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PAPER

CRIMINALISTICS

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Signature Profiling and Classification of Illicit Heroin by GC-MS Analysis of Acidic and Neutral Manufacturing Impurities

ABSTRACT: The illicit manufacture of heroin results in the formation of trace level acidic and neutral impurities. These impurities are detectable in illicit heroin and provide valuable information about the manufacturing process used. The isolation, derivatization, and semiquantitative analysis of neutral and acidic heroin manufacturing impurities by programmed temperature vaporizing injector-gas chromatography-mass spectrometry (PTV-GC-MS) is described. Trace acidic and neutral heroin impurities were isolated from basic fractions using liquid–liquid extraction. Extracted impurities were treated with *N*-Methyl-*N*-trimethylsilyltrifluoroacetamide followed by PTV-GC-MS analyses. Semiquantitative data were obtained using full scan mass spectrometry utilizing unique ions or ion combinations for 36 trace impurities found in crude and/or highly refined heroin samples. Minimum detection limits for acidic and neutral impurities were estimated to be at the 10^{-7} level relative to total morphine. Over 500 authentic heroin samples from South America, Mexico, Southwest Asia, and Southeast Asia were analyzed. Classification of illicit heroin based on the presence or absence and relative amounts of acidic and neutral impurities is presented.

KEYWORDS: forensic science, gas chromatography, mass spectrometry, derivatization, ion trap, heroin, morphine, heroin signature analysis, heroin profiling

Analytical methodology used to determine major and minor manufacturing impurities in illicit heroin provide useful data to differentiate heroin produced in different source countries/regions worldwide, and may also be used for the comparative analyses of heroin samples (1–8). Commonalities determined by the in-depth analysis of authentic heroin samples from known source regions form the basis for differentiation of unknown samples. Currently, the United States Drug Enforcement Administration's Special Testing and Research Laboratory (STRL) uses three distinct analytical methods to classify heroin samples: a capillary electrophoresis (CE) procedure that quantifies heroin and major basic impurities (9), a static headspace gas chromatographic mass spectrometric (SHS-GC-MS) procedure that quantifies trace occluded solvents (10), and a programmed temperature vaporizing injector-gas chromatography-mass spectrometry (PTV-GC-MS) procedure that measures trace acidic and neutral impurities. This PTV-GC-MS procedure replaces a gas chromatographic flame ionization detection (GC-FID) method that was previously used at this laboratory.

The illicit manufacture of heroin results in the formation of trace level acidic and neutral impurities that arise from the reaction of acetic anhydride with morphine and other alkaloids found in opium. The presence or absence, and relative amounts of these impurities in illicit heroin, are a direct result of the manufacturing process used and, to a lesser extent, the origin of the opium itself. Previous work has found that these impurities are related to, or arise from, a variety of compounds including narcotine, norlaudanosine, thebaine, and the tetrahydrobenzylisoquinolines (11–14). While some of the acidic and neutral impurities have been characterized, few have been isolated in sufficient quantities to serve as primary standards for a fully quantitative procedure. Accordingly, the methodology described here yields semiquantitative data.

Previous analytical approaches to determine acidic and neutral heroin impurities have included gas chromatography with flame ion detection (15), gas chromatography with electron capture detection (GC-ECD) (16), and capillary electrophoresis (17). Prior to the adoption of the new PTV-GC-MS procedure, STRL had successfully used a methodology related to the GC-FID method described by Neumann (15). However, the GC-FID and other procedures, while sensitive and robust, lacked the specificity needed at times to correctly assign peaks and allow the tracking of additional uncharacterized acidic and neutral impurities. In addition, utilization of a PTV inlet reduced potential analyte decomposition and provided better chromatography for late eluting impurities than a conventional split/splitless injection technique.

This paper describes a procedure to isolate, separate, identify, and determine acidic and neutral manufacturing impurities in heroin. Results from the analysis of authentic heroin samples are discussed relative to the classification of samples and the formation of sub-classifications of the major known growing/processing regions using the presence or absence and relative amounts of acidic and neutral impurities.

Materials and Methods

Drug Materials and Reagents

High purity methylene chloride and ethyl acetate were obtained from American Burdick and Jackson (Muskegon, MI). Reagent grade sulfuric acid (96.8%, specific gravity 1.84) and petroleum

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ether (20–40°C), were obtained from J.T. Baker Chemical Company (Phillipsburg, NJ). The derivatization reagent MSTFA (*N*-Methyl-*N*-trimethylsilyltrifluoroacetamide), was obtained in 1 mL glass ampules from Pierce Chemical Company (Rockford, IL). Sodium sulfate was obtained from Fisher Scientific (Fairlawn, NJ). Helium used as a GC carrier gas and MS buffer gas was zero grade or better. Nitrogen used to evaporate organic extraction solvents was zero grade or better.

Disposable 15 mL centrifuge tubes and phenolic caps used for liquid/liquid extractions and extract derivatizations were obtained from Kimble (Vineland, NJ). Autosampler vials (1.8 mL glass crimpable) and the corresponding 6 × 29 mm 250 µL inserts (silanized) were obtained from SUN-SRI (Rockwood, TN). Crimp caps for autosampler vials (11 mm) and 10 mil PTFE septa were obtained from SUN-SRI (Rockwood, TN).

All internal standards and authentic heroin calibration samples were obtained from the STRL authentic reference collection.

Gas Chromatography and Mass Spectrometry

PTV-GC-MS analysis was performed using a Thermo Electron Trace gas chromatograph outfitted with a PTV injection port and coupled with a Thermo Electron Polaris-Q ion trap mass spectrometer. The PTV injection port was configured in solvent elimination mode and was outfitted with a 2 mm ID Siltek® deactivated glass liner (Restek, Bellefonte, PA). The PTV was programmed as follows: initial base temperature, 85°C; splitless time, 1.5 min; solvent surge mode, 0.5 psi; inject pressure, 8.0 psi; inject time, 1.5 min; vent flow, 50 mL/min; evaporation rate, 2.0°C/sec; evaporation temperature, 85°C; evaporation time, 0.2 min; transfer pressure, 35.0 psi; transfer rate, 3.0°C/sec; transfer temperature, 310°C; transfer time, 12.0 min; clean rate, 14°C/min; clean temperature, 325°C; clean time, 23.0 min; and clean flow, 70.0 mL/min. Injection volumes were 1 µL for crude samples and 3 µL for refined samples.

The Trace GC was fitted with an Agilent DuraGuard 30 m × 0.25 mm ID fused-silica capillary column coated with DB-1 (0.25 µm) with an integrated 10 meter deactivated precolumn (Agilent Technologies, Santa Clara, CA). The oven temperature was programmed as follows: initial temperature, 60°C; initial hold, 6.0 min; temperature program rate, 40.0°C/min; final temperature, 200°C; temperature program rate 2, 6.3°C/min; final temperature 2, 227°C; temperature program rate 3, 2.0°C/min; final temperature 3, 274°C; temperature program rate 4, 2.3°C/min; final temperature 4, 298°C; final hold, 10.5 min. Helium carrier gas was maintained at a constant flow of 1.5 mL/min.

The Polaris-Q mass spectrometer was operated in full scan mode with the following parameters: source temperature, 245°C; transfer line, 295°C; multiplier offset, 0 volts; start time, 10 min; micro scans, 6; max ion time, 30 ms; first mass, 43; last mass, 575; damping gas flow, 0.3 mL/min.

Stock Internal Standard Solution (SISS)

The procedure utilizes six structurally related internal standards (to selected analytes) that are added to the extraction solutions. The internal standard stock solution was prepared by accurately weighing 15.0 mg of m-Meconin, 3-Methoxy-4-acetylphenanthrene, d_6–3,6-dimethoxy-4-acetyloxy-5-(2-*N*-methylacetamido)ethylphenanthrene (d_6-1–395), d_9-triacetylnormorphine (d_9-TANM), and *N*-propionylnorlaudanosine (NPNL), and 25.0 mg of *N*-propionylnornarcotine (NPNN) and making up to volume with methylene chloride in a 250 mL glass-stoppered volumetric flask. The sealed solution was refrigerated at 4°C when not in use and is viable for 6 months.

Extraction Solution for Crude Samples (ESCS)

Exactly 60 mL of the SISS was added to a glass-stoppered 1000 mL Erlenmeyer flask. To this, 600.0 mL of petroleum ether (20–40°C) and 340.0 mL of methylene chloride was added. This solution was used for the preparation of crudely refined heroin samples which required a greater dilution due to higher concentrations of neutral and acidic impurities. This solution is viable for up to 3 months.

Extraction Solution for Refined Samples (ESRS)

Exactly 100.0 mL of the ESCS was transferred to a glass 1000 mL Erlenmeyer flask. The solution was diluted to volume with 540.0 mL of petroleum ether (20–40°C) and 360.0 mL of methylene chloride. This solution was used for the preparation of highly refined heroin samples containing lower levels of neutral and acidic impurities. This solution is viable for up to 3 months.

2.0 N H_2SO_4 plus 10% Sodium Sulfate Extraction Solution (2.0N H_2SO_4/10% Na_2SO_4)

The solution was prepared by adding 57.6 mL of concentrated sulfuric acid to a 1 L glass volumetric flask containing 700 mL of distilled water. While stirring, 100 g of anhydrous sodium sulfate was added to the flask and mixed until all of the sodium sulfate was dissolved and the solution had cooled. It was then diluted to volume with distilled water.

20% MSTFA Derivatization Solution

This solution was prepared daily in a 15 mL centrifuge tube by adding the contents of a 1 mL ampule of MSTFA and 4.0 mL of ethyl acetate. The centrifuge tube was capped and the solution was agitated to ensure thorough mixing. The solution was usable for 48 h.

Weighing Heroin Samples

Since neutral and acidic impurity levels vary according to the extent of cleanup performed by illicit heroin laboratory operators, it was necessary to weigh different amounts of sample according to estimated sample type (e.g., crude or refined). To better estimate the sample type, a quantitative analysis by CE (9) was used to determine the amount of basic compounds present including heroin, O^6-monoacetylmorphine (O^6-MAM), O^3-monoacetylmorphine (O^3-MAM), acetylcodeine, papaverine, noscapine, codeine, and morphine. With these data, an initial signature assignment was established for each sample, which dictated the amount of sample to use. There were four target weights used depending upon the type of sample; 10, 20, 30, or 45 mg/equivalents to total morphine. The total morphine value was calculated using the amounts of heroin, O^6-MAM, O^3-MAM, and morphine. The appropriate amount was determined using the following criteria:

- 10 mg/equivalent—Used if the sample was suspected to be crudely refined. Crude samples include all Mexican (MEX) samples (often black tar) and specific Southwest Asian (SWA) that were suspected to be crudely refined. Characteristics of SWA-crude samples include high noscapine levels, heroin present as the free base, and coloration ranging from tan to brown.

- 20 mg/equivalent—Used for samples suspected to be highly refined, but noscapine and/or papaverine are present at levels greater than 1%. These were typically South American (SA) heroin hydrochloride samples which were tan in color.
- 30 mg/equivalent—Used for samples that were thought to be highly refined, but had detectable noscapine and/or papaverine less than 1%. This primarily included SWA moderately refined samples. These samples were typically the hydrochloride salt and tan in color.
- 45 mg/equivalent—Used if the sample was suspected to be very highly refined, and there was no detectable papaverine or noscapine. These include Southeast Asian (SEA), SWA, or SA heroin hydrochloride samples that were off-white to white in color.

Once the appropriate milligram equivalent was determined via CE, specific target weights were calculated using previously determined quantitation values for heroin, O^6-MAM, O^3-MAM, and morphine using the following formula:

$$\begin{aligned}\text{Target Weight (in mg)} = n/[&((\%\text{Heroin Base}/100)0.77)\\ &+ ((\%O^6 - \text{MAM Base}/100\\ &+ \%O^3 - \text{MAM Base}/100)0.87)\\ &+ (\%\text{Morphine Base}/100)],\end{aligned}$$

where n = 10, 20, 30, or 45.

Samples were accurately weighed into 15 mL disposable centrifuge tubes within ±0.1 mg of the target weight.

Preparation of Crude Samples

This procedure was used if samples had been weighed as a 10 mg/equivalent to morphine (i.e., suspected SWA-crude or MEX), and for the SWA-crude and MEX calibration standards. Samples are ideally run immediately after preparation, but may be stored for up to 12 h. Crude sample preparation involved two steps:

Step 1: Liquid/Liquid Extraction of Crude Samples—Exactly 5.0 mL of the ESCS solution for crude samples was added to the 15 mL centrifuge tube containing the accurately weighed sample or standard. The tube was capped and vortexed on the high setting for 1 min. To this, exactly 4.0 mL of the 2.0N H_2SO_4/10% Na_2SO_4 solution was added, followed by recapping and vortexing on the high setting for 2 min. The tube was centrifuged at 1718×*g* for 10 min, followed by the transfer of the top organic layer containing the neutral and acidic target impurities (and internal standards) to a new 15 mL centrifuge tube. Care was taken to maximize the removal of the organic solution without carryover of any of the acidic solution. The centrifuge tube containing the organic extract was then placed into a heating block set to 75°C and was evaporated to dryness under a low nitrogen stream.

Step 2: Derivatization and Dilution of Crude Samples—The extracted residue contained in the 15 mL centrifuge tube was treated with 250 µL of the 20% MSTFA Derivatization Solution followed by vortexing on the medium setting for 1 min. The capped tube was then heated for 15 min at 75°C in a heating block. After heating, crude samples and standards were diluted with 2.0 mL of ethyl acetate. The capped tube was vortexed on the medium setting for 1 min. A portion of this solution was transferred to a 2 mL autosampler vial and crimp capped using a PTFE cap.

Preparation of Refined Samples

This procedure was used for samples weighed as 20, 30, or 45 mg/equivalents to morphine (i.e., suspected SA, SWA-refined, or SEA) and for the SEA calibration standard. Samples were

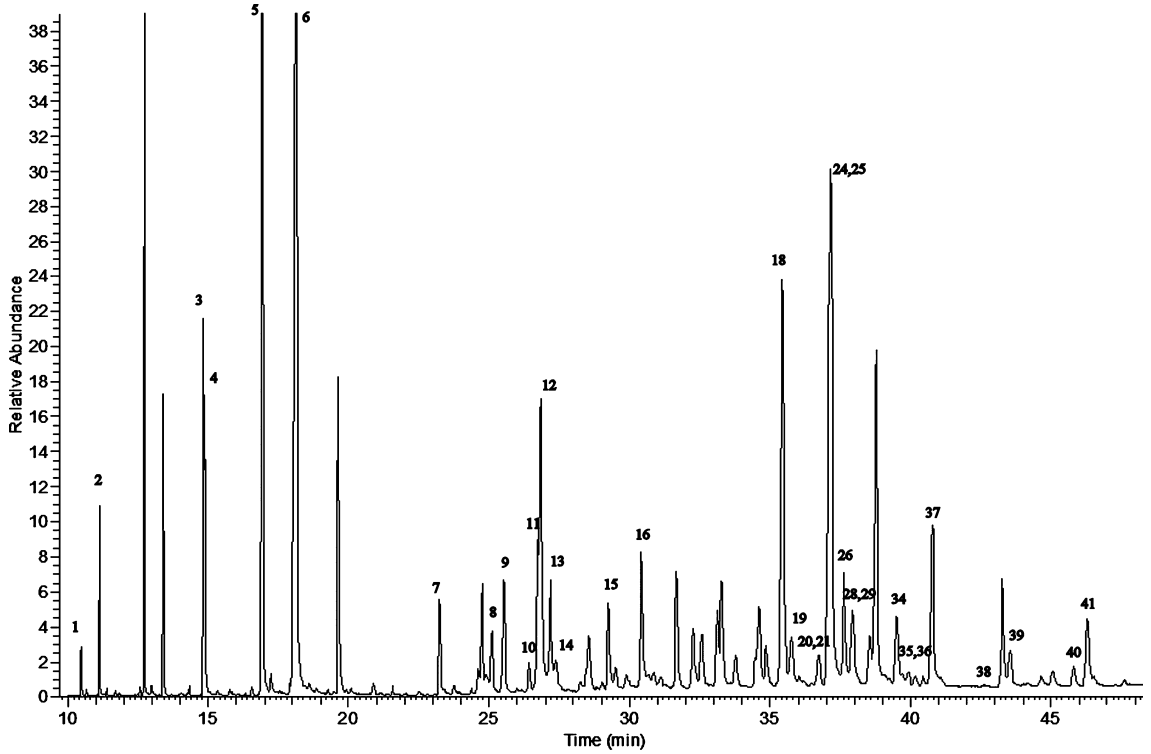

FIG. 1—*Reconstructed TIC for SWA-crude heroin standard. Peak labels correspond to peak identities given in Table 1.*

ideally run immediately after preparation, but were viable for up to 12 h. Refined sample preparation involved two steps:

Step 1: Liquid/Liquid Extraction of Refined Samples—This procedure was the same as the previously described Liquid/Liquid Extraction of Crude Samples except that exactly 5.0 mL of the refined extraction solution (ESRS) was added to the 15 mL centrifuge tube containing accurately weighed samples or standards.

Step 2: Derivatization of Refined Samples—The extracted residue contained in the 15 mL centrifuge tube was treated with 250 µL of the 20% MSTFA Derivatization Solution followed by vortexing on the medium setting for 1 min. The solution was

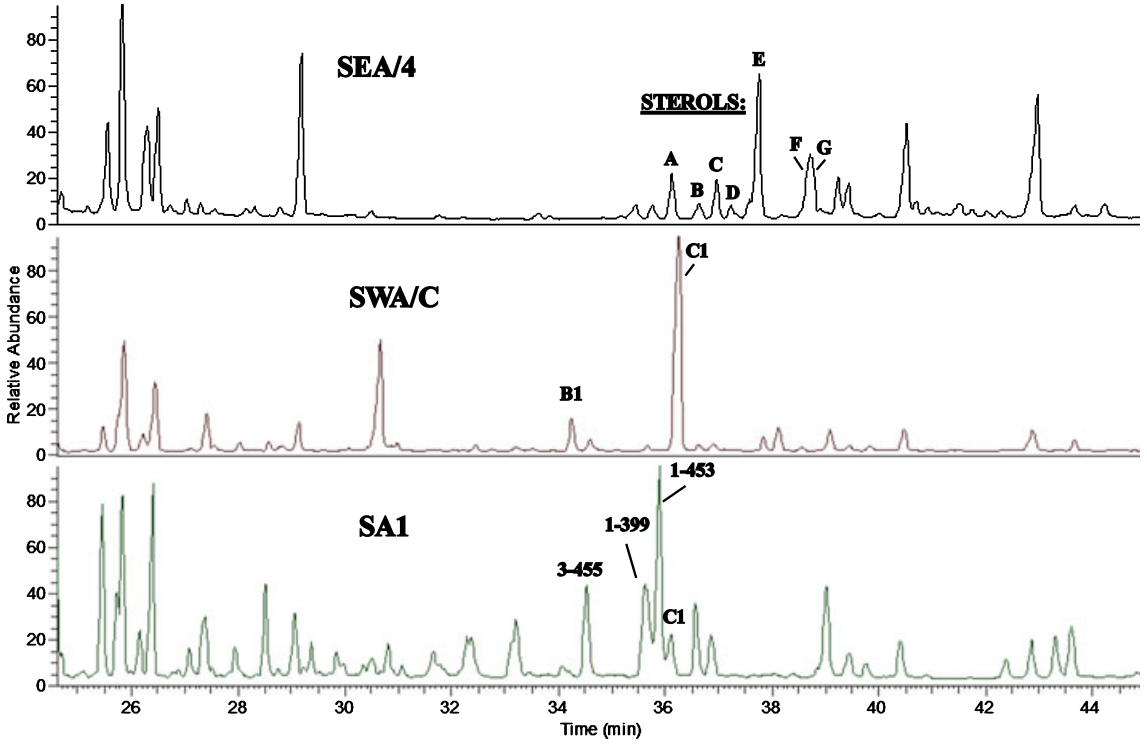

FIG. 2—*Partially reconstructed TIC of SEA/4, SWA/C, and SA1 type heroin.*

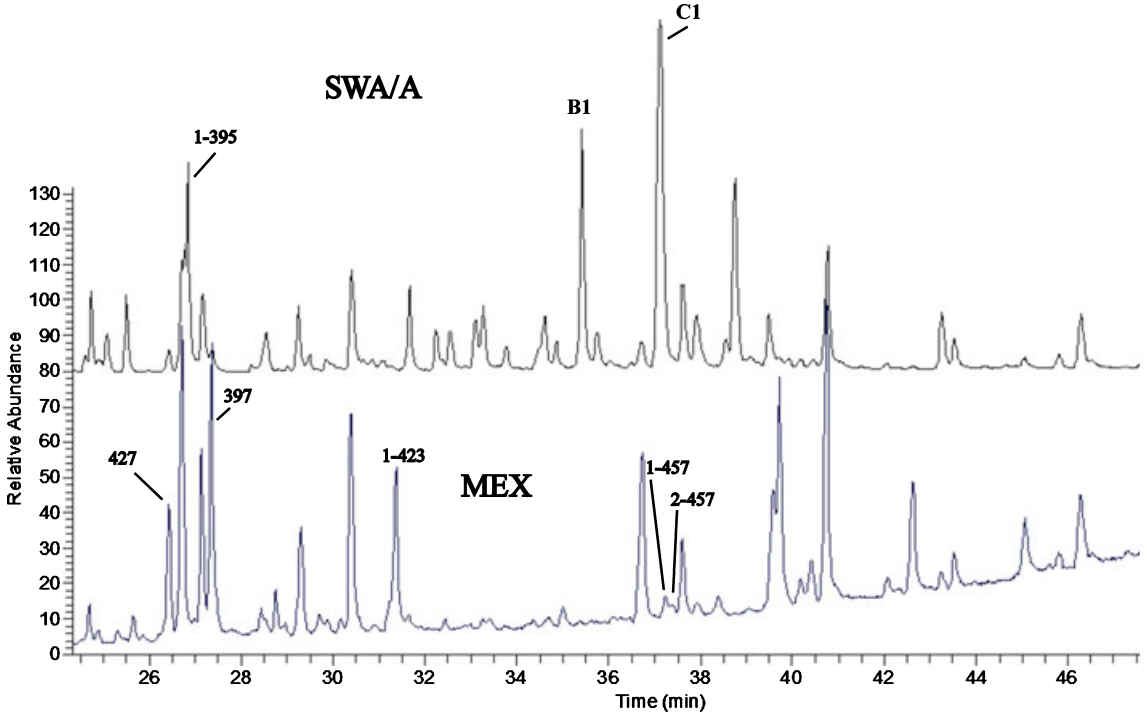

FIG. 3—*Partially reconstructed TIC of SWA/A and MEX type heroin.*

TABLE 1—*Semiquantitation parameters for target compounds and internal standards.*

Peak#	Compound Name*	Retention Time (min)	Internal Standard	Calibration Level	Quantitation Ion(s) (m/z)	Calibration Amount (μg)†
1	Meconin (194)	10.46	Meta-meconin	SWA	147,165,176,193	25.2
2	Meta-meconin ISTD	11.10	N/A	N/A	165,194	1.0
3	3-Methoxy-4-acetylphenanthrene (3M4AP) ISTD	14.82	N/A	N/A	181,209,224,266	1.0
4	3,6-Dimethoxy-4,5-epoxyphenanthrene (252)	14.88	3M4AP	SWA	194,237,252	14.0
5	Thebaol (254)	16.92	3M4AP	SWA	281,296,326	58.8
6	Acetylthebaol (296)	18.10	3M4AP	SWA	239,254,296	130.0
7	UNK 1-425	23.24	d9-TANM	SWA	262,324,352,425	10.5
8	Diacetylnorcodeine (2–369)	24.91	d9-TANM	SWA	223,224,250,327,369	11.0
9	3,6-Dimethoxy-4-acetyloxy-9,10-dihydro-9-(*N*-methylacetamido) phenanthrene (3–369)	25.52	3M4AP	SWA	239,254,296	10.0
10	Diacetylnormorphine (427)	26.41	d9-TANM	SWA	266,281,427	5.8
11	d6-3,6—Dimethoxy-4-acetyloxy-5-[2-(*N*-methylacetamido)ethyl]-phenanthrene (d6-1-395) ISTD	26.72	N/A	N/A	292,357,358,401	1.0
12	3,6—Dimethoxy-4-acetyloxy-5-[2-(*N*-methylacetamido)ethyl]-phenanthrene (1-395)	26.83	d6-1-395	SWA	353,354,395	35.7
13	d9-Triacetylnormorphine (d9-TANM) ISTD	27.19	N/A	N/A	405,406,407	1.0
14	Triacetylnormorphine (397)	27.38	d9-TANM	SWA	295,313,355,397	6.4
15	*n*-Acetylnorlaudanosine (385)	29.23	NPNL	SWA	192,193,234,235	18.0
16	*n*-Propionylnorlaudanosine (NPNL) ISTD	30.41	N/A	N/A	192,193,248,249	1.0
17	UNK 1-423	31.37	NPNL	MEX	251,266,308,381,423	35.7
18	UNK B1	35.41	NPNN	SWA	394,410,453,500,528,543	36.6
19	UNK 3-455	35.77	NPNN	SWA	294,340,413,455	3.1
20	UNK 1-399	36.70	NPNN	SWA	326,399	2.5
21	UNK 1-453	36.72	NPNN	SWA	296,297,369,411,453	1.2
22	Sterol-A	36.87	NPNN	SEA	281,393,483	0.5
23	Sterol-B	37.07	NPNN	SEA	391,466	0.3
24	UNK C1	37.14	NPNN	SWA	338,379,423,442,453,470	72.7
25	UNK 1-457	37.24	NPNN	MEX	151,353,384,457	0.3
26	UNK 2-457	37.48	NPNN	MEX	209,385,457	5.0
27	Sterol-C	37.61	NPNN	SEA	189,190,393,408,498	0.5
28	3,6-Dimethoxy-4-acetyloxy-8-[2-(*N*-methylacetamido)ethyl]phenanthrene (2-395)	37.61	NPNN	SWA	280,281,353,354,395	15.5
29	UNK 3-515	37.91	NPNN	SWA	442,515,516	2.5
30	Sterol-D	38.04	NPNN	SEA	189,190,391,393,453,468	0.2
31	Sterol-E	38.29	NPNN	SEA	393,453	1.4
32	Sterol-F	38.91	NPNN	SEA	218,219	1.1
33	Sterol-G	39.01	NPNN	SEA	189,190	1.1
34	*n*-Acetylnornarcotine (441)	39.50	NPNN	SWA	206,207,248,249	15.0
35	UNK 2-427	39.72	NPNN	SWA	151	2.5
36	UNK 3-427	39.83	NPNN	SWA	137,179	2.5
37	*n*-Propionylnornarcotine (NPNN) ISTD	40.78	N/A	N/A	206,262,263	1.0
38	UNK 4-455	42.61	NPNN	MEX	298,340,454,455	1.1
39	*cis-n*-Acetylanhydronornarceine (1-455)	43.53	NPNN	SWA	193, 382, 383, 455	5.0
40	erythro-*n*-Acetyl-1-acetyloxynornarceine (1-515)	45.81	NPNN	SWA	252, 280, 281, 455	5.3
41	*trans-n*-Acetylanhydronornarceine (2-455)	46.27	NPNN	SWA	193, 382, 383, 455	11.7

*Number in parentheses and otherwise are apparent molecular weights.
†Semiquantitative mass estimate, based on the GC-MS response relative to the prior-eluting ISTD.

TABLE 2—*Precision and minimum detection limits for target compounds.*

Compound Name	Precision (%RSD)*	MDL†
Meconin (194)	5.7	54.9
3,6-Dimethoxy-4,5-epoxyphenanthrene (252)	4.7	15.5
Thebaol (254)	5.4	3.8
Acetylthebaol (296)	4.8	69.4
UNK 1-425	3.9	32.3
Diacetylnorcodeine (2-369)	4.1	41.4
3,6-Dimethoxy-4-acetyloxy-9, 10-dihydro-9-(*N*-methylacetamido) phenanthrene (3-369)	4.7	2.9
Diacetylnormorphine (427)	2.2	10.3
3,6—dimethoxy-4-acetyloxy-5-[2-(*N*-methylacetamido)ethyl]-phenanthrene (1-395)	3.6	56.6
Triacetylnormorphine (397)	1.7	9.2
n-Acetylnorlaudanosine (385)	1.6	38.1
UNK 1-423	3.9	9.7
UNK B1	8.4	8.5
UNK 3-455	4.6	2.2
UNK 1-399	5.4	9.5
UNK 1-453	2.7	2.4
Sterol-A	6.6	0.9
Sterol-B	5.8	0.3
UNK C1	8.3	1.5
UNK 1-457	5.5	0.6
UNK 2-457	5.5	0.7
Sterol-C	6.6	1.5
3,6-Dimethoxy-4-acetyloxy-8-[2-(*N*-methylacetamido)ethyl] phenanthrene (2-395)	2.8	23.2
UNK 3-515	5.9	0.2
Sterol-D	5.1	0.4
Sterol-E	4.8	0.5
Sterol-F	4.9	0.8
Sterol-G	6.0	0.8
n-Acetylnornarcotine (441)	8.7	42.8
UNK 2-427	5.0	0.1
UNK 3-427	2.6	0.2
UNK 4-455	5.7	9.9
cis-n-Acetylanhydronornarceine (1-455)	4.7	12.9
erythro-*n*-Acetyl-1-acetyloxynornarceine (1-515)	7.4	33.2
trans-n-Acetylanhydronornarceine (2-455)	6.5	41.8

*Replicate analyses ($N = 9$) from uniform SWA-crude, MEX, or SEA calibration samples.

†Data is presented as $\times 10^{-6}$ w/w percent relative to total morphine. Semiquantitative estimate based on GC-MS response relative to the prior eluting ISTD

allowed to derivatize at room temperature for 10 min (slightly less time was required to derivatize these samples). Further dilution was not typically required. A portion of this solution was transferred to a 2 mL autosampler vial containing a deactivated 250 μL insert and was crimp capped with a PTFE cap.

Preparation of Calibration Standards

Since sufficient quantities of individual target compounds were not available as primary standards, homogenous SWA-crude, MEX, and SEA samples were identified and corresponding target compound levels were estimated by peak ratio versus available primary standard materials using GC-MS and GC-FID. Daily preparation of the SWA-crude and MEX calibration standards was accomplished by accurately weighing 20.9 and 21.1 mg (±0.1 mg), respectively, into 15 mL centrifuge tubes and following the above described procedure (Preparation of Crude Samples). The target weights were calculated based upon a 10 mg/equivalent to morphine for the crudely refined SWA heroin base and the crudely refined MEX heroin hydrochloride.

Preparation of the SEA calibration standard was accomplished by accurately weighing 75.6 mg (±0.1 mg) into a 15 mL centrifuge tube and following the above described procedure (Preparation of Refined Samples). The target weight of 75.6 mg was calculated based upon a 45 mg/equivalent to morphine for the highly refined SEA heroin hydrochloride authentic.

Results and Discussion

Qualitative

The reconstructed total ion chromatogram (TIC) for the GC-MS analysis of a SWA-crude standard, containing the majority of the target compounds, is illustrated in Fig. 1. A partially reconstructed TIC of three types of refined heroin is shown in Fig. 2. A partially reconstructed TIC of the two remaining crude heroin types is illustrated in Fig. 3. Most target compounds and internal standards were well resolved; however, those that exhibited overlap were easily distinguished by differing mass spectral fragmentation patterns. For example, unresolved d_6-1-395 and 1-395 were identified and targeted using ions m/z 357, 358, and 401 and ions m/z 353, 354, 395 respectively. The procedure demonstrated sufficient sensitivity and resolution to detect new manufacturing impurities as well as commonly encountered neutral and acidic adulterants found in illicit heroin. New impurities included UNK 3-455, UNK 1-399 and UNK 1-453, prominent in SA samples and UNK 1-423 present in MEX samples. In addition, the acquisition of MS data in full scan mode proved to be invaluable in daily peak confirmation.

Frequently, illicit heroin samples contain adulterants and diluents ranging from trace level to a major constituent. Common adulterants encountered in illicit heroin that are known to coextract with acidic and neutral impurities include caffeine, acetaminophen, phenacetin, phenobarbital, and diltiazem. However, these adulterants did not interfere with the identification or quantitation of key acidic and neutral impurities. In addition, common diluents and basic adulterants did not extract with the acidic/neutral impurities and, therefore, presented no analytical problems.

Semiquantitative Analysis

A daily calibration of the GC-MS system was performed using three authentic samples that collectively contained the specific neutral and acidic target compounds of interest. The specific SWA-crude, MEX, and SEA calibration samples were selected based on the presence of key manufacturing impurities, sample homogeneity, and sufficient sample reserve. Table 1 illustrates peak number, compound name, retention time, internal standard, calibration level, quantitation ions, and calibration amount. In addition to system calibration, the daily analysis of the three calibration samples served as an extraction and GC-MS system check prior to sample analysis.

Overall, acidic and neutral impurities ranged from over 1% to less than 10^{-6}%, relative to total morphine. Method reproducibility was determined over several days utilizing several instruments by performing nine replicate analyses on the SWA-crude, MEX, and SEA calibration samples. Results are presented in Table 2. Method precision for the target compounds ranged from 1.6% to 8.7% RSD, despite the low levels encountered.

Minimum detection limits (MDL) for the target compounds were estimated using data from sample analyses with minimum levels of acidic and neutral impurities. MDL values, based on semi-

TABLE 3—*Sub-classification types and descriptions.*

Classification	Refinement Level	Distinguishing Characteristics
SWA/A	Crude	Almost all target impurities present (excluding sterols & 1-423) Average levels at 10^{-3} to 0.1% relative to total morphine 1-395 > 397 Predominant UNK B1 and UNK C1
MEX	Crude	Absence of many target impurities including B1 Average levels at 10^{-3} to 0.1% relative to total morphine Presence of 1-423 1-395 < 397 Presence of acetylated sugars
SWA/C	Refined	Almost all target impurities present (excluding sterols & UNK 1-423) Average levels at 10^{-3} to 10^{-5}% relative to total morphine 1-395 > 397 Presence of UNK B1 and UNK C1
SA3	Refined	Moderate number of target impurities present (excluding sterols & 1-423) Average levels at 10^{-3} to 10^{-5}% relative to total morphine 397 > 10^{-2}% relative to morphine Presence of UNK 3-455, UNK 1-399, and UNK 1-453 greater than UNK B1 and UNK C1
SA1	Refined	Moderate number of target impurities present (excluding sterols & 1-423) Average levels at 10^{-3} to 10^{-5}% relative to total morphine 397 < 10^{-2}% relative to morphine Presence of UNK 3-455, UNK 1-399, and UNK 1-453 greater than UNK B1 and UNK C1
SEA/4	Highly refined	Few target impurities present Presence of sterols Average levels at 10^{-4} to 10^{-6}% relative to total morphine
SA2	Highly refined	Few target impurities present Absence of sterols Average levels at 10^{-3} to 10^{-6}% relative to total morphine May contain small amounts of UNK 3-455, UNK 1-399, and UNK 1-453 Currently indistinguishable from SWA/B
SWA/B	Highly refined	Few target impurities present Absence of sterols Average levels at 10^{-3} to 10^{-6}% relative to total morphine May contain small amounts of UNK B1 and UNK C1 Currently indistinguishable from SA2

quantitative results, were calculated as the percent relative to total morphine (w/w) and are presented in Table 2. Overall MDL values ranged from 1.0×10^{-7} to 6.9×10^{-5}% relative to morphine. These MDL levels were found to be sufficient to obtain necessary semiquantitative information on the most highly refined illicit heroin samples.

Sample Classification

Using the described procedure, in-depth qualitative and semiquantitative examinations were performed on 643 authentic heroin samples from the four known manufacturing regions: SA (n = 211), MEX (n = 68), SWA (n = 160), and SEA (n = 204). The qualitative and semiquantitative determinations of these authentics provide the template by which unknown heroin samples are classified. Within each manufacturing region, multiple illicit processing techniques are employed, resulting in different impurity profiles of acidic and neutral compounds. Therefore, as needed, several sub-classifications were established for some of the known manufacturing regions based upon the presence or absence of key impurities, as well as the level of impurities detected.

Crude Samples

Crudely prepared heroin samples have particularly high levels of acidic and neutral impurities, ranging from the 10^{-3}% to over 1% relative to total morphine. Samples classified as crude include all Mexican heroin (MEX) and Southwest Asian heroin type A (SWA/A). Figure 3 contains a stacked, partially reconstructed TIC of a SWA/A and MEX sample. In addition, Table 3 contains specific characteristics of crudely refined samples. MEX samples typically contain significant levels of UNK 1-423, and also often have a series of acetylated sugars which are a direct result of the addition of sugars to the morphine acetylation reaction. Acetylated sugars were not specifically targeted by this procedure, but when present, were easily identified by their corresponding mass spectra. SWA/A heroin samples are noted for their high number and high levels of acidic and neutral impurities.

Refined Samples

Overall, refined heroin samples have a moderate number of acidic and neutral impurities present at the 10^{-3} to 10^{-5}% level relative to total morphine. Refined samples include Southwest Asian type C (SWA/C) and South American types 1 and 3 (SA1 and SA3). SWA/C heroin contains many of the same target compounds, as SWA/A, but at an order of magnitude lower concentration. Characteristics of SA1 and SA3 type heroins include significant amounts of UNK 3-455, UNK 1-399, and UNK 1-453. Typically, SA1 and SA3 heroin are qualitatively similar, but are distinguished by the greater level of impurities encountered in SA3 heroin, as indicated by the amount of triacetylnormorphine (397). Figure 2 contains the stacked, partially reconstructed TIC of SA1, SWA/C, and SEA/4 heroin, while Table 3 contains a summary of distinguishing characteristics of refined heroin types.

Highly Refined Samples

Highly refined samples typically contain very few acidic and neutral impurities, at levels ranging from 10^{-3} to 10^{-6}% relative to

morphine. Heroin classified as highly refined includes Southeast Asian type 4 (SEA/4), South American type 2 (SA2), and Southwest Asian type B (SWA/B). SEA/4 heroin is easily distinguished by the presence of sterols (not of opium origin) present at the 10^{-4}% or lower level, which have not been encountered in the other highly refined heroin types. Figure 2 contains a partially reconstructed TIC for an SEA/4 sample, illustrating the characteristic sterol pattern. Heroin samples classified as SA2 and SWA/B have few acidic and neutral impurities and are currently indistinguishable by this technique. Additional notable characteristics of highly refined samples are detailed in Table 3.

Conclusions

The described methodology determines trace level acidic and neutral impurities in illicit heroin. It yields reproducible, qualitative, and semiquantitative data which are essential for the classification of unknown heroin samples. Authentic heroin from known source regions allowed for the determination of characteristic profiles to classify and sub-classify unknown heroin samples. To date, the procedure has proven to be robust and reproducible in the analysis of thousands of unknown heroin samples submitted to STRL. Additionally, method reproducibility and precision allows for the examination of heroin samples submitted for sample-to-sample comparative analysis.

Additional work is ongoing to further identify the origin of unknown acidic and neutral manufacturing impurities. This work may enhance our existing Heroin Signature Program by providing additional specific origin information. Also, the application of multivariate analysis program (i.e., neural network or cluster analysis software) could lead to quicker sample classification and further detection of sub-classifications.

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