

Identification of Levamisole and Lidocaine Acetylation Reaction Impurities Found in Heroin Exhibits

Ellen M. Casale, Ph.D.* and John F. Casale, B.S.

U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166

[email address withheld at corresponding author's request]

ABSTRACT: Five (5) compounds, S-[2-(3-acetyl-2-oxo-4-phenylimidazolidin-1-yl)ethyl] ethanethioate; 3-acetyl-4-phenyl-1-(2-sulfanylethyl)imidazolidin-2-one; N-acetyl-lidocaine; N-(2,6-dimethylphenyl)acetamide; and N-acetyl-N-(2,6-dimethylphenyl)acetamide were identified as impurities present in a heroin exhibit containing levamisole and lidocaine. Spectroscopic and chromatographic data are provided for characterization of these products. The presence of these impurities suggests that levamisole and lidocaine were added to morphine base prior to acetylation of morphine with acetic anhydride that produced heroin and the aforementioned compounds.

KEYWORDS: heroin, levamisole, lidocaine, acetylated products, mass spectrometry, forensic science

The Drug Enforcement Administration's Special Testing and Research Laboratory conducted an analysis of an exhibit as a part of its Heroin Signature Program (HSP), and determined that the exhibit contained 78.4% heroin, 2.3% levamisole, 1.4% lidocaine, and other typical heroin-related alkaloids. In addition to the heroin, levamisole, and lidocaine identified in the exhibit, four other unidentified compounds of significant concentration and one compound at a trace level were observed. Examination of the total ion chromatographic profile indicated the presence of fragment ions for two of those compounds that appear to be related to levamisole impurities found in some cocaine exhibits [1]. Furthermore, fragment ions for three other compounds present in the profile indicated the possible presence of acetylated lidocaine and two lidocaine-related compounds. Although levamisole, an antineoplastic, has been a known cocaine adulterant for more than five years [2], recently, it has become more prevalent in heroin exhibits. Some researchers have suggested that levamisole may enhance the effects of cocaine [3,4]; however, it is rarely found in heroin exhibits. Lidocaine, an anesthetic, has been a known adulterant of heroin for many years. Lidocaine and levamisole-related impurities have not been reported previously in heroin exhibits. A second heroin exhibit containing suspected levamisole-related impurities was also identified.

The results presented herein reveal the presence of levamisole and lidocaine acetylation by-products formed during illicit heroin processing. Lidocaine and levamisole were subjected to acetylation reactions, preparative isolation, gas chromatographic-mass spectrometric, and nuclear magnetic resonance analyses. These acetylation by-products were characterized as S-[2-(3-acetyl-2-oxo-4-phenylimidazolidin-1-yl)ethyl] ethanethioate; 3-acetyl-4-phenyl-1-(2-sulfanylethyl)imidazolidin-2-one; N-acetyl-lidocaine; N-(2,6-dimethylphenyl)acetamide; and N-acetyl-N-(2,6-dimethylphenyl)acetamide. Reaction mechanisms are proposed for the formation of these by-products.

Experimental

Solvents, Chemicals, and Materials

All solvents were distilled-in-glass products of Burdick and Jackson Laboratories (Muskegon, MI). All other chemicals were of reagent-grade quality and were products of Sigma-Aldrich Chemical (Milwaukee, WI). Alumina (basic) was deactivated slightly by adjusting the water content to 4% (w/w). Levamisole HCl, lidocaine HCl, heroin HCl, and morphine base were part of the authentic reference collection of the DEA Special Testing and Research Laboratory. A reference standard of 2,6-dimethylacetanilide (N-(2,6-dimethylphenyl)acetamide) was obtained from Sigma-Aldrich Chemical.

Gas Chromatography/Mass Spectrometry (GC/MS)

GC/MS analyses were performed using an Agilent (Palo Alto, CA) Model 5973 quadrupole mass-selective detector (MSD) interfaced with an Agilent Model 6890 gas chromatograph. The GC system was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with DB-1 (0.25 μ m) (J & W Scientific, Rancho Cordova, CA). The oven temperature was programmed as follows: initial temperature, 100°C; initial hold, 0.0 min; program rate, 6°C/min; final temperature, 300°C; final hold, 5.67 min. The injector was operated in the split mode (21.5:1) and at a temperature of 280°C. The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34-700 mass units, and a scan rate of 1.34 scans/s. The auxiliary transfer line to the MSD and the source were maintained at 280°C and 230°C, respectively.

Nuclear Magnetic Resonance Spectroscopy (NMR)

Proton (^1H) spectra were obtained on a Varian (Palo Alto, CA) Inova 600 MHz NMR using a 5 mm Varian Nalorac Z-Spec broadband, variable temperature, pulse field gradient (PFG) probe. The compounds were dissolved in deuteriochloroform (CDCl_3) containing 0.03% v/v tetramethylsilane (TMS) as the 0 ppm reference compound.

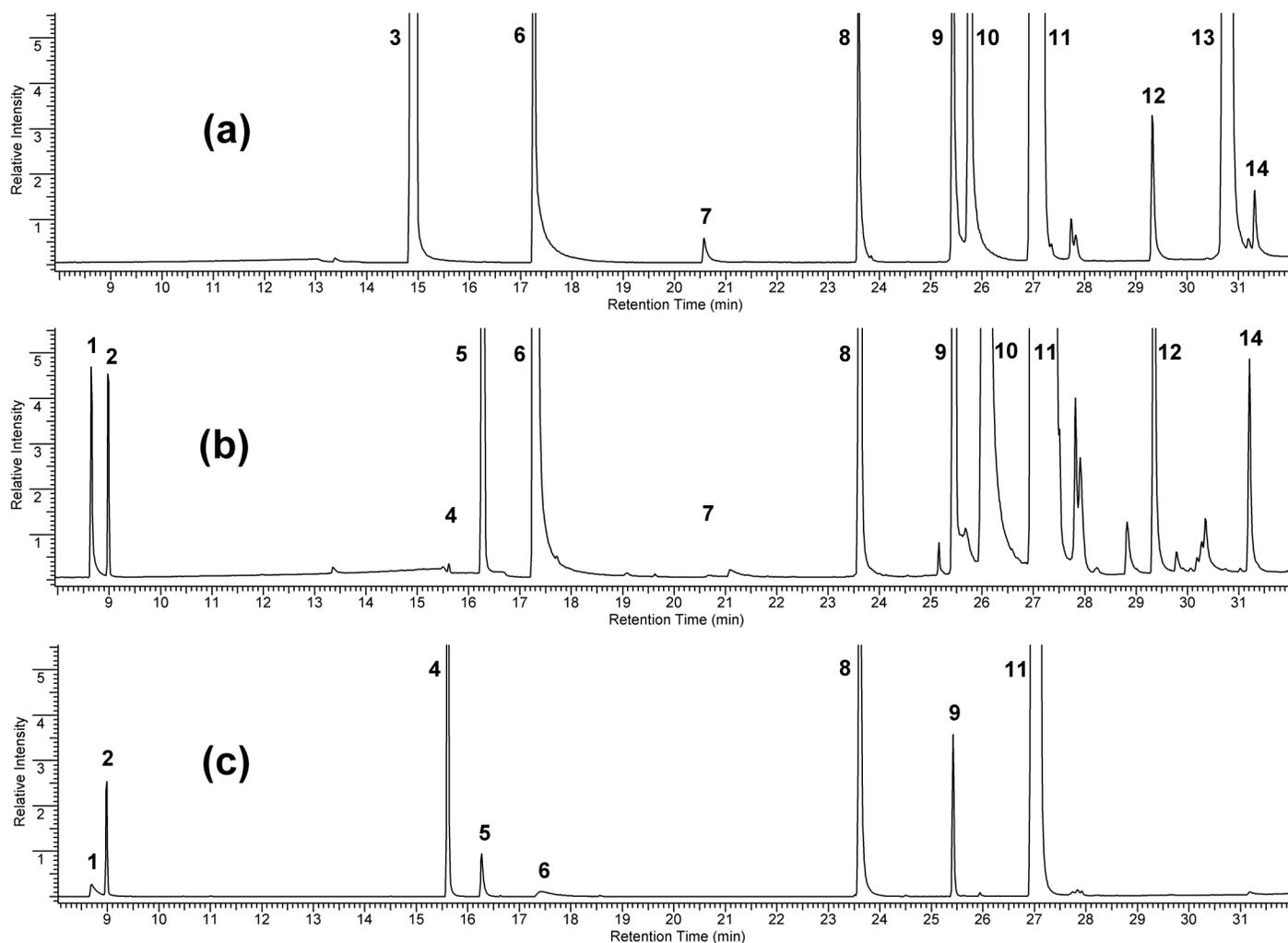


Figure 1 - Partial reconstructed total ion chromatograms of heroin exhibits. (a) Heroin exhibit containing levamisole and levamisole acetylation by-products, (b) Heroin exhibit containing levamisole and lidocaine and their acetylation by-products, (c) Heroin product produced from acetylation of a mixture of morphine, levamisole, and lidocaine. Peak identification can be found in Table 1.

The temperature of the samples was maintained at 25°C. Standard Varian pulse sequences were used to acquire the proton spectra. Processing of data was performed using software from Applied Chemistry Development (ACD/Labs, Toronto, Canada).

Synthesis

S-[2-(3-acetyl-2-oxo-4-phenylimidazolidin-1-yl)ethyl] ethanethioate: Levamisole HCl (440 mg, 1.83 mmol) was heated at 75°C with acetic anhydride (2.0 mL, 27.3 mmol) in a 15 mL capped centrifuge tube for 35 hrs. The reaction was cooled and quenched with 30 mL of water, solid Na₂CO₃ was added until pH = 9 was measured, and the reaction was extracted with CHCl₃ (2 x 75 mL). The CHCl₃ was washed with 0.36 N H₂SO₄ (to remove unreacted levamisole), dried over anhydrous sodium sulfate, and evaporated *in vacuo* to an orange-amber oil (120 mg crude material). The crude material was eluted with CHCl₃ on a glass chromatographic column (25 cm x 1.0 cm i.d.) containing 10 g of basic alumina (150 mesh). The first 20 mL of eluate was collected and evaporated to dryness to give the target compound as 32 mg of clear oil (yield not calculated).

3-acetyl-4-phenyl-1-(2-sulfanylethyl)imidazolidin-2-one: Levamisole HCl (3.0 g, 12.5 mmol) and acetic anhydride (15.0 mL, 204 mmol) were refluxed in a 100 mL round-bottom flask equipped with a reflux condenser for 35 hrs. The reaction was transferred to a 250 mL flask containing 150 mL of 0.36 N H₂SO₄ and stirred for 24 hrs at 75°C. The reaction was extracted with CHCl₃ (3 x 75 mL), extracts combined, dried over anhydrous sodium sulfate, and evaporated *in vacuo* to a crude mixture. The crude material was eluted on a glass chromatographic column (90 cm x 4.5 cm i.d.) containing 300 g of basic alumina (150 mesh). The column was eluted with CHCl₃ (500 mL), followed by a mixture of CHCl₃/acetone (1:1). The first 200 mL of the CHCl₃/acetone (1:1) eluate was collected and evaporated to dryness to give 1.14 grams of material containing the desired compound at *ca.* 20% purity. Approximately 280 mg of this material was chromatographed again on a glass chromatographic column (25 cm x 1.0 cm i.d.) containing 15 g of basic alumina (150 mesh). The column was eluted with the following solvent series: 30 mL Et₂O/CHCl₃ (1:1), 10 mL Et₂O/CHCl₃ (4:6), 10 mL Et₂O/CHCl₃ (3:7), 10 mL Et₂O/CHCl₃ (2:8), 10 mL Et₂O/CHCl₃ (1:9), 50 mL CHCl₃, 20 mL CHCl₃/acetone (19:1), 20 mL CHCl₃/acetone (18:2),

Table 1 - Retention times (R_t) and Relative Retention Times (RR_t) of levamisole, lidocaine, and related impurities resulting from acetylation^a.

Compound	R_t (min)	RR_t	GC/MS Peak #
163 compound ^b	8.66	0.50	1
205 compound ^c	8.99	0.52	2
caffeine	14.95	0.87	3
N-acetyl-lidocaine	15.61	0.90	4
lidocaine	16.30	0.94	5
levamisole	17.25	1.00	6
264 compound ^d	20.56	1.91	7
306 compound ^e	23.64	1.37	8
acetylcodeine	25.47	1.48	9
O ⁶ -monoaetylmorphine	26.16	1.52	10
heroin	27.20	1.58	11
papaverine	29.35	1.70	12
diltiazem	30.69	1.78	13
Triacetylnormorphine	31.20	1.81	14

^aConditions given in experimental section.

^bN-(2,6-dimethylphenyl)acetamide

^cN-acetyl-N-(2,6-dimethylphenyl)acetamide

^d3-acetyl-4-phenyl-1-(2-sulfanylethyl)imidazolidin-2-one

^eS-[2-(3-acetyl-2-oxo-4-phenylimidazolidin-1-yl)ethyl] ethanethioate

20 mL CHCl_3 /acetone (17:3), and 20 mL CHCl_3 /acetone (16:4). Fractions of 10-mL were collected and monitored via gas chromatography. Fractions 8-11 were combined and evaporated to dryness to give the target compound as 8 mg of clear oil (yield not calculated).

N-acetyl-N-(2,6-dimethylphenyl)acetamide: 2,6-dimethylacetanilide (100 mg, 0.61 mmol) was heated at 120°C with acetic anhydride (2.0 mL, 27.3 mmol) in a 15 mL capped centrifuge tube for 22 hrs. The reaction was cooled and quenched with 10 mL of water, solid Na_2CO_3 was added until pH = 9 was measured, and the reaction was extracted with CHCl_3 (1 x 10 mL). The CHCl_3 was dried over anhydrous sodium sulfate, and evaporated *in vacuo* to 110 mg of a clear oil (yield not calculated).

N-acetyl-lidocaine: Repeated attempts to acetylate lidocaine yielded mixtures of acetyl-lidocaine, 2,6-dimethylacetanilide, and N-acetyl-N-(2,6-dimethylphenyl)acetamide which could not be separated. A typical reaction was as follows: lidocaine (130 mg, 0.48 mmol) was heated at 75°C with acetic anhydride (1.0 mL, 13.6 mmol) in a 15 mL capped centrifuge tube for 8 days. The reaction was cooled and quenched with 8 mL of 0.36 N H_2SO_4 and extracted with CHCl_3 (1 x 6 mL). The CHCl_3 was dried over anhydrous sodium sulfate, and evaporated *in vacuo* to yield a mixture of acetyl-lidocaine, 2,6-dimethylacetanilide, and N-acetyl-N-(2,6-dimethylphenyl)acetamide. Although N-acetyl-lidocaine (33% purity via GC-FID) could not be isolated from the by-products, its mass spectrum was obtained (yield not calculated).

GC-MSD Analytical Artifact Experiments

A solution of levamisole HCl (0.13 mg/mL), lidocaine HCl (0.13 mg/mL), and heroin HCl (1.07 mg/mL) was made in CHCl_3 . An identical solution was prepared in MeOH. Each solution was injected under the conditions detailed previously. The resulting chromatographic profiles were examined for the formation of analytical artifacts related to acetylated levamisole and lidocaine.

Acetylation of a Morphine, Lidocaine, and Levamisole Mixture

A mixture of levamisole HCl (102 mg, 0.42 mmol), lidocaine HCl (102 mg, 0.35 mmol), and illicit morphine base (729 mg, 2.56 mmol) was heated at 120°C with acetic anhydride (3.0 mL, 40.9 mmol) in a 15-mL capped centrifuge tube for 2 hrs. The reaction was cooled and quenched with 40 mL of water, solid Na_2CO_3 was added until pH = 9 was measured, and the precipitated product was captured via suction filtration. The precipitate was washed further with 40 mL of water and then allowed to dry overnight at room temperature. The precipitated product and filtrate (after CHCl_3 extraction) were each examined via GC-MSD for acetylated products of levamisole and lidocaine.

Results and Discussion

Initial GC-MSD analysis of two heroin signature exhibits demonstrated the presence of peaks in their reconstructed total ion chromatograms (Figure 1a and 1b, Table 1) that could not be identified without further analysis. Two unknown

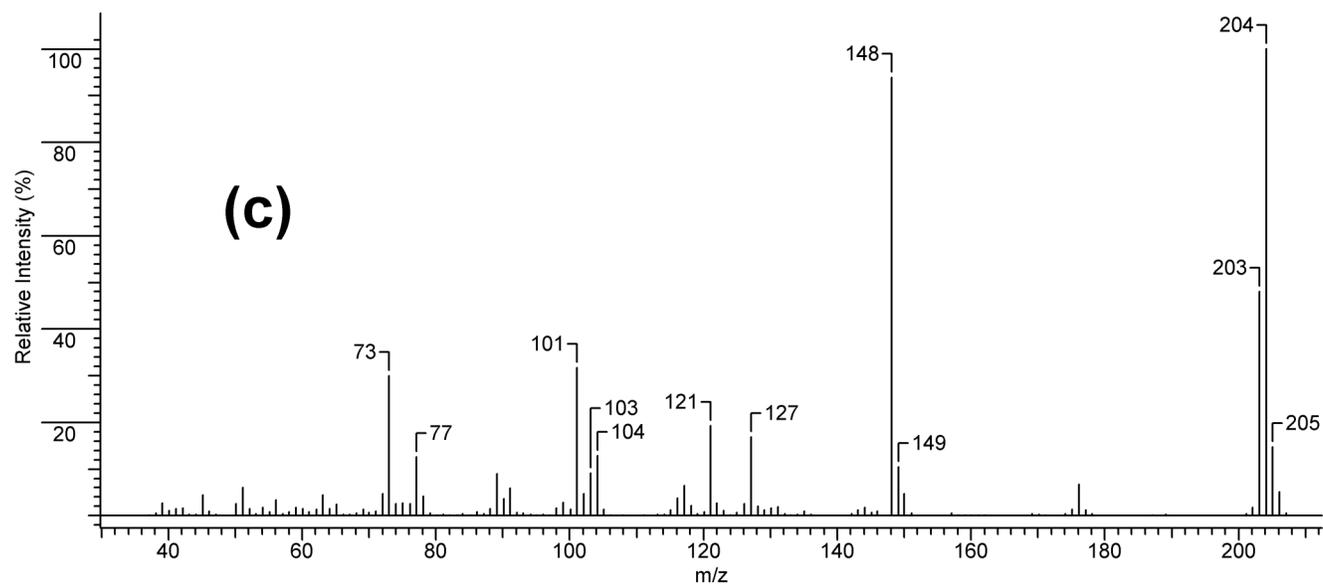
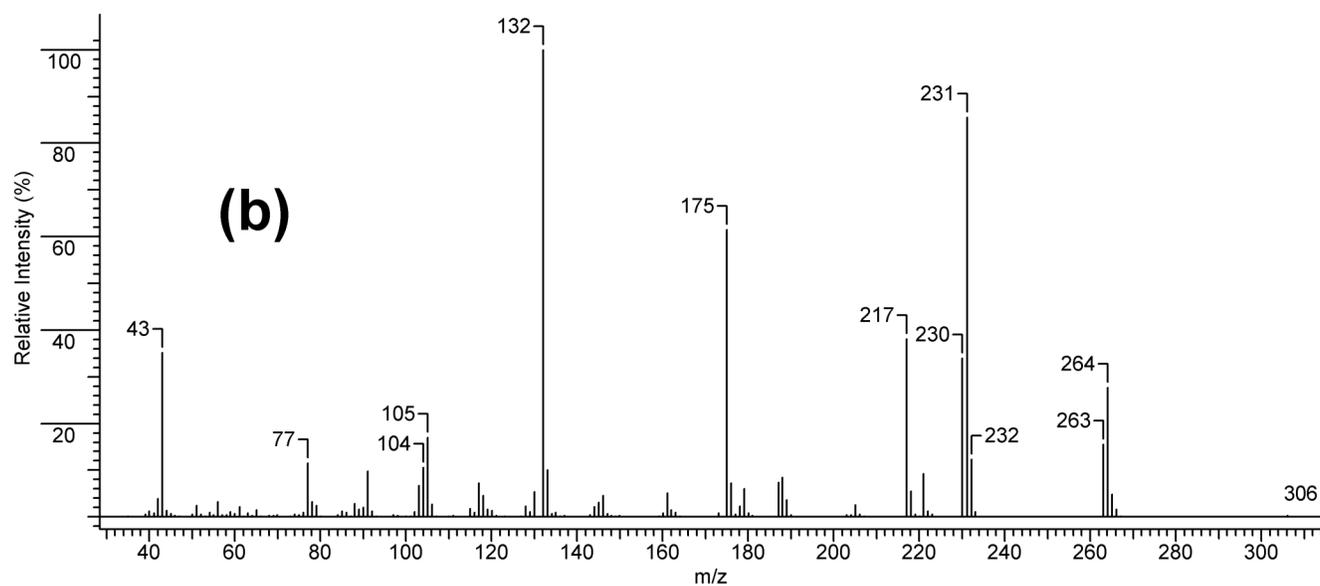
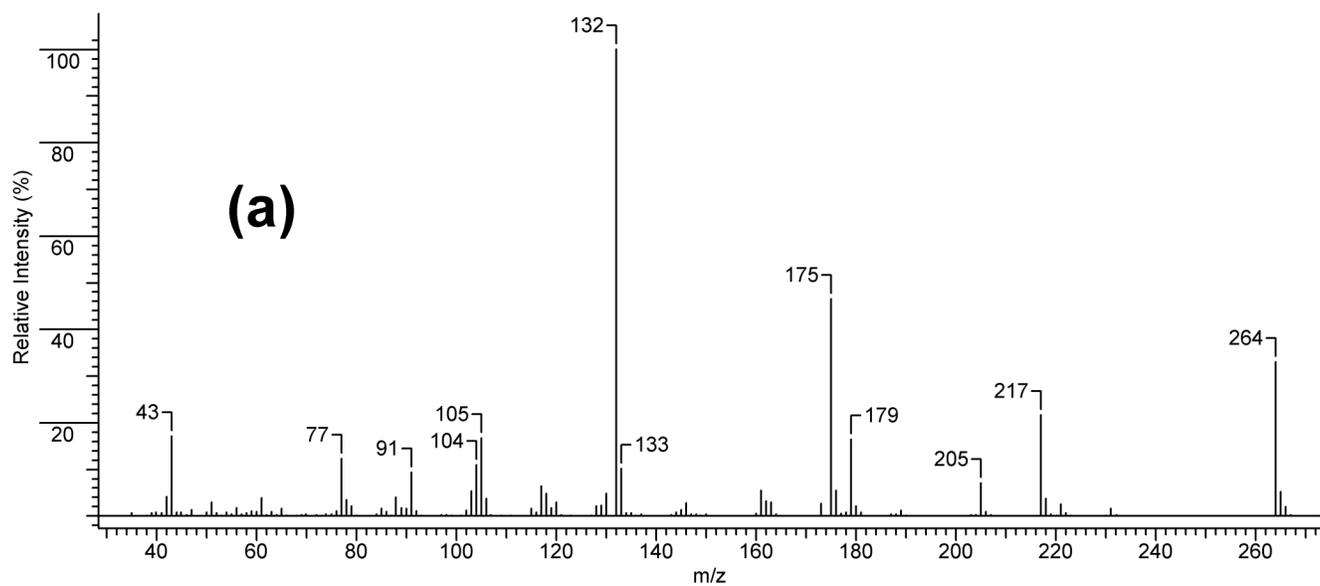


Figure 2 - Electron ionization mass spectrum of (a) 3-acetyl-4-phenyl-1-(2-sulfanylethyl)imidazolidin-2-one (264 compound), (b) S-[2-(3-acetyl-2-oxo-4-phenylimidazolidin-1-yl)ethyl] ethanethioate (306 compound), (c) levamisole.

Table 2 - NMR Data for levamisole and its acetylated products^a.

position	(6R)-6-phenyl-2,3,5,6-tetrahydroimidazo[2,1-b][1,3]thiazole (levamisole)			(4R)-3-acetyl-4-phenyl-1-(2-sulfanylethyl)-imidazolidin-2-one			S-{2-[(4R)-3-acetyl-2-oxo-4-phenylimidazolidin-1-yl]ethyl}ethanethioate		
		proton	carbon		proton	carbon		proton	carbon
Phenyl ring									
quaternary		-	142.8		-	141.1		-	141.1
ortho		7.34 m	126.5		7.29 d	125.5		7.26 d	125.5
meta		7.34 m	128.5		7.35 t	129		7.35 t	128.9
para		7.26 m	127.7		7.3 t	128.2		7.29 t	128.1
Imidazolidine ring									
N1	Bonded to C2, C5, and C6								
C2	N1-C(-S)=N3	-	174.3	C=O	-	155.13	C=O	-	155
N3	Bonded to C2 and C4			N1-C(=O)-CH3	-	170	N1-C(=O)-CH3	-	170
				N1-C(=O)- CH3	2.52 s	23.9	N1-C(=O)- CH3	2.51 s	23.9
C4 -phenyl	CH	5.46 t	76.9	CH	5.33 dd	54.3	CH	5.31 dd	54.3
C5	CH2	3.68 t, 2.99 t	58.4	CH2	3.34 dd, 3.90 t	50.5	CH2	3.33 dd, 3.89 t	50.4
N1-CH2-CH2-S									
C6	N1- CH2 -CH2-S	3.13 ddd, 3.37 ddd	49.1	N1- CH2 -CH2-SH	3.44 ddd, 3.61 ddd	46.7	N1- CH2 -CH2-S	3.41 ddd, 3.57 ddd	43.1
C7	N1-CH2- CH2 -S	3.53 ddd, 3.65 ddd	34.1	N1-CH2- CH2 -SH	2.73 m	22.4	N1-CH2- CH2 -S	3.07 m, 3.09 m	26.7
S	bonded to C2 and C7			SH bonded to C7 only			S-C(=O)-CH3		
							S-C(=O)- CH3		2.31 s 30.6

Proton abbreviations: d = doublet, m = multiplet, s = singlet, t = triplet

^aIUPAC Names using Advanced Chemistry Development, Inc., ACD/Name, version 12.00, Toronto, Canada.

compounds (Peaks #7 and #8) were observed having apparent molecular ions at m/z 264 and m/z 306, respectively, as illustrated in Figure 2a and 2b. These compounds appeared to be related to levamisole based on the presence of ions found at m/z 132 and m/z 175. Although these two fragment ions do not directly indicate levamisole, other reported levamisole degradation products contain these ions [1]. Further examination of the spectra for peaks #7 and #8 (Figure 2a and 2b), showed the presence of an ion at m/z 43 that is indicative of an acetyl loss. The mass spectral data for these two compounds suggested that each compound was an acetylated levamisole by-product. Levamisole was acetylated as outlined in the experimental section and produced two compounds, each with identical mass spectra to peaks #7 and #8. Both synthetic compounds were isolated and their structures were elucidated via NMR (Table 2) and MS analysis. The levamisole acetylation by-products, S-[2-(3-acetyl-2-oxo-4-phenylimidazolidin-1-yl)ethyl] ethanethioate and 3-acetyl-4-phenyl-1-(2-sulfanylethyl)imidazolidin-2-one (Figure 3) were verified for peaks #7 and #8.

Additionally, two other compounds (Figure 1b, Peaks #1 and #2) with apparent molecular ions at m/z 163 and m/z 205, respectively, were found. Peak #1 was identified by its mass spectrum (Figure 4a) as N-(2,6-dimethylphenyl)acetamide after comparison to a known standard. Both peaks #1 and #2 appeared to be lidocaine related, based on the 2,6-dimethyl aromatic substitution (also found in lidocaine, Figure 5). Peak #2 produced a mass spectrum (Figure 4b) of 42 Daltons greater

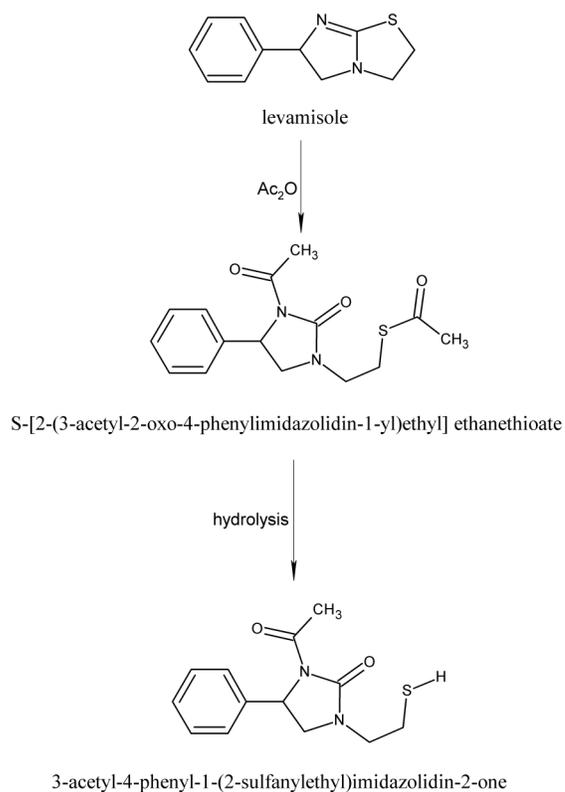


Figure 3 - Structural formulae of levamisole and related acetylation by-products.

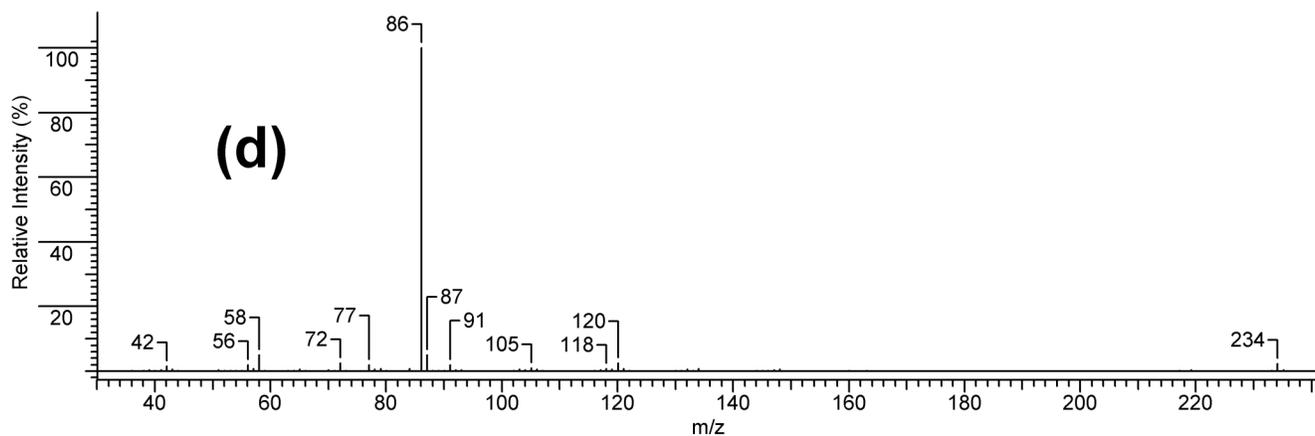
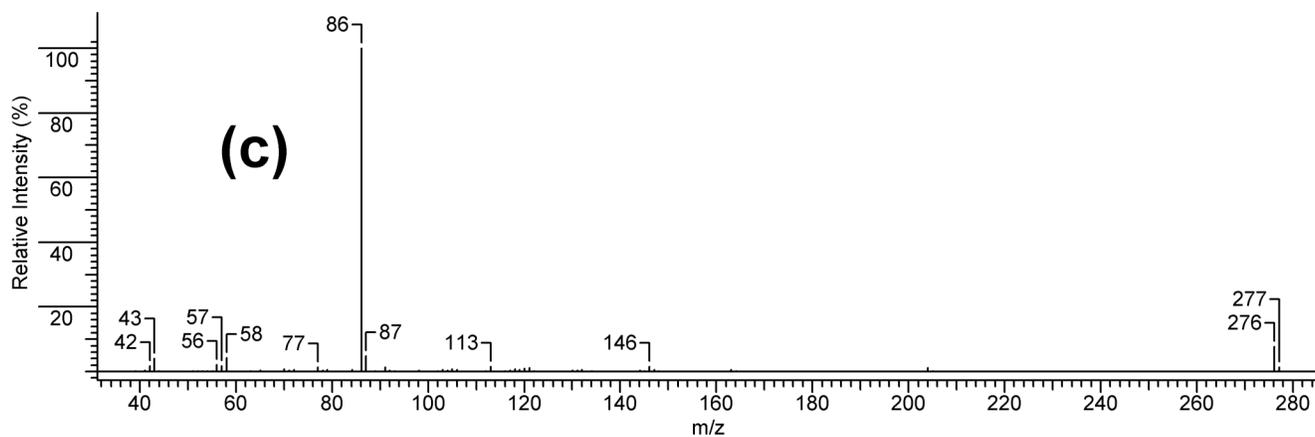
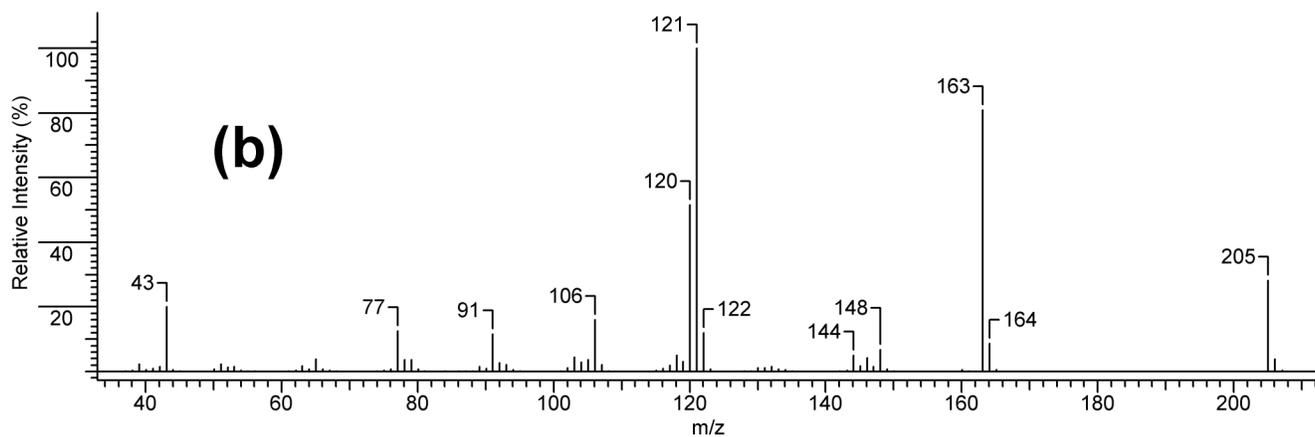
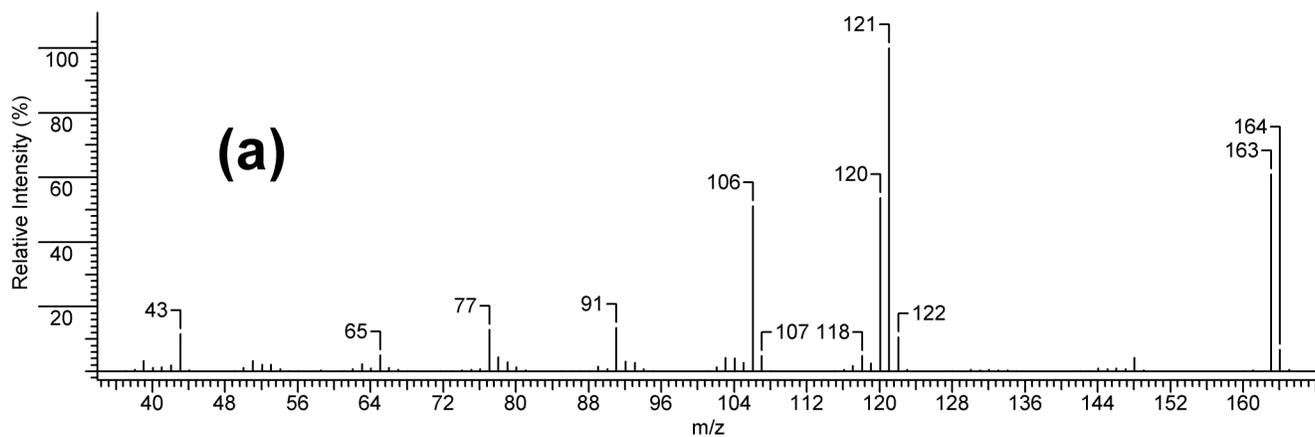


Figure 4 - Electron ionization mass spectrum of (a) N-(2,6-dimethylphenyl)acetamide (163 compound), (b) N-acetyl-N-(2,6-dimethylphenyl)acetamide (205 compound), (c) N-acetyl-lidocaine (276 compound), (d) lidocaine.

Table 3 - NMR data for lidocaine and its acetylated degradation products^a.

position	N-acetyl-N-(2,6-dimethylphenyl)acetamide			N-(2,6-dimethylphenyl)acetamide (major rotamer)			N-(2,6-dimethylphenyl)acetamide (minor rotamer)		
		proton	carbon		proton	carbon		proton	carbon
benzene ring									
1	C	-	137.6	C	-	136.7	C	-	135
2, 6	C	-	135.6 (2)	C	-	135.5 (2)	C	-	133.9 (2)
2, 6	C- <u>CH3</u>	2.14 s (6)	17.8 (2)	C- <u>CH3</u>	2.26 s (6)	18.4 (2)	C- <u>CH3</u>	2.21 s (6)	18.4 (2)
3, 5	CH	7.15 d (2)	129.0 (s)	CH	7.05 d (2)	128.1 (2)	CH	7.12 d (2)	128.6 (2)
4	CH	7.22 dd (1)	129.0	CH	7.08 dd (1)	127.4	CH	7.16 dd (1)	128.2
N attached to C1									
	N- <u>C</u> (=O)-CH3	-	172.6 (2)	N- <u>C</u> (=O)-CH3	-	168.6 (1)	N- <u>C</u> (=O)-CH3	-	173.1 (1)
	N-C(=O)- <u>CH3</u>	2.25 s (6)	26.1 (2)	N-C(=O)- <u>CH3</u>	2.17 s (3)	23.1 (1)	N-C(=O)- <u>CH3</u>	1.74 s (3)	19.8 (1)

Proton abbreviations: d = doublet, m = multiplet, s = singlet, t = triplet

^aIUPAC Names using Advanced Chemistry Development, Inc., ACD/Name, version 12.00, Toronto, Canada.

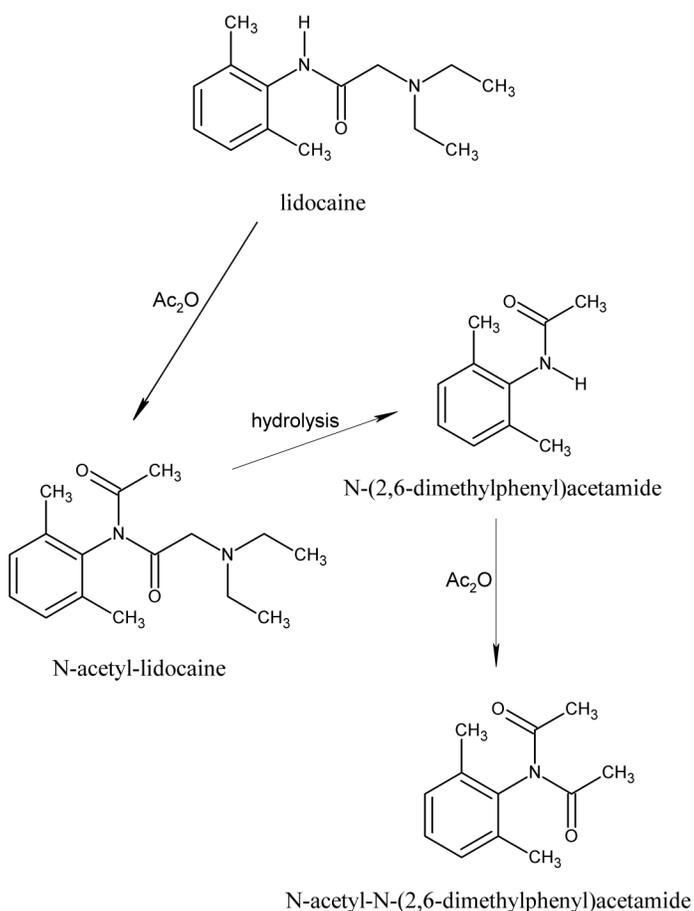


Figure 5 - Formulae of lidocaine and related acetylation by-products.

than N-(2,6-dimethylphenyl)acetamide (Figure 4a), suggesting it was the acetyl derivative of peak #1. N-(2,6-dimethylphenyl)acetamide was acetylated as outlined in the experimental section to produce N-acetyl-N-(2,6-dimethylphenyl)acetamide (Figure 5). The resulting mass spectrum was identical to peak #2; its NMR spectrum was consistent with the expected resonances (Table 3). Finally, lidocaine was acetylated with

acetic anhydride to form N-acetyl-lidocaine. The resulting mass spectrum (Figure 4c) was identical to peak #4 (Figure 1b). Additionally, acetylation of lidocaine produced N-(2,6-dimethylphenyl)acetamide and N-acetyl-N-(2,6-dimethylphenyl)acetamide.

In order to demonstrate that the target compounds are not formed as analytical artifacts, solutions containing heroin, lidocaine, and levamisole were examined via GC/MS using chloroform and methanol as separate injection solvents. Examination of the resulting chromatographic profiles did not indicate the presence or formation of any acetylated lidocaine or levamisole by-products.

Finally, a mixture of illicit morphine, levamisole, and lidocaine were reacted with acetic anhydride to produce heroin. The resulting heroin sample was examined via GC/MS (Figure 1c). One acetylated levamisole by-product, S-[2-(3-acetyl-2-oxo-4-phenylimidazolidin-1-yl)ethyl] ethanethioate (306 compound), as well as all three acetylated lidocaine products were produced and detected in the resulting heroin. Additionally, these four compounds were also detected in higher concentrations in the precipitation filtrate. The presence of 3-acetyl-4-phenyl-1-(2-sulfanylethyl)imidazolidin-2-one (264 compound) was not detected since it is a degradation product of S-[2-(3-acetyl-2-oxo-4-phenylimidazolidin-1-yl)ethyl] ethanethioate (306 compound).

Conclusions

Characterization of the five impurities present in the heroin exhibits, in concert with the performed acetylation experiments, demonstrate that levamisole and lidocaine were added to the illicit morphine prior to the acetylation reaction that produced the heroin. It is unlikely that lidocaine and levamisole were purposely added to the morphine to enhance the effect of the final heroin product since cutting agents or adulterants are typically added to the final heroin product prior to trafficking or distribution.

Acknowledgement

The authors are indebted to Patrick A Hays of this laboratory for his assistance in acquiring the NMR data.

References

1. Casale JF, Corbeil EM, Hays PA. Identification of Levamisole Impurities Found in Illicit Cocaine Exhibits. *Microgram J* 2008;6(3-4):82-9.
2. Valentino AMM, Fuentecilla K. Levamisole. An analytical profile. *Microgram J* 2005;3(3-4):134-7.
3. Zhu NY, LeGatt DF, Turner AR. Agranulocytosis after consumption of cocaine adulterated with levamisole. *Ann Internal Med* 2009;150:287-9.
4. Raymon LP, Isenschmid DS. The Possible Role of Levamisole in Illicit Cocaine Preparations. *J Anal Toxicol* 2009;33:620-2.