Journal of Chromatography, 281 (1983) 355–361
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,223

Note

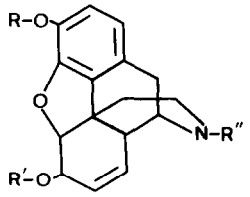
Detection of selected heroin manufacturing impurities using fused-silica capillary and electron capture–gas chromatography

JAMES M. MOORE

Special Testing and Research Laboratory, Drug Enforcement Administration, 7704 Old Springhouse Road, McLean, VA 22102-3494 (U.S.A.)

(Received August 9th, 1983)

The characterization of trace manufacturing impurities in illicit drugs sometimes requires sensitive methods such as gas chromatography–electron capture detection (GC–ECD). The detection and quantitation of trace impurities in heroin from illicit sources using packed column GC–ECD has been previously described[1–3]. This paper reports on a preliminary study that utilizes fused-silica capillary columns in the GC–ECD analysis of some manufacturing impurities present in illicit heroin samples. These impurities, studied in their standard form, include codeine (Ia), morphine (Ib), O^6-acetylmorphine (Ic), O^3-acetylmorphine (Id), norcodeine (Ie), normorphine (If), O^6-acetylmorphine (Ig) and O^3,O^6-diacetylnormorphine (Ih). A brief discussion of the preliminary results for the capillary GC–ECD analysis of unadulterated heroin samples is also presented.



(I)

(a)	$R=R''=CH_3$, $R'=H$;	(a′)	$R'=$HFB
(b)	$R=R'=H$, $R''=CH_3$;	(b′)	$R=R'=$HFB
(c)	$R=H$, $R'=$Ac, $R''=CH_3$;	(c′)	$R=$HFB
(d)	$R=$Ac, $R'=H$, $R''=CH_3$;	(d′)	$R'=$HFB
(e)	$R=CH_3$, $R'=R''=H$;	(e′)	$R'=R''=$HFB
(f)	$R=R'=R''=H$;	(f′)	$R=R'=R''=$HFB
(g)	$R=R''=H$, $R'=$Ac;	(g′)	$R=R''=$HFB
(h)	$R=R'=$Ac, $R''=H$;	(h′)	$R''=$HFB

0021-9673/83/$03.00

In this study the heroin impurities were subjected to derivatization with heptafluorobutyric anhydride (HFBA) followed by a one-step extraction into an isooctane–ethyl ether mixed solvent. The isolated HFB derivatives were chromatographed on non-polar and polar fused-silica capillary columns using the splitless injection technique. All derivatized impurities exhibited good peak symmetry at the picogram level.

EXPERIMENTAL

Apparatus

All chromatography was performed on a Hewlett-Packard 5880A gas chromatograph equipped with a ^{63}Ni electron capture detector and interfaced with a Hewlett-Packard level four data processor. The gas chromatograph was fitted with a 12 m × 0.20 mm I.D. cross-linked fused-silica capillary column coated with OV-1 (Hewlett-Packard, Avondale, PA, U.S.A.) and an 11 m × 0.25 mm I.D. fused-silica capillary column coated with OV-17 (Alltech, Deerfield, IL, U.S.A.). The injector and detector temperatures were maintained at 250°C. The oven temperature was multilevel-programmed for both columns as follows. Level 1: initial temperature, 90°C; initial hold, 1.8 min; temperature programme rate, 25°C/min; final temperature, 160°C; final hold, 1.0 min; Level 2: temperature programme rate, 4°C/min; final temperature, 275°C. Helium ("Zero Grade") (Air Products, Allentown, PA, U.S.A.) was used as the carrier gas at a flow-rate of *ca.* 45 cm/sec and measured at an oven temperature of 90°C. An argon–methane mixture (95:5) was used as the detector make-up gas at a flow-rate of 30 ml/min. The septae used were Thermogreen LB-1 (Supelco, Bellefonte, PA, U.S.A.). All chromatography was recorded at an attenuation of 2^6 and at a chart speed of 0.5 cm/min. During the splitless injection, the solvent was vented after a 0.6 min hold.

Solvents and chemicals

Pyridine was obtained from Matheson, Coleman and Bell (Los Angeles, CA, U.S.A.). All other solvents were distilled in glass and were products of Burdick & Jackson (Muskegon, MI, U.S.A.). Heptafluorobutyric anhydride (HFBA), supplied in 1-ml sealed glass ampules, was obtained from Pierce (Rockford, IL, U.S.A.). All other chemicals were of reagent grade quality.

Standards

Morphine and codeine were obtained from S. B. Penick (Lyndhurst, NJ, U.S.A.). The O^3-acetylmorphine was prepared by the method of Welsh[4]. Normorphine, norcodeine, O^3,O^6-diacetylnormorphine and O^6-acetylnormorphine standards were prepared using the procedures of Rice *et al.*[5,6]. The method of Wright[7] was used in the synthesis of O^6-acetylmorphine. The internal standard, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane (*p,p'*-DDT), was obtained from Supelco. Dioctyl phthalate internal standard was supplied by Aldrich (Milwaukee, WI, U.S.A.).

Method

Methanolic solutions of mixed standards Ia–d and Ie–h were prepared and aliquots equivalent to 5–50 μg of each standard were evaporated to dryness in two

separate 13-ml conical glass-stoppered centrifuge tubes. To each tube was added 1.0 ml of acetonitrile, 50 μl of pyridine and 50 μl of HFBA. After the contents of the tube were throughly mixed, derivatization was allowed to proceed at room temperature for 10 min. To each tube was added 5.0 ml of isooctane–ethyl ether (85:15) (containing *p,p'*-DDT at 100 pg/μl and dioctyl phthalate) and 5.0 ml of an aqueous solution saturated with sodium bicarbonate. The tubes were shaken vigorougly for 5–10 sec and then centrifuged. An aliquot of the organic layer from each tube was diluted to an appropriate volume with additional extraction solvent containing internal standards. About 2–3 μl of each solution were injected into the gas chromatograph operating under conditions described previously.

RESULTS AND DISCUSSION

Fused-silica capillary versus packed column GC–ECD

In previous studies of illicit heroin samples, packed column GC–ECD was used for the detection and quantitation of some major heroin manufacturing impurities, such as O^6-acetylmorphine (Ic), O^3-acetylmorphine (Id), codeine (Ia) and morphine (Ib)[1,2]. This method also allowed for the detection of several other theretofore unreported heroin impurities, including O^6-acetylnormorphine (Ig) and O^3,O^6-diacetylnormorphine (Ih) as well as desoxymorphine-A^3. For the in-depth analyses of ultratrace impurities in heroin samples though, it was necessary to reduce detection limits and improve column efficiency to an extent not possible with packed column GC–ECD.

With the recent introduction of fused-silica capillary columns and improved ECD systems, the forensic drug chemist has been afforded the opportunity to improve markedly the analyses of trace manufacturing in drugs. In investigations of heroin impurities, we have found the fused-silica capillary column to offer several distinct advantages over packed column GC–ECD. These include the obvious enhancements in sensitivity and resolution as well as the improved capability of detecting very early- and late-eluting impurities. Additionally, after subjecting the system to daily injections of HFB derivatives for about one year, no significant loss in detector standing current was noted. This was in contrast to packed column GC–ECD, in which extensive detector maintenance was required about every six months. Finally, because of the enhanced resolution and sensitivity, capillary GC–ECD was more suited for the analysis of highly refined heroin samples than packed column GC–ECD. Though the quantitative applications of packed column GC–ECD have been established for certain heroin impurities[1], no such data are presently available from the work using capillary column GC–ECD.

Split versus splitless injection

In this study, both the split and splitless modes of injection were investigated. Though split capillary (50:1) offered the obvious advantage in resolution over packed column and was satisfactory in the analysis of crudely processed heroin, it was not adequate for the analysis of ultratrace impurities in highly refined heroin samples. To analyze these samples in the split mode, a concentration of the extraction solvent containing the HFB derivatives was usually required. Unfortunately, losses of low-level amounts of some HFB derivatives occurred during these evaporation and re-

constitution steps. When using the splitless mode, a concentration step was avoided in most cases. In this mode we have observed at least a 10–20-fold enhancement for most derivatives when compared to packed column GC–ECD. When compared to packed column GC–FID, an enhancement of 500–1000-fold for some HFB derivatives was observed. Finally, the splitless mode offered the advantage of improved chromatography for very early eluting derivatives due to the "solvent effect" phenomenon[8].

Column selection, chromatography, retention and response data for HFB derivatives

HFB derivatives were studied initially using a fused-silica column coated with OV-101. Though providing acceptable chromatography throughout most of the study, column performance began to deteriorate after 9–12 months. Furthermore, because of the relatively high bleed-rate of OV-101, daily adjustments in temperature programme parameters were required to maintain constant retention times for the HFB derivatives. For these reasons, the recently developed and more stable cross-linked OV-1 replaced the OV-101 column in subsequent work. To study the effects of substrate polarity on the chromatography and elution order of the HFB derivatives, a non-cross-linked OV-17 was chosen as the other column.

Illustrated in Figs. 1 and 2 are capillary chromatograms of the HFB derivatives of selected standard heroin impurities. For reasons that will be discussed subsequently, the standards were chromatographed in two separate groups, one representing tertiary amines (Ia–d) and the other secondary amines (Ie–h). The amount of each

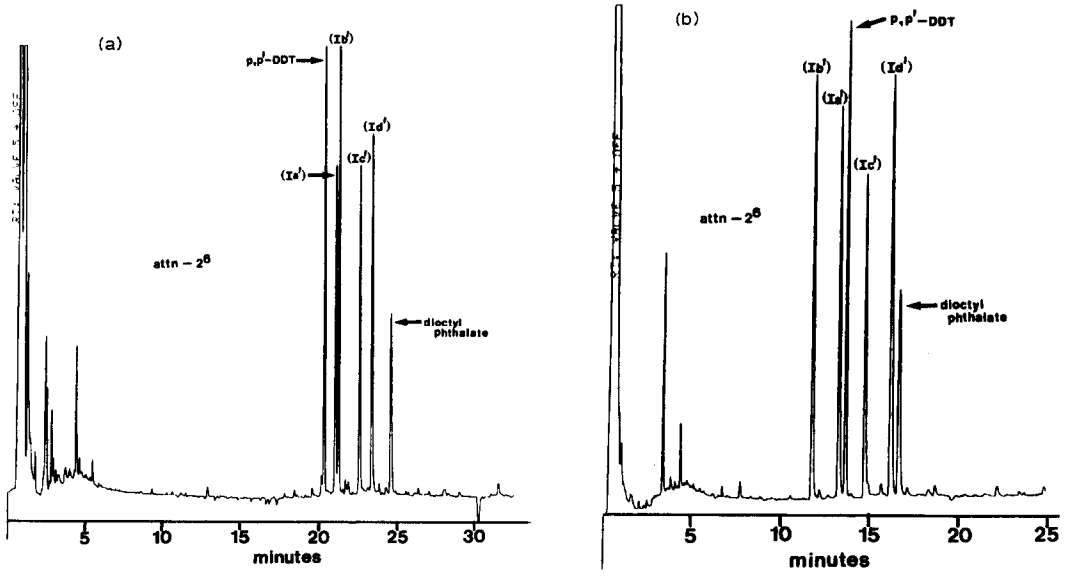


Fig. 1. Chromatograms of some HFB derivatives of tertiary amine manufacturing impurities found in illicit heroin. A GC–ECD equipped with a cross-linked OV-1 (a) or OV-17 (b) capillary column and operating in the splitless mode was used. Method and chromatographic conditions are given under Experimental. The designations for all peaks are referred to in the body of the paper. All amounts injected on-column have been calculated with respect to Ia–d: *p,p'*-DDT, 200 pg; (Ia'), 800 pg; (Ib'), 160 pg; (Ic'), 800 pg; (Id'), 800 pg.

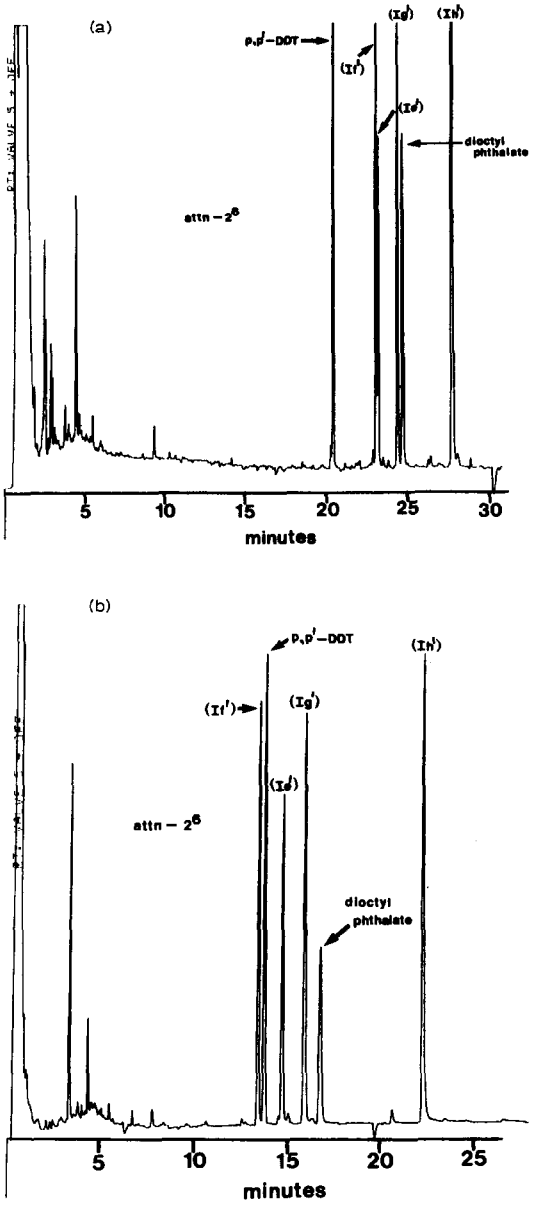


Fig. 2. Chromatograms of some HFB derivatives of secondary amine manufacturing impurities found in illicit heroin. A gas chromatograph–electron capture detector equipped with a cross-linked OV-1 (a) or OV-17 (b) capillary column and operating in the splitless mode was used. Method and chromatographic conditions are given under Experimental. The designations for all peaks are referred to in the body of the paper. All amounts injected on-column have been calculated with respect to Ie–h: *p,p'*-DDT, 200 pg; (Ie'), 120 pg; (If'), 65 pg; (Ig'), 108 pg; (Ih'), 544 pg.

standard injected on-column, as given in Figs. 1 and 2, refers to Ia–h and assumes complete derivatization to Ia'–h' and quantitative extraction. The facile formation of HFB derivatives and their use in quantitative GC–ECD has been addressed previously[1].

The enhanced efficiency of the capillary columns is demonstrated by comparing the chromatograms in Figs. 1 and 2 with previous packed column studies[1–3]. The cross-linked OV-1 capillary column also provided separation of the HFB derivatives of codeine (Ia') and morphine (Ib'), not possible using non-polar packed column substrates. This separation was even more pronounced on OV-101, suggesting a slightly greater polarity associated with the cross-linked OV-1. This increase in polarity of cross-linked OV-1 compared to OV-101 was best demonstrated by the fact that the elution order of the HFB derivatives of norcodeine (Ie') and normorphine (If') were reversed on these two columns. The non-polar character that HFB groups impart to a molecule is obvious in Figs. 1 and 2 when comparing absolute retention times and retention order relative to *p,p'*-DDT for the polar and non-polar columns.

Though not determined in this study, the minimum detectable amount for all derivatives appear to be at low picogram and high femtogram levels. As expected, the N-HFB derivatives exhibited a modest increase in response compared to their O-HFB counterparts. Table I lists response and retention data for HFB derivatives.

Sample analyses

Having established method and chromatographic conditions suitable for the detection of heroin impurity standards, a preliminary study of unadulterated heroin samples was conducted. The impurities in these samples were conveniently divided into three groups: (A) tertiary amines, including Ia–d, (B) secondary amines, including Ie–h, and (C) acidics and neutrals. Initially, these impurities were derivatized, extracted and chromatographed together. Unfortunately, the chromatograms were

TABLE I

RESPONSE AND RETENTION DATA FOR HFB DERIVATIVES*

Compound	*Amount injected (pg)***	*Peak height response (mm)***	t_R *(min)*	
			OV-1	*OV-17*
p,p'-DDT	–	–	20.43	13.73
Codeine $(HFB)_1$-(Ia')	200	77	21.11	13.34
Morphine $(HFB)_2$-(Ib'	40	101	21.31	11.82
O^6-Acetylmorphine $(HFB)_1$-(Ic')	200	79	22.70	14.80
O^3-Acetylmorphine $(HFB)_1$-(Id')	216	100	23.49	16.24
Norcodeine $(HFB)_2$-(Ie')	26	69	23.29	14.73
Normorphine $(HFB)_3$-(If')	13	91	23.15	13.40
O^6-Acetylnormorphine $(HFB)_2$-(Ig')	26	83	24.48	15.91
O^3,O^6-Diacetylnormorphine $(HFB)_1$-(Ih')	51	85	27.89	22.30
Dioctyl phthalate	–	–	24.76	16.74

* See Figs. 1 and 2 for chromatograms and refer to Experimental for method and chromatographic conditions.

** Data generated on 12 m × 0.20 mm I.D. fused-silica capillary column coated with OV-101 and instrument attenuation of 2^3.

dominated by the presence of impurities in Group A, especially Ib and Ic. This resulted in the potential masking of some peaks representing the HFB derivatives in Groups B and C. More importantly, microgram amounts of heroin were unavoidably and concomitantly injected with the HFB derivatives, resulting in column overloading and causing anomalous chromatographic behavior. This problem was solved by extracting the iso-octane–ethyl ether extraction solvent, containing the HFB derivatives and heroin, with dilute sulfuric acid prior to injection. This treatment effectively removed the bulk of heroin, pyridine and the HFB derivatives of Group A impurities. This allowed for improved chromatography and the facile detection of impurities in Groups B and C. Because of column overloading due to heroin, the impurities in Group A were not studied using capillary chromatography.

A total of 25 unadulterated, highly refined heroin samples were subjected to analysis using the revised method. In each case 15 mg of sample were subjected to derivatization and extraction and with a final dilution of 5 ml. The chromatographic conditions were the same as those used for the standards, except for a decreased instrument attenuation of 2^4. A review of the chromatograms revealed that all samples generated a significant number of peaks during a 40-min run. Some of the off-scale peaks were assigned to Group B impurities, while others were attributed to Group C or remained unidentified. Preliminary results indicated that the procedure was reproducible, an important consideration if attempting to apply the methodology to sample comparison cases. Of special significance was the ability to generate peak-enriched chromatograms for highly refined samples while excluding undesirable evaporation steps.

CONCLUSIONS

This paper presents the preliminary results of the application of fused-silica capillary GC–ECD in the analysis of trace manufacturing impurities in illicit heroin. The impurity standards studied, as HFB derivatives, exhibited good chromatography at the picogram level. The minimum detectable amounts for most derivatives appeared to be at low picogram or high femtogram levels. The chromatographic system performed well during a 12-month period with no significant deterioration in detector performance. A survey of unadulterated heroin samples indicated that the method, using the dilute sulfuric acid back-extraction, was reproducible and suitable for group B and C impurities for even the most highly refined heroin samples. This suggests its utility in forensic drug applications such as sample comparison cases.

REFERENCES

1 J. M. Moore, *J. Chromatogr.*, 147 (1978) 327.
2 J. M. Moore and M. Klein, *J. Chromatogr.*, 154 (1978) 76.
3 J. M. Moore, in M. Klein, A. V. Kruegel and S. P. Sobol (Editors), *Instrumental Applications in Forensic Drug Chemistry*, U.S. Government Printing Office, Washington, DC, 1978, pp. 180–201.
4 L. H. Welsh, *J. Org. Chem.*, 19 (1954) 1409.
5 K. C. Rice and E. L. May, *J. Heterocyclic Chem.*, 14 (1977) 665.
6 K. C. Rice and A. E. Jacobson, *J. Med. Chem.*, 18 (2975) 1033.
7 C. I. Wright, *J. Pharmacol. Exp. Ther.*, 71 (1941) 164.
8 L. S. Ettre, *Introduction to Open Tubular Columns*, Perkin-Elmer Corp., Norwalk, CT, 1978 (Order No: 6CD-46).