Journal of Chromatography, 265 (1983) 293–300
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 15,892

USE OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY TO QUANTITATE THE OPIATE AND SUGAR CONTENT OF ILLICIT HEROIN PREPARATIONS

P. C. WHITE*, I. JANE, A. SCOTT and B. E. CONNETT
Metropolitan Police Forensic Science Laboratory, 109 Lambeth Road, London SE1 7LP (Great Britain)
(Received March 31st, 1983)

SUMMARY

Two high-performance liquid chromatographic systems can be used to obtain the opiate and sugar content of illicit heroin preparations. The combined quantitative results can then be used to determine if the samples originate from a common source.

INTRODUCTION

In about half of the illicit heroin preparations that are encountered in Great Britain, the sugars glucose, sucrose and lactose are used either individually or as mixtures to dilute the active ingredient. For forensic purposes it is important to be able to quantitate both active components and diluents to determine if the samples originated from a common source. This paper describes a dual-column high-performance liquid chromatographic (HPLC) approach to this problem.

Gas chromatography can be used for the analysis of sugars after they have been silylated with N-trimethylsilylimidazole[1]. However, the heroin and the other alkaloids interfere because they cannot be silylated with this reagent[2]. HPLC has been widely employed for the analysis of sugars[3], and an HPLC method previously developed in this laboratory[4] was adapted to determine the sugars present in the illicit heroin samples. Chloride ions from the heroin hydrochloride were found to produce an interference problem, but this study shows that after their removal by treatment with silver sulphate and barium hydroxide, reproducible quantitation of glucose, sucrose and lactose can be obtained.

Although the system for separating the sugars is unable to resolve the active components in heroin mixtures, many HPLC methods for this analysis have been reported using silica[5,6], reversed-phase[7,8], reversed-phase ion-pair[9–11] and cation-exchange packing materials[12]. Since the majority of these gave either poor separations or poor peak shapes of heroin, monoacetyl morphine, codeine and acetyl codeine, accurate quantitation could not be obtained. With the system developed by Huizer *et al.*[6], quantitation of diamorphine and morphine could not be achieved in a single chromatographic run, but the work reported here shows that by modifying the solvent conditions this problem can be resolved, and reproducible quantitation can be obtained.

0021-9673/83/$03.00

EXPERIMENTAL

Sugar analysis

The analyses were performed on a 12.5 cm × 4.9 mm I.D. stainless steel column packed with irregular shaped silica of *ca.* 5-μm diameter, average pore diameter 13 nm, and surface area 320 m^2 g^{-1}. The column was initially conditioned with eluent consisting of acetonitrile–water (75:25) containing 0.1% (v/v) pentaethylenehexamine for 0.5 h at 3 ml min^{-1}. The eluent was then changed to contain 0.01% (v/v) of the amine and was pumped thereafter at 2 ml min^{-1}. The eluent was monitored with a Model 750/13 refractive index detector (Applied Chromatography Systems, Luton, Great Britain). Injections were made under continuous flow conditions via an injection valve (Negretti and Zambra, Southampton, Great Britain), fitted with a 25-μl volume injection loop.

Pure sugar standards were prepared by dissolving glucose (100 mg), sucrose (100 mg) and lactose (150 mg) in distilled water (10 ml), and suitable dilutions of this standard were used for calibration.

The illicit heroin samples were finely ground and then prepared for analysis by vortexing an accurately weighed amount (*ca.* 10 mg) with a silver sulphate solution (400 μl; 200 mg in 30 ml of water). After heating at 50°C for 1 min the mixture was cooled and treated with barium hydroxide solution (100 μl; 200 mg in 10 ml of water), vortexed and allowed to stand for 0.25 h. After centrifuging, aliquots of the supernatant were analysed.

Opiates analysis

Initial studies were performed on a 15-cm column but a 25-cm column was used finally for the sample comparison work. Both columns were packed with silica of the type described above. Separations were obtained with isooctane–diethyl ether–methanol–diethylamine water (400:325:225:0.5:15) at 2 ml min^{-1}. To obtain stable eluents pyrogallol was removed from the ether by passing it through a column of Florisil (60–80 mesh; BDH, Poole, Great Britain). A UV detector (Model CE202; Cecil Instruments, Cambridge, Great Britain) was used to monitor the eluent at 279 nm. Injections were performed under the same conditions as described for the sugar analysis. Quantitation was conducted with a Model 308 computing integrator (LDC, Stone, Great Britain).

Samples of finely ground illicit preparations were accurately weighed (*ca.* 10 mg) into a 25-ml volumetric flask and dissolved in methanol. Aliquots of these solutions were analysed to determine the alkaloid composition. Samples of morphine hydrochloride and diamorphine hydrochloride were obtained from McFarlan Smith, Edinburgh, Great Britain, and monoacetyl morphine was supplied by the Home Office Central Research Establishment, Aldermaston, Great Britain. Acetyl codeine was produced in the laboratory by acetylation of codeine, and its purity was established by gas chromatography–mass spectrometry.

RESULTS AND DISCUSSION

Sugar analysis

When the sugar separation system previously developed in this laboratory[4]

was applied to the analysis of illicit heroin preparations, a broad peak was detected with a retention time which coincided with that of sucrose, and this was shown to be due to chloride ions released by the dissolution of heroin hydrochloride. Attempts to resolve the chloride peak from the sugars by varying the eluent composition, amine concentration and pH were unsuccessful. In agreement with Hendrix *et al.*[13], it was found that if the eluent pH was less than pH 9, or if the amine concentration exceeded 0.02% (v/v), negative peaks were produced.

The possibility of removing the chloride ions by the addition of organic bases was investigated. Triethylamine, diethylamine, trimethylamine and pyridine were used but only triethylamine removed the interference. Unfortunately negative deviations in the baseline were produced which precluded the use of this amine.

The classic method for detecting chloride by the addition of silver ions to precipitate silver chloride was investigated. Although silver nitrate eliminated the chloride peaks, it could not be used because the nitrate ions produced a peak with a retention time similar to that of glucose. With silver sulphate, the chloride ions were removed but sulphate ions produced a very broad deviation in the baseline which encompassed the retention range of all the sugars. Although the sugars could be quantitated against this background it was preferable to remove the sulphate ions,

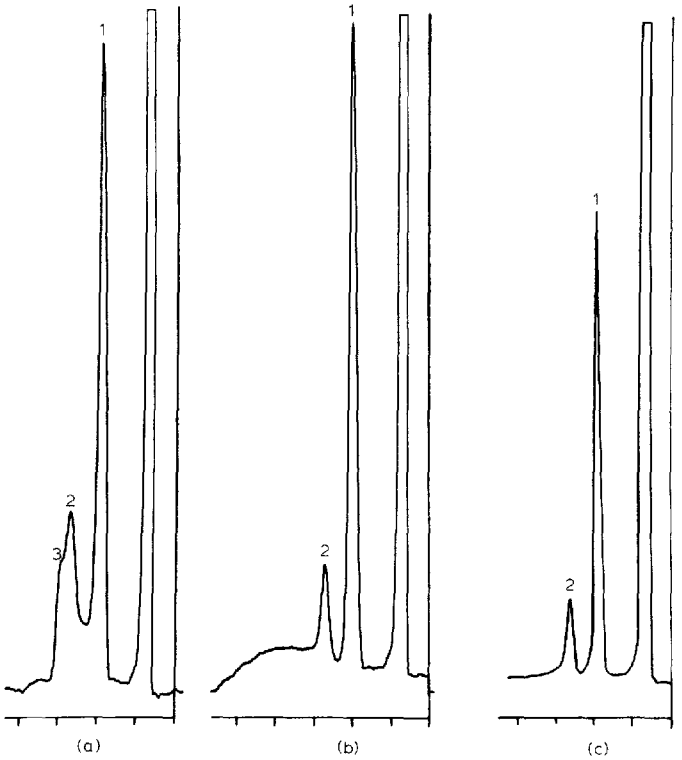

Fig. 1. (a) Analysis of an aqueous illicit heroin sample. (b) Sample after treatment with silver sulphate. (c) Sample after treatment with silver sulphate and barium hydroxide. 1 = Glucose; 2 = sucrose; 3 = chloride interference. Chromatography conditions are as stated in the text. Scale graduations represent 2-min intervals.

and this was successfully accomplished by their precipitation with barium hydroxide. The chromatogram obtained from an aqueous illicit heroin sample is shown in Fig. 1a and Fig. 1b and c illustrate the improvements obtained by using silver sulphate and silver sulphate–barium hydroxide respectively.

Linear calibration curves of peak height *versus* the weight of sugar injected were obtained from aqueous mixed standards containing 0–260 μg of each sugar. Sucrose and lactose both gave a lower peak height response in comparison with glucose because of their longer retention times. Calibration curves derived from peak area measurements gave responses which were all similar to that obtained for the glucose peak height calibration curve, but the peak height method was preferred because greater accuracy could be obtained.

Reproducibility of this method for the quantitation of sugars was determined by analysing a heroin sample that was made up in this laboratory. This standard was prepared by grinding together known amounts of heroin hydrochloride, glucose, sucrose and lactone. Ten accurately weighed amounts of this preparation were then treated as outlined in the experimental section and each sample was analysed in duplicate. From peak height measurements the weight of each sugar was determined from the respective calibration curve and the percentage weight of sugar in each sample was calculated. The statistical results are shown in Table I.

Overall the reproducibility results of the individual and total sugar content of the standard compared favourably with the actual values, and therefore the method was suitable for the quantitation of sugars in illicit heroin samples.

Opiate analysis

Heroin (diamorphine) is produced by acetylation of morphine obtained from opium. In clandestine laboratories the purification of the morphine is not particularly efficient, and the heroin produced contains significant amounts of the opium alkaloids and their acetylated products. Monoacetylmorphine (6-MAM) can arise from either incomplete acetylation of the morphine, or by partial decomposition of diamorphine if it is present. The HPLC system must be capable of resolution of all of these compounds. With the system that has been used in this laboratory for several years[14] (System 1), complex mixtures were difficult to analyse due to poor resolution between morphine and codeine, and diamorphine, 6-MAM and acetylcodeine.

TABLE I

RESULTS OF REPRODUCIBILITY STUDY

	Total sugars	*Sugar content*		
		Glucose	*Sucrose*	*Lactose*
Actual % weight	41.2	11.7	11.8	17.7
Mean % weight	44.4	12.1	12.9	19.3
Relative standard deviation (%)*	3.9	4.4	12.9**	2.6

* Result from duplicate analysis of ten samples.

** Exclusion of two high results reduce the R.S.D. to 6.3%. This value was found to be similar to that obtained from the analysis of illicit preparations.

An isooctane–diethyl ether–methanol–diethylamine eluent system has been described previously for the analysis of opiate alkaloids in illicit heroin preparations[6]. However, under these conditions morphine gave a long retained asymmetric peak. Our initial experiments with this system indicated that when a small amount of water was added and the methanol level was increased (System 2), morphine was eluted within 10 min from a 15-cm column and the retention times of the other alkaloids were more reproducible. In order to improve the morphine peak shape still further the diethylamine, water and methanol contents were varied, and Figs. 2a and 2b illustrate the influence of two of these variables.

An increase in diethylamine content produced a decrease in retention of the opiates without improving the peak shape. Increasing the water content of the eluent improved the peak shapes of all the components, and produced relatively small decreases in their retention. From this information it was possible to develop an eluent system which gave good peak shapes and an acceptable analysis time. The composition of this eluent was: isooctane–diethyl ether–methanol–diethylamine–water (400:325:225:0.5:15), (System 3). Chromatograms obtained from the analyses of an opiates standard with the three eluent systems that have been described are shown in Fig. 3, and illustrate the improvements that were obtained by modifying the eluent composition.

Several compounds encountered in illicit heroin preparations are insoluble in some organic solvents. In particular, morphine salts and diamorphine phosphate have limited solubility in chloroform. Our studies have shown that methanol was the preferred solvent.

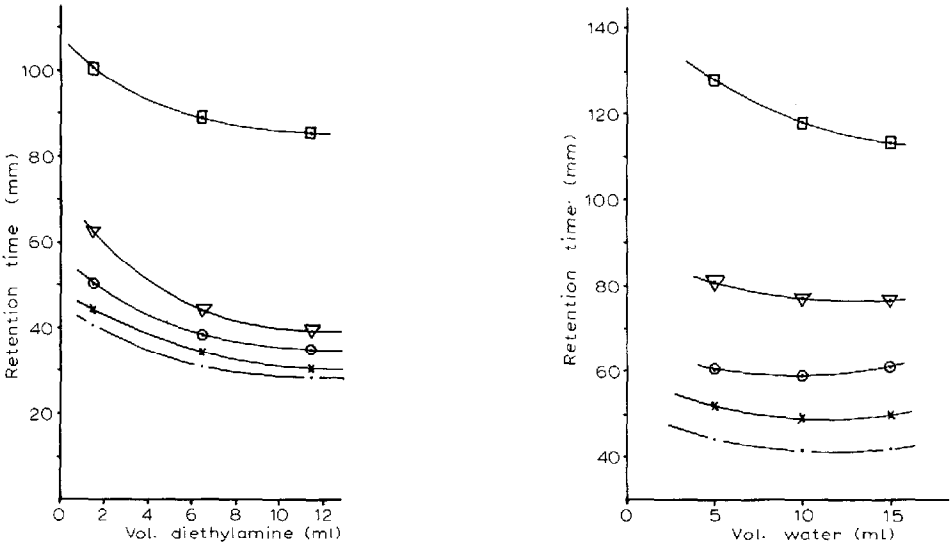

Fig. 2. Effects of changing the diethylamine and water content on the retention of the opiates. (a) Variation of diethylamine content. Eluent, isooctane–diethyl ether–methanol–diethylamine–water (400:325:225:*X*:0.5). (b) Variation of water content. Eluent, isooctane–diethyl ether–methanol–diethylamine–water (400:325:200:1.5:*X*). Results obtained on a 25-cm column, and other conditions as stated in text. ● = cetylcodeine; × = diamorphine; ○ = 6-MAM; ▽ = codeine; □ = morphine.

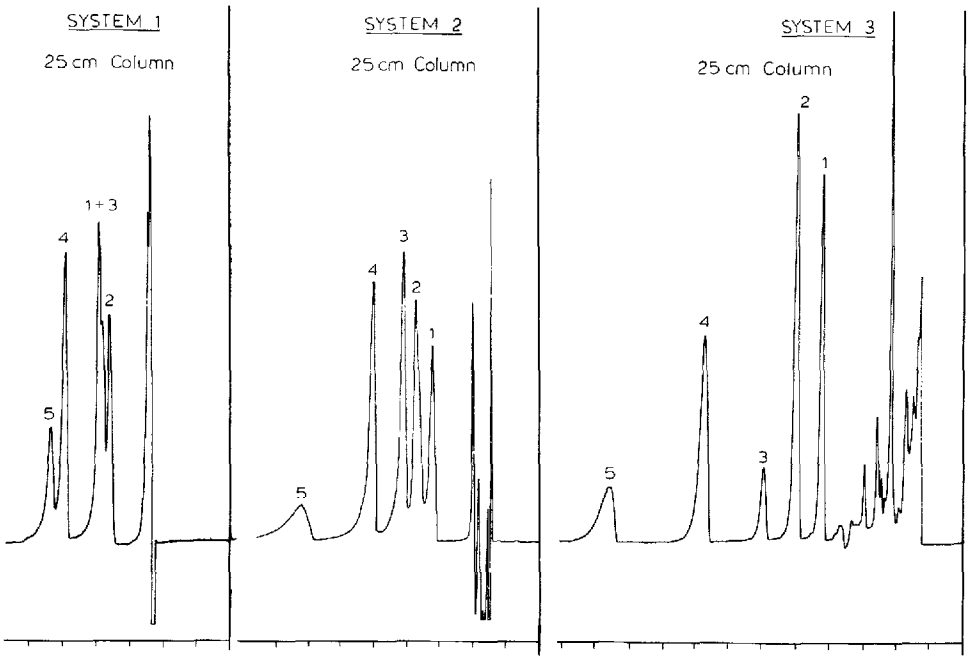

Fig. 3. Improvements obtained by modifying the composition of the eluent. System 1, methanol–2 *N* ammonia solution–1 *N* ammonium nitrate (27:2:1); other conditions as described in ref. 13. System 2, iso-octane–diethyl ether–methanol–diethylamine–water (400:325:175:1.5:0.5). System 3, same as system 2 but composition altered to 400:325:225:0.5:15. Other conditions as described in the text. Scale graduations represent 1-min intervals. 1 = Acetylcodeine; 2 = diamorphine; 3 = 6-MAM; 4 = codeine; 5 = morphine.

TABLE II

RETENTION DATA FOR THE OPIATE ALKALOIDS AND OTHER DRUGS THAT HAVE BEEN DETECTED IN ILLICIT HEROIN SAMPLES

Compound	*k′ value*	*Relative retention time, w.r.t. diamorphine**
Noscapine	0.37	0.34
Cocaine	0.71	0.42
Procaine	0.79	0.44
Amitriptyline	0.93	0.47
Bisacodyl	1.00	0.49
Methadone	1.00	0.49
Papaverine	1.33	0.58
Caffeine	1.61	0.64
Acetylcodeine	2.41	0.85
Quinine	2.71	0.95
Diamorphine	3.00	1.00
Methyl amphetamine	3.50	1.12
Thebaine	3.53	1.15
Amphetamine	3.53	1.15
6-Monoacetylmorphine	3.79	1.22
Ethylmorphine	4.93	1.54
Codeine	6.36	1.57
Morphine	7.86	2.16
Strychnine	15.43	4.34

* Retention time of diamorphine = 4.9 min (25-cm column).

The retention data for the opiates and other drugs that have been detected in illicit heroin samples with this new eluent are given in Table II.

With the analytical conditions that have been described a linear calibration existed over a range of 0–0.5 mg ml^{-1} for both morphine and diamorphine, with correlation coefficients based upon peak height measurements of 0.999 for both morphine and diamorphine. A relative standard deviation of less than 2% was obtained from 25 peak-height measurements of a morphine and diamorphine standard, and so these opiates were quantitated by calculating the sample to standard peak height ratio without the use of an internal standard.

Reproducibility was established by analysing ten accurately weighed samples of an illicit heroin preparation that contained diamorphine. Duplicate injections of these samples were followed by duplicate injections of a pure diamorphine standard, and the purity of each sample was determined by comparing peak heights with that of

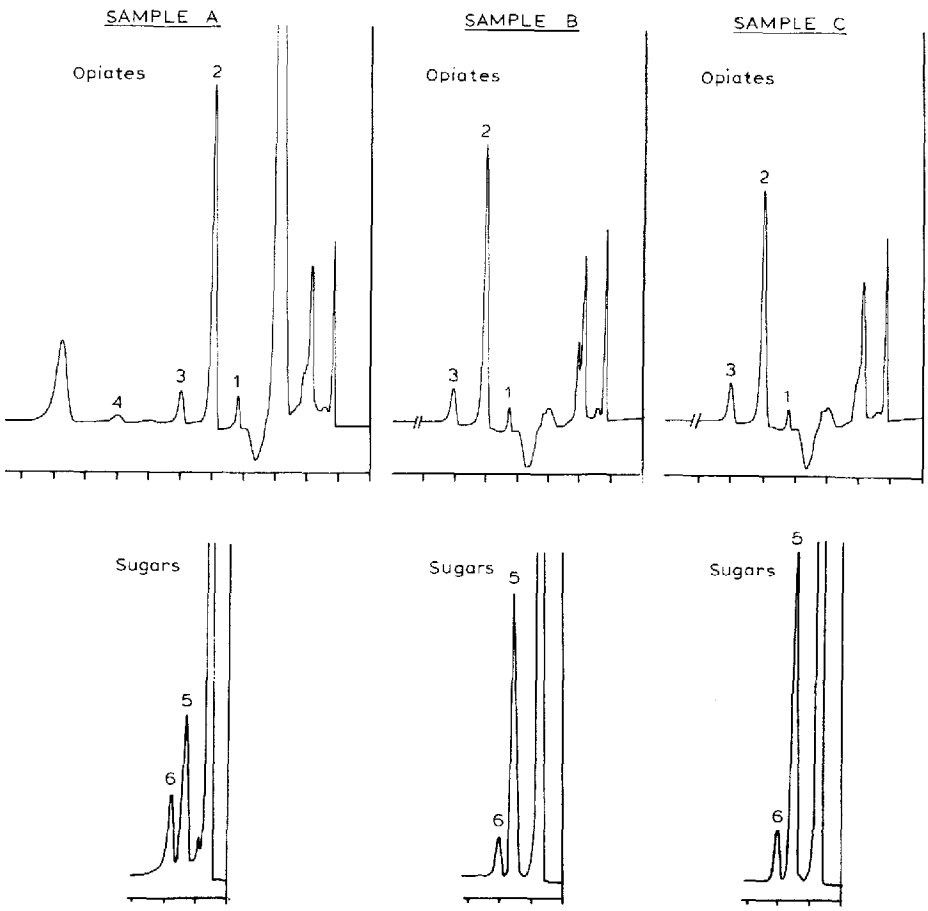

Fig. 4. Opiate and sugar analysis of some illicit heroin samples. Identity of components: 1 = acetylcodeine; 2 = diamorphine; 3 = 6-MAM; 4 = codeine; 5 = glucose; 6 = sucrose. Conditions of analysis are described in the text, and scale graduations represent 1 min for opiate and 2 min for sugar analyses.

the standard. A relative standard deviation of 1.3% was recorded, and therefore this method of quantitation was shown to be both reproducible and rapid.

Normally only diamorphine and morphine are estimated quantitatively, and if quantitation of the other components is required it is obtained in a similar manner by comparison with pure standards.

The quantitative results obtained from the sugar and opiate analyses when combined were found to offer an excellent method for the comparison of illicit heroin samples, and some are illustrated in Fig. 4. From the chromatograms it is evident that samples A and B have completely different compositions, and do not originate from a common source. We believe that samples B and C do come from a common source, because there is excellent agreement both qualitatively and quantitatively.

CONCLUSIONS

To quantitate sugars in illicit heroin samples by HPLC, chloride ions must be removed prior to analysis otherwise interference arises due to the anion-exchange properties of the chromatographic column. Good reproducibility based upon peak-height measurements can be obtained after the samples have been treated with silver sulphate to remove the chloride ions and barium hydroxide to remove the sulphate ions.

An eluent system that has been devised for the analysis of the opiates in illicit heroin produces excellent separation of all the components and gives very good peak shapes. Comparison of the peak heights of morphine and diamorphine *versus* standards of known concentration gives rapid and reproducible quantitative results.

The quantitative results obtained from the sugar and opiate analyses offer an excellent method for the comparison of illicit heroin samples.

REFERENCES

1 G. D. Brittain and L. Schewe, in I. I. Domsky and J. A. Perry (Editors), *Recent Advances in Gas Chromatography*, Marcel Dekker, New York, NY, 1971.
2 J. B. Metcalfe, personal communication.
3 L. A. Th. Verhaar and B. F. M. Kuster, *J. Chromatogr.*, 220 (1981) 313.
4 B. B. Wheals and P. C. White, *J. Chromatogr.*, 176 (1979) 421.
5 G. K. Poochikian and J. C. Cradock, *J. Chromatogr.*, 171 (1979) 371.
6 H. Huizer, H. Logtenberg and A. J. Steenstra, *Bull. Narc.*, 29 (1977) 65.
7 J. L. Love and L. K. Pannell, *J. Forensic Sci.*, 25 (1980) 320.
8 J. A. Reuland and W. A. Trinler, *Forensic Sci.*, 11 (1978) 195.
9 P. B. Baker and T. A. Gough, *J. Chromatogr. Sci.*, 19 (1981) 483.
10 I. Lurie, *J. Ass. Offic. Anal. Chem.*, 60 (1977) 1035.
11 S. K. Soni and S. M. Dugar, *J. Forensic Sci.*, 24 (1979) 437.
12 P. J. Twitchett, *J. Chromatogr.*, 104 (1975) 205.
13 D. L. Hendrix, R. E. Lee Jr. and J. G. Baust, *J. Chromatogr.*, 210 (1981) 45.
14 I. Jane, *J. Chromatogr.*, 111 (1975) 227.