Determination of Neutral Manufacturing Impurities in Heroin by Capillary Gas Chromatography with Electron Capture Detection after Reduction with Lithium Aluminum Hydride and Derivatization with Heptafluorobutyric Anhydride

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Neutral byproducts associated with the manufacture of illicit heroin are many and include meconin, 4-acetoxy-3,6-dimethoxyphenanthrene, *N*,O^6-diacetylnorcodeine, 4-acetoxy-3,6-dimethoxy-5-[2-(*N*-methylacetamido)ethyl]phenanthrene, *N*,O^3,O^6-triacetylnormorphine, and 4-acetoxy-3,6-dimethoxy-8-[2-(*N*-methylacetamido)ethyl]phenanthrene. These N- and O-acetylated impurities, and meconin, are easily isolated from the bulk heroin matrix, after which they are subjected to reduction with lithium aluminum hydride ($LiAlH_4$) followed by derivatization with heptafluorobutyric anhydride (HFBA) in the presence of pyridine. The resultant heptafluorobutryl (HFB) electrophiles are detected on-column at picogram (pg) levels by using capillary column gas chromatography/electron capture detection (CC-GC/ECD) in the splitless mode. The method is applicable for the in-depth analyses of crudely processed and highly refined heroin samples.

The characterization of manufacturing impurities in illicit drugs is important for forensic purposes, especially in sample comparison, precursor, and geographical origin studies. We, and others, have previously described the detection, quantitation, and structural elucidation of numerous byproducts associated with the manufacture of illicit heroin (*1–5*). In recent years dynamic conditions associated with heroin manufacture, such as the production of a more highly refined product, have necessitated the development of more sensitive methodology for the low-level detection of heroin byproducts. We have successfully applied packed column gas chromatography/electron capture detection (PC-GC/ECD) (*6, 7*) and, more recently, CC-GC/ECD (*2, 3*) for the detection of trace impurities in heroin.

This study describes the CC-GC/ECD determination of selected neutral manufacturing impurities in illicit heroin. Our laboratory has applied packed column gas chromatography/flame ionization detection (PC-GC/FID) for the detection of these impurities in crudely processed heroin samples (*8*). This method was subsequently modified by others using capillary column gas chromatography/flame ionization detection (CC-GC/FID) (*9*). Unfortunately, these procedures lacked sufficient sensitivity for the more refined heroin samples. Our current investigations demonstrate that CC-GC/ECD offers a significant sensitivity enhancement for the detection of acidic and neutral heroin impurities when compared to CC-GC/FID. Additionally, there is an improved capability for the detection of heretofore unreported heroin impurities.

The impurities reported in this study are N- and O-acetylated and/or decomposition products associated with some major alkaloids of *Papaver somniferum* L., including codeine, morphine, thebaine, and noscapine. When subjected to acetylation conditions associated with heroin manufacture, these alkaloids yield small amounts of numerous byproducts, (Figure 1), including meconin (I), 4-acetoxy-3,6-dimethoxyphenanthrene (IV), *N*,O^6-diacetylnorcodeine (VI), 4-acetoxy-3,6-dimethoxy-5-[2-(*N*-methylacetamido)ethyl]-phenanthrene (V), *N*,O^3,O^6-triacetylnormorphine (VII), and 4-acetoxy-3,6-dimethoxy-8-[2-(*N*-methylacetamido)ethyl]-phenanthrene (IX). The neutral character associated with these, and other impurities, permits their facile extraction from the heroin matrix. Many of the isolated impurities are readily reduced with $LiAlH_4$ to yield compounds with hydroxyl groups, which allows for their rapid derivatization with HFBA in the presence of pyridine to yield HFB derivatives suitable for CC-GC/ECD analysis. All HFB electrophiles exhibited good chromatography at the picogram level when chromatographed in the splitless mode on a bonded, nonpolar, fused silica capillary column interfaced with a ^{63}Ni electron capture detector. The method is applicable to adulterated as well as unadulterated heroin samples. Some preliminary quantitative results are described.

EXPERIMENTAL SECTION

Gas Chromatography/Electron Capture Detection. All standard and sample chromatograms were generated in the splitless mode with a Hewlett-Packard 5880A gas chromatograph fitted with a 15 m × 0.25 mm i.d. fused silica capillary column coated with DB-1 (J and W Scientific, Inc., Rancho Cordova, CA) at a film thickness of 0.25 μm. The GC was equipped with a ^{63}Ni electron capture detector (15 mCi) and interfaced with a Hewlett-Packard Level IV data processor. The oven temperature was multilevel programmed as follows: (level 1) initial temperature, 80 °C; initial hold, 5.5 min; temperature program rate, 25 °C/min; final temperature, 160 °C; final hold, 1.0 min; (level 2) temperature program rate, 4.0 °C/min; final temperature, 275 °C; final hold, 15 min. Injector and detector temperatures were maintained at 275 °C and 300 °C, respectively. Hydrogen (Zero Grade; Air Products, Tamaqua, PA) was used as the carrier gas at a velocity of about 40 cm/s and measured at an oven temperature of 80 °C. An argon/methane (95/5) mixture was used as the detector makeup gas at a flow rate of 30 mL/min. The septa used were Thermogreen LB-1 (Supelco, Inc., Bellefonte, PA). Chromatograms were recorded at a chart speed of 0.75 cm/min and at an attenuation of 2^6. During the splitless injection the solvent was vented after a 1.0-min hold.

Reagents. All solvents, including pyridine, were Distilled in Glass products of Burdick and Jackson Laboratories (Muskegon, MI) and were peroxide- and ethanol-free. HFBA, supplied in 1-mL sealed glass ampules, was obtained from Pierce Chemical Co. (Rockford, IL). $LiAlH_4$ was a product of Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were of reagent grade quality.

Lithium Aluminum Hydride Solution. An ether solution saturated with $LiAlH_4$ was prepared in the following manner. To 2 g of $LiAlH_4$ in a flask was added 40 mL of ethyl ether (stored over 4-Å molecular sieve). With frequent mixing, the suspension was *gently* evaporated to a volume of about 20 mL. After cooling,

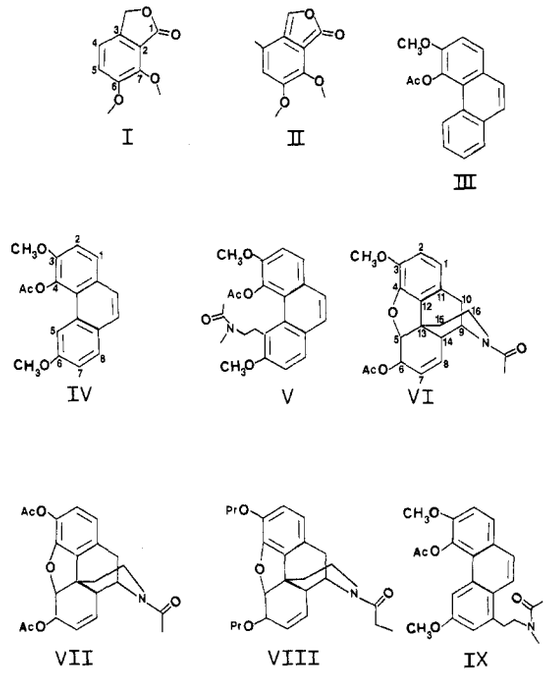


Figure 1. Heroin impurities and structurally related internal standards.

the suspension was transferred to a conical centrifuge tube and centrifuged at 2000 rpm to clarify. The supernatant was used for subsequent reduction reactions (note: $LiAlH_4$ solution was stable for at least several weeks).

Standards. The structures for the following standards are illustrated in Figure 1. The standards 4-acetoxy-3,6-dimethoxyphenanthrene (IV), 4-acetoxy-3,6-dimethoxy-5-[2-(*N*-methylacetamido)ethyl]phenanthrene (V) and 4-acetoxy-3,6-dimethoxy-8-[2-(*N*-methylacetamido)ethyl]phenanthrene (IX) were prepared and purified as described by Allen et al. (*4*). N,O^6-Diacetylnorcodeine (VI) and N,O^3,O^6-triacetylnormorphine (VII) were obtained by acetylating norcodeine and normorphine, respectively, in acetic anhydride. The internal standard N,O^3,O^6-tripropionylmorphine (VIII) was synthesized by the treatment of normorphine and propionic anhydride. The internal standards 4-methylmeconin (II) and 4-acetoxy-3-methoxyphenanthrene (III) were prepared as outlined by Bhattacharjee and Popp (*10*) and Holmes (*11*), respectively. Standard meconin (I) was obtained by use of a modification of Wilson et al. (*12*) and is described below. The internal standard 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p'*-DDT) was a product of Supelco, Inc. [Bellefonte, PA).

Synthesis of Meconin. Concentrated hydrochloric acid (1500 mL) was heated to boiling while stirring. To the acid was added 2,3-dimethoxybenzoic acid (15 g) and then HCl gas was bubbled into the mixture. After saturation with HCl, formaldehyde (37%, 6.8 g) was added dropwise over a 30 min period (note: it is essential that high dilution, rapid stirring, and dropwise addition be maintained to minimize the formation of 4-chloromethyl-6,7-dimethoxyphthalide). After the addition of formaldehyde, the solution was maintained at boiling for 30 min. After cooling, the solution was diluted to 3 L with ice water and the product extracted with three 100-mL volumes of chloroform. After extraction with sodium carbonate, the combined chloroform extracts were evaporated to dryness, the residue was dissolved in ethyl ether, and the solution was then chromatographed on neutral alumina. Removal of the ether eluate yielded meconin (3.5 g, 20% yield).

Internal Standards Solution. An acetone solution containing II, III, and VIII, each at a concentration of 1.0 mg/mL, was prepared.

Mixed Standards Solution. A chloroform solution of I (0.025 mg/mL), II (0.025 mg/mL), III (0.050 mg/mL), IV (0.050 mg/mL, V (0.10 mg/mL), VI (0.20 mg/mL), VII (0.050 mg/mL), and VIII (0.050 mg/mL) was prepared.

Reduction, Derivatization, and Chromatography of Standards. A 200-μL aliquot of the above mixed standards solution was transferred to a 13-mL conical glass-stoppered centrifuge tube. To the tube was added 4 mL of dry ethyl ether (dried over 4-Å molecule sieve) and 0.10 mL of the $LiAlH_4$ solution. After vortex mixing, the volume was reduced to about 0.5 mL at a temperature of 50–60 °C. To the tube was added 3 mL of ethyl ether saturated with water to consume excess $LiAlH_4$. After vortex mixing, the solution was evaporated to dryness at 80 °C. To the dry residue was added 1.0 mL of acetonitrile and 50 μL of HFBA. After vortex mixing, the solution was heated at 80 °C for 5 min. To the tube was added 100 μL of acetonitrile (containing 10 μL of pyridine) and the heating continued for 2 min. After cooling, 5.0 mL of isooctane (containing 50 pg/μL of *p,p'*-DDT) and 5 mL of a saturated aqueous solution of sodium bicarbonate were added to the tube. Without delay, the tube was shaken vigorously for 5–10 s and then centrifuged. A 1.0-mL aliquot of the upper layer was diluted to 5.0 mL with isooctane (containing *p,p'*-DDT at 50/ pg/μL) and then 2 μL injected into the GC-ECD under conditions described previously. The chromatogram of this mixed standard is illustrated in Figure 2.

Sample Analysis. (*a*) *Crudely Processed Heroin (Heroin with Relatively High Levels of Byproducts and Manufacturing Impurities).* An amount of sample equivalent to 50 mg of heroin was placed into a 15-mL conical, glass-stoppered centrifuge tube. To the sample was added 7 mL of 1 N H_2SO_4 and 50 μL of the internal standards solution. After thorough mixing, the solution was extracted with two 5-mL aliquots of ethyl ether. The combined ether extracts were diluted to 10.0 mL with additional ethyl ether. A 1.0-mL aliquot of this solution was evaporated to dryness in another centrifuge tube. To the residue was added 200 μL of chloroform and, after vortex mixing, the sample was treated as described above from the standards beginning with the second sentence of the preceding section. Figure 3 illustrates the chromatogram of a crudely processed Pakistani heroin sample.

(*b*) *Highly Refined Heroin.* An amount of sample equivalent to 50 mg of heroin was placed into a 15-mL conical, glass-stoppered centrifuge tube. To the sample was added 7 mL of 1 N H_2SO_4 and 50 μL of a diluted internal standards solution (note: the internal standards solution described above was diluted 1-to-50 with acetone). After thorough mixing, the solution was extracted with two 5-mL aliquots of ethyl ether. The combined ether extracts were evaporated to dryness in another centrifuge tube. To the tube was added 200 μL of chloroform. After vortex mixing, the samples was treated as above for the standards beginning with the second sentence of the preceding section, except 3.0 mL of isooctane (containing 50 pg/μL *p,p'*-DDT) was used for extraction of the HFB derivatives and no further dilution was made. Furthermore, after 19 min into the chromatographic run, the attenuation was changed from 2^6 to 2^4.

RESULTS AND DISCUSSION

Rationalization of the Presence of Selected Neutral Heroin Impurities. It is important for intelligence purposes to structurally characterize peaks represented in the CC-GC/ECD chromatograms. Furthermore, once characterized, it is desirable to relate these impurities to components of *Papaver somniferum* L. and/or the manufacturing process. Thus, the presence of I in heroin samples can be attributed to the partial degradation of noscapine during the acetylation process. The presence of IV, V, and IX has been previously shown to be the result of the decomposition of thebaine during the acetylation step (*4*). The occurrence of VI and VII may result from the acetylation of codeine *N*-oxide and morphine *N*-oxide, respectively. The *N*-oxide can be present in the morphine precursor material or formed as a transient intermediate during the acetylation step. Figure 3 illustrates the chromatogram of heroin impurities that have been identified and some yet to be characterized. It should also be recognized that there are a number of neutral impurities that cannot be detected by using this methodology, e.g., certain acetylated noscapine and thebaine byproducts.

Extraction, Reduction, Derivatization, and Chromatography of Heroin Impurities. The selection of ethyl ether as an extraction solvent for neutral and acidic impurities resulted from the investigation of other solvents and solvent

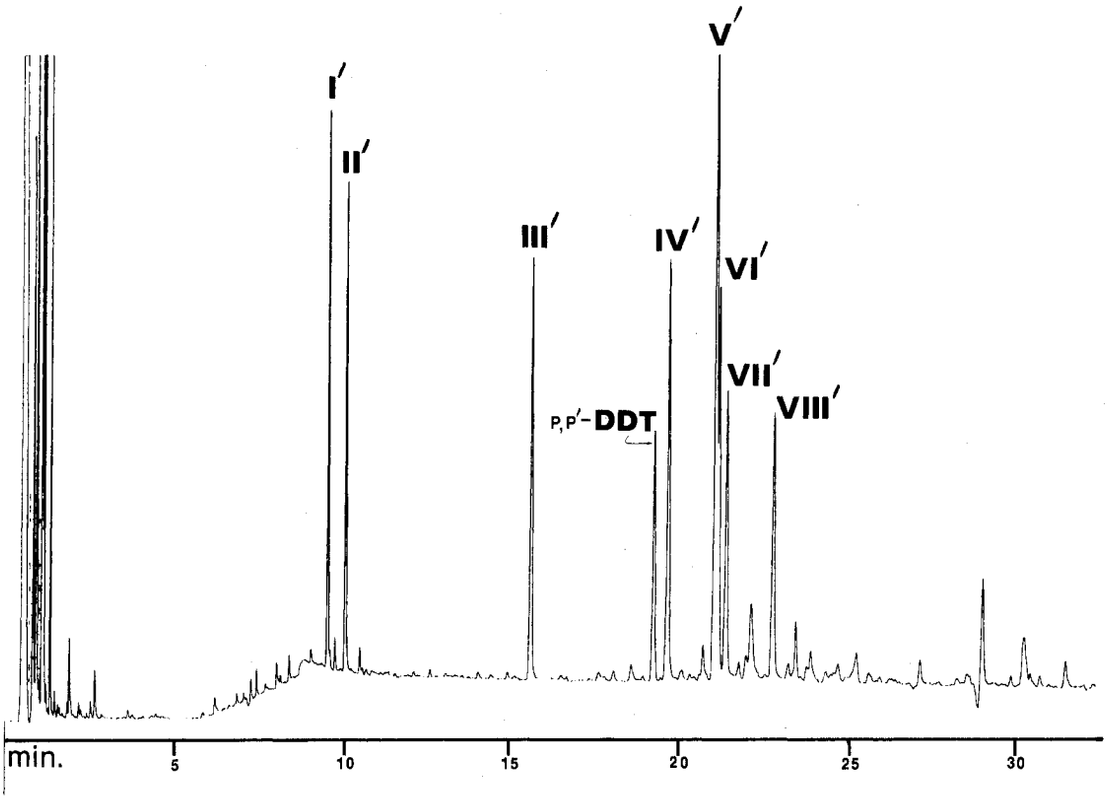
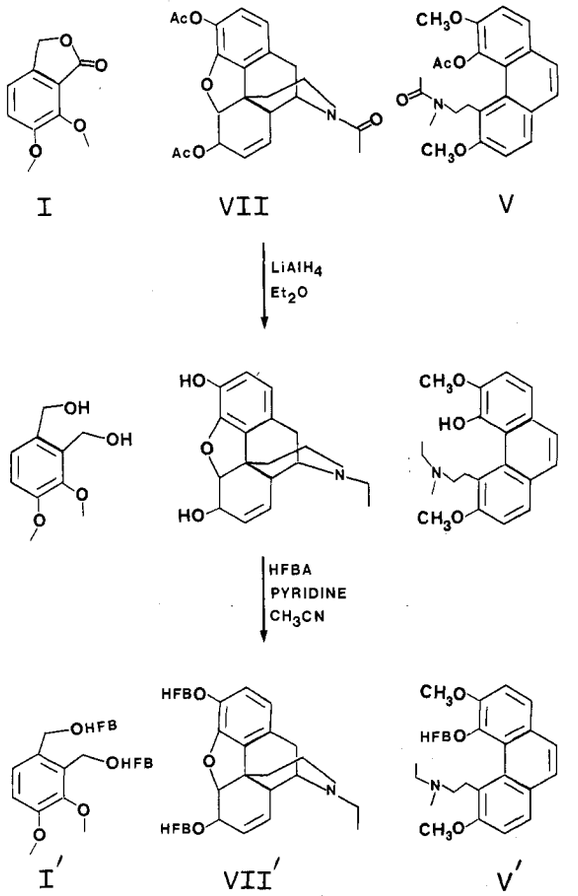


Figure 2. Splitless capillary GC/ECD chromatogram of a mixed standard after $LiAlH_4$ reduction and derivatization with HFBA.

Scheme I. $LiAlH_4$ Reduction and HFBA Derivatization of Meconin (I), 4-Acetoxy-3,6-dimethoxy-5-[2-(*N*-methylacetamido)ethyl]phenanthrene (V), and N,O^3,O^6-Triacetylnormorphine (VII)



combinations. Because of its high purity and volatility, its lighter-than-water characteristics, and extraction efficiency, ethyl ether was considered an ideal extraction solvent. The ether used should be peroxide free so as to minimize the degradation of certain impurities.

The most critical step in the procedure described appears to be the $LiAlH_4$ reduction. For most impurities studied, a reduction yield of greater than 50% was realized at the microgram level. Of special significance was the quality of the $LiAlH_4$ used in the preparation of the ethereal solution. $LiAlH_4$ having a dark gray coloration resulted in lower reduction yields than material possessing white or cream coloration. The $LiAlH_4/Et_2O$ solution was found to be stable for at least 2–3 weeks. After the reduction step, the HFB derivatization should be carried out on the dry residue without delay. Scheme I illustrates the reduction and HFB derivatization for compounds I, V, and VII.

Heptafluorobutyrylation of the reduced impurities proceeded rapidly and in high yield. Although many impurities provided HFB derivatives in good yield without base assistance, phenanthrene derivatives such as IV required the presence of pyridine to effect near complete HFB derivatization.

Most impurities exhibited good chromatography at picogram levels. Although columns of varying polarities were studied, the nonpolar and bonded DB-1 proved the most suitable in terms of peak resolution and chromatographic behavior for HFB derivatives. Figures 2 and 3 illustrate the CC-GC/ECD chromatograms of a mixed standard and a sample, respectively. Table I lists retention data for the HFB derivatives studied.

Selection of Internal Standards. The appropriate use of internal standards is critical in the implementation of this methodology. Due to the structural variations associated with heroin impurities, their extraction, reduction, derivatization, and chromatographic characteristics can vary. The three "methodology" internal standards selected are structurally related to phenanthrene (III), morphine (VIII), and meconin (II) moieties. Addition of these internal standards to the sample prior to the extraction of impurities allows for rea-

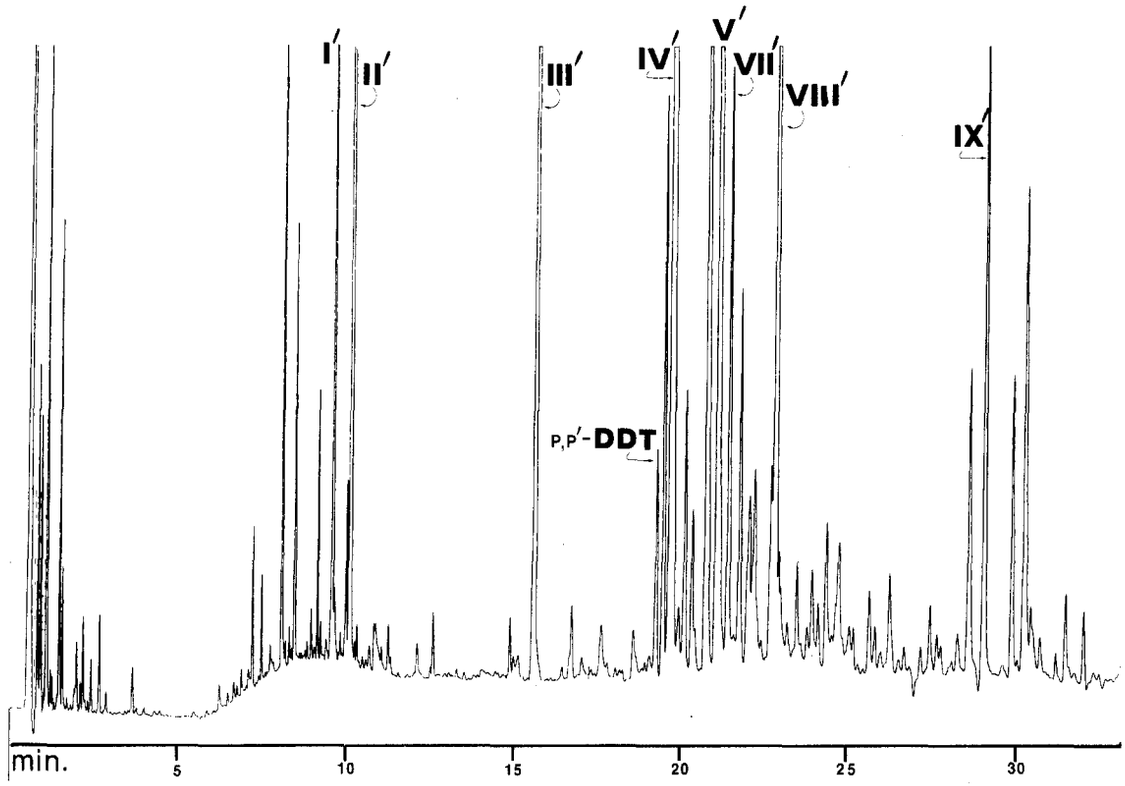


Figure 3. Splitless capillary GC/ECD chromatogram of neutral impurities in a crudely processed heroin sample from Pakistan after reduction with $LiAlH_4$ and derivatization with HFBA.

Table I. Retention Times of $LiAlH_4$-Reduced and HFBA-Derivatized Neutral Heroin Impurities and Internal Standard

compound	retention time, min
3,4-dimethoxy-*o*-phenylenedimethylene bis(heptafluorobutyrate) (I′)	9.59
3,4-dimethoxy-6-methyl-*o*-phenylenedimethylene bis(heptafluorobutyrate) (II′) (internal standard)	10.12
4-[(heptafluorobutyryl)oxy]-3-methoxyphenanthrene (III′) (internal standard)	15.69
p,p′-DDT (internal standard)	19.34
3,6-dimethoxy-4-[(heptafluorobutyryl)oxy]phenanthrene (IV′)	19.78
3,6-dimethoxy-4-[(heptafluorobutyryl)oxy]-5-[2-(*N*-methylethyl)ethyl]phenanthrene (V′)	21.16
N-ethyl-O^6-(heptafluorobutyryl)norcodeine (VI′)	21.26
N-ethyl-O^3,O^6-bis(heptafluorobutyryl)normorphine (VII′)	21.51
N-propyl-O^3,O^6-bis(heptafluorobutyryl)normorphine (VIII′)	22.90
3,6-dimethoxy-4-[(heptafluorobutyryl)oxy]-8-[2-(*N*-methylethyl)ethyl]phenanthrene (IX′)	29.16

sonable quantitative results. The compound *p,p′*-DDT was selected as the "instrumental" internal standard. By use of *p,p′*-DDT in conjunction with the "methodology" internal standards, response ratios can be established which can be used to monitor instrumental and methodology performances. Thus, any problem associated with either the extraction, reduction, or derivatization step would result in a higher response ratio of *p,p′*-DDT to the "methodology" internal standards. Such a condition would require corrective action before evaluation of the sample chromatogram is made.

Analysis of Adulterated and Unadulterated "Blind" Heroin Samples. A total of 22 highly adulterated and six unadulterated heroin samples were subjected to this methodology on a "blind" basis. The adulterated samples were "cut" to the 2% and 4% heroin levels with common adulterants such as quinine hydrochloride, procaine hydrochloride, lactose, mannitol, dextrose, and sucrose. In all cases we were able to differentiate between samples of Southeast Asian origin from those of Southwest Asian origin. Furthermore we were able to chemically subclassify heroin samples from these sources.

In summary, the method described herein has been used to differentiate refined heroin samples from Southeast Asia from refined heroin samples of Pakistani and European origin. The method is also applicable for more highly refined samples. There is evidence to suggest that when using this method in conjunction with the O^6-acetylcodeine content, Indian samples can be differentiated from all others. Crudely processed samples from Pakistan have levels of neutral impurities of at least 100 times greater than refined samples from Southeast Asia, Pakistan, India, and Europe. Finally, the method has been shown to be applicable for the analysis of adulterated, refined samples.

Registry No. I, 569-31-3; I′, 100334-40-5; IV, 47192-97-2; IV′, 100334-41-6; V, 89469-84-1; V′, 100334-42-7; VI, 89493-70-9; VI′, 100334-43-8; VII, 65846-34-6; VII′, 100334-44-9; IX, 91295-74-8; IX′, 100334-45-0; HFBA, 336-59-4; $LiAlH_4$, 16853-85-3; 2,3-dimethoxybenzoic acid, 1521-38-6; formaldehyde, 50-00-0; heroin, 561-27-3.

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Liquid Chromatography of Tin-Reduced Technetium Hydroxyethylidene Diphosphonate Complexes for On-Line Spectral Characterization and Double Isotope Labeling

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An anion-exchange high-performance liquid chromatographic separation has been developed for tin-reduced technetium hydroxyethylidene diphosphonate complexes, Tc-HEDP, at millimolar concentrations of technetium-99. The separation facilitates the acquisition of ultraviolet-visible spectra of the Tc-HEDP complexes on-line with a diode array spectrophotometer. A precolumn backflush technique has been devised to remove unreacted pertechnetate from the chromatographic system after each injection, thus preventing on-column reactions from subsequent samples containing Sn(II). The Tc-HEDP complexes were prepared at pH 2.5, 7.3, and 10.0 to demonstrate the effect of pH on the distribution of complexes within the separation. The reactions produce from four to five primary complexes, which vary as a function of pH. Some 13 additional complexes are present in trace amounts. The reactions were carried out with ^{113}Sn to determine if tin is incorporated into the complexes. Only one minor complex was found to contain tin. The ultraviolet-visible spectral characteristics of 10 Tc-HEDP complexes are reported.

Technetium diphosphonates are extensively employed as bone imaging radiopharmaceuticals in the field of nuclear medicine (*1*). Synthesis of bone imaging agents involves the reduction of pertechnetate (TcO_4^-) with a reductant (e.g., $SnCl_2$, $NaBH_4$, etc.) in the presence of a complexing diphosphonate ligand, such as hydroxyethylidene diphosphonate (HEDP).

$$TcO_4^- + \text{reductant} + \text{HEDP} \rightarrow \text{Tc-HEDP}$$

When Sn(II) is used as a reductant, previous investigators have presumed the reaction yielded a mixture of metal-ligand, mixed metal-ligand, and reductant-ligand complexes (*2*, *3*). These proposed complexes could be represented by the general formulas $Tc_qO_p(OH)_r(HEDP)_s$, $Tc_qO_p(OH)_r(HEDP)_s(Sn)_t$, and $Sn_t(HEDP)_s$ (*4*, *5*). It has been observed that the nature of the reducing agent affects the relative composition of the Tc-HEDP complexes within reaction mixtures, as indicated in chromatographic separations (*5-8*). Because of these variations, it is customary to indicate the reductant when referring to a given Tc-HEDP reaction; thus in comparison of tin to sodium borohydride reductions, the Tc-HEDP {Sn} or Tc-HEDP {$NaBH_4$} notation will be used.

Francis and Tofe (*2*) first postulated Tc-Sn-HEDP species based upon in vivo and in vitro studies involving the adsorption of a Tc-HEDP {Sn} reaction mixture, containing excess HEDP and $^{113}SnCl_2$, onto either synthetic or skeletal hydroxyapatite. The results indicated the ^{113}Sn adsorbed onto the hydroxyapatite. The presumption that the ^{113}Sn-HEDP alone would not adsorb onto the hydroxyapatite led these investigators to conclude that tin must be incorporated into the technetium complexes. Since that time, it has been demonstrated that ^{113}Sn-HEDP will uptake onto hydroxyapatite (*9*). The hypothesis that tin may be incorporated into the Tc-HEDP {Sn} complexes has been supported by the existence of a mixed metal Tc-Sn-dimethylglyoxime complex, evidenced by a crystal structure determination (*10*), and the triple isotope (^{99}Tc, ^{32}P, ^{113}Sn) labeling of a Tc-HEDP {Sn} reaction mixture by Van den Brand (*5*). In this latter investigation, low-performance size exclusion separations were unable to resolve ^{113}Sn-HEDP from several Tc-HEDP {Sn} fractions; thus the incorporation of tin within the Tc-HEDP complexes has not been previously established.

The realization that the reduction of pertechnetate in the presence of HEDP produces a mixture of complexes is significant, because biodistributions of various Tc-HEDP complexes have been found to differ (*11-14*). The physical characteristics of these complexes dictate the nature of their biodistribution; therefore, the elucidation of their chemical composition and physical properties is necessary to propose in vivo distribution mechanisms. Isolation of the Tc-HEDP {Sn} complexes in pure form is required for such determinations; thus high-performance liquid chromatographic separations of the complexes must be achieved. Low-performance separations of technetium radiopharmaceuticals are routinely accomplished by paper or thin-layer chromatography (*15*, *16*), but these quality control methods lack the high resolution necessary to separate all the complexes within these complicated reaction mixtures.

Liquid chromatographic separations have been developed for ^{99m}Tc-HEDP {Sn} complexes prepared without addition of the technetium-99 carrier isotope (i.e., at "no carrier added" concentrations). In each case with these "no carrier added" preparations only a limited number of ^{99m}Tc-HEDP {Sn} complexes have been resolved. Van den Brand (*5*) has reported the separation of four ^{99m}Tc-HEDP {Sn} complex fractions by gel permeation chromatography, while Srivastava et al. (*8*) have reported the separation of six ^{99m}Tc-HEDP {Sn} complexes by anion-exchange HPLC and eight components by reversed-phase HPLC. These previous separations were monitored with Na(Tl) solid scintillation detectors, which measured the γ emission from the ^{99m}Tc isotope. Since the micromolar concentrations of these "no carrier added" preparations are inadequate for acquisition of ultraviolet-visible spectra of the Tc-HEDP {Sn} complexes, new separation methods are required to accommodate millimolar ^{99}Tc concentrations of tin-reduced preparations.

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