml, O^3-monoacetylmorphine and noscapine and papaverine were determined at levels of 0.1% relative to heroin. Values for O^6-monoacetylmorphine and acetylcodeine were 0.40% relative to heroin.

Quantitative values of the various basic impurities relative to heroin in the samples analyzed were found to vary over a large range and formed a basis for comparing illicit heroin samples from various source areas. An example of a chromatogram of a heroin sample seized in the Middle East is presented in Figure 7.

CONCLUSION

An excellent separation of heroin, selected basic impurities and adulterants was obtained via reversed-phase liquid chromatography. Methodology amenable to automation was developed which allowed the simultaneous quantitation of heroin, acetylcodeine, O^3-monoacetylmorphine, O^6-monoacetylmorphine, noscapine, and papaverine in illicitly manufactured unadulterated heroin samples. Certain impurities such as O^3-monoacetylmorphine, noscapine, and papaverine were measured at levels of at least 0.1% relative to heroin. Peak purity as well as peak identity were confirmed by a photodiode array detector.

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