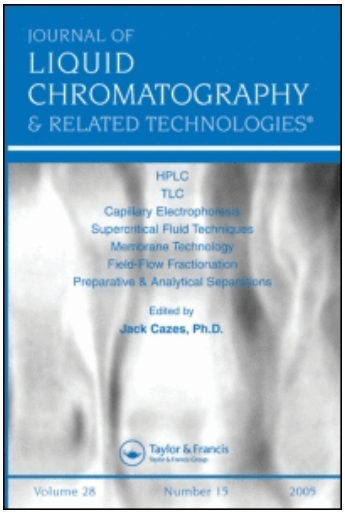

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THE QUANTITATION OF HEROIN AND SELECTED BASIC IMPURITIES VIA REVERSED PHASE HPLC. II. THE ANALYSIS OF ADULTERATED SAMPLES

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ABSTRACT

Methodology is presented for the quantitation of heroin, O^6-monoacetylmorphine, acetylcodeine, noscapine and papaverine in adulterated illicit heroin samples. Reversed phase chromatography was employed using an HS-5 C18 column with a gradient system. This methodology used a multimode detection scheme which consisted of a photodiode array detector in series with a dual electrochemical detector in the parallel mode. Owing to its high specificity for O^6-monoacetylmorphine, electrochemical detection via the oxidation mode proved necessary for the quantitation of this compound in samples highly adulterated with quinine. The use of relative retention times, UV spectra and dual electrochemical response ratios for the screening of adulterants in heroin is discussed.

INTRODUCTION

For forensic chemists the simultaneous quantitation of heroin and basic impurities in illicit heroin samples can be important for intelligence purposes. The results of these assays can form the basis

for comparative analysis of exhibits. GLC and HPLC procedures have been reported for the quantitative determination of heroin and/or basic impurities in both unadulterated and adulterated samples (1-13). The analysis of the above compounds in adulterated samples can be difficult because of possible interferences. Limits of detection for basic impurities can be higher in adulterated samples due to column overload. This is a particular problem with GLC methods using dilute-and-shoot methods. There are few reports dealing with the analysis of such samples.

This paper reports a reversed phase procedure for the simultaneous quantitation of O^6-monoacetylmorphine, acetylcodeine, heroin, noscapine and papaverine in the presence of large quantities of common adulterants such as sugars, polyhydric alcohols, procaine, acetylprocaine, caffeine and quinine. The chromatographic conditions which have been previously reported (13) for the analysis of unadulterated samples have been modified. In addition, a multimode detection scheme consisting of photodiode array detection in series with dual electrochemical detection in the parallel mode was employed. The feasibility of screening for possible adulterants present in heroin samples via the use of relative retention times, UV spectra and dual electrochemical response ratios was examined. The use of the photodiode array detector for general drug screening in toxicological samples has previously been reported (14). In addition, response ratios obtained from coupling a single wavelength UV detector with a single channel electrochemical detector has been used for drug screening (15). Twelve broad based categories based on ultraviolet and electrochemical response ratios were used in the study by Jane et. al.

EXPERIMENTAL

Materials

Methanol was acquired from Burdick and Jackson (Muskegon, MI, U.S.A.). Propanophenone was obtained from Pierce Chemical (Rockford, IL, U.S.A.). Reagent grade hexylamine was acquired from Eastman Chemicals (Rochester, NY, U.S.A.). 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 amine-phosphate buffer. The preparation of the hexylamine-phosphate buffer and the injection solvent has been described (13).

Equipment

The liquid chromatographic system consisted of a Series 4 liquid chromatograph (Perkin-Elmer, Norwalk, CT, U.S.A.), an ISS-100 autosampler fitted with a 50 ul loop (Perkin-Elmer); an HS-5 C18 column (12.5 cm x 4.6 mm I.D.) (Perkin-Elmer); a Model 1040a photodiode array detection system (Hewlett-Packard, Waldbronn, FRG); a Model LC-4B dual amperometric detector fitted with a dual TL-5 glassy carbon electrode cell in the parallel mode, and an Ag/AgCl reference detector with the top half of the cell serving as the auxiliary electrode (Bioanalytical Systems, Inc., West Lafeyette, IN, U.S.A.); four Model LCI-100 laboratory computing integrators (Perkin-Elmer) interfaced to a Model 7500 data station equipped with Chromatographics 3 software (Perkin-Elmer); and a Model PR-210 printer (Perkin-Elmer).

Standard Preparation

Accurately weigh into a 100 ml volumetric flask 1.0 mg each of noscapine base and papaverine base; 1.5 mg of O^6-monoacetylmorphine base, 3.0 mg of acetylcodeine base and 90 mg of heroin base. Before diluting to volume with injection solvent, add to the flask nalorphine and propanophenone internal standards in injection solvent to give final concentrations of 0.01 mg/ml and 0.04 mg/ml, respectively. Inject 50 ul onto the liquid chromatograph.

Sample Preparation

Accurately weigh into a volumetric flask an amount of heroin which results in an approximate heroin base concentration of 0.90 mg/ml after dilution to volume. Before diluting to volume with injection solvent, add to the flask nalorphine and propanophenone internal standards in injection solvent to give final concentrations of 0.01 mg/ml and 0.04 mg/ml, respectively. Inject 50 ul onto the liquid chromatograph.

Chromatographic Conditions

Gradient elution conditions were a modification of a previously described system (13). This modification allowed for the elution of highly lipophilic adulterants. The initial conditions consisted of 5% methanol, 95% phosphate buffer (0.023 M hexylamine, pH 2.2). A

linear gradient was employed for 20 minutes with the final conditions of the first gradient step consisting of 30% methanol, 70% phosphate buffer. The latter conditions were held for six minutes. Next, a linear gradient was employed for 10 minutes with the final conditions of this second gradient step consisting of 60% methanol, 40% phosphate buffer. These final conditions were held for nine minutes. A re-equilibration time of 10 minutes was found to be satisfactory. A flow rate of 1.5 ml/min was employed throughout. Detection wavelengths were identical to those previously employed on the photodiode array detector (13). For the quantitation of O^6-monoacetylmorphine and general screening, oxidation potentials of 1.0 V and 1.1 V or equivalent were used in the electrochemical detector.

RESULTS AND DISCUSSION

Quantitation of Highly Adulterated Samples

In order to determine whether the compounds of interest could be determined in the presence of adulterants, unadulterated samples which were spiked with large quantities of cutting agents were analyzed before and after being adulterated. As table 1 indicates, O^6-monoacetylmorphine, acetylcodeine, heroin, noscapine and papaverine could be determined in the presence of sugars and polyhydric alcohols even when present relative to heroin in a weight ratio of 100 to 1. This determination was facilitated by the lack of any UV absorbance by sugars and polyhydric alcohols at the wavelengths used for analysis.

Table 1

Results of Analysis via UV Detection of Two Samples Cut with Various Compounds

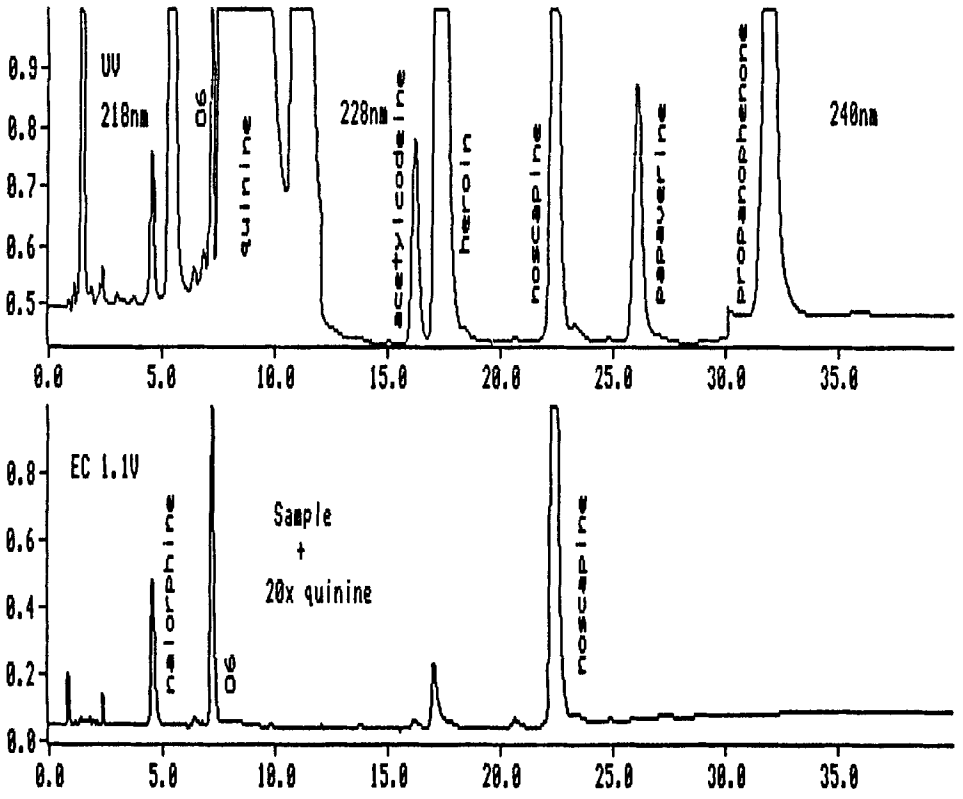
Sample	Heroin (%)	O^6- (%)	Acetylcodeine (%)	Noscapine (%)	Papaverine (%)
uncut	71.6	2.8	6.6	2.7	2.5
100x[1] dextrose	72.7	2.8	6.8	2.7	2.6
100x sucrose	71.1	2.7	6.6	2.6	2.5
100x lactose	72.7	2.7	6.7	2.7	2.5
100x mannitol	73.8	2.8	6.9	2.8	2.6
100x innositol	72.4	2.7	6.8	2.7	2.6
uncut	93.1	0.5	5.6	N.D.[2]	N.D.
20x caffeine	93.7	0.6	5.9	N.D.	N.D.
20x procaine and 20x acetylprocaine	93.5	0.5	5.6	N.D.	N.D.

Heroin determined via peak area. O^6-monoacetylmorphine, acetylcodeine, noscapine and papaverine determined via peak height. In the case of 20x procaine + 20x acetylprocaine, O^6-monoacetylmorphine determined via peak area. All compounds determined at 228 nm except O^6-monoacetylmorphine which is determined at 218 nm.
(1) Denoting a weight ratio of 100:1 cutting agent to sample.
(2) Not detected.

As table 1 also indicates, the compounds of interest, if present, could be determined in the presence of a 20:1 weight ratio of caffeine, procaine and acetylprocaine relative to heroin.

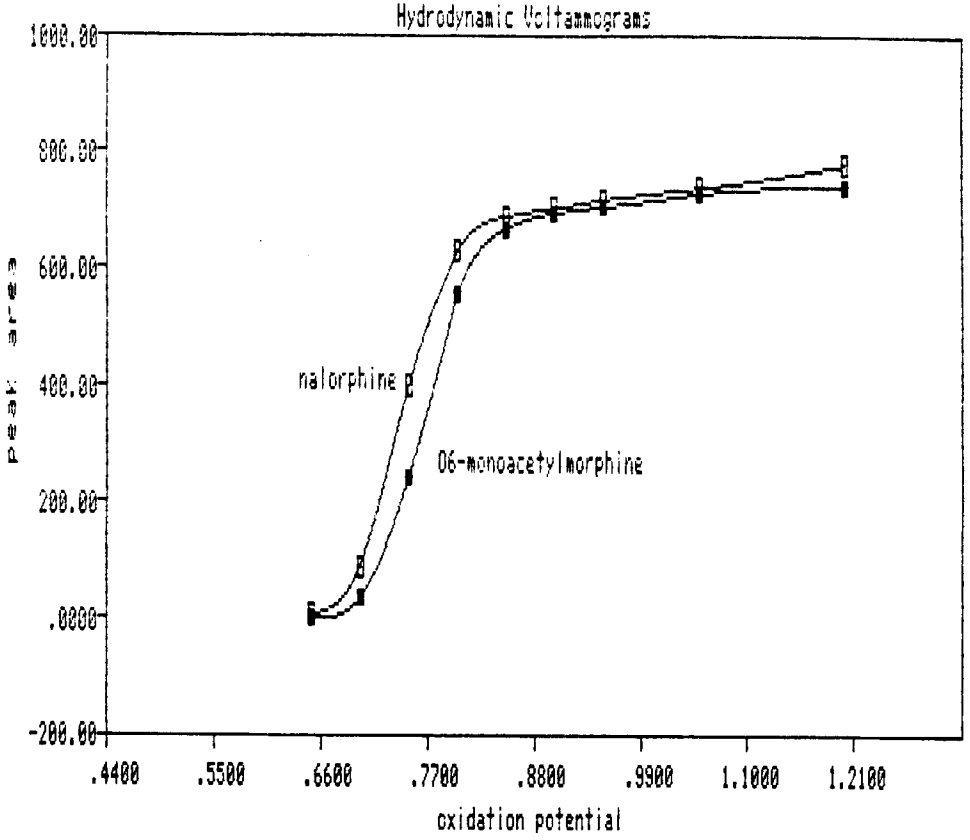
The determination of the compounds of interest in the presence of large amounts of the adulterant quinine is illustrated in table 2. It is not uncommon for this adulterant to be present in ratios as high as 60-80X relative to heroin. As table 2 and figure 1 indicate, UV detection is viable up to approximately a 20:1 weight ratio of quinine to heroin. For larger amounts of quinine, electrochemical detection would be the method of choice due to the great selectivity of detection for O^6-monoacetylmorphine.

Good precision and accuracy were obtained via both UV and electrochemical detection as shown in table 2. The favorable results for electrochemical detection were obtained by careful selection of both the working potential and the internal standard. Hydrodynamic voltammograms for O^6-monoacetylmorphine and nalorphine, a structurally related compound, are shown in figure 2. Nalorphine, which was found to be chromatographically suitable for use as an internal standard in the present study, was used as an internal standard for the electrochemical determination of structurally similar morphine (16). For greatest reproducibility it was desirable to work at the plateau region of the hydrodynamic voltammogram where responses varied little with changes in applied potential. These changes could arise due to drifts in the redox potential of the reference electrode. At the plateau region the responses of O^6-monoacetylmorphine and nalorphine were essentially similar. Also at the plateau region overpotential



1. Chromatogram of heroin sample containing 20X quinine to heroin.

effects, which could result in nonlinearity of detection of O^6-monoacetylmorphine, were minimized. At higher solute concentrations, uncompensated resistances could occur which would result in the compound being detected at an effectively lower potential and possibly with a smaller response depending on the operating potential. It was also desirable to work at the plateau region to minimize temperature dependence effects on the solute response (17). Although small changes



2. Hydrodynamic voltammograms of 1.04 mg/ml nalorphine and 0.986 mg/ml O^6-monoacetylmorphine.

could occur in this region due to the temperature dependence of the diffusion coefficients of the solutes, they are compensated by the use of a structurally similar internal standard (17). Another advantage electrochemically for the use of the internal standard would be compensation for any electrode passification. At an oxidation potential of 1.1 V, the O^6-monoacetylmorphine peak area response normalized to internal

Table 2

Results of Analysis of a Sample Cut with Quinine

Ratio of Sample to Quinine	Heroin (%)	O^6- (%)	O^6- (%)	Acetyl-codeine (%)	Noscapine (%)	Papaverine (%)
Detector	UV[1]	UV	EC[2]	UV	UV	UV
Parameters	228 nm	218 nm	1.1V	228 nm	228 nm	240 nm
uncut	61.1	1.2	1.2	4.2	8.3	1.7
1:20	60.0	1.1	1.2	4.1	8.2	1.6
	60.6	1.1	1.2	4.2	8.0	1.6
	60.1	1.1	1.2	4.1	8.0	1.7
	60.6	1.1	1.3	4.2	8.0	1.6
	60.5	1.2	1.2	4.2	8.0	1.6
1:80	60.5	*	1.3	4.1	8.4	1.6

*O^6-monoacetylmorphine peak not resolved.
All *compounds determined via peak area.*
(1) Ultraviolet.
(2) Electrochemical.

standard was linear between 0.525 and 304 ug/ml. For the linearity determination, a correlation coefficient of 0.9998 was obtained with the plot passing through the origin.

As shown in tables 1 and 2, peak area was used in certain instances. It is known (13) that because of the possibility of obtaining minor interferences in the complex chromatograms obtained and higher

precision at low levels, peak height is normally preferred over peak area. However for the present study, if large amounts of basic adulterants were present, the linear isotherm for compounds of interest was altered so that the use of peak height was no longer viable.

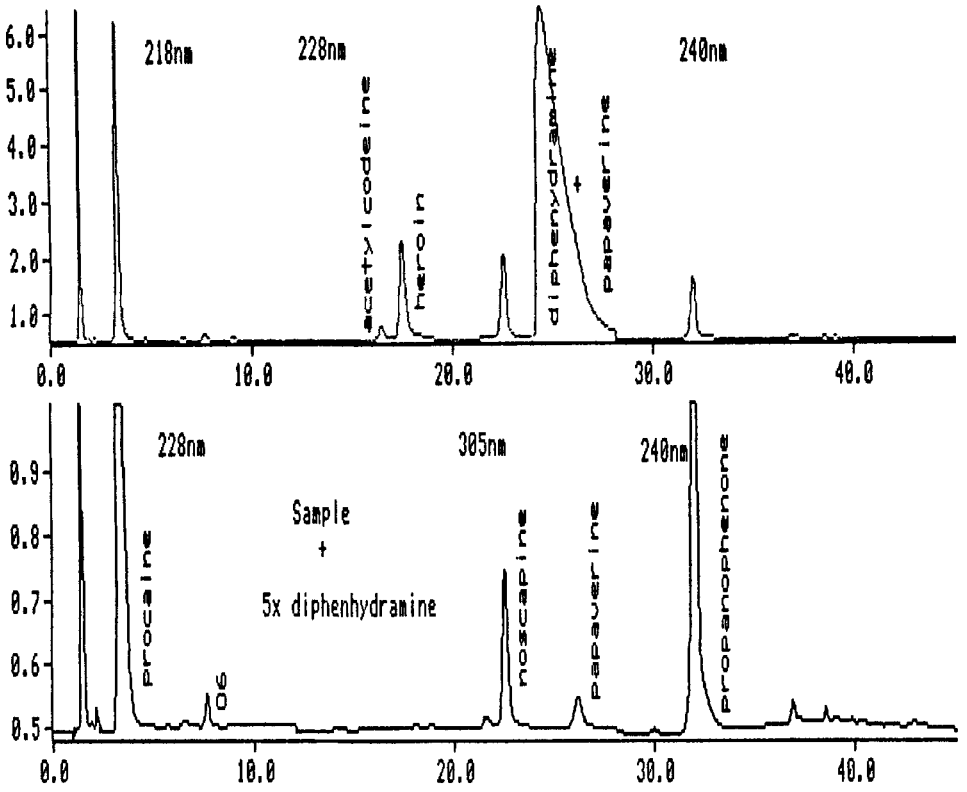
For the quantitation of other adulterants, alternative wavelengths of detection or the use of electrochemical detection was found to be useful due to selective detection. For example, as figure 3 illustrates, papaverine can be determined in the presence of diphenhydramine. For certain samples, electrochemical detection could provide selective detection for O^6-monoacetylmorphine or noscapine.

Screening

The methodology reported here is applicable to the screening of upwards of 50 compounds whose presence has been reported in heroin samples.

The qualitative identification of these compounds at levels of 1.0% or greater could be accomplished by the use of relative retention times, UV spectra and dual electrochemical response factors.Table 3 presents relative retention times and dual electrochemical response ratios for these compounds.

Acetylcodeine was chosen as the reference compound since it is present in every sample at relatively high concentration levels, it elutes



3. Chromatogram of heroin sample containing 5X diphenhydramine to heroin.

in the middle of the chromatogram, and its k' value is relatively constant over column lifetime.

The UV spectra were evaluated using vendor software "EVALUP" which measures peak purity by examining normalized UV spectra at the upslope, apex and downslope of a peak.

Table 3

Retention Relative to Acetylcodeine and Dual Electrochemical Response Ratios for Compounds Present in Heroin Samples

Compound	RRT	A_{EA} / A_{EB}
tartaric acid	0.0607	1
nicotinimide	0.0697	1
morphine	0.149	3
ephedrine	0.163	1
procaine	0.175	2
dipyrone	0.204	2
aminopyrene	0.217	2
tripelennamine	0.218	1
methapyrilene	0.221	1
methamphetamine	0.256	1
nalorphine	0.267	3
phenmetrazine	0.267	1
codeine	0.325	1
pyrilamine	0.341	2
lidocaine	0.342	1
acetaminophen	0.380	3
O^3-monoacetylmorphine	0.414	1
O^6-monoacetylmorphine	0.454	3
quinidine	0.465	1
acetylprocaine	0.550	1
quinine	0.570	1
ethylmorphine	0.603	1
barbital	0.742	1
strychnine	0.745	1
salicilamide	0.814	2
tropacocaine	0.829	1
cocaine	0.864	1
caffeine	0.887	1
benztropine	0.890	1
thebaine	0.94	1
acetylcodeine	1.000 (16.56 min.)	1
antipyrene	1.010	1
heroin	1.050	1
meconin	1.190	1
aspirin	1.270	1
noscapine	1.410	2
phenobarbital	1.440	1
phenacetin	1.480	2
papaverine	1.490	1
salicylic acid	1.510	2
tetracaine	1.540	2
diphenydramine	1.660	1
propanophenone	2.080	1
propoxyphene	2.160	1
amitriptyline	2.240	*
secobarbital	2.250	1
methaqualone	2.270	1
phenolphthalein	2.270	3
diazepam	2.420	1

#Peak area normalized to nalorphine at electrode A divided by peak area normalized to nalorphine at electrode B. All compounds were measured at a concentration of 0.01 to 0.05 mg/ml.

1. Response before rising portion of hydrodynamic voltammogram.
2. Response at rising portion of hydrodynamic voltammogram.
3. Response at plateau region of hydrodynamic voltammogram.

* Coelutes with gradient artifact.

Dual electrochemical response ratios were reported as one of three broad categories in order to minimize changes in ratios which result from drifts in the reference electrode redox potential, sample overpotential effects or changes in temperature. For the analysis of platinum-derived cancer chemotherapy drugs, Ding and Krull report on the difficulty of obtaining reproducible absolute response ratios on a day-to-day basis (18). Peak area responses normalized to the internal standard nalorphine were used in order to minimize effects due to electrode passification. The three categories were as follows:

1. Responses before the rising portion of hydrodynamic voltammogram, i.e., a dual response ratio of essentially zero. In practice any normalized response at the higher ratio electrode potential of 0.2 or less was used as a cutoff to place a sample in category 1.

2. Responses at the rising portion of hydrodynamic voltammogram, i.e., a ratio of 1.3 or greater with the normalized response at the higher electrode potential greater than 0.2.

3. Responses at the plateau region of a hydrodynamic voltammogram, i.e., a response ratio of less than 1.3.

Based on a five sigma window for relative retention times and the comparison of UV spectra, a single component from the list in table 3

was uniquely identified. Categories from the dual electrochemical response ratios gave additional independent confirmation. If more than one compound was present in the same relative retention time window, distinguishing them via UV spectra could be difficult depending on relative concentrations and/or similarity of UV spectra. The presence of an electrochemical response ratio could aid in identification of mixtures. For example, it is difficult to distinguish via UV spectroscopy phenolphthalein in the presence of methaqualone. However, phenolphthalein has an electrochemical response ratio of approximately 1.0, while methaqualone has no response at either potential.

A test mixture of five representative compounds gave a single day average coefficient of variation of relative retention times of 0.25%. Over a period of four weeks the average percent variation for ten test compounds was 4.5%. The changes which occur during column lifetime have been previously explained (13). Reproducibility of UV spectra was dependent on whether the instrument was correctly calibrated, which was determined by examining spectra of standard components on a daily basis. Reproducibility of broad-based electrochemical response factors was dependent on the frequency with which voltammograms for O^6-monoacetylmorphine were obtained. Changes could most likely occur for those compounds whose ratios occur near the boundaries of the categories. For best results, suspected compounds should be confirmed by standard injection on the same day of analysis.

REFERENCES

1. S. P. Sobol and A. R. Sperling, in G. Davies (Editor), Forensic Science--American Chemical Society Symposium Series No. 12, Washington, D.C., 1975, p. 170.

2. T. A. Gough and P. B. Baker, J. Chromatogr. Sci., 19 (1981) 227.

3. M. Gloger and H. Neumann, Forensic Sci. Int., 22 (1983) 63.

4. B. Law, J. R. Joyce, T. S. Bal, C. P. Goddard, M. Japp, and I. J. Humphreys, Anal. Proc., 20 (1983) 611.

5. J. M. Moore, The Application of Derivatization Techniques in Forensic Drug Analysis, in Instrumental Applications in Forensic Drug Chemistry (M. Klein, A. V. Kruegel and S. P. Sobol, eds.) U.S. Government Printing Office, Washington, D.C., (1978) 180.

6. J. M. Moore, J. Chromatogr., 147 (1978) 327.

7. J. M. Moore and M. Klein, J. Chromatogr., 154 (1978) 327.

8. H. Huizer, H. Logtenberg, and A. J. Steenstra, Bulletin on Narcotics, 29 (1977) 65.

9. H. Huizer, J. Forensic Sci., 28 (1983) 32.

10. H. Huizer, J. Forensic Sci., 28 (1983) 40.

11. P. C. White, I. Jane, A. Scott, and B. E. Connett, J. Chromatogr., 265 (1983) 293.

12. P. B. Baker, and T. A. Gough, J. Chromatogr. Sci., 19 (1981) 483.

13. I. S. Lurie and S. M. Carr, J. Liquid Chromatogr., in press.

14. F. Overzet, A. Rurak, H. Van Der Voet, B. F. H. Drenth, R. T. Ghijsen and R. A. De Zeeuw, J. Chromatogr., 267 (1983) 329.

15. I. Jane, A. McKinnon and R. J. Flanagan, J. Chromatogr., 323 (1985) 191.

16. R. D. Todd, S. H. Muldoon and R. L. Watson, J. Chromatogr., 232 (1982) 101.

17. D. J. Miner, M. J. Skibic and R. J. Boop, J. Liq. Chromatogr., 6 (1983) 2209.

18. X. D. Ding and I. S. Krull, J. Liq. Chromatogr., 6 (1983) 2173.