Journal of Chromatography, 147 (1978) 327–336

CHROM. 10,269

RAPID AND SENSITIVE GAS CHROMATOGRAPHIC QUANTITATION OF MORPHINE, CODEINE AND O⁶-ACETYLMORPHINE IN ILLICIT HEROIN USING AN ELECTRON CAPTURE DETECTOR

JAMES M. MOORE

Drug Enforcement Administration, Special Testing and Research Laboratory, 7704 Old Springhouse Road, McLean, Va. 22101 (U.S.A.)

(Received May 24th, 1977)

SUMMARY

Morphine, codeine and O⁶-acetylmorphine are reacted with heptafluorobutyric anhydride, rendering them suitable for electron capture detection and quantitation. The fluorinated derivatives are extracted from an acetonitrile–sodium bicarbonate solution into light petroleum in a rapid one-step extraction procedure. The derivatives are chromatographed on a stationary phase of 3% OV-17 on Gas-Chrom Q. Morphine, codeine and O⁶-acetylmorphine can be readily quantitated in heroin at levels as low as 0.001%, 0.01% and 0.01% respectively. Reproducibility, linearity and recovery studies are described.

INTRODUCTION

Illicit drugs that are produced clandestinely often contain trace impurities that are associated with the manufacturing process. The characterization of these impurities is of importance for forensic purposes[1–5]. In addition to a qualitative analysis of these impurities, a quantitative determination is often desirable[1].

A number of trace compounds in illicit heroin are associated with its manufacturing process. These include codeine and morphine and their acetylated products, namely, acetylcodeine and O⁶-acetylmorphine. Previous methods used to quantitate these substances have utilized gas chromatography with flame ionization detection (FID)[1,2]. However, the amounts of codeine and morphine in illicit heroin are often at levels that render quantitation using FID inaccurate. Therefore, a rapid method was needed to determine these substances at levels below 0.1%.

This study reports the use of the more sensitive electron capture detector (ECD) for the quantitation of morphine and codeine in illicit heroin. Since these compounds contain labile protons, they are susceptible to derivatization using perfluorinated anhydrides. Earlier studies have reported the excellent response of ECD towards perfluorinated compounds[6–8]. This paper describes the use of heptafluorobutyric anhydride in the derivatization and quantitation of morphine and codeine. Since O⁶-acetylmorphine is associated with these substances, it is included in the quantitation.

EXPERIMENTAL

Chromatographic apparatus

A Perkin-Elmer 990 gas chromatograph was used in this study. It was equipped with a ^{63}Ni electron capture detector and interfaced with an Infotronics CRS 208 integrator. The gas chromatograph was fitted with a coiled glass column (1.83 m × 4 mm I.D.) packed with 3% OV-17 on Gas-Chrom Q (100–120 mesh), obtained from Supelco (Bellefonte, Pa., U.S.A.). Temperature, flow-rates and column conditioning parameters are described in the body of paper.

Solvents and reagents

Light petroleum and acetonitrile were distilled in glass and obtained from Burdick & Jackson Labs. (Muskegon, Mich., U.S.A.). Heptafluorobutyric anhydride (HFBA), supplied in 1-ml sealed glass ampules, was obtained from Pierce (Rockford, Ill., U.S.A.). Methanol was supplied by Eastman-Kodak (Rochester, N.Y., U.S.A.). Sodium bicarbonate was obtained from Matheson, Coleman & Bell (Los Angeles, Calif., U.S.A.). Silyl-8 was a product of Pierce.

Standards

Morphine and codeine hydrochlorides were obtained from S. B. Penick and Co. (Lyndhurst, N.J., U.S.A.). Heroin and O^6-acetylmorphine hydrochlorides were supplied by the Special Testing and Research Laboratory, Drug Enforcement Administration (McLean, Va., U.S.A.). Aldrin internal standard was obtained from Julius Hyman and Co. (Denver, Colo., U.S.A.). Chlorpromazine hydrochloride internal standard was obtained from Smith, Kline & French Labs. (Philadelphia, Pa. U.S.A.).

Method

Column conditioning. The column was conditioned at 175° for 1 h by injecting 5 × 5 μl of Silyl-8 and 5 × 5 μl of light petroleum containing O^6-acetylmorphine $(HFB)_1$ at a concentration of 5 mg/ml (for preparation of HFB derivative see below). The temperature was then increased to 285° and maintained for 48 h. A nitrogen flow-rate of *ca.* 100 ml/min was maintained.

Preparation of saturated sodium bicarbonate solution. Sodium bicarbonate was added to *ca.* 200 ml distilled water at 60–70° until saturation was achieved. The solution was cooled to room temperature before using. The saturated solution had a pH of 8.7 and was prepared fresh daily.

Standards preparation and derivatization. Individual standards in methanol were prepared for codeine·HCl (0.25 mg/ml), morphine·HCl (0.50 mg/ml) and O^6-acetylmorphine·HCl (2.0 mg/ml). Exactly 50.0 μl of the codeine solution, 50.0 μl of the morphine solution and 250.0 μl of the O^6-acetylmorphine solution were dispensed in duplicate into the bottom of individual 13-ml conical glass-stoppered centrifuge tubes. The methanol was evaporated to dryness at 50–60° under an air current.

To each tube was added exactly 1.0 ml of acetonitrile and 50 μl of HFBA. (The acetonitrile used in codeine standard contained 0.20 mg/ml chlorpromazine·HCl internal standard.) The tubes were agitated to dissolve the HFBA and then swirled occasionally for 15 min. To each tube was added exactly 2.0 ml of light petroleum.

(The light petroleum used for morphine and O⁶-acetylmorphine standards contained 0.30–20 μg/ml aldrin internal standard.) To each tube was added 5.0 ml of saturated sodium bicarbonate solution and then shaken vigorously 5–10 sec. The tubes were vented carefully to release the pressure and then centrifuged to clarify the layers.

Duplicate 1, 2 and 4 μl injections of the light petroleum layer containing the codeine standard were made under the conditions described in Fig. 1. The morphine and O⁶-acetylmorphine standards were diluted with additional light petroleum to yield final concentrations of 2 μg/ml and 20 μg/ml, respectively. Aliquots of 1, 2 and 4 μl of each solution were injected under the conditions described in Fig. 2.

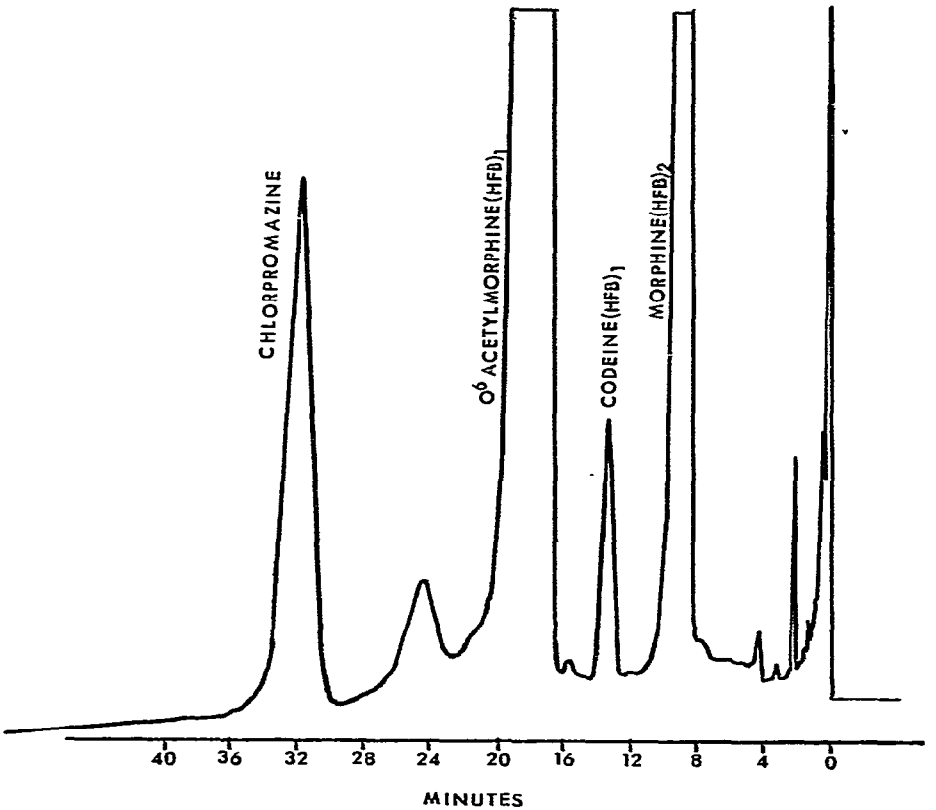

Fig. 1. Codeine quantitation. Chromatogram of illicit heroin sample containing codeine (0.06%), morphine 0.33%) and O⁶-acetylmorphine (5.2%). Temperatures: column, 200–220°; injector, 275°; detector, 300°. Nitrogen flow-rate, 100–120 ml/min. Sensitivity: amplifier, 1×; integrator, 20×; standing current, 2.0 nA.

Sample analysis

Codeine quantitation. Weigh accurately no more than 10 mg of heroin into the bottom of a 13-ml conical centrifuge tube. Add 1.0 ml of acetonitrile containing chlorpromazine internal standard and swirl tube to dissolve sample. Add 50 μl HFBA and proceed as above for codeine standard. Inject appropriate volume of light

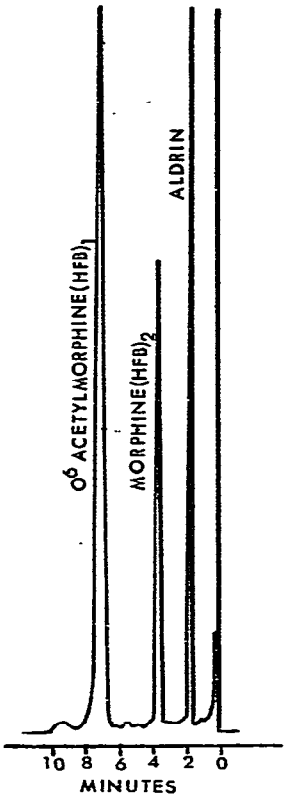

Fig. 2. Morphine and O⁶-acetylmorphine quantitation. Chromatogram of illicit heroin containing codeine (0.06%), morphine (0.33%) and O⁶-acetylmorphine (5.2%). Temperatures: column, 220–240°; injector, 275°; detector, 300°. Nitrogen flow-rate, 100–120 ml/min. Sensitivity: amplifier, 2×; integrator, 50×; standing current, 2.0 nA.

petroleum to give codeine response within standard range. (Note: heroin is a weak electrophile and gives an ECD response at *ca.* 90 min under the conditions given in Fig. 1.)

Morphine and O⁶-acetylmorphine quantitation. Weigh accurately 1–10 mg of heroin into the bottom of a 13-ml conical centrifuge tube. (Note: no more than 1 mg of O⁶-acetylmorphine should be in tube.) Add 1.0 ml of acetonitrile and swirl tube to dissolve sample. Add 50 μl of HFBA and proceed as above for morphine and O⁶-acetylmorphine standards. Dilute with light petroleum, if necessary, and make appropriate gas chromatography injection to give responses within standard range.

RESULTS AND DISCUSSION

Selection of derivatizing reagent

In addition to heptafluorobutyric anhydride, other anhydrides and acid chlorides were investigated for their suitability as derivatizing reagents for codeine, morphine and O⁶-acetylmorphine. These included heptafluorobutyryl chloride, tri-

fluoroacetic anhydride, chloroacetic anhydride and pentafluorobenzoyl chloride. The reaction rate using heptafluorobutyryl chloride was considerably slower than its anhydride. Though trifluoroacetic anhydride reacted rapidly, the corresponding ester apparently hydrolyzed significantly during extraction. This was in agreement with previously published studies[7,8]. Chloroacetic anhydride and pentafluorobenzoyl chloride did not yield derivatives suitable for rapid gas–liquid chromatographic (GLC) quantitation.

Chromatographic behavior of derivatives

GLC stationary phases studies included OV-1, OV-17, OV-25, OV-210 and OV-225. Of these, OV-17 proved most suitable. On OV-1, codeine $(HFB)_1$ and morphine $(HFB)_2$ were not resolved. Though the resolution on OV-25 was acceptable, peak width at half height was greater than on OV-17. The greater "bleed" rates of the highly polar OV-210 and OV-225 precluded their use with an electron capture detector.

A rather interesting and unusual phenomenon was noted with the chromatography of the codeine derivative. On stationary phases using Chromosorb W HP as the solid support, a significant pre-inflection was noted in the codeine $(HFB)_1$ peak. When using Gas-Chrom Q, this inflection was less significant. The size of the inflection appeared to increase as a function of column age. The reason for this phenomenon is not clear. It is recommended, though, that Gas-Chrom Q be used as the solid support and the column be conditioned as described earlier.

Reaction rates

The reaction rate of codeine, morphine and O⁶-acetylmorphine with heptafluorobutyric anhydride was studied. Authentic samples of 10 mg of 0.05% codeine hydrochloride, 0.25% morphine hydrochloride, 5% O⁶-acetylmorphine hydrochloride and 94% heroin hydrochloride were prepared. This sample was analyzed using reaction times from 5 min to 1 h. No significant variation in reaction yield was noted over this time period.

Recovery studies

Several authentic heroin samples of varying composition were prepared and analyzed by this method. The recoveries are given in Table I. The amount of heroin

TABLE I

RECOVERY STUDIES

Sample composition: A = 94% heroin hydrochloride–0.05% codeine hydrochloride–0.20%, morphine hydrochloride–5.0% O⁶-acetylmorphine hydrochloride; B = 94% heroin hydrochloride–0.50% morphine hydrochloride–5.0% O⁶-acetylmorphine hydrochloride; C = 89% heroin hydrochloride–1.0% morphine hydrochloride–10.0% O⁶-acetylmorphine hydrochloride.

Sample	*Weight (mg)*	*Recovery (%)*		
		Codeine	*Morphine*	*O⁶-Acetylmorphine*
A	10.0	103	—	—
B	10.0	—	102	96
C	10.0	—	99	90

hydrochloride in the reaction tube was limited to 10 mg. Though all the HFB derivatives were extracted in high yield, the amount of O^6-acetylmorphine recovered decreased as its concentration in the reaction tube increased. Therefore, a maximum of 1 mg of O^6-acetylmorphine in the reaction tube was established.

Reproducibility and comparative assay studies

A number of illicit heroin samples were analyzed in replicate by this method for morphine and O^6-acetylmorphine and compared to a silyl procedure using flame ionization detection[1]. The coefficient of variation was calculated for each sample analyzed by the HFB method. The results are given in Table II. Given in Table III are typical codeine results using the ECD method. They compare favorably with a modified silyl procedure[9].

TABLE II

REPRODUCIBILITY AND COMPARATIVE ASSAY RESULTS FOR MORPHINE AND O^6-ACETYLMORPHINE IN ILLICIT HEROIN

Sample	*Weight (mg)*	*No. of assays*	*Average morphine content (%)*			*Average O^6-acetylmorphine content (%)*		
			This method	*(CV)**	*Silyl method***	*This method*	*(CV)**	*Silyl method*
1	10	8	0.182	(2.63)	0.136	3.10	(3.30)	2.99
2	10	8	0.332	(2.37)	0.326	4.96	(4.27)	5.24
3	10	9	0.184	(5.22)	0.228	4.55	(4.76)	5.09
4	10	9	0.186	(3.89)	0.186	4.27	(3.77)	5.10
5	5	10	0.348	(3.04)	0.330	14.56	(3.81)	15.83
6	5	10	0.802	(2.58)	0.798	14.85	(4.50)	15.80
7	10	8	0.051	(2.34)	0.056	1.77	(4.50)	1.85
8	10	8	0.041	(3.81)	0.061	1.39	(5.91)	1.84

* Coefficient of variation (no quantitative data rejected in statistical treatment).
** Ref. 1.

TABLE III

COMPARATIVE ASSAY RESULTS FOR CODEINE IN ILLICIT HEROIN

Sample	*Weight (mg)*	*Codeine content (%)*	
		This method	*Silyl method**
1	10	0.045	0.043
2	10	0.033	0.029
3	10	0.080	0.087
4	10	0.012	N.D.**
5	10	0.013	N.D.**

* Ref. 9.
** N.D. = None detected.

Response and retention data for HFB derivatives

Table IV gives response and retention data for the HFB derivatives and aldrin internal standard. Fig. 3 illustrates the corresponding chromatogram. Since the morphine derivative contains two HFB groups, its response is greater (*ca.* 7 times)

TABLE IV

RESPONSE AND RETENTION DATA FOR HFB DERIVATIVES

See Fig. 3 for chromatographic conditions.

Compound	*Amount injected (ng)*	t_R *(min)*	*Peak height response (mm)*
Aldrin	0.53	1.9	90
Morphine $(HFB)_2$	3.34	4.1	85
Codeine $(HFB)_1$	16.7	6.1	50
O^6-Acetylmorphine $(HFB)_1$	16.7	8.0	62

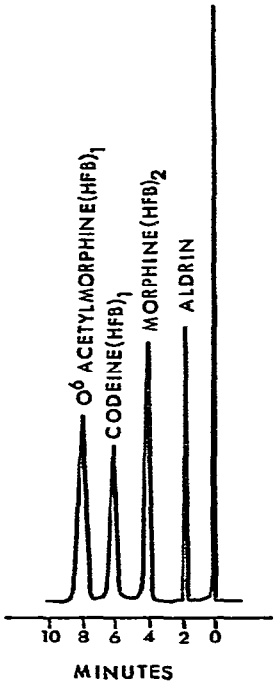

Fig. 3. Chromatogram illustrating response and retention data given in Table IV. Amount (ng) injected: aldrin, 0.534; morphine, 3.34; codeine, 16.66; O^6-acetylmorphine, 16.66. Temperatures: column, 220–240°; injector, 275°; detector, 300°. Nitrogen flow-rate, 100–120 ml/min. Sensitivity: amplifier, 4×; integrator, 50×; standing current, 2.0 nA.

than either the codeine or O^6-acetylmorphine derivative. However, the relative responses of the derivatives can vary depending upon the condition of the column stationary phase.

The lowest quantifiable amount of morphine and O^6-acetylmorphine depends primarily upon a favorable signal-to-noise ratio for each derivative as well as a linearity factor. It is *ca.* 0.001% for morphine and 0.01% for O^6-acetylmorphine. The lowest quantifiable amount of codeine, however, depends largely upon the quantity of morphine in the sample. Morphine $(HFB)_2$ has about a seven-fold response enhancement over an equivalent amount of codeine $(HFB)_1$ and it is usually

present in illicit heroin in larger amounts. Since morphine $(HFB)_2$ precedes codeine $(HFB)_1$ in chromatographic elution order, it can tail significantly into codeine. As a consequence, the minimum amount of codeine that can be quantitated increases as the morphine-to-codeine content increases. For this reason chromatographic conditions described in Fig. 1 were used for codeine analysis in order to achieve acceptable codeine $(HFB)_1$–morphine $(HFB)_2$ baseline resolution.

Linearity of HFB derivatives

Stock solutions of codeine $(HFB)_1$, morphine $(HFB)_2$ and O^6-acetylcodeine $(HFB)_1$ were prepared in light petroleum as described earlier. Serial dilutions were made and injected in the GC under conditions given in Figs. 1 and 2. Fig. 4 shows the results of this study. As expected, when the amounts of HFB derivatives injected exceeded those in Fig. 4, the slope of the response curve began to decrease. It is recommended, therefore, that sample and standard injections fall within the range shown in Fig. 4.

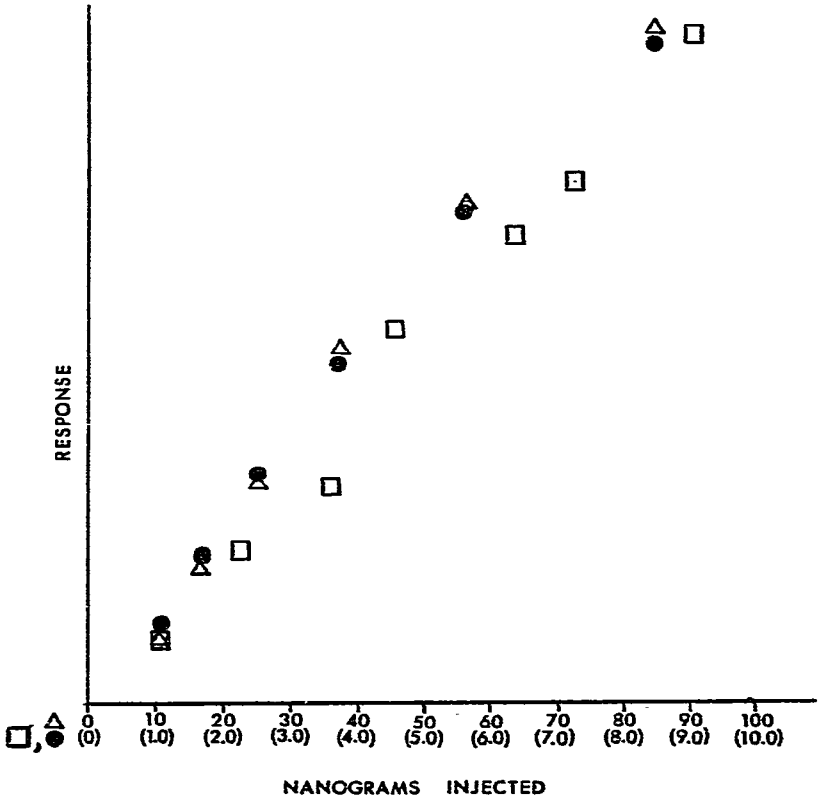

Fig. 4. Linearity of HFB derivatives of morphine (●), codeine (□) and O^6-acetylmorphine (△) on OV-17. Conditions are the same as used in sample analysis.

Reaction yield and extraction efficiency of derivatives versus component concentration

Varying amounts of morphine, codeine and O^6-acetylmorphine were subjected to reaction and extraction conditions described under *Method*. In order to evaluate

the effect heroin hydrochloride had on the reaction yield and extraction efficiency of the derivatives, it was added to one series of samples at the 10 mg level and omitted in another series. All extracted derivatives were diluted and injected to yield a response within the linear portion of the curve in Fig. 4 (*ca.* 50 ng for O⁶-acetylmorphine and 5 ng for morphine and codeine).

It can be seen from Fig. 5 that reaction yield and extraction efficiency were independent of component concentration over a wide range. It is also evident that hydrolysis of heroin during the derivatization is negligible.

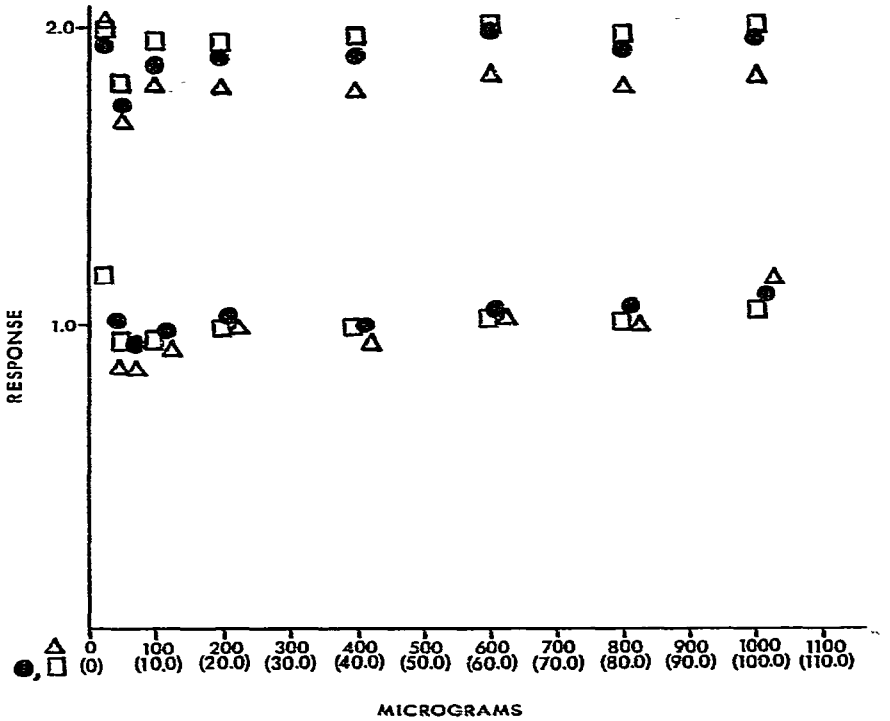

Fig. 5. Reaction yield and extraction efficiency of derivative *versus* component concentration. Chromatographic conditions used given in Fig. 3. ●, Morphine; □, codeine; △, O⁶-acetylmorphine derivatives. Heroin hydrochloride (10 mg) added to series of samples at response 2.0 level and omitted at response 1.0 level. Response levels 1.0 and 2.0 offset electronically for readability.

Derivative stability

The stability of the HFB derivative in light petroleum was observed during the course of this study. All derivatives were stable over a wide concentration range for at least 8 h. It is recommended though that, after derivatization is complete, the analysis be completed without delay.

Minimum detectable quantity

Stock solutions of the HFB derivatives of morphine, codeine and O⁶-acetylmorphine were prepared. Dilution of each derivative was made until detection by GC, operating at maximum sensitivity with acceptable signal-to-noise levels, was not possible. Under these conditions, the minimum detectable quantity wa *ca.* 20 pg for

morphine, 80 pg for codeine and 100 pg for O^6-acetylmorphine. Except for sensitivity parameters, all other conditions used are given in Fig. 3.

Detector performance

Injections of heroin samples and HFB derivatives were made frequently for a period of *ca.* 4 months. During this time no significant detector contamination or decrease in standing current was noted. Additionally, no baseline drift was noted at nominal sensitivity settings.

Other manufacturing by-products

During the quantitation of codeine in illicit heroin using the HFB procedure, the gas chromatograms of all samples revealed numerous unidentified peaks. The majority of these are believed to represent substances associated with clandestine heroin manufacture. Work is progressing in the identity and subsequent quantitation of these substances.

Adulterated heroin samples

The work described in this paper has been limited primarily to uncut heroin samples. Illicit heroin samples are commonly diluted with such adulterants as procaine, quinine, lactose, dextrose, caffeine and barbital. Preliminary investigations have shown that, when present, some of these substances can cause significant interferences in the gas chromatograms of the HFB derivatives. The magnitude of these interferences vary, depending upon the diluent concentrations and also their relative reactivities towards HFBA. Barbital and caffeine do not react with HFBA. Interference caused by sugars can be minimized by reducing HFBA reaction time. Procaine, however, is highly reactive and causes significant interferences in the chromatograms of the HFB derivatives.

CONCLUSIONS

The procedure described in this paper is the most sensitive to date for the quantitation of codeine, morphine and O^6-acetylmorphine in "uncut" illicit heroin samples. It is more accurate for codeine and morphine and comparable in speed to existing methods.

ACKNOWLEDGEMENT

I would like to thank Mrs. Lani Hidalgo for typing the final manuscript.

REFERENCES

1 S. P. Sobol and A. R. Sperling, *A.C.S. Symposium Ser.*, No. 13, 1975, p. 170.
2 E. P. J. van der Slooten and H. J. van der Helm, *J. Forensic Sci.*, 6 (1975) 83.
3 J. M. Moore, *J. Chromatogr.*, 101 (1974) 215.
4 R. P. Barron, A. V. Kruegel, J. M. Moore and T. C. Kram, *J. Ass. Offic. Anal. Chem.*, 57 (1974) 1147.
5 J. M. Moore, *J. Ass. Offic. Anal. Chem.*, 56 (1973) 1199.
6 D. A. Saelens, T. Walle and P. J. Privitera, *J. Chromatogr.*, 123 (1976) 185.
7 F. K. Kawahara, *Anal. Chem.*, 40 (1968) 1009.
8 E. Anggard and G. Seduall, *Anal. Chem.*, 41 (1969) 1251.
9 Private communication.