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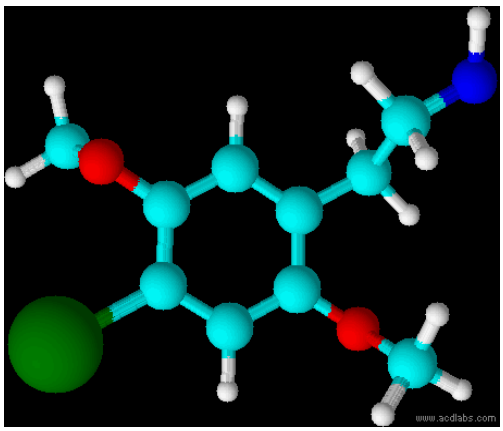

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Cover Art: "Ball and Stick" Model of 2,5-Dimethoxy-4-iodophenethylamine (2C-I; Courtesy of Patrick A. Hays, DEA Special Testing and Research Laboratory, Dulles, VA).

A Specific Screening Color Test for Diazepam

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ABSTRACT: A new, highly specific color test for the screening/presumptive identification of diazepam is reported. The test is a variant of the McKibben test for flunitrazepam. Treatment of diazepam with alkaline dimethylsulfoxide produces a reddish color which gradually changes to yellow with passage of time. The color instantly vanishes upon addition of water or attempted extraction with organic solvents, suggesting that the color is due to a transient charge-transfer complex. Somewhat unexpectedly, the test does not produce color with powder scraped from diazepam-containing tablets - in such cases, a chloroform extraction is required. The test is negative for other controlled substances, including other benzodiazepines, and also for various diluents and binders typically present in tablets (62 compounds were tested). The LODs were 20 µg for diazepam extracted from tablets, and 2 µg for diazepam standard. The test is particularly useful for the rapid screening of illicit Lemmon 714 (Quaalude) mimic tablets, which contain diazepam as a substitute for methaqualone.

KEYWORDS: Diazepam, Dimethylsulfoxide, Screening, Color Test, Forensic Chemistry

Introduction

Diazepam (Figure 1), most commonly known by its trade name Valium, is a benzodiazepine and a controlled substance (Schedule IV in the United States [1]). It is a potent sedative - hypnotic (CNS depressant), and is one

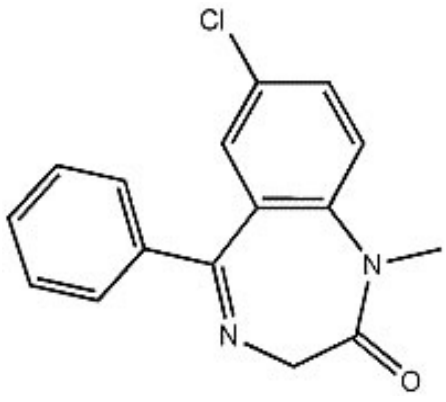

Figure 1. Diazepam (7-Chloro-1-methyl-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one); $C_{16}H_{13}ClN_2O$; m.w. (Base) = 284.7; mp = 131.5-134.5^{O}C

of the most prescribed drugs in the world. It is also one of the top five most abused benzodiazepines, and misuse can lead to both psychological dependence and/or physical addiction [2].

In addition to Valium and numerous other licit (prescription) formulations, diazepam is found as an adulterant in heroin and as a substitute in various mimic drugs (most notably as a substitute for methaqualone in Lemmon 714 (Quaalude) mimic tablets [3]). Because its synthesis is challenging, its presence in illicit drug markets is almost universally due to diversion of pharmaceutical stocks.

There are numerous analytical methods for the identification of diazepam in forensic and toxicological laboratories [4,5], including a number of color tests; in general, however, the latter are not sensitive or specific (*vide infra*). Herein, a new presumptive color test for the preliminary screening of diazepam is reported. The test, which is a variant of the McKibben test for flunitrazepam [6], is simple, easy to perform, highly sensitive, and highly specific.

Experimental

Materials: Dimethylsulfoxide (hereafter DMSO) and sodium hydroxide were both acquired from Merck (Whitehouse Station, NJ). The diazepam standard was acquired from Roche Pakistan Ltd. (Lahore). Tablets containing other benzodiazepines and other tested compounds, and standard (pure) materials, were purchased from various pharmaceutical companies (see Table 1). All other chemicals used were of analytical grade or better.

Methods: Test Reagent A was prepared by adding 50 parts of DMSO and one part of 3M sodium hydroxide. [Note: Use of sodium hydroxide solutions below 2M or over 3M was not as effective.] Test Reagent B was prepared by adding solid sodium hydroxide to DMSO and vortexing it for approximately 2 minutes. In the latter case, the supernatant was transferred to another tube and used as the test reagent. In this study, both test reagents were freshly prepared just before use; however, subsequent studies indicated that the reagents were stable for at least 4 days. Both test reagents are colorless (see Photo 1).

Photo 1 - Reagent

Detection of Diazepam: A Valium tablet containing 2 mg of diazepam was ground to a fine powder. A small portion of powder (approximately 0.5 mg) was added to 1 mL of chloroform, and the resulting mixture was vortexed for approximately one minute and then centrifuged at 13,200 rpm for 3 minutes. The supernatant chloroform was isolated and evaporated to dryness, and 3 - 4 drops of the test reagent was added to the remaining residue. An immediate reddish color developed (see Photo 2). If Test Reagent A was used, the color faded to yellow after approximately 1 minute; if Test Reagent B was used, the color persisted for at least 20 minutes before gradually fading to yellow. Repeating the sequence with the diazepam standard gave the same results. Addition of 2 - 3 drops of water to either the reddish or yellow test solutions caused the color to instantly vanish. None of the other compounds tested (Table 2) gave similar results.

Limit of Detection (LOD) from Tablets: The above analysis was repeated on a second Valium tablet, except that five sequential extractions were performed on the same powder, and each extract isolated in separate test tubes. Upon testing, only the first three extracts displayed color; it was therefore assumed that the 2.0 mg of diazepam in the original tablet was quantitatively extracted by three serial extractions. Another tablet was extracted in the

same manner, and the first three extracts were combined to form the "stock solution." Aliquots containing 70, 60, 50, 40, 30, 20, 10, and 5 μg of diazepam were each evaporated to dryness, and tested. The results indicate a detection limit of 20 μg.

LOD from Diazepam Standard: Stock solutions containing 50, 30, 20, 10, 5, 3, 2, and 1 μg of reference grade diazepam were prepared in acetone. The solutions were evaporated to dryness, and the remaining residue was tested as detailed above. The reddish color was noted down to 2 μg - one tenth the LOD for diazepam extracted from tablets. The order of magnitude difference is thought to be due to the sensitivity of the test to co-extracted impurities from the tablets (i.e., that are not present in the standard).

Analysis of Other Substrates: All other compounds were either analyzed as standards (pure), or were extracted from tablets using water or an organic solvent (see Table 1).

Results and Discussion

Previous Studies

As noted in the Introduction, a number of color tests have been reported for the presumptive identification of diazepam; however, most are neither specific or sensitive, and others were not tested against other benzodiazepines or other controlled substances. Formaldehyde + H_2SO_4 [7], Zimmerman's reagent (*meta*-dinitrobenzene + benzyltrimethylammonium hydroxide [8]), and the Janovsky reagent (*meta*-dinitrobenzene + KOH [9]) have been used as general screens for benzodiazepines. Of the three reagents, Zimmerman's reagent is the most specific, producing violet/purple colors with keto-benzodiazepine derivatives such as diazepam, fludiazepam, and flurazepam. The Wagner's test (acidic KI_3) gives a brown solution with a brown-black precipitate; however, similar results are obtained for many alkaloids, including cocaine hydrochloride [10,11]. In a commercial test kit (ingredients proprietary), a presumptive test was designed to identify the presence of diazepam, flunitrazepam, or ketamine [12]. After breaking and agitation of the two ampoules in the kit, a pale lavender color will develop for either diazepam and flunitrazepam, and a darker color for ketamine; it is unknown how this kit performs with other benzodiazepines or other controlled substances. Diazepam gives an intense red color product with the addition of picric acid (2,4,6-trinitrophenol), 3,5-dinitrobenzoic acid, or 3,4-dinitrobenzoic acid [13]; however, no other benzodiazepines or drugs were studied. Bromocresol green has been used to produce an orange colored ion-association complex with diazepam [14]; again, no other benzodiazepines or drugs were studied. Treatment of diazepam, bromazepam, and clonazepam with methanolic potassium hydroxide produces a yellow color, which could be analyzed by spectrophotometry [15]; however, a similar coloration is obtained for almost any benzodiazepine.

Alkaline Dimethylsulfoxide

McKibben was the first to report the use of alkaline DMSO for color testing, for screening of flunitrazepam [6]; interestingly, alkaline dimethylformamide (DMF) also worked. There were three variants of the test; in the first, the sample and the reagent were placed in a flint glass/soda lime test tube and heated at 100^{O}C, giving a deep purple color within four minutes. In the second, the sample, reagent, and either barium oxide, barium hydroxide, or finely ground flint glass/soda lime glass were placed in a regular test tube and heated at 100^{O}C, again giving a deep purple color within four minutes. In the third, the sample was dissolved in either DMSO or DMF and a small amount of solid sodium hydroxide added, resulting in immediate formation of a red-purple color (different than that observed in the first two variants). In all three cases, (cautious) addition of concentrated hydrochloric acid resulted in an immediate canary-yellow color.

McKibben tested over 100 different drugs, including diazepam, and determined that the tests were highly specific for flunitrazepam. Diazepam gave no color in either of the heated variants, but gave a dark orange color in the

third (unheated) variant. In all, 30 of the compounds tested by McKibben using the third (unheated) variant gave colors, including greens, yellows, and oranges.

In the current study, markedly different results were obtained, with only diazepam (reddish, see Photo 2), flunitrazepam (purple, see Photo 3), flurazepam (yellow), nitrazepam (yellow), and temazepam (green) giving colors (Table 2); however, fewer compounds (62) were tested, and the tested substrates included many non-drug compounds. Nonetheless, none of the other compounds that were tested displayed a reddish color, and the purple color displayed by flunitrazepam was similar but distinct from the reddish color produced by diazepam. Somewhat surprisingly, and in direct contrast to the McKibben reagents, the test failed to produce any color when DMF was substituted for DMSO. [Note: The colors observed with flurazepam, nitrazepam, and temazepam were not further investigated in this study; however, based on the compounds tested, the green color observed for temazepam also appears to be unique, and may constitute another definitive test. McKibben also observed a light green color for temazepam (Test Variant 3) - but noted similar colors for alprazolam, estazolam, lorazepam, and scopolamine.]

In order to further differentiate diazepam and flunitrazepam, their respective positive test solutions were each further treated (cautiously!) with 2 drops of concentrated hydrochloric acid. The reddish solution from diazepam instantly produced a faint yellow solution (see Photo 4), while the purple solution from flunitrazepam instantly produced a distinct orange solution (see Photo 5). Thus, although we feel that the initial colors allow for differentiation of diazepam from flunitrazepam, if desired this second step would rigorously confirm the identification.

The only difference between the McKibben Test/Variant 3 and the Test Reagent B used in this study is the point at which the solid sodium hydroxide is added. In the McKibben test, the sample is first dissolved in the DMSO, and then the base is added. In this study, the base is first dissolved in the DMSO, and then the sample is added. It is unclear how such a simple variation can have such a profound impact; however, the (fortuitous) difference allows for a specific test for diazepam.

Attempted Identification of the Colored Specie: In order to attempt identification of the colored specie, the reddish test solutions resulting from diazepam were extracted with a variety of organic solvents (acetic anhydride, acetone, acetone and chloroform, chloroform, ethyl acetate, formaldehyde, and petroleum ether). The color not only failed to extract into any of the organic solvents, in every case it vanished altogether. In addition, and as was noted in the Experimental section, addition of water to the reddish colored solutions also caused the color to instantly vanish (and the rapid fading of the reddish color when Test Reagent A was used is very likely due to the small percentage of water in that reagent). The results suggest that the reddish color results from formation of a transient charge-transfer complex that is sensitive to virtually any change in polarity or concentration. This sensitivity may explain why the test is so specific to diazepam, why the test fails with powder from tablets, why the LOD is so much higher for diazepam extracted from tablets, and finally why it fails if DMF is substituted for DMSO.

Screening of Lemmon 714 (Quaalude) Tablets: Actual Lemmon 714 tablets contain only methaqualone, whereas nearly all Lemmon 714 mimic tablets seen over the past 25 years have contained diazepam, sometimes adulterated with diphenhydramine [3]. Diazepam is also (uncommonly) substituted for methaqualone in Mandrax mimic tablets (widely abused in South Africa [16]). In a 1982 patent, Fischer and Morris reported a screening test to differentiate tablets containing methaqualone or mecloqualone from mimic tablets containing diazepam or diphenhydramine. Addition of about 7 drops of 85% formic acid to a sample containing methaqualone or mecloqualone, followed by 5 drops of 5% sodium nitrite, then 10 drops of chloroform, results in a yellow color that extracts into the chloroform layer; if diazepam is present, however, it will give a yellow color that is *not* extracted into the chloroform layer [17]. In the present test, methaqualone did not display any color. Thus, the two tests perfectly complement each other for rapid, facile screening of Lemmon 714 (Quaalude) or Mandrax tablets.

Photo 2 - Reddish Color from Diazepam

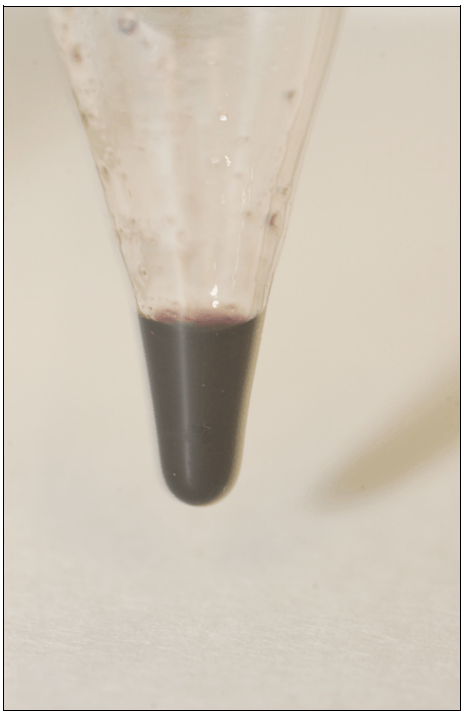

Photo 3 - Purple Color from Flunitrazepam

Photo 4 - Faint Yellow Color from Addition of Conc. HCl to the Reddish Solution (i.e., from Diazepam)

Photo 5 - Distinct Orange Color from Addition of Conc. HCl to the Purple Solution (i.e., from Flunitrazepam)

Acknowledgments

We are highly grateful to the Higher Education Commission of Pakistan for providing finances for the survey of illegal drugs in Pakistan. We also appreciate the help provided by the Abdul Rehman and Nasir Siddique, M.Phil. Scholars, Centre for Excellence in Molecular Biology Lahore, Pakistan.

References

1. Code of Federal Regulations, Title 21; 1308.14 (c).

2. Diazepam (See: http://en.wikipedia.org/wiki/diazepam (most recently accessed 1/15/09)).

3. Anonymous. Quaalude LEMMON 714 mimic tablets (containing diazepam). Microgram Bulletin 2007;40(1):5-6.

4. Moffat AC, Osselton MD, Widdop B, Galichet LY, eds. Clarke's Analysis of Drugs and Poisons. 3rd ed. London: Pharmaceutical Press, 2004; 897-898.

5. Mills III T, Roberson JC. Instrumental data for drug analysis. 3rd ed., Vol. 2. New York, NY: Taylor and Francis, 2006; 884-885.

6. McKibben T. Simple and rapid color screening tests for flunitrazepam (Rohypnol). Journal of Forensic Sciences 1999;44(2):396-400.

7. See Reference 4, p. 286.

8. Organic Name Reaction #206. The Merck Index. 14th ed. Whitehouse Station, NJ: Merck & Co., Inc., 2006; ONR-49.

9. Satoshi K, Katoshi F, Toshiko I. Color test of benzodiazepine type psychotropic drugs using Zimmermann's reagent. Reports of the Central Customs Laboratory 2000;40:67-71.

10. Monograph #4292. The Merck Index. 5th ed. (reprinted version). Rahway, NJ: Merck & Co., Inc., Originally Published 1940; 956.

11. Kidwell DA, Athanaselis SA. Cocaine: Methods of forensic analysis. In: Handbook of Forensic Drug Analysis. New York, NY: Elsevier Academic Press, 2005; 243.

12. ODV Narco Pouch #925 (See: http://www.odvinc.com/Web%20Tubes_Pouches_Art/test_prod_desc.html (most recently accessed 1/15/09)).

13. El-Hawary WF, Issa YM, Talat A. Spectrophotometric determination of diazepam in pure form, tablets, and ampoules. International Journal of Biomedical Science 2007;3(1):50-55.

14. Sadeghi S, Takjoo R, Haghgoo S. Qualitative determination of diazepam in pharmaceutical preparation by using a new extractive - spectrophotometric method. Analytical Letters 2002;35(13):2119-2131.

15. Salem AA, Barsoum BN, Izake EL. Spectrophotometric and fluorimetric determination of diazepam, bromazepam, and clonazepam in pharmaceuticals and urine samples. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 2004,60(4):771-780.

16. Methaqualone (See: http://en.wikipedia.org/wiki/methaqualone (most recently accessed 1/15/09)).

17. Fischer JF, Morris WA. Field test for methaqualone and mecloqualone. U.S. Patent 4313736 (1982).

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Table 1. Tested Compounds, Sources, Extraction Solvents, and Manufacturers.

	Compound	Source	Extraction Solvent	Manufacturer
1	Acetaminophen	Cold and Sinus Tablet	Acetone	Equal USA
2	*alpha*-Lipoic Acid	-	A/G	Standard
3	Alprazolam	Xanax	Ethanol	Pharmacia Pakistan Ltd.
4	Aspirin Sodium Salt	Disprin	Water	Reckitt Benckiser Pakistan
5	Benzophenone	-	A/G	BDH
6	Bromazepam	Lexotanil	Chloroform	Roche Pakistan Ltd.
7	Calcium Carbonate	-	A/G	Sigma
8	Chlorazepate Dipotassium	Tranxene	Water	Searle Pakistan (pvt.) Ltd.
9	Chlordiazepoxide	Librium	Ethanol	Ethical Pharmaceutical Pakistan
10	Chlorpheniramine Maleate	Pritone	Water	Glaxo Smith Kline Ltd. Pakistan
11	Clonazepam	Rivotril	Acetone	Roche Pakistan Ltd.
12	Cocaine	-	Chloroform	Sigma
13	Codeine	Cocodamol	Water	Alpharma Barnstaple UK
14	Dextrose	Splenda	Water	McNeil Nutritionals LLC F. Washington
15	Diazepam	Valium	Chloroform	Roche Pakistan Ltd.
16	Diclofenac Na	Dicloran	Acetone	Sami Pharmaceutical Pakistan
17	Diphenhydramine HCl	Cold and Sinus Tablet	Water	Equal USA
18	Flunitrazepam	Rohypnol	Chloroform	Sigma
19	Flurazepam	Dalmane	Chloroform	Sigma
20	Folic Acid	-	A/G	Sigma
21	Glucose	-	A/G	Merck
22	Guanidine	-	A/G	Sigma
23	Heroin	-	Chloroform	Sigma
24	Ibuprofen	Bruphen	Chloroform	Equal USA
25	Indomethacin	Indomethacin cap.	Chloroform	Chongqing Medicine Pakistan
26	Kanamycin Monosulfate	-	A/G	MP Biomedicals, Inc. Ohio, France.
27	Ketamine	Ketalar	Chloroform	Sigma
28	Lactose	-	A/G	Merck
29	L-Cysteine	-	A/G	Acros
30	L-Glutamine	-	A/G	Sigma
31	Lorazepam	Aitivan	Acetone	Spencer Pharma (pvt) Ltd. Pakistan
32	Mefenamic Acid	Ponstan	Chloroform	Reckitt Benckiser Pakistan
33	Magnesium Sulfate	-	A/G	Merck
34	Maltodextrin	Splenda	Water	McNeil Nutritionals LLC F. Washington
35	Mannitol	-	A/G	Difco Laboratory
36	Methaqualone	-	Chloroform	Sigma
37	Myo-Inositol	-	A/G	Sigma
38	Nalbuphine HCl	Loricin injection	Water	Medicina Pharma Pakistan
39	Naproxen	Synflex	Chloroform	ICI Karachi Pakistan
40	Nicotinic Acid	-	A/G	Sigma

41	Nitrazepam	Mogadon	Chloroform	Sigma
42	Oxazepam	Murelax	Chloroform	Sigma
43	Pentazocine	-	A/G	Standard
44	Phenolphthalein	-	A/G	BDH
45	Phenylephrine HCl	Cold and Sinus Tablet	Water	Equal USA
46	Prednisolone	Prednisolone cap.	Ethanol	Ethical Pharmaceutical Pakistan
47	Propoxyphene	Algaphan	Water	Efroze Chemical Industries Ltd. Pakistan
48	Pyridoxine	-	A/G	Sigma
49	Salicylic Acid	-	A/G	Sigma
50	Sorbitol	-	A/G	Sigma
51	Sodium Bicarbonate	-	A/G	Merck
52	Sodium Gluconate	-	A/G	GPR*
53	Sodium Glutamate	-	A/G	Merck
54	Sorbic Acid	-	A/G	Merck
55	Starch	-	A/G	Merck
56	Succinic Acid	-	A/G	Kodak
57	Sucralose	Splenda	Water	McNeil Nutritionals LLC F. Washington
58	Sucrose	-	A/G	Merck
59	Temazepam	Restoril	Acetone	Novartis Pharmaceutical Pakistan
60	Triazolam	Halcion	Chloroform	Sigma
61	Tyrothricin	-	A/G	Sigma
62	Vitamin D	Calcium D Tablet	Chloroform	Spring Valley by Schiff Nutrition Group Inc., USA

* GPR = General Purpose Reagents; A/G = Analytical Grade (Not Extracted).

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[Table 2 Follows.]

Table 2. Test Results.

	Compound	Color		Compound	Color
1	Acetaminophen	N/C	32	Mefenamic Acid	N/C
2	*alpha*-Lipoic Acid	N/C	33	Magnesium Sulfate	N/C
3	Alprazolam	N/C	34	Maltodextrin	N/C
4	Aspirin	N/C	35	Mannitol	N/C
5	Benzophenone	N/C	36	Methaqualone	N/C
6	Bromazepam	N/C	37	Myo-Inositol	N/C
7	Calcium Carbonate	N/C	38	Nalbuphine HCl	N/C
8	Chlorazepate Dipotassium	N/C	39	Naproxen	N/C
9	Chlordiazepoxide	N/C	40	Nicotinic Acid	N/C
10	Chlorpheniramine Maleate	N/C	41	Nitrazepam	Yellow
11	Clonazepam	N/C	42	Oxazepam	N/C
12	Cocaine	N/C	43	Pentazocine	N/C
13	Codeine	N/C	44	Phenolphthalein	N/C
14	Dextrose	N/C	45	Phenylephrine HCl	N/C
15	Diazepam	Red	46	Prednisolone	N/C
16	Diclofenac Na	N/C	47	Propoxyphene	N/C
17	Diphenhydramine HCl	N/C	48	Pyridoxine	N/C
18	Flunitrazepam	Purple	49	Salicylic acid	N/C
19	Flurazepam	Yellow	50	Sorbitol	N/C
20	Folic acid	N/C	51	Sodium Bicarbonate	N/C
21	Glucose	N/C	52	Sodium Gluconate	N/C
22	Guanidine	N/C	53	Sodium Glutamate	N/C
23	Heroin	N/C	54	Sorbic Acid	N/C
24	Ibuprofen	N/C	55	Starch	N/C
25	Indomethacin	N/C	56	Succinic Acid	N/C
26	Kanamycin Monosulfate	N/C	57	Sucralose	N/C
27	Ketamine	N/C	58	Sucrose	N/C
28	Lactose	N/C	59	Temazepam	Green
29	L-Cysteine	N/C	60	Triazolam	N/C
30	L-Glutamine	N/C	61	Tyrothricin	N/C
31	Lorazepam	N/C	62	Vitamin D	N/C

N/C = No Color.

* * * * *

An In-Depth Study of the Peruvian *Base Llavada* ("Washed Base") Technique for Purification of Crude Cocaine Base

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ABSTRACT: An in-depth study of the Peruvian *base llavada* ("washed base") technique for cleanup of crude cocaine base with ethanol is presented. Used as a substitute method for the traditional potassium permanganate purification process, the technique selectively decreases or removes many alkaloid and colored impurities. Authentic crude Peruvian cocaine base was subjected to the method, and the resulting washed base samples and their respective ethanolic washings were examined immediately thereafter and again 14 months later. The results confirm that the technique gives a whiter appearing but only slightly more pure base versus standard (unwashed) base. The fate of several alkaloid impurities is tracked. The presence of cocaethylene in illicit cocaine (resulting from transesterification of cocaine with ethanol) *may* be indicative of use of the *base llavada* technique; however, ethanol is known to be utilized in several other variants of illicit cocaine processing, so the presence of cocaethylene alone does not confirm the use of the *base llavada* technique.

KEYWORDS: Cocaine, Cocaine Impurities, Cocaethylene, Ethanol, Chromatographic Signature Analysis, Forensic Chemistry

Introduction

In traditional illicit cocaine production, coca leaf is processed to give crude cocaine base, which is purified with potassium permanganate to give a whiter, more refined base, which is then converted to cocaine hydrochloride [1]. Although the potassium permanganate purification is reasonably effective, the method is somewhat time intensive and technique sensitive, and potassium permanganate is both costly and difficult to acquire in cocaine processing regions. Recent interviews of South American cocaine processors indicated that a new technique is being used by some Peruvian chemists to purify their crude cocaine base. In this new variant, referred to locally as the *base llavada* or "washed base" technique, the crude base is first mixed with ethanol until a dough-like consistency is achieved, then wrapped in cloth and hydraulically compressed in a hydraulic press to force out as much of the ethanol solution (containing dissolved impurities) as possible. The process results in a whiter and slightly more pure product, similar in appearance to that produced by the potassium permanganate purification technique.

Prior work at this laboratory demonstrated how the use of the *base llavada* technique affects the signature profile of the resulting cocaine [2]. In the current study, the technique was performed on numerous samples of authentic Peruvian crude cocaine base in order to better understand what is occurring to give the whiter appearing product. The selected samples were analyzed at the time of the process (both before and after the washing), and again 14 months later. The residual ethanol/impurities solutions pressed from the samples were also analyzed both at the time of the process, and again 14 months later. This study also investigated the slow production of cocaethylene in washed samples during storage, resulting from transesterification of cocaine by the residual ethanol still remaining after the wash and pressing.

Experimental

Materials and Solvents: Ethanol and chloroform were distilled-in-glass products of Burdick and Jackson Laboratories (Muskegon, MI). N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was obtained from Pierce Chemical (Rockford, IL). All other reagents and chemicals were reagent-grade quality products of Sigma-Aldrich Chemical Company (Milwaukee, WI). The authentic crude Peruvian cocaine base used in this study was from the reference collection of the DEA Special Testing and Research Laboratory.

Gas Chromatography / Mass Spectrometry (GC/MS): An Agilent (Palo Alto, CA) Model 5973 quadrupole mass-selective detector (MSD) interfaced with an Agilent Model 6890 gas chromatograph was used to conduct all GC/MS analyses. The MSD was operated in the electron ionization mode with an ionization potential of 70 eV, scan range of 34 - 700 mass units at 1.34 scans/s. The GC system was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 µm DB-1 (J&W Scientific). Helium (99.999% UHP) was used as a carrier gas at an average linear velocity of 40 cm/s. The oven temperature was programmed as follows: Initial temperature, 100^{O}C; initial hold, 0.0 min; program rate, 6^{O}C/min; final temperature, 300^{O}C; final hold, 5.67 min. Samples (1 µL) were injected in the split mode (21.5 : 1) by an Agilent 7683 Series Auto Injector. The injection temperature and the auxiliary transfer line to the MSD were both maintained at 280^{O}C.

Gas Chromatography / Flame Ionization Detection (GC/FID) and Gas Chromatography / Electron Capture Detection (GC/ECD): GC/FID and GC/ECD analyses were performed using an Agilent 6890N gas chromatograph. Prepared solutions were placed into an autosampler vial and analyzed using previously published methods and conditions [3,4]. MSTFA was utilized as the derivatization reagent for GC/FID analyses.

Headspace - Gas Chromatography / Mass Spectrometry (HS-GC/MS): Determination of occluded ethanol was accomplished using the method described by Morello *et al.* [5].

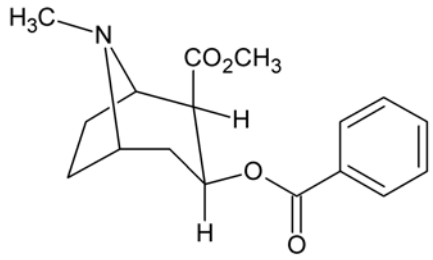
Preparation of Base Llavada Samples: Seven samples from an authentic Peruvian crude cocaine base exhibit were subjected to the *base llavada* technique, using 90% ethanol. The amount of sample and amount of ethanol used were varied slightly from sample to sample (Table 1). The residual ethanol/impurities solution from each pressing was collected for analysis. The ethanol was evaporated *in vacuo* to a dark brown oil prior to analysis. All samples were then subjected to chromatographic impurity analysis to quantitatively determine the alkaloids present. All samples were stored at room temperature for 14 months and then re-examined to determine any changes that had occurred.

* * * * *

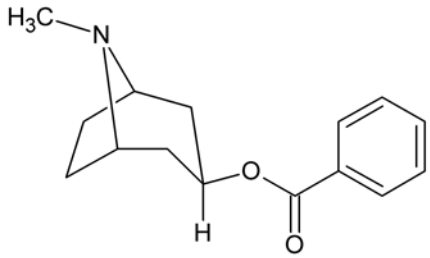
Table 1. Preparation of *Base Llavada* Samples.

Sample	Amount of Cocaine Base Used* (g)	Amount of Ethanol Used (mL)
1	300	45
2	600	90
3	500	75
4	400	60
5	500	75
6	500	75
7	456	65

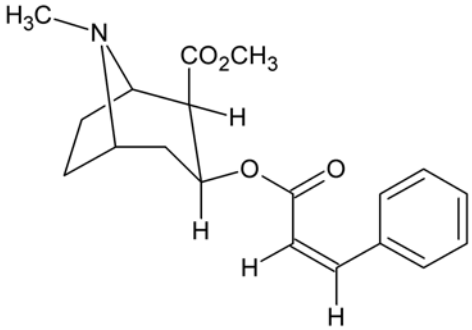
* Authentic Crude Peruvian Cocaine Base.



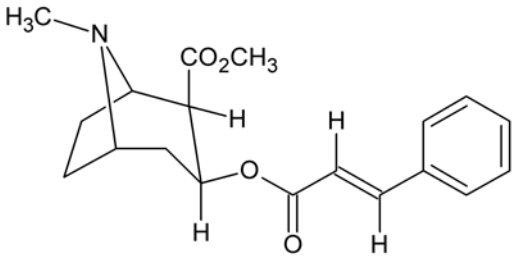
Cocaine



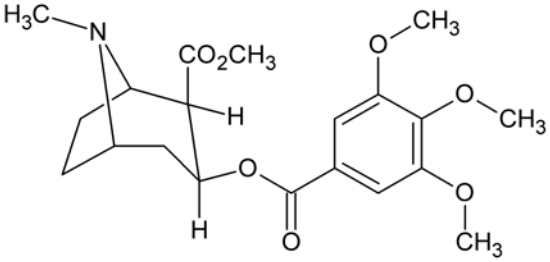
Tropacocaine



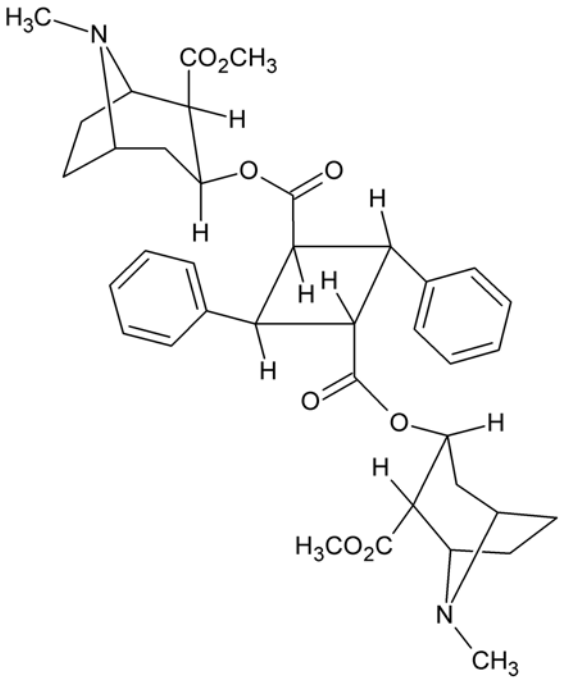
cis-Cinnamoylcocaine



trans-Cinnamoylcocaine



Trimethoxycocaine



α-Truxilline

Figure 1. Structures of Major Alkaloids Found in Illicit Cocaine.

Results and Discussion

The *base llavada* technique experiments resulted in a minor (4.5% average) increase in cocaine base purity, primarily due to the partial removal of the cinnamoylcocaines, truxillines, tropacocaine, and trimethoxycocaine (Figure 1). In addition (and in accordance with the information provided by the Peruvian cocaine processors), the resulting product was also noticeably whiter in appearance after the wash, due to the partial removal of the cinnamoylcocaines and other colored impurities (as noted above, the residual ethanol/impurities solution is dark brown in color). Partial reconstructed chromatographic profiles of the crude cocaine base, washed cocaine base, and residual ethanol/impurities solution are illustrated in Figure 2. The retention times of the target compounds are given in Table 2. As seen in Figures 2a and 2b, the cinnamoylcocaine content was reduced in the washed sample. Table 3 illustrates the effects of the wash on the relative concentrations of tropacocaine, *cis*-cinnamoylcocaine, *trans*-cinnamoylcocaine, trimethoxycocaine, and total truxillines. These naturally occurring alkaloids are more soluble than cocaine in ethanol, and as a result, are selectively extracted from the illicit cocaine (with minimal loss due to co-extraction of cocaine). Quantitative determinations confirmed decreases ranging from 21 - 78% relative to cocaine. The truxilline concentration was the least affected, while the trimethoxycocaine concentration was the most affected. Re-analysis of the samples 14 months later showed further decreases in alkaloid concentrations, ranging from 32 - 100%, versus the original crude cocaine base values, indicating further hydrolysis of those alkaloids over time (Table 3).

The residual ethanol/impurities solutions were also examined immediately after completion of the *base llavada* technique and not surprisingly were found to contain significant amounts of tropacocaine, *cis*-cinnamoylcocaine, *trans*-cinnamoylcocaine, trimethoxycocaine, and total truxillines relative to cocaine (Figure 2c). While the residual oil also contained some cocaine, the bulk of the material was primarily comprised of the alkaloidal impurities, approaching an order of magnitude concentration increase over their respective starting values relative to cocaine in the original crude base.

The hydrolysis of the cocaine was also examined. Hydrolysis of cocaine occurs at both ester bonds, giving ecgonine, ecgonine methyl ester, and benzoylecgonine (Figure 3). Hydrolysis of *cis*-cinnamoylcocaine and *trans*-cinnamoylcocaine will also occur, giving *cis*-cinnamoylecgonine, *trans*-cinnamoylecgonine, and ecgonine (Figure 3). The respective concentrations of the hydrolysis products was determined immediately after completion of the *base llavada* technique and again 14 months later. As shown in Table 4, the cleanup immediately increased these hydrolysis products from 18% to nearly 400% relative to cocaine in the resulting washed base. The residual oil also contained an increased concentration of the hydrolysis products. In addition, the ethanol remaining in the washed base samples continued to hydrolyze the cocaine, *cis*-cinnamoylcocaine, and *trans*-cinnamoylcocaine during the 14 month storage period, resulting in even more significant increases in the concentrations of *cis*-cinnamoylecginine, *trans*-cinnamoylecgonine, and benzoylecgonine. When compared to the original samples, after 14 months, the hydrolysis products had increased from 57% to nearly 1,400% relative to cocaine.

Finally, although not quantitatively determined, the transesterification of cocaine to cocaethylene due to the presence of residual ethanol was also observed during storage [6]. Both the original sample and the washed base (immediately following the wash) showed no traces of cocaethylene. However, storage of the sample for 14 months allowed transesterfication to occur, resulting in a measurable quantity of cocaethylene; the ethyl esters of *cis*- and *trans*-cinnamoylcocaine were also detected (Figure 5). Thus, the presence of cocaethylene in illicit cocaine *may* be indicative of use of the *base llavada* technique - however, ethanol is known to be utilized in several other variants of illicit cocaine processing, so the presence of cocaethylene alone does not confirm the use of the *base llavada* technique.

Although increasingly utilized in Peru, the *base llavada* method is currently not known to be in use in either Bolivia or Colombia.

Conclusions

The *base llavada* technique is a partially effective purification technique. Ethanol selectively removes significant amounts of major alkaloid impurities from crude cocaine base, due to their higher solubility versus cocaine. In contrast to the more time intensive and costly potassium permanganate purification method, the *base llavada* technique is relatively easy and inexpensive to perform. Although this new methodology results in only a moderate increase in cocaine base purity, it does give a noticeably whiter product.

References

1. Casale JF, Klein RFX. Illicit production of cocaine. Forensic Science Review 1993;5(2):95-107.

2. Casale JF, Boudreau DK, Jones LM. Tropane ethyl esters in illicit cocaine: Isolation, detection, and determination of new manufacturing by-products from the clandestine purification of crude cocaine base with ethanol. Journal of Forensic Sciences 2008;53(3):661-667.

3. Casale JF, Waggoner RW. A chromatographic impurity signature profile analysis for cocaine using capillary gas chromatography. Journal of Forensic Sciences 1991;36(5):1312-1330.

4. Moore JM, Casale JF, Cooper DA. Comparative determination of total isomeric truxillines in illicit, refined, South American cocaine hydrochloride using capillary gas chromatography - electron capture detection. Journal of Chromatography A 1996;756:193-201.

5. Morello DR, Meyers RP. Qualitative and quantitative determination of residual solvents in illicit cocaine HCl and heroin HCl. Journal of Forensic Sciences 1995;40(6):957-63.

6. Casale JF, Moore JM. An in-depth analysis of pharmaceutical cocaine: Cocaethylene and other impurities. Journal of Pharmaceutical Sciences 1994;83(8):1186.

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Table 2. Relative Retention Times (RRT) of Coca Alkaloids.

Compound	Peak #	GC/FID RT	GC/MS RT
Ecgonine-di-TMS	1	5.24	----
Tropacocaine	2	10.96	17.51
para-Fluorococaine	3	14.92	----
Cocaine	4	15.81	21.38
Benzoylecgonine-TMS	5	16.18	----
cis-Cinnamoylcocaine	6	18.56	23.80
cis-Cinnamoylecgonine-TMS	7	18.90	----
trans-Cinnamoylcocaine	8	20.32	25.61
trans-Cinnamoylecgonine-TMS	9	20.52	----
Trimethoxycocaine	10	24.59	----
Cocaethylene	11	16.41	22.09
cis-Cinnamoylecgonine ethyl ester	12	----	24.55
Benzoylecgonine	13	----	25.95
trans-Cinnamoylecgonine ethyl ester	14	----	26.31

Table 3. Average Change in Alkaloid Values and (Percent Change) Relative to Percent Cocaine.

	Original Values	Day 1	14 Months Later	Enriched Ethanolic Oil
% Cocaine	79.5	83.0 (4.4)	84.3 (6.0)	32.5 (-59.1)
% Tropacocaine	0.12	0.07 (-41.7)	0.06 (-50.0)	1.02 (750.0)
% *cis*-Cinnamoyl-cocaine	6.36	4.31 (-32.2)	3.08 (-51.6)	47.13 (641.0)
% *trans*-Cinnamoyl-cocaine	4.08	2.68 (-34.3)	1.88 (-53.9)	32.94 (707.4)
% Trimethoxycocaine	0.18	0.04 (-77.8)	0.00 (-100)	1.02 (466.7)
% Truxillines	4.41	3.49 (-20.9)	3.01 (-31.7)	40.04 (87.9)
% EtOH relative to Cocaine	n/a	<0.1%	<0.1%	n/a

* * * * *

Table 4. Average Change in Hydrolysis Product Values and (Percent Change) Relative to Percent Cocaine.

	Original Values	Day 1	14 Months Later	Enriched Ethanolic Oil
% *cis*-Cinnamoyl-ecgonine	0.08	0.38 (375.0)	1.19 (1387.5)	4.49 (5512.5)
% *trans*-Cinnamoyl-ecgonine	0.00	0.18 (18.0)	0.57 (57.0)	1.45 (145)
% Ecgonine	0.81	1.20 (48.1)	1.51 (86.4)	19.02 (2248.1)
% Methylecgonine	0	0	0	0
% Benzoylecgonine	0.12	0.48 (300.0)	1.57 (1208.3)	5.23 (4258.3)

* * * * *

[Figures 2 - 5 Follow.]

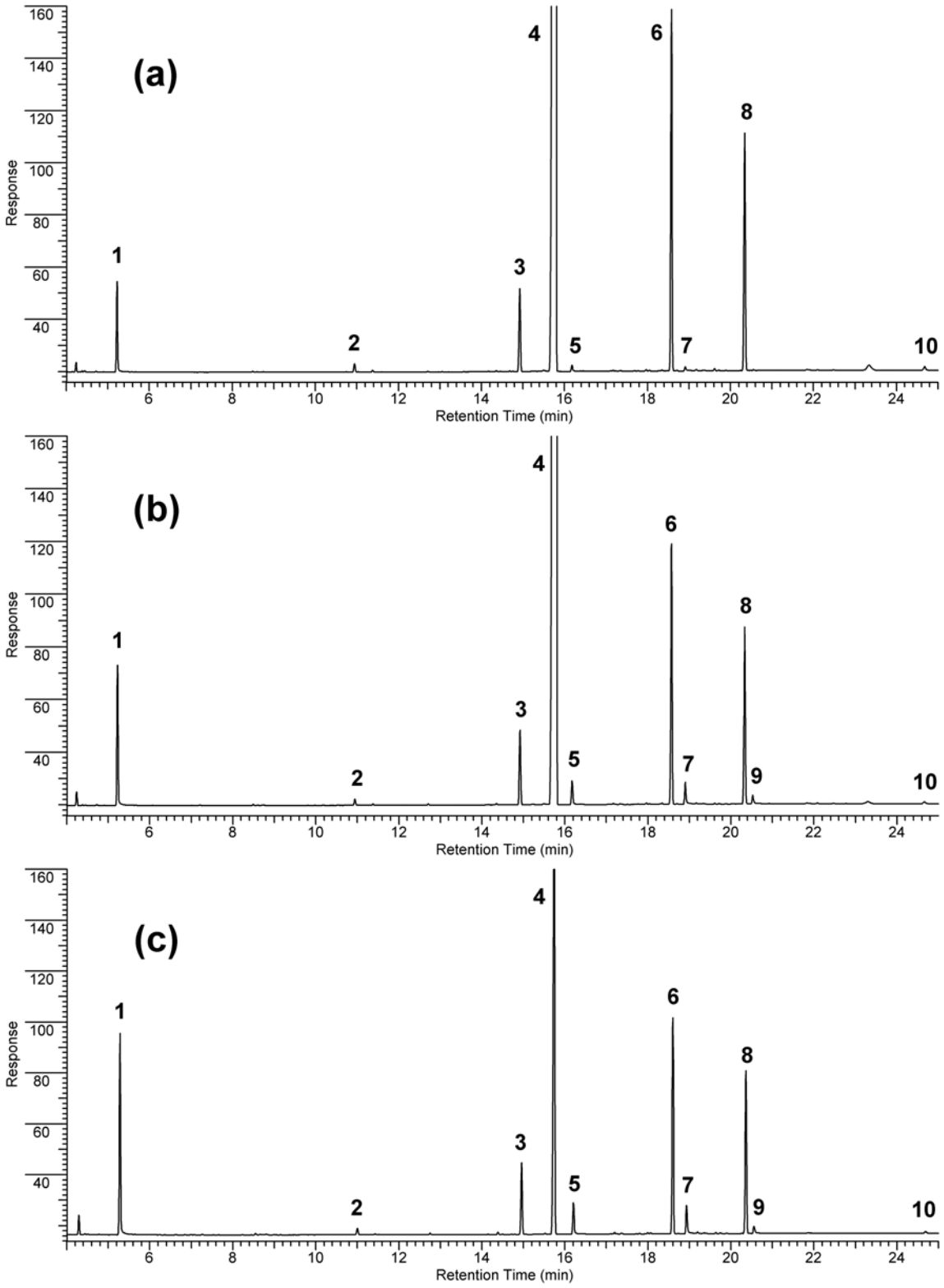
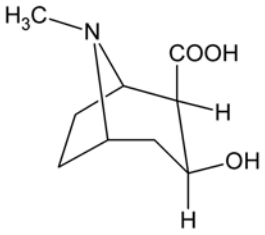
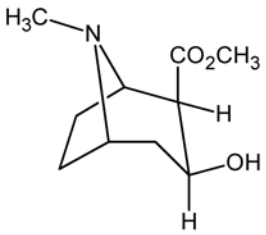


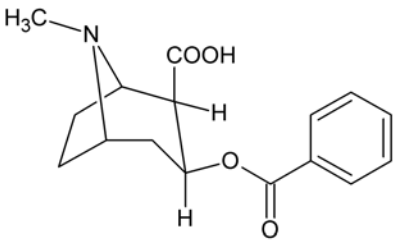
Figure 2. Partial Reconstructed GC/FID Chromatograms of: (A) Crude Cocaine Base; (B) Washed Cocaine Base; and (C) Enriched Oil. See Table 2 for Peak Identification.



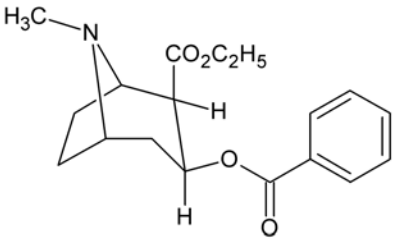
Ecgonine



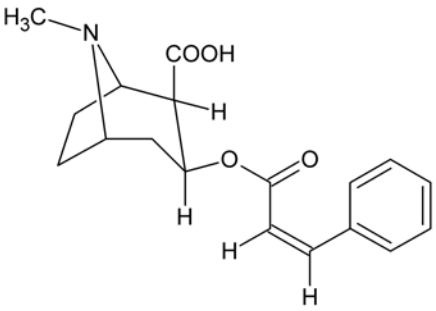
Ecgonine methyl ester



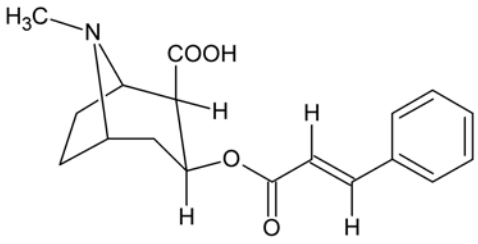
Benzoylecgonine



Cocaethylene



cis-Cinnamoylecgonine



trans-Cinnamoylecgonine

Figure 3. Structures of Hydrolysis Products.

* * * * *

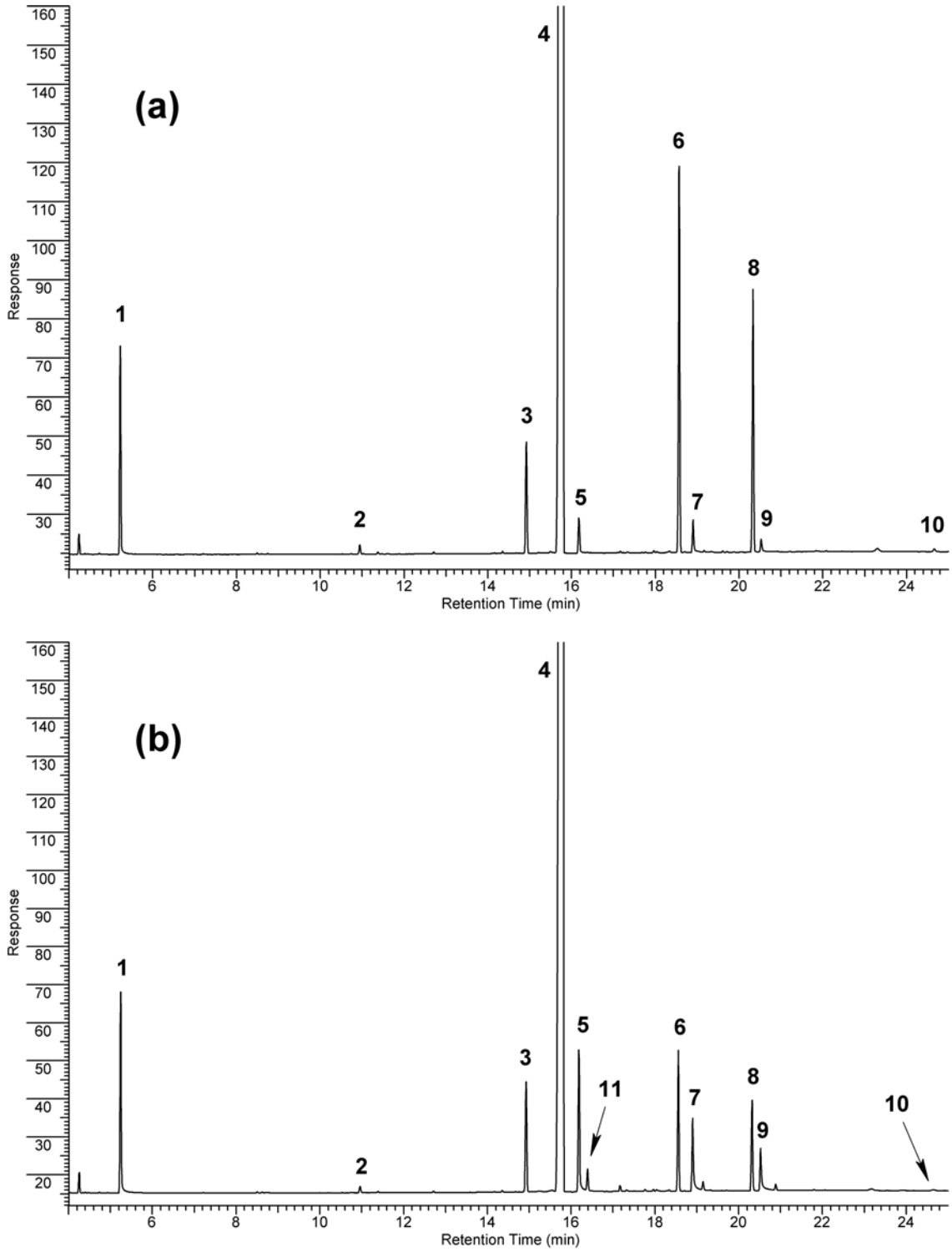


Figure 4. Partial Reconstructed GC/FID Chromatograms of: (A) Washed Cocaine Base after 1 Day; and (B) Washed Cocaine Base after 14 Months. See Table 2 for Peak Identification.

* * * * *

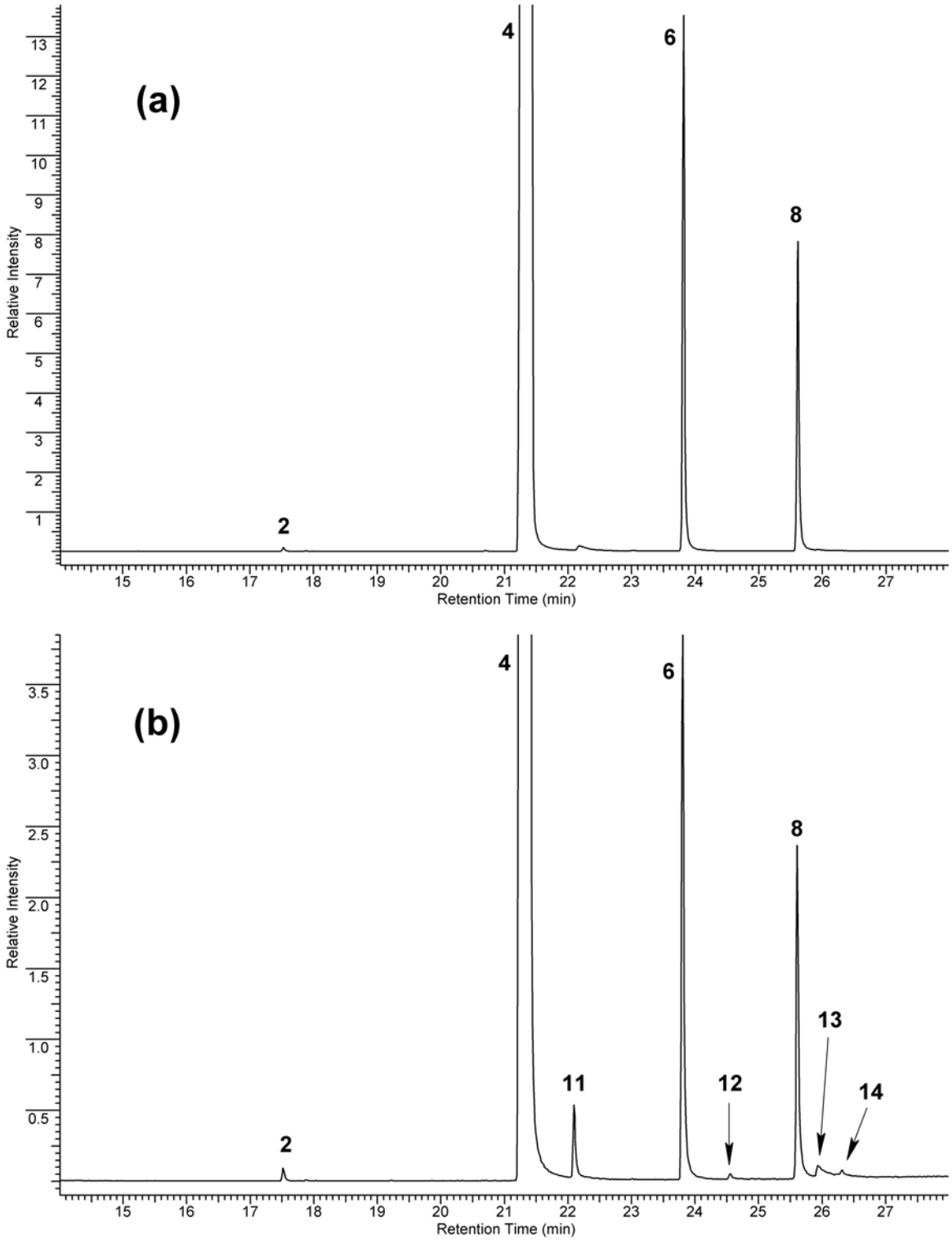


Figure 5. Partial Reconstructed Total Ion Chromatograms Showing Cocaethylene's Development Over Time: (A) Day One; and (B) 14 Months Later. See Table 2 for Peak Identification.

* * * * *

Technical Note

Identification of Levamisole Impurities Found in Illicit Cocaine Exhibits

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ABSTRACT: 6-Phenyl-2,3-dihydroimidazo[2,1b]thiazole and 3-(2-mercaptoethyl)-5-phenylimidazolidine-2-one, known levamisole degradation products, were identified in a "crack" cocaine exhibit. Spectroscopic and chromatographic data are provided for both compounds, and their presence in the sample is discussed.

KEYWORDS: Levamisole, Degradation, Cocaine Base, "Crack" Cocaine, Impurities, 6-phenyl-2,3-dihydroimidazo[2,1b]thiazole, 3-(2-mercaptoethyl)-5-phenylimidazolidine-2-one, Forensic Chemistry

Introduction

This laboratory recently received a 1 gram portion of a "crack" cocaine (cocaine base) exhibit from another laboratory for the purpose of identifying an unknown component. The exhibit contained 79% cocaine base, 6% levamisole, and 3% of an unknown compound. The unknown had an apparent molecular weight of 202 Daltons based on the mass spectrum generated by the original laboratory, and was suspected to be a levamisole-related impurity. Upon screening, the exhibit was found to also contain trace amounts of a second unknown compound, suspected to be another levamisole-related impurity. Levamisole (Figure 1), an antineoplactic (cancer chemotherapy drug), has been a cocaine adulterant for nearly 5 years [1], but this is the first report of suspected levamisole impurities in illicit cocaine. The prevalence of levamisole in cocaine hydrochloride bricks has increased dramatically over the past year, and is currently found in 30% of all seizures (Figure 2). Herein, we report the preparative isolation, gas and liquid chromatographic-mass spectrometry, and nuclear magnetic resonance spectroscopy of both impurities. The major unknown compound (6-phenyl-2,3-dihydroimidazo[2,1b]thiazole) was confirmed via comparison to an authentic standard, while the trace unknown compound (3-(2-mercaptoethyl)-5-phenyl-imidazolidine-2-one) was synthesized to verify its identity.

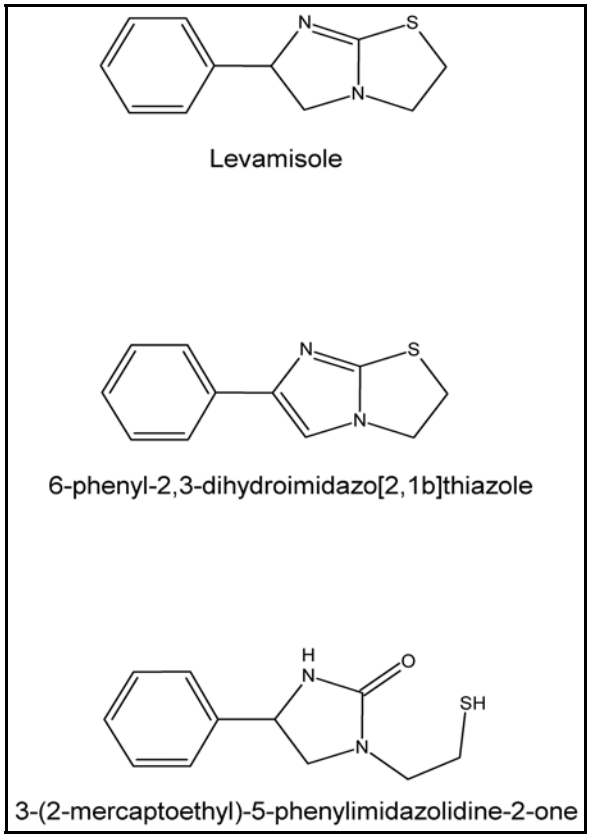

Figure 1.

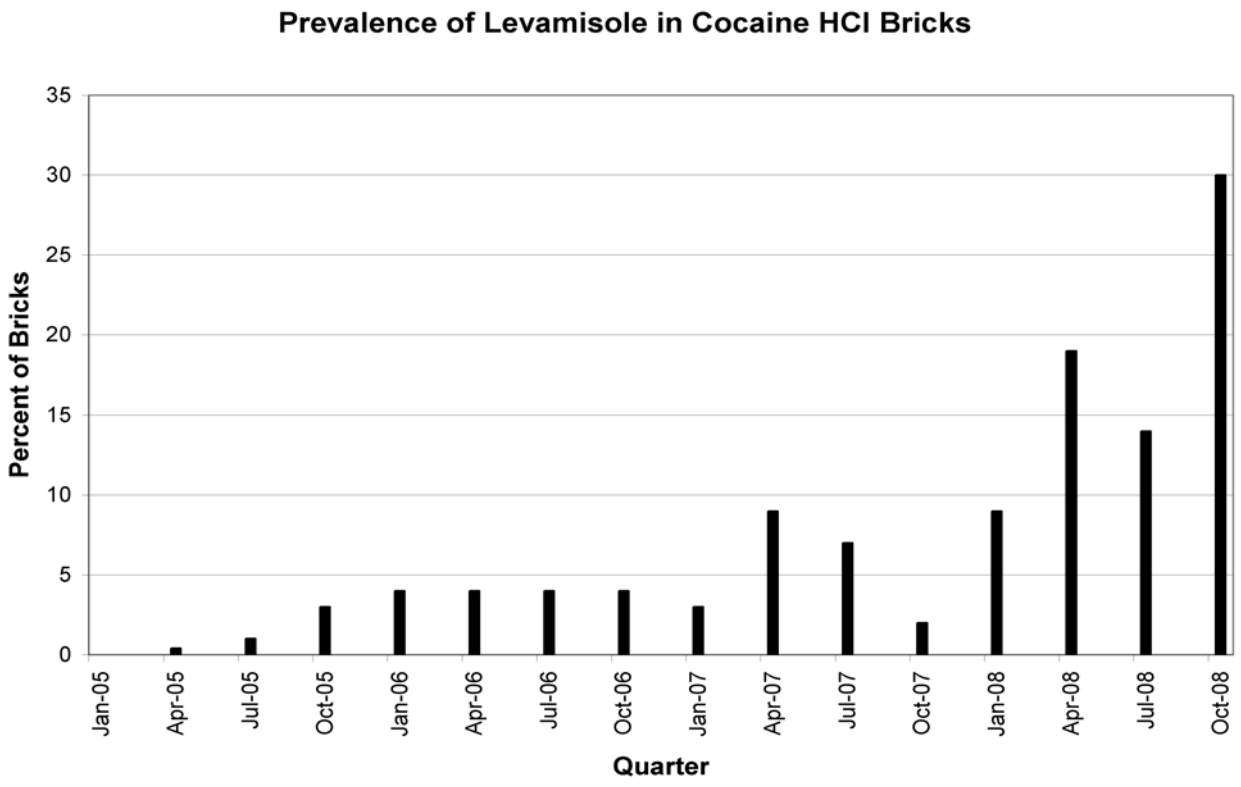

Figure 2. Prevalence of Levamisole in Cocaine Hydrochloride Bricks over the past 4 Years.

Experimental

Solvents, Chemicals, and Materials: All solvents were distilled-in-glass products of Burdick and Jackson Laboratories (Muskegon, MI). N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was obtained from Pierce Chemical (Rockford, IL). 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 was part of the authentic reference collection of the DEA Special Testing and Research Laboratory. A reference standard of 6-phenyl-2,3-dihydroimidazo[2,1b]thiazole was obtained from LGC Standards (Luckenwalde, Germany).

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 0.25 µm DB-1 (J&W Scientific, Rancho Cordova, CA). The oven temperature was programmed as follows: Initial temperature, 100^{O}C (no initial hold); program rate, 6^{O}C/min; final temperature, 300^{O}C; final hold, 5.67 min. The injector was operated in the split mode (21.5 : 1) and at a temperature of 280^{O}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^{O}C and 230^{O}C, respectively.

Liquid Chromatography/Mass Spectrometry (LC/MS): Molecular weight information derived from $[M+H]^+$ was obtained using a Waters (Milford, MA) 2525 HPLC pump fitted with a XTerra MS 150 mm x 4.8 mm, 5 µm, C-18 column. The sample was diluted to a concentration of approximately 0.2 mg/mL, and the injection volume

was 0.5 mL per run. The flow was optimized at 1.0 mL/min, using the following reversed-phase gradient solvents: (A) Water containing 0.1% trifluoroacetic acid, and (B) Acetonitrile. The linear gradient started at 95% A and 5% B; changed to 75% A and 25% B over 20 min, held 15 min; then changed to 5% A and 95% B over 25 min; and finally returned to 95% A and 5% B for 1 min. The HPLC eluent was introduced into a Waters Micromass ZQ single quadrupole mass spectrometer using Electrospray Ionization (ESI) with positive ion detection. The detector operated in the scan range of 40 - 500 mass units, a scan time of 0.5 sec, and an inter-scan delay of 0.1 sec.

Preparative Isolation of 6-Phenyl-2,3-dihydroimidazo[2,1b]thiazole via Alumina Column Chromatography: Approximately 900 mg of the illicit cocaine sample containing about 3% (~27 mg) of target compound was dissolved in a minimal amount of $CHCl_3$ and eluted 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 20 mL each of the following solvent combinations: 1) $CHCl_3$, 2) $CHCl_3$/acetone (85 : 15), 3) $CHCl_3$/acetone (1 : 1), and acetone. Ten mL fractions were collected and examined via GC/MS. Fractions 2 and 3 contained 17 - 22% pure target compound and were combined and evaporated to dryness. The residue was dissolved into a minimal amount of $CHCl_3$/hexane (1 : 1) and was chromatographed again on 15 g of basic alumina (150 mesh). The column was eluted with 20 mL each of the following solvent combinations: 1) $CHCl_3$/hexane (1 : 1), 2) $CHCl_3$, 3) $CHCl_3$/acetone (40 : 1), 4) $CHCl_3$/acetone (20 : 1), 5) $CHCl_3$/acetone (15 : 1), 6) $CHCl_3$/acetone (10 : 1), and 7) $CHCl_3$/acetone (6 : 1). Ten mL fractions were collected and examined via GC/MS. Fraction 5 contained the majority of the target compound (but also contained some levamisole and cocaine), and was evaporated to dryness. The residue was washed with 2 - 3 mL of petroleum ether (20 - 40^{O}C boiling range) to remove the cocaine, and was then dried to provide a white powder (20 mg, 67% 6-phenyl-2,3-dihydroimidazo[2,1b]thiazole and 33% levamisole).

Nuclear Magnetic Resonance Spectroscopy (NMR): Proton (^{1}H) NMR 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 deuterochloroform ($CDCl_3$) containing 0.03% v/v tetramethylsilane (TMS) as the 0 ppm reference. The temperature of the samples was maintained at 25^{O}C. Standard Varian pulse sequences were used to acquire the spectra. Data processing was performed using Applied Chemistry Development software (ACD/Labs, Toronto, Canada).

Synthesis of 3-(2-Mercaptoethyl)-5-phenylimidazolidine-2-one: Levamisole hydrochloride (25 mg, 0.104 mmol) was dissolved in water (5 mL), adjusted to pH 8 with aqueous $NaHCO_3$ (1 mL), and microwaved at 1200 watts until all the water had boiled off (about 2 - 3 minutes). The residue was dissolved in $CHCl_3$ (10 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated *in vacuo* to give the title compound as a white powder (22 mg, 95%). The reaction was repeated at pH 10 using aqueous Na_2CO_3, and gave identical results.

Results and Discussion

GC/MSD analysis of the exhibit was first conducted as a cursory assessment. Examination of the reconstructed total ion chromatogram (Figure 3a, Table 1) indicated a compound (Peak #4) closely related to levamisole was present. Peak #4 represented approximately 3% of the total ion current. Its mass spectrum (Figure 4a) produced an apparent molecular ion at *m/z* 202. The spectrum was markedly similar to levamisole (Figure 4b), with fragment ion shifts of minus one to two mass units for several ions, thus suggesting a levamisole-like compound with incorporation of another double bond. It did not form a TMS derivative, indicating no labile protons within the molecule. The molecular weight for this compound was confirmed via LC/MS, yielding a $[M+H]^+$ at *m/z* 203, consistent with the molecular weight assignment of 202. This compound was semi-isolated as described in the Experimental section and examined via ^{1}H-NMR. The chemical shifts obtained were consistent with the loss of two protons within the imidizole ring and suggested that the compound was 6-phenyl-2,3-dihydro-imidazo[2,1b]thiazole. A reference standard of this compound was obtained, and its retention time and mass spectrum were identical to the unknown (Figure 1).

A trace component (Figure 3a, Peak #3) was also noted in the GC/MSD analysis, having an apparent molecular ion at *m/z* 222 (Figure 5a). Upon derivatization with MSTFA, this compound formed both a mono-TMS and di-TMS derivative (Figure 3b, Peaks #2 & #6), with molecular ions at *m/z* 294 (Figure 5b) and *m/z* 366 (Figure 5c), respectively. These results indicated that two labile protons were present. A mass difference of +18 Daltons from levamisole, coupled with two labile protons, suggested that the compound was an oxidation by-product of levamisole. Since 3-(2-mercaptoethyl)-5-phenylimidazolidine-2-one had been previously reported as an oxidative by-product of levamisole in aqueous solutions [2-4], it was synthesized as described in the Experimental section. The retention times and mass spectra of the synthesized standard (both underivatized and derivatized) were identical to the unknown (Figure 1).

Some Colombian-run cocaine hydrochloride laboratories have been adding levamisole to cocaine hydrochloride for nearly 5 years. Over that time period, the levamisole has always appeared to be of pharmaceutical-grade quality. Significant amounts of process impurities are rarely encountered in pharmaceutical drug products. This exhibit contained 6-phenyl-2,3-dihydroimidazo[2,1b]thiazole at a concentration of about 50% relative to levamisole, which is quite remarkable. This impurity (and the trace amount of 3-(2-mercaptoethyl)-5-phenyl-imidazolidine-2-one) may have arisen from two possible sources: 1) A poorly processed or waste batch of pharmaceutical levamisole; and/or 2) Degradation of levamisole during the conversion of cocaine hydrochloride into "crack" cocaine.

The formation of significant amounts of 6-phenyl-2,3-dihydroimidazo[2,1b]thiazole from levamisole during the conversion of cocaine hydrochloride into "crack" cocaine as currently practiced seems unlikely. The stability of levamisole in aqueous solutions has been studied at length [2-4]. In those works, the formation of up to four degradation products were tracked as a function of pH and temperature. 6-Phenyl-2,3-dihydroimidazo[2,1b]-thiazole was not detected even when levamisole was boiled to dryness in basic aqueous solutions (i.e., at pH 7 to 10). However, 6-phenyl-2,3-dihydroimidazo[2,1b]thiazole is also a synthetic by-product from the pharmaceutical production process, due to an undesired cyclization of the first intermediate product [5]. Due to the very high relative concentration of this compound to levamisole in this sample, it appears that an impure or waste batch of levamisole made its way into the illicit drug trade.

However, as was demonstrated in the Experimental section, 3-(2-mercaptoethyl)-5-phenylimidazolidine-2-one is nearly quantitatively created from levamisole when boiled to dryness in basic aqueous solutions (i.e., at either pH 8 or 10). Therefore, the trace amount of 3-(2-mercaptoethyl)-5-phenylimidazolidine-2-one in the sample may be attributed to its formation as a by-product from levamisole during the conversion of cocaine hydrochloride into "crack" cocaine. In this case, the water was not boiled off during the "crack process," or all of the levamisole would have been converted to this compound. The presence of large amounts of 3-(2-mercaptoethyl)-5-phenyl-imidazolidine-2-one in "crack" cocaine exhibits would be evidence of boiling the "crack" cocaine conversion solution to dryness.

The physiological effects and consequences of smoking "crack" cocaine adulterated with levamisole and contaminated with 6-phenyl-2,3-dihydroimidazo[2,1b]thiazole and 3-(2-mercaptoethyl)-5-phenylimidazol-idine-2-one are unknown.

References

1. Valentino AMM, Fuentecilla K. Levamisole: An analytical profile. Microgram Journal 2005;3(3-4):134-7.

2. Dickinson NA, Hudson HE, Taylor PJ. Levamisole: Its stability in aqueous solutions at elevated temperatures - Part I. Isolation and identification of decomposition products formed in aqueous solutions of levamisole stored under Nitrogen and Oxygen at 100^{O}C. Analyst 1971;96:235-43.

3. Dickinson NA, Hudson HE, Taylor PJ. Levamisole: Its stability in aqueous solutions at elevated temperatures - Part II. An assay specific for levamisole and applicable to stability studies. Analyst 1971;96:244-7.

4. Hanson KA, Nagel DL, Heidrick ML. Immunomodulatory action of levamisole - I. Structural analysis and immunomodulating activity of levamisole degradation products. International Journal of Immunopharmacology 1991;13(6):655-68.

5. Romanov NN, Kovtun YP, Kochergin PM. Methods of synthesis and technology of production of drugs: Levamisole and analogs. Pharmaceutical Chemistry Journal 1989;23(2):167-81.

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Table 1. Retention Times (RT) and Relative Retention Times (RRT) of Levamisole and Related Impurities [a.]

Compound	RT (min)	RRT (min)
Levamisole	17.25	0.81
222-mono-TMS*	19.09	0.90
222*	19.77	0.93
202**	20.99	0.98
Cocaine	**21.30**	**1.00**
222-di-TMS*	22.39	1.05

[a] Conditions Detailed in the Experimental Section.
* 3-(2-Mercaptoethyl)-5-phenylimidazolidine-2-one
** 6-Phenyl-2,3-dihydroimidazo[2,1b]thiazole

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[Figures 3 - 5 Follow.]

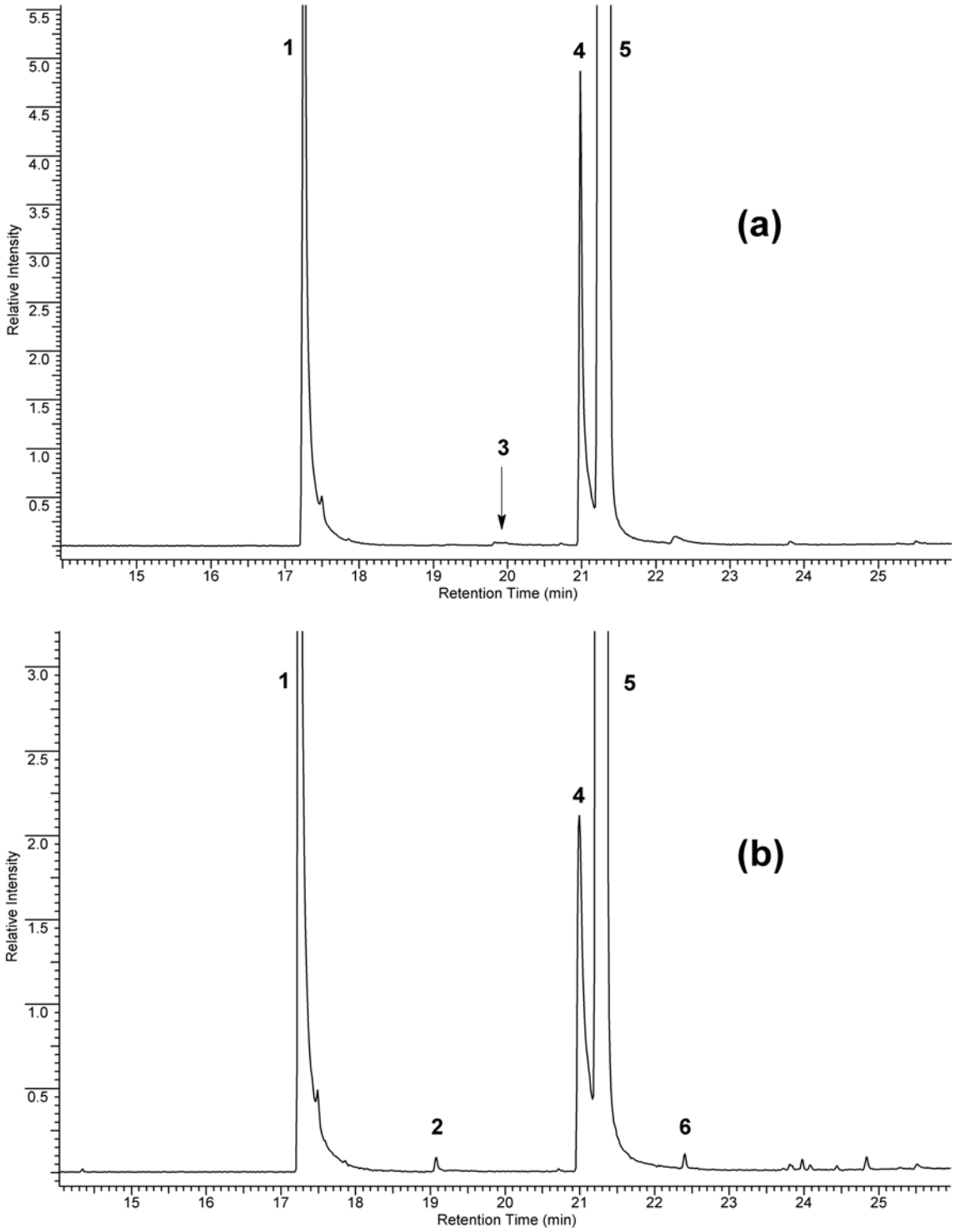

Figure 3. Partial Reconstructed Total Ion Chromatograms of a Cocaine Base Exhibit Containing Levamisole Impurities. Upper (A) Is Underivatized and Lower (B) Is Derivatized. Peak Identification: 1 = Levamisole, 2 = TMS Derivative of 3-(2-Mercaptoethyl)-5-phenylimidazolidine-2-one, 3 = 3-(2-Mercaptoethyl)-5-phenylimidazolidine-2-one, 4 = 6-Phenyl-2,3-dihydroimidazo[2,1b]thiazole, 5 = Cocaine, 6 = di-TMS Derivative of 3-(2-Mercaptoethyl)-5-phenylimidazolidine-2-one.

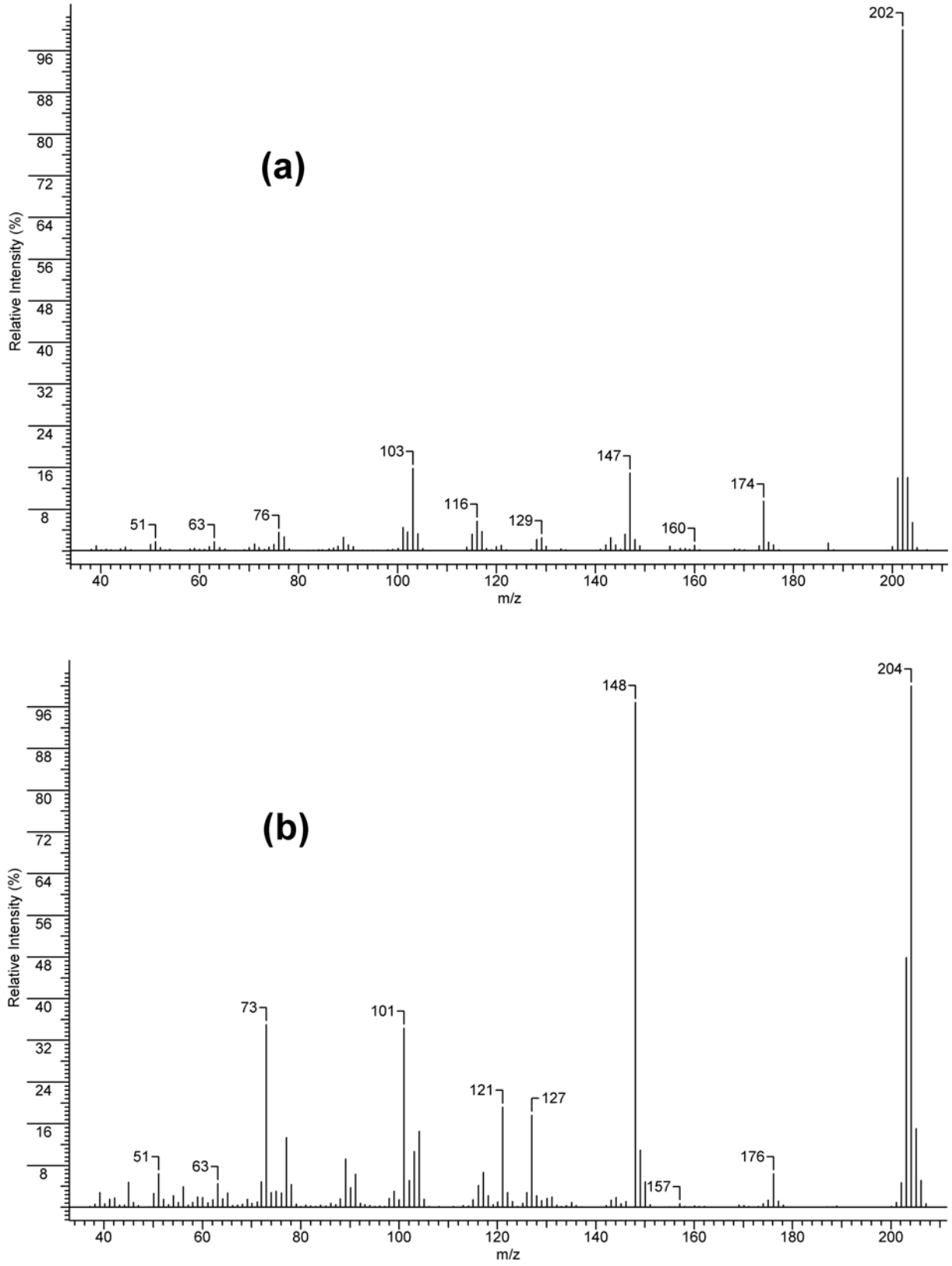

Figure 4. Electron Ionization Mass Spectrum of (A) 6-Phenyl-2,3-dihydro-imidazo[2,1b]thiazole; and (B) Levamisole.

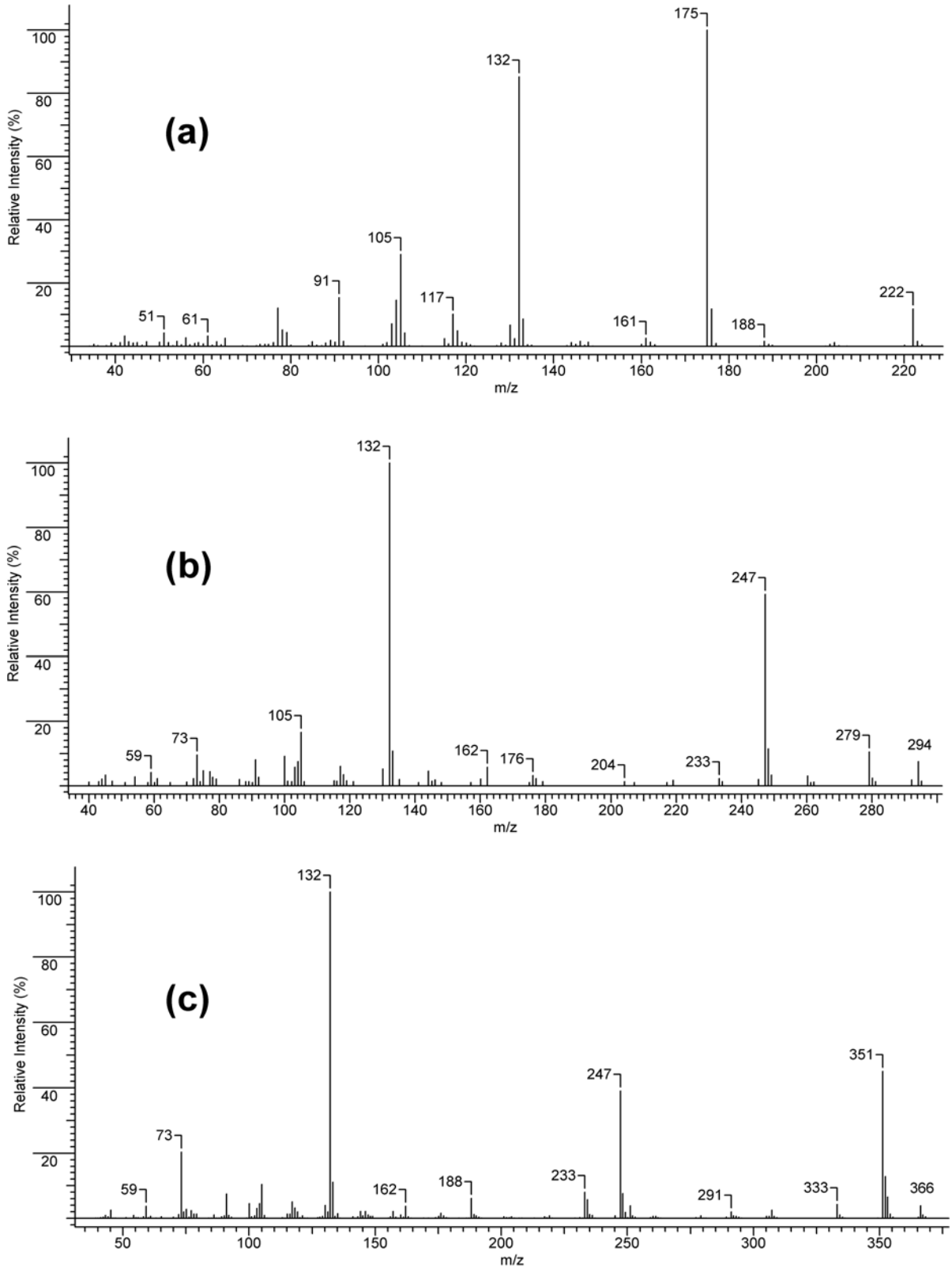

Figure 5. Electron Ionization Mass Spectrum of 3-(2-Mercaptoethyl)-5-phenylimidazolidine-2-one: (A) Underivatized, (B) mono-TMS Derivative, and (C) di-TMS Derivative.

Identification of Diltiazem Impurities / Artifacts during the Analyses of Illicit Cocaine Exhibits Containing Diltiazem

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ABSTRACT: Desacetyldiltiazem and an uncharacterized artifactual compound with an apparent mass of 354 Daltons have been observed in gas chromatographic profiles of cocaine exhibits containing diltiazem. The use of methanol as an injection solvent for cocaine samples containing sodium bicarbonate causes the formation of these compounds in the injection port; however, the use of chloroform as an injection solvent does not result in their formation. Spectroscopic and chromatographic data are provided for diltiazem, desacetyldiltiazem, and 2,3-dehydrodesacetyldiltiazem. Desacetyldiltiazem is a *bona fide* impurity in some cocaine exhibits, but it can also be produced as an analytical artifact. The artifact with an apparent mass of 354 Daltons is not (as postulated) 2,3-dehydrodesacetyldiltiazem, and remains unidentified.

KEYWORDS: Diltiazem, Desacetyldiltiazem, 2,3-Dehydrodesacetyldiltiazem, Injection Port Artifacts, Cocaine, Chemical Analysis, Gas Chromatography, Forensic Chemistry

Introduction

Over the past 5 years, DEA laboratories have received increasing numbers of both cocaine hydrochloride and cocaine base ("crack") exhibits adulterated with diltiazem (Figure 1) [1]. Diltiazem is a potent vasodilator used in the treatment of angina pectoris, arrhythmia, hypertension, and related heart ailments [1]. Identification of diltiazem has typically involved GC/MS, with comparison of spectra and retention time to a standard. There are four stereoisomers of diltiazem; the isomer being utilized to adulterate cocaine is the pharmaceutical product, (+)-*cis*-diltiazem.

In the course of identifying suspected diltiazem in cocaine samples, slight differences between the mass spectra and retention times of the presumed diltiazem in the sample and the diltiazem standard were sometimes observed. Additionally, some samples exhibited two unknown compounds eluting just after diltiazem (Figure 2). These observations suggest that the diltiazem is degrading to other compounds during the analysis. Known diltiazem degradation products include desacetyldiltiazem, N-demethyldiltiazem, N-demethyldesacetyldiltiazem, and O-demethyldesacetyldiltiazem [2-5].

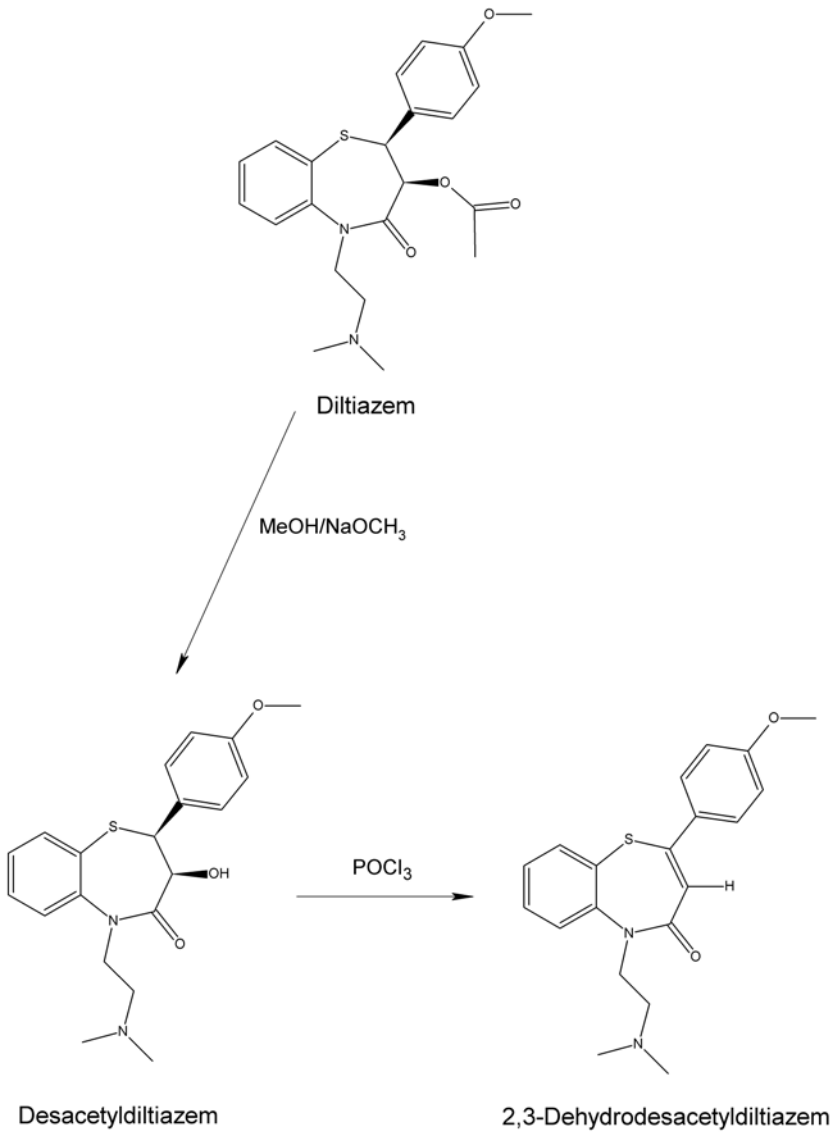

Figure 1. Synthesis and Structural Formulas of Diltiazem and Related Compounds.

* * * * *

Interestingly, it was noted that significant amounts of the two unknown compounds were observed when analyzing many - but not all - cocaine base ("crack") samples, and furthermore were rarely observed when analyzing cocaine hydrochloride samples. The two unknowns had apparent molecular ion at *m/z* 372 and 354, respectively (Note: The molecular weight of diltiazem is 414). Since significant amounts of the first unknown compound (Figure 2, peak #2) were observed in many "crack" exhibits that contained excess sodium bicarbonate, hydrolysis to desacetyldiltiazem (414 - 42 = 372) was suspected. The instability of diltiazem in solution, and its hydrolysis to desacetyldiltiazem (Figure 1), are well documented [4-7]. Significant amounts of the second unknown compound (Figure 2, peak #3) were also observed in these same "crack" exhibits. Its apparent molecular ion (372 - 18 = 354) suggested that the compound is derived via elimination of water from desacetyldiltiazem to form 2,3-dehydrodesacetyldiltiazem (Figure 1). Of note, in most instances the two unknown components were only detected when methanol was incorporated as the injection solvent for GC/MS analyses.

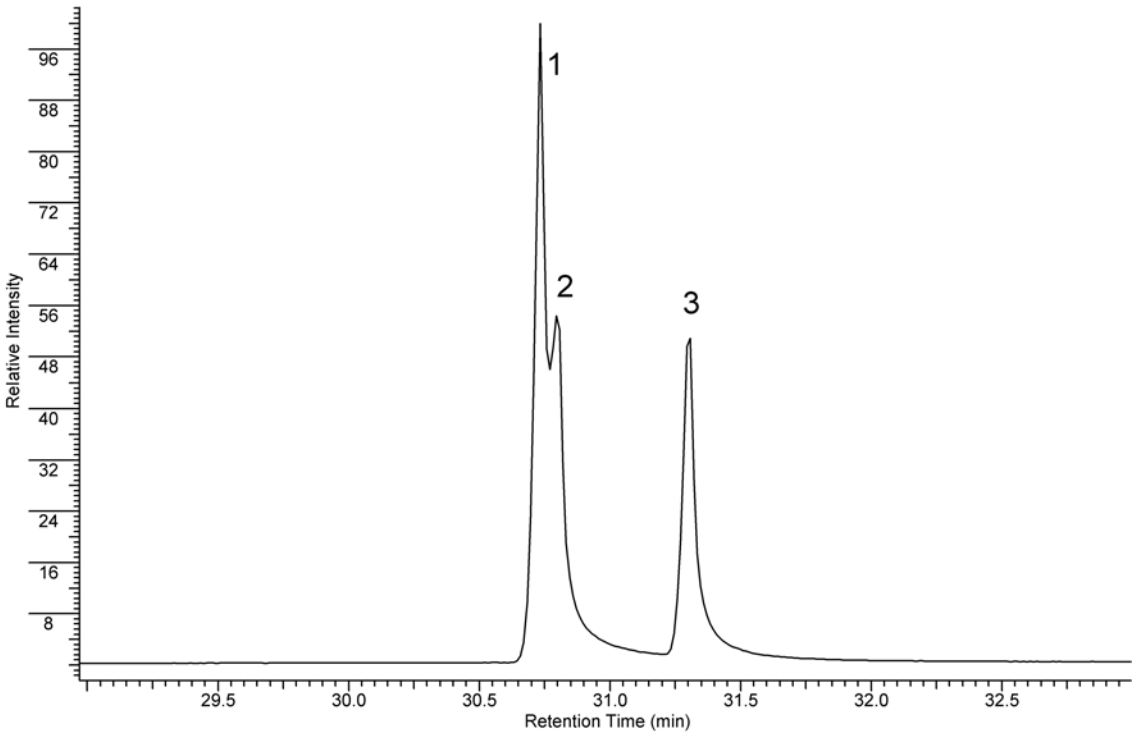

Figure 2. Partial Reconstructed Total Ion Chromatogram Containing Diltiazem and Suspected Impurities/Artifacts. Peak Identification: 1 = Diltiazem, 2 and 3 = Suspected Diltiazem Impurities/Artifacts.

* * * * *

Herein, we report the synthesis and characterization (GC/FID, GC/MS, DESI MS, and NMR) of desacetyl-diltiazem and 2,3-dehydrodesacetyldiltiazem. To identify and characterize the two unknowns, and to determine if they existed as true impurities or were only analytical artifacts, a series of chromatographic experiments were conducted on illicit cocaine samples that contained diltiazem.

Experimental

Solvents, Chemicals, and Materials: All solvents were distilled-in-glass products of Burdick and Jackson Laboratories (Muskegon, MI). N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was obtained from Pierce Chemical (Rockford, IL). All other chemicals were reagent-grade quality and were products of Sigma-Aldrich Chemical (Milwaukee, WI). The standard of (+)-*cis*-diltiazem was also obtained from Sigma-Aldrich Chemical. The illicit cocaine base and cocaine hydrochloride samples were obtained from seized exhibits. Standards of desacetyldiltiazem and 2,3-dehydrodesacetyldiltiazem were synthesized at the DEA Special Testing and Research Laboratory (*vide infra*).

Standard Solutions for Quantitative Determination of Diltiazem and Desacetyldiltiazem: Individual $CHCl_3$/MSTFA solutions containing 38, 95, 189, 473, 946, and 1892 µg/mL of diltiazem hydrochloride and 14, 34, 69, 172, 344, and 688 µg/mL of desacetyldiltiazem hydrochloride, respectively, were prepared. Each solution also contained 100 µg/mL of *para*-fluorococaine as the internal standard (ISTD). Linearity was confirmed over the concentration ranges for each component and linear regression analysis determined that the correlation coefficient (R^2) exceeded 0.9998 for each.

Instrumentation

Gas Chromatography / Flame Ionization Detection (GC/FID): All purity determinations of cocaine, diltiazem, and desacetyldiltiazem were performed on an Agilent (Palo Alto, CA) Model 6890 gas chromatograph. Sample preparation and chromatographic parameters for diltiazem and desacetyldiltiazem were identical to those reported by Casale and Waggoner [8], except that MSTFA was utilized as the derivatizing reagent (instead of BSA). Chromatographic parameters for all cocaine purity determinations were identical to those reported by Piñero and Casale [9].

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 0.25 µm DB-1 (J&W Scientific, Rancho Cordova, CA). The oven temperature was programmed as follows: Initial temperature, 100^{O}C; initial hold, 0.0 min; program rate, 6^{O}C/min; final temperature, 300^{O}C; final hold, 5.67 min. The injector was operated in the split mode (21.5 : 1), at 280^{O}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^{O}C and 230^{O}C, respectively.

Desorption Electrospray Ionization Mass Spectrometry (DESI MS): Molecular weight information derived from $[M+H]^+$ and MS/MS data were obtained using a Thermo (Madison, WI) Accela Liquid Chromatograph coupled with an LCQ (Madison, WI) Advantage MAX Ion Trap Mass Spectrometer. The non-ground sample was positioned near the entrance to the mass spectrometer in the 100% methanol LC eluent spray with a 100 µL/min flow rate. Atmospheric Pressure Ionization (API) parameters included a 5.0 kV spray voltage, 40 psi sheath gas, 300^{O}C capillary temperature, and positive polarity. Full MS data was collected with a scan range of 90 - 500 *m/z*. MS/MS data for 415 *m/z* and 373 *m/z* were collected at 35% *cid* with scan ranges of 110 - 500 *m/z* and 100 - 500 *m/z*, respectively.

Nuclear Magnetic Resonance Spectroscopy (NMR): One and two dimensional NMR analyses were performed on a Varian (Palo Alto, CA) VNMRS 600 MHz NMR using a 3 mm triple resonance Varian indirect detection probe. The samples were prepared in deuterated chloroform containing tetramethylsilane ($CDCl_3$ with TMS, Aldrich Chemical Co., Milwaukee, WI). Gradient versions of the two dimensional NMR experiments HSQC (one bond correlation of hydrogens directly bonded to carbon) and HMBC (correlation of hydrogens 2, 3, or 4 bonds from a carbon) were performed to make the assignments listed in Table 1.

Syntheses

Desacetyldiltiazem Hydrochloride: Diltiazem hydrochloride (1.00 g, 2.22 mmol) and $NaOCH_3$ (0.21 g, 3.89 mmol) were dissolved into MeOH (15 mL) and heated at 75^{O}C overnight in a sealed tube. The MeOH was evaporated to a minimal volume (about 0.5 mL) and then diluted with 5 mL of water and 0.25 mL of saturated aqueous Na_2CO_3. The solution was extracted with $CHCl_3$ (2 x 5 mL). The combined extracts were washed with water (2 x 10 mL) and dried over anhydrous Na_2SO_4, filtered, and evaporated *in vacuo* to a semi-crystalline mass. The product was dissolved in 300 mL diethyl ether, precipitated as the hydrochloride ion-pair with the addition of sufficient ethereal hydrochloric acid, filtered, and dried to provide a white powder (666 mg, 70% yield, 97+% purity).

2,3-Dehydrodesacetyldiltiazem Base: Desacetyldiltiazem hydrochloride (150 mg, 0.37 mmol) and $POCl_3$ (1.0 mL, 10.9 mmol) were heated at 75^{O}C for 9 hours in a sealed tube. The reaction was cooled to 0^{O}C and carefully quenched with cold water (2 mL), and then cold concentrated NaOH until a pH of 9 was achieved. The solution was extracted with $CHCl_3$ (2 x 5 mL). The combined extracts were dried over anhydrous Na_2SO_4, filtered, and evaporated *in vacuo* to provide an off-white powder (150 mg, 71% yield, 98+% purity).

Results and Discussion

Two cocaine base exhibits (Base #1 and Base #2) and one cocaine hydrochloride exhibit were examined for cocaine, diltiazem, and desacetyldiltiazem by GC/FID, as detailed in the Experimental section. The base exhibits both contained sodium bicarbonate, and were specifically selected because they gave differing responses for diltiazem by GC/FID vs. GC/MS. The cocaine hydrochloride exhibit was specifically selected because it contained a relatively high level of diltiazem and trace amounts of suspected desacetyldiltiazem. Partial reconstructed GC/FID chromatograms for the diltiazem/desacetyldiltiazem determinations are illustrated in Figure 3. The quantitative data and relative retention times are given in Tables 2 and 3, respectively.

Base #1 contained 7.1% diltiazem and 0.67% desacetyldiltiazem by GC/FID utilizing $CHCl_3$/MSTFA. However, when analyzed by GC/MS with methanol as the injection solvent (Figure 4a), no diltiazem was detected. Instead, two unknown compounds were observed. The first was identified as desacetyldiltiazem via comparison of its mass spectrum (Figure 5b) and retention time with the synthesized standard. The second had an apparent molecular ion at *m/z* 354 (hereafter referred to as the "354" compound; Figure 6a). We had postulated that the "354" compound was 2,3-dehydrodesacetyldiltiazem (Figure 1), resulting from elimination of water from desacetyldiltiazem. This is analogous to the formation of methyl ecgonidine (anhydroecgonine methyl ester) from cocaine [10]. However, when the "354" compound's mass spectrum and retention time were compared to the synthetic standard, the spectra (Figure 6a and 6b) were dissimilar, and the retention time differed by 1.5 minutes (Table 3). Thus, the "354" compound remains unidentified at this time.

When Base #1 was examined by GC/MS using $CHCl_3$/MSTFA as the injection solvent (Figure 4b), diltiazem was identified by its mass spectrum (Figure 5a), as well as a lower level of desacetyldiltiazem as its TMS derivative (Figure 5c). However, the "354" compound was not detected. When Base #1 was examined by DESI MS, only a small $[M+H]^+$ at *m/z* 373 (consistent with desacetyldiltiazem (mw = 372)) was detected relative to a $[M+H]^+$ at *m/z* 415 (diltiazem). The collective results indicate that use of methanol as the injection solvent for this exhibit results in quantitative degradation of diltiazem to desacetyldiltiazem and the "354" compound.

Base #2 was determined to contain trace diltiazem and 1.3% desacetyldiltiazem via GC/FID utilizing $CHCl_3$/MSTFA. When examined by GC/MS using methanol as the injection solvent (Figure 7a), trace desacetyldiltiazem was identified but no diltiazem or "354" compound were detected. When this exhibit was examined by GC/MS using $CHCl_3$/MSTFA as the injection solvent (Figure 7b), desacetyldiltiazem was easily identified as its TMS derivative, but again, no diltiazem or "354" compound were detected.

GC artifacts are well known when analyzing cocaine base ("crack") exhibits that contain sodium bicarbonate. In this study, sodium methoxide and methanol were used to synthesize desacetyldiltiazem (from diltiazem) in high yield. Since the use of methanol as an injection solvent, coupled with the presence of sodium bicarbonate, will produce sodium methoxide in the injection port [10], the observed degradation of diltiazem to desacetyldiltiazem is not surprising. Since the "354" compound was not identified, the mechanism for its formation is unknown.

The cocaine hydrochloride exhibit was determined to contain 12.0% diltiazem and 0.27% desacetyldiltiazem via GC/FID. When examined by GC/MS using methanol as the injection solvent (Figure 8a), only diltiazem was identified. In this case, use of methanol did not cause degradation of diltiazem because the exhibit contained no sodium bicarbonate. Finally, when the exhibit was examined by GC/MS using $CHCl_3$/MSTFA as the injection solvent (Figure 8b), diltiazem and trace desacetyldiltiazem as its TMS derivative were identified, consistent with the GC/FID analysis (Figure 3c); the "354" compound was not detected in either analysis.

The cocaine hydrochloride exhibit was then converted into "crack" cocaine using the traditional process (i.e., with water and sodium bicarbonate). The quantitative GC/FID data for this exhibit is given in Table 2. The converted sample contained essentially the same percentage of cocaine, diltiazem, and desacetyldiltiazem as was found in the original hydrochloride sample. This indicates that the "crack" conversion process did not cause hydrolysis

of diltiazem to desacetyldiltiazem. However, it is likely that prolonged storage of "crack" cocaine containing diltiazem and sodium bicarbonate (or a stronger base (e.g., Na_2CO_3 or NaOH)) would cause slow hydrolysis to desacetyldiltiazem. Finally, as expected, when this "crack" exhibit was examined by GC/MS using methanol as the injection solvent (Figure 9), only desacetyldiltiazem and the "354" compound were detected.

The physiological effects and consequences of smoking "crack" cocaine adulterated with diltiazem and sodium bicarbonate are unknown.

Conclusions

Desacetyldiltiazem and an uncharacterized artifact (the "354" compound) can be formed as analytical artifacts in gas chromatographic profiles of cocaine exhibits containing diltiazem and sodium bicarbonate. The use of methanol as the injection solvent for these samples causes the formation of these compounds in GC injection ports. However, the use of $CHCl_3$ or $CHCl_3$/MSTFA as injection solvents does not promote the formation of these artifacts. Although desacetyldiltiazem can be present at detectable levels as a *bona fide* impurity in some cocaine exhibits, analysts should be aware that degradation of diltiazem to desacetyldiltiazem and the "354" compound will occur in GC injection ports when analyzing cocaine samples containing diltiazem and sodium bicarbonate, when using methanol as the injection solvent.

References

1. Peters DE. Diltiazem HCl: An analytical profile. Microgram Journal 2004;2(1-4):11-16.

2. Quaglia MG, Donati E, Fanali S, Bossu E, Montinaro A, Buiarelli F. Analysis of diltiazem and its related substances by HPLC and HPLC/MS. Journal of Pharmaceutical and Biomedical Analysis 2005;37:695-701.

3. Grech-Belanger O, Leboeuf E, Langlois S. Assay of diltiazem and desacetyldiltiazem by capillary gas chromatography. Journal of Chromatography 1987;417:89-98.

4. Muszalska I, Jamszol L, Grzeskowiak D. Kinetics of hydrolysis of diltiazem hydrochloride in aqueous solutions. Acta Poloniae Pharmaceutica - Drug Research 2003;60(3):163-8.

5. Dube LM, Mousseau N, McGilveray IJ. High-performance liquid chromatographic determination of diltiazem and four of its metabolites in plasma: Evaluation of their stability. Journal of Chromatography 1988;430:103-111.

6. Andrisano V, Hrelia P, Gotti R, Leoni A, Cavrini V. Photostability and phototoxicity studies on diltiazem. Journal of Pharmaceutical and Biomedical Analysis 2001;25:589-97.

7. Chafetz L, Shah KP. Stability of diltiazem in acid solution. Journal of Pharmaceutical Sciences 1991;80(2):171-2.

8. Casale JF, Waggoner RW. A chromatographic impurity signature profile analysis for cocaine using capillary gas chromatography. Journal of Forensic Sciences 1991;36(5):1312-30.

9. Piñero EL and Casale JF. Quantitation of cocaine by gas chromatography - flame ionization detection utilizing isopropylcocaine as a structurally related internal standard. Microgram Journal 2006;4(1-4):47-53.

10. Casale JF. Methyl esters of ecgonine: Injection port produced artifacts from cocaine base (crack) exhibits. Journal of Forensic Sciences 1992;37(5):1295-1310.

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Table 1. NMR Chemical Shift (in ppm) Data for Proton and Carbon.

position	*cis*-diltiazem HCl		desacetyldiltiazem		2,3-dehydrodesacetyldiltiazem	
benzothiazepin	proton	carbon	proton	carbon	proton	carbon
2	5.02 d	54.1	4.83 d	56.5	-	116.6 **
3	5.12 d	71.2	4.29 d	69.2	7.80 s	134.3
4	-	168.2	-	172.6	-	162.2
5a	-	144.2	-	143.7	-	136.8 **
6	7.52 dd	124.6	7.45 dd	124.6	7.15 dd	117.9
7	7.58 dt	132.1	7.47 dt	131.5	7.21 dt	127.5
8	7.32 dt	128.5	7.25 dt	128.3	7.00 dt	123.3
9	7.72 dd	135.7	7.64 dd	135.5	7.23 dd	127.1
9a	-	127.6	-	128.0	-	119.5 **
3-acetyl C=O	-	169.8	-	-	-	-
3-acetyl CH3	1.90 s	20.4	-	-	-	-
phenyl						
1	-	126.0	-	125.8	-	126.5 **
2,6	7.37 d	130.6	7.24 d	131.1	7.60 d	132.0
3,5	6.90 d	113.9	6.82 d	113.8	6.95 d	113.9
4	-	159.9	-	159.9	-	160.0
methoxy	3.83 s	55.3	3.74 s	55.3	3.85 s	55.3
(CH3)2-N-CH2-CH2	2.84 d	43.5	2.75 d	43.6	2.36 s	45.8
(CH3)2-N-CH2-CH2	2.92 d	43.0	2.83 d	43.1	2.36 s	45.8
(CH3)2-N-CH2-CH2	3.25 ddd	54.4	3.18 ddd	54.2	2.69 dd	56.0
(CH3)2-N-CH2-CH2	3.50 ddd	54.4	3.46 ddd	54.2	2.69 dd	56.0
(CH3)2-N-CH2-CH2	4.42 ddd	44.9	4.26 ddd	44.3	4.20 dd	44.2
(CH3)2-N-CH2-CH2	4.58 ddd	44.9	4.54 ddd	44.3	4.20 dd	44.2

d = doublet, dd = doublet of doublets, ddd = doublet of doublet of doublets, dt = doublet of triplets, s = singlet, ** indicates uncertainty with assignment of quaternary carbons

* * * * *

Table 2. Quantitative Data for Cocaine Exhibits Containing Diltiazem.

Sample	Cocaine%	Diltiazem%	Desacetyldiltiazem%
Base #1	44.2	7.1	0.67
Base #2	54.5	trace	1.3
Hydrochloride	79.9	12.0	0.27
Base #3 [a]	77.3	12.7	0.32

[a] Produced from the Cocaine Hydrochloride Sample.

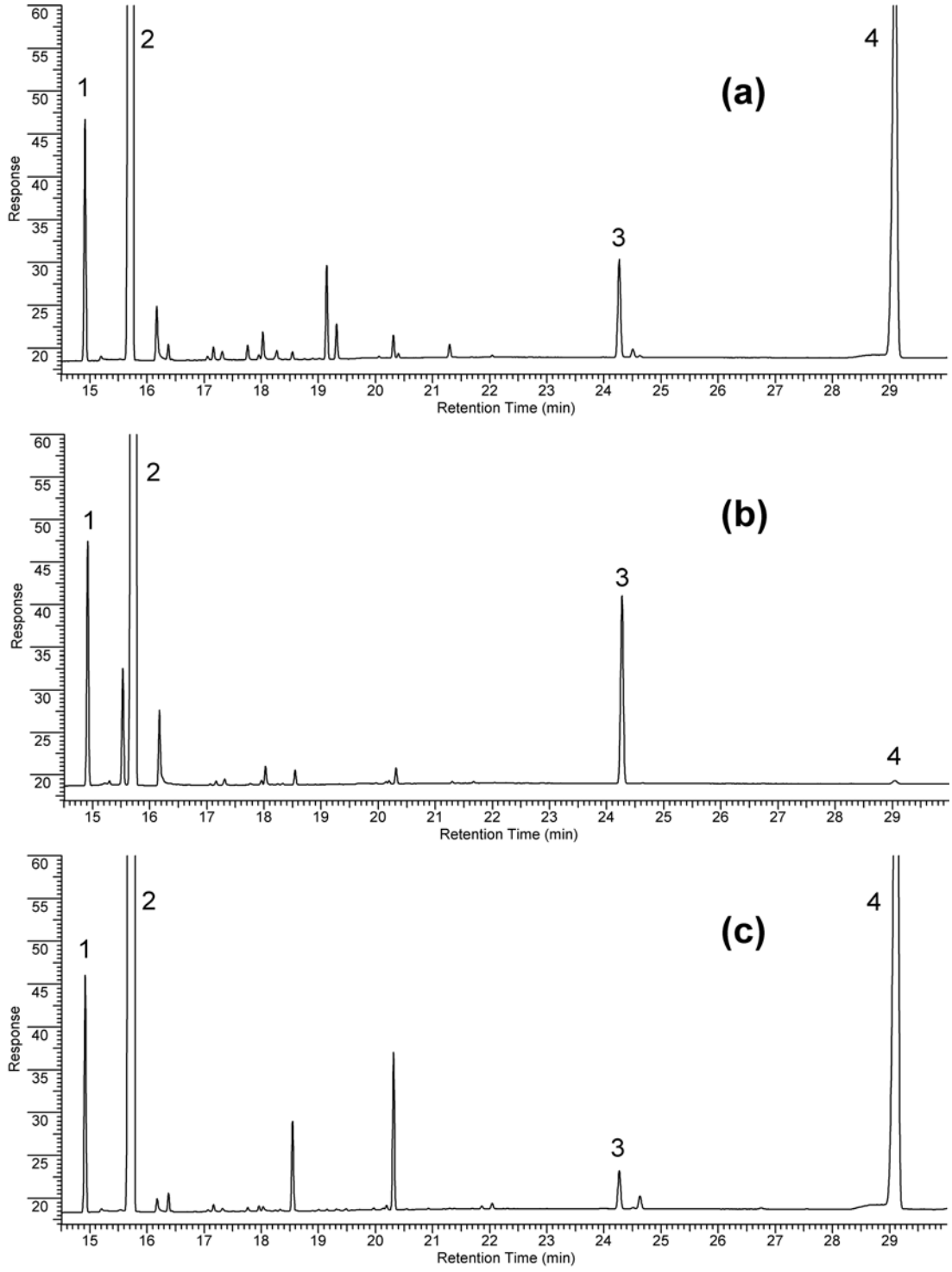

Figure 3. Partial Reconstructed GC/FID Chromatograms of: (A) Cocaine Base Exhibit #1 Containing 44.2% Cocaine, 0.67% Desacetyldiltiazem, and 7.1% Diltiazem; (B) Cocaine Base Exhibit #2 Containing 54.5% Cocaine, 1.3% Desacetyldiltiazem, and Trace Diltiazem; (C) Cocaine Hydrochloride Exhibit Containing 79.9% Cocaine, 0.27% Desacetyldiltiazem, and 12.0% Diltiazem. Peak Identification: 1 = *para*-Fluorococaine; 2 = Cocaine; 3 = Desacetyl-diltiazem-TMS Derivative; and 4 = Diltiazem. $CHCl_3$/MSTFA was Utilized as the Injection Solvent.

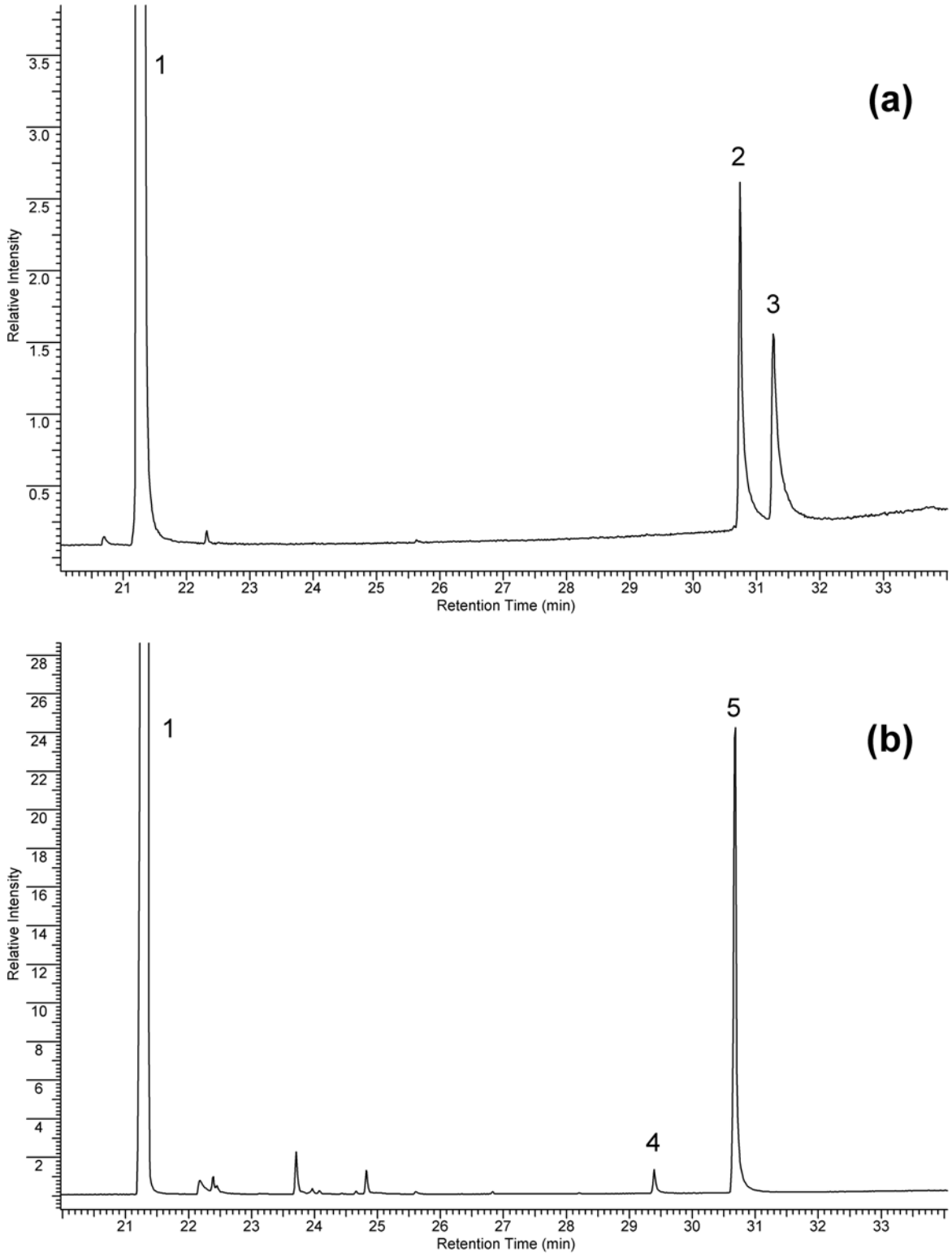

Figure 4. Partial Reconstructed Total Ion Chromatograms of Cocaine Base Exhibit #1 Using: (A) Methanol as Injection Solvent; and (B) $CHCl_3$/MSTFA as Injection Solvent. Peak Identification: 1 = Cocaine, 2 = Desacetyldiltiazem, 3 = Diltiazem Artifact, 4 = Desacetyldiltiazem-TMS, and 5 = Diltiazem.

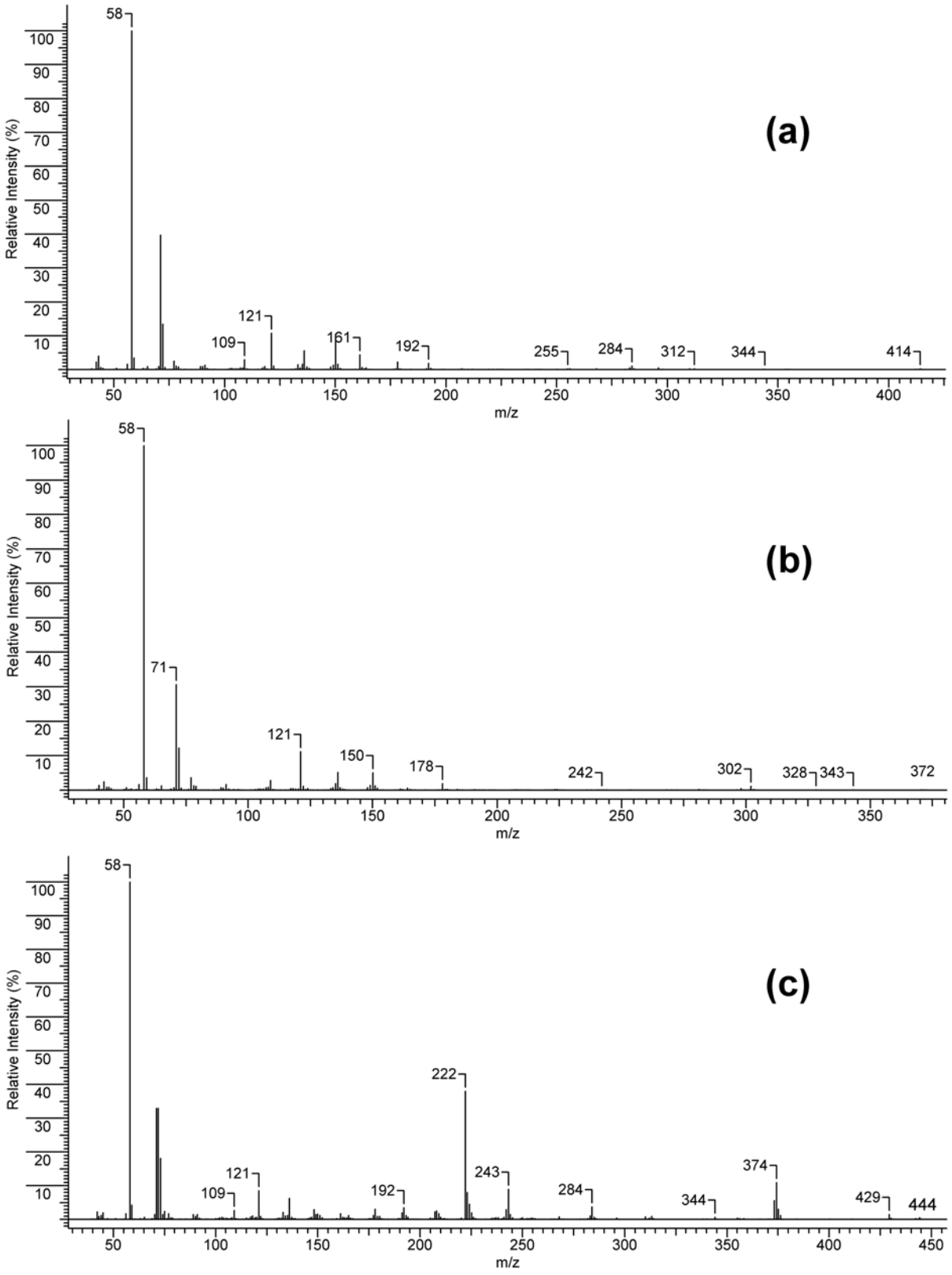

Figure 5. Electron Ionization Mass Spectrum of: (A) Diltiazem; (B) Desacetyldiltiazem; and (C) Desacetyldiltiazem-TMS Derivative.

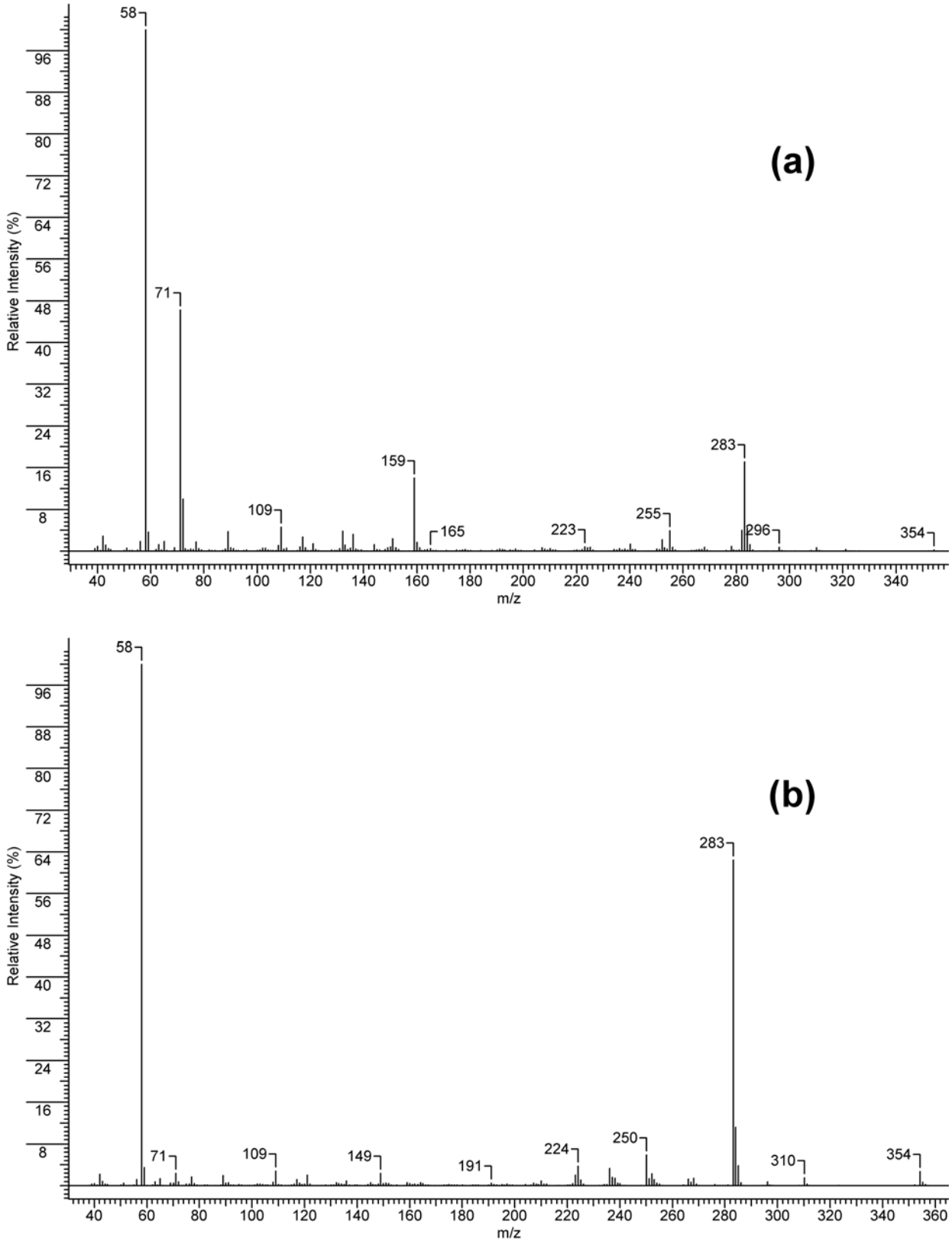

Figure 6. Electron Ionization Mass Spectrum of (A) Diltiazem Artifact; and (B) 2,3-Dehydrodesacetyldiltiazem.

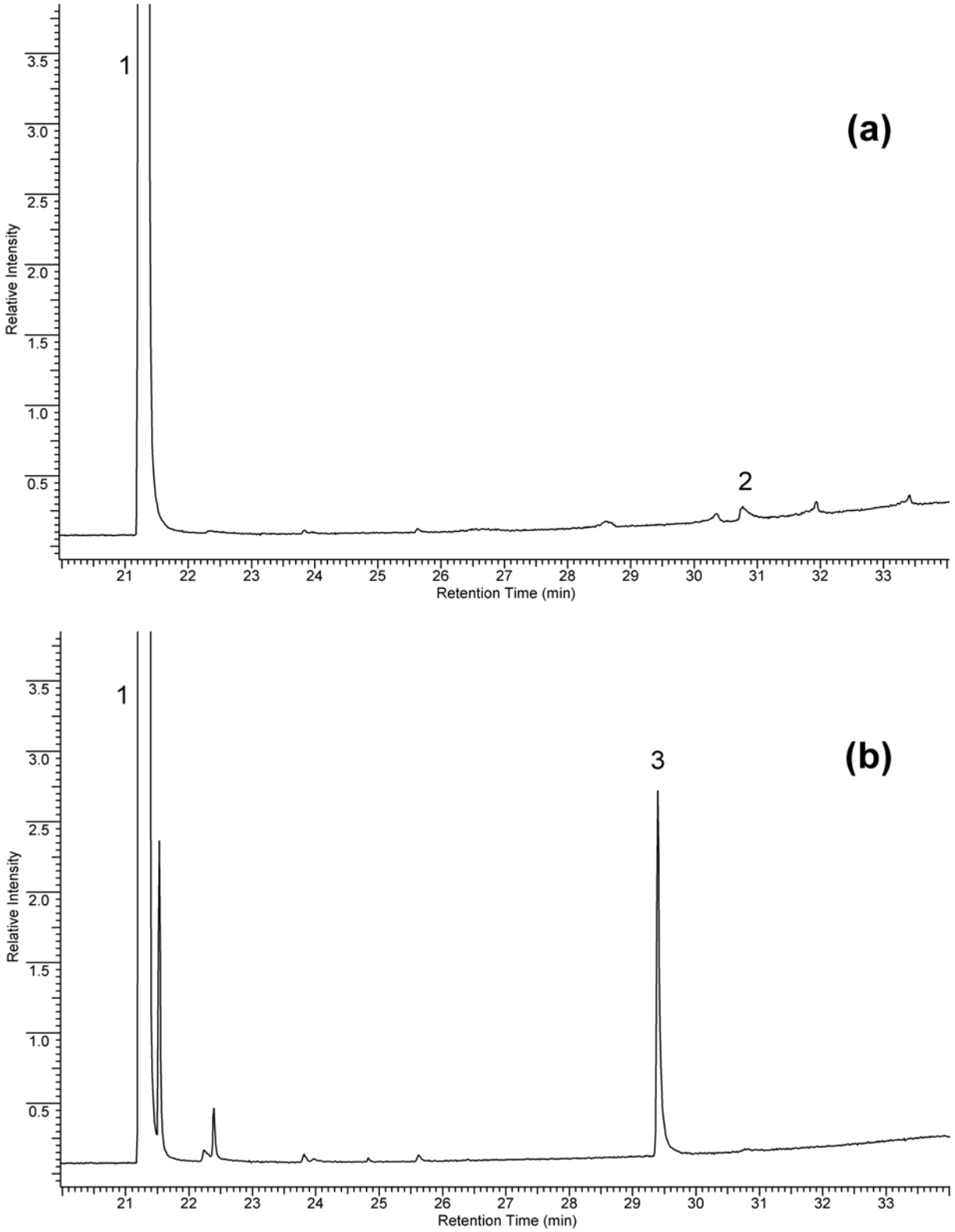

Figure 7. Partial Reconstructed Total Ion Chromatograms of Cocaine Base Exhibit #2 Using: (A) Methanol as Injection Solvent; and (B) $CHCl_3$/MSTFA as Injection Solvent. Peak Identification: 1 = Cocaine; 2 = Desacetyldiltiazem; and 3 = Desacetyldiltiazem-TMS Derivative.

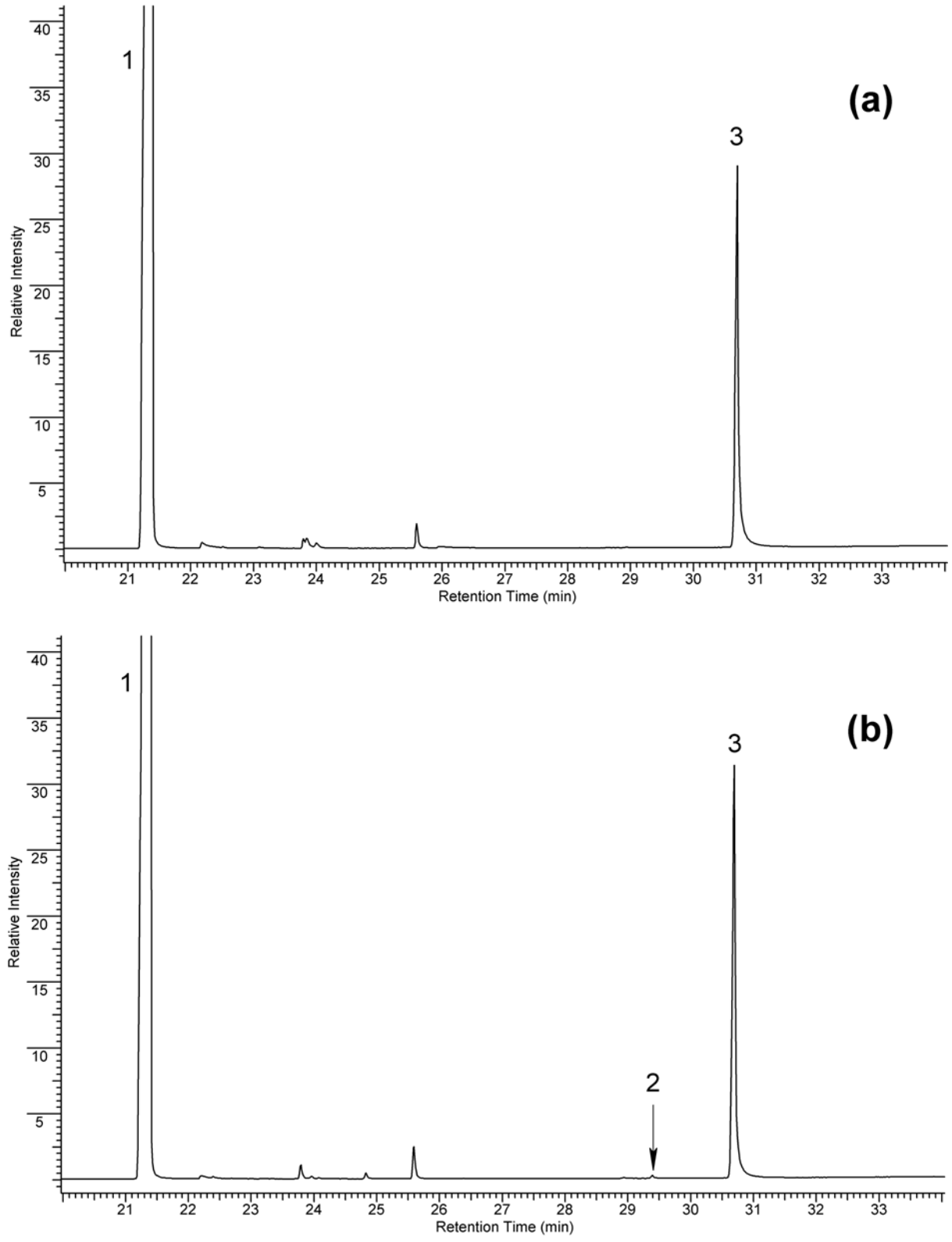

Figure 8. Partial Reconstructed Total Ion Chromatograms of Cocaine Hydrochloride Exhibit Using (A) Methanol as Injection Solvent; and (B) $CHCl_3$/MSTFA as Injection Solvent. Peak Identification: 1 = Cocaine, 2 = Desacetyldiltiazem-TMS, and 3 = Diltiazem.

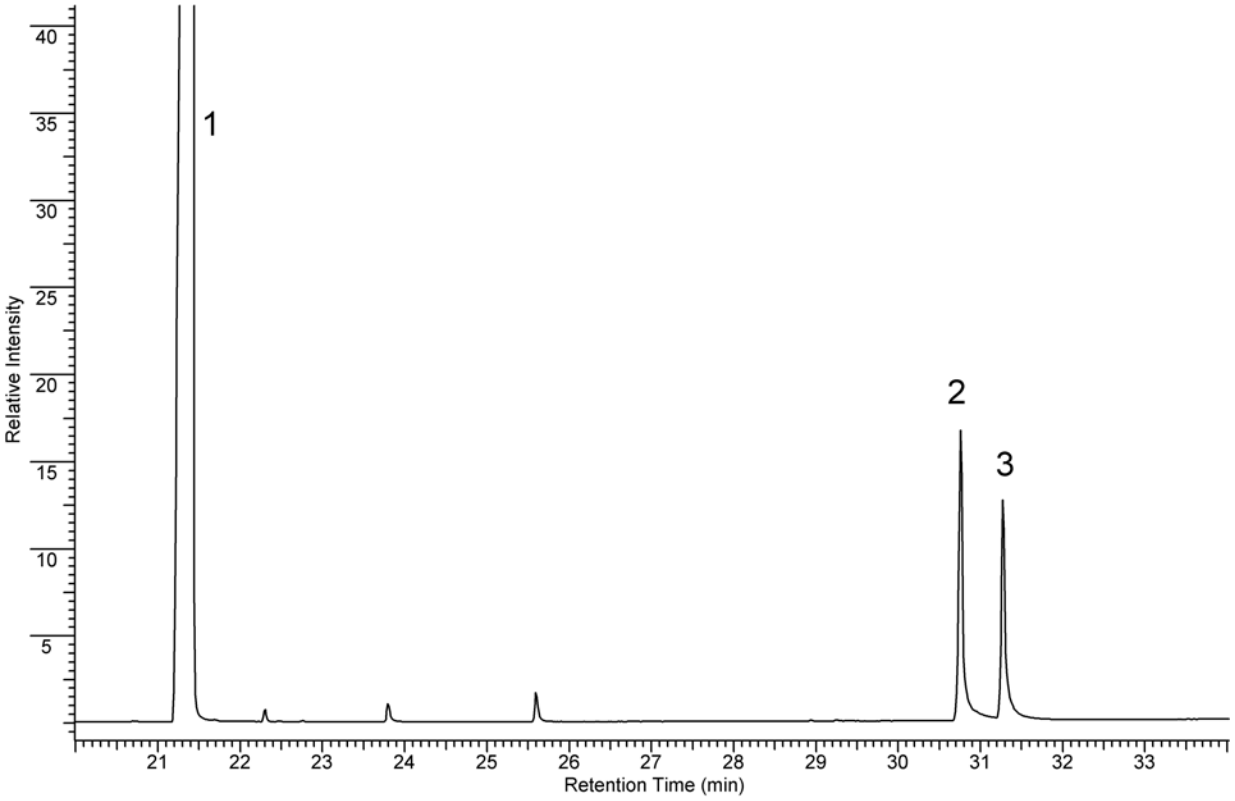

Figure 9. Partial Reconstructed Total Ion Chromatogram of Cocaine Base ("Crack") Produced from the Cocaine Hydrochloride Exhibit. Peak Identification: 1 = Cocaine; 2 = Desacetyldiltiazem; and 3 = Diltiazem Artifact.

* * * * *

Table 3. Retention Times (RT) Diltiazem and Related Impurities [a]

Compound	GC/FID RT	GC/MS RT
para-Fluorococaine [b]	14.91	N/A
Cocaine	15.74	21.43
Desacetyldiltiazem-TMS	24.27	29.40
Diltiazem	29.10	30.68
Desacetyldiltiazem	N/A	30.77
"354" Compound	N/A	31.28
2,3-Dehydrodesacetyldiltiazem	N/A	32.76

[a] Conditions Given in Experimental Section. RT Values Given in Minutes.
[b] Internal Standard.

* * * * *

Technical Note

Etodolac: An Analytical Profile

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ABSTRACT: Etodolac (Lodine) has been identified in various submissions of illicit heroin seizures in the northeast region of the United States. Etodolac is a nonsteroidal anti-inflammatory drug used in the treatment of mild to moderate pain, and helps relieve symptoms of arthritis, such as inflammation, swelling, stiffness, and joint pain. Analytical data, including gas chromatography, infrared spectroscopy, Raman spectroscopy, mass spectroscopy and proton nuclear magnetic resonance spectroscopy are presented.

KEYWORDS: Etodolac, Heroin, Adulteration, NSAID, Analysis, Forensic Chemistry

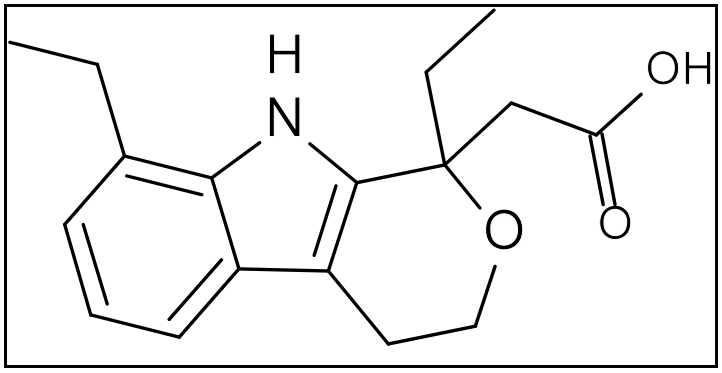

Figure 1. Structure of Etodolac ($C_{17}H_{21}NO_3$; mw = 287.4).

Introduction

The presence of pharmacologically active adulterants and inactive diluents as cutting agents in illicit heroin exhibits is common, and dynamic. Over the past 9 years, this laboratory has received increasing numbers of heroin submissions containing varying amounts of etodolac (trade name Lodine, Figure 1), 1,8-diethyl-1,3,4,9-tetrahydropyranol[3,4-b]indol-1-acetic acid, a prescription nonsteroidal anti-inflammatory drug (NSAID) [1,2] (see Figure 1). Approved by the U.S. Food and Drug Administration in 1997 for acute and long term use in the management of osteoarthritis and rheumatoid arthritis, etodolac is produced by multiple pharmaceutical companies in both capsule and tablet forms [3]. Herein, standard analytical data (GC/FID, FTIR/ATR, Raman, GC/MS, and ^{1}H-NMR) is presented for etodolac.

Experimental

Etodolac Standard: Sigma-Aldrich, Inc. (St. Louis, MO); Lot #121K4049. Because etodolac is *de facto* an indole propionic acid (see Figure 1), it is presumed to be a zwitterionic compound.

Gas Chromatography / Flame Ionization Detector (GC/FID):

Instrument	Agilent 6890N with a flame ionization detector
Column	HP-5, 30 m x 0.25 mm x 0.25 μm film thickness
Injector Temperature	270^{O}C
Oven Temperature	175^{O}C for 1.0 min, ramped 15^{O}C/min to 280^{O}C for 3.0 min
Carrier Gas	Hydrogen ramped flow 2.5 mL/min for 5 min to 3.5 mL/min; split ratio 50 : 1

Utilizing the above experimental parameters, etodolac breaks down into four peaks, three minor peaks followed by one major peak. The retention time for the three minor peaks are 3.940, 4.974, and 5.068 minutes followed by the major peak at 6.056 minutes. The retention times relative to heroin are 0.525, 0.659, 0.676, and 0.807, respectively.

Fourier Transform Infrared Spectroscopy (FTIR/ATR):

Instrument	Perkin Elmer Spectrum One
Number of Scans	16
Resolution	4.000 cm^{-1}
Wavenumber Range	4000 cm^{-1} to 650 cm^{-1}

Data was obtained by direct analysis using an attenuated total reflectance (ATR) attachment on FTIR. The data was not ATR corrected [Figure 2].

Fourier Transform Raman Spectroscopy (FT Raman):

Instrument	Thermo Nicolet Nexus 670 FTIR
Number of Scans	8
Resolution	8.000 cm^{-1}
Wavenumber Range	3701 cm^{-1} to 100 cm^{-1}

Data was obtained by direct analysis using a Smart Golden Gate ZnSe Accessory on FTIR. The data was corrected with the automatic smooth function [Figure 3].

Gas Chromatography / Mass Spectrometry (GC/MS):

Instrument	Agilent 5973
Column	HP-5 MS, 30 m x 0.25 mm x 0.25 μm film thickness
Injector Temperature	255^{O}C
Oven Temperature	90^{O}C for 1.35 min, 35^{O}C/min to 290^{O}C
Carrier Gas	Helium with split ratio = 35 : 1
MS Quad	150^{O}C
MS Source	230^{O}C
Scan Range	40 - 550 amu

Electron impact mass spectrometry data shows a molecular ion at *m/z* 287 and a base ion at *m/z* 228 [Figure 4].

Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR):

Data was obtained using a Varian Mercury 400 MHz NMR. The sample was prepared at a final concentration of 25.2 mg/mL in deuterated methanol (CD_3OD) containing TMS (tetramethylsilane, $Si(CH_3)_4$) as the 0 ppm reference. The spectrum was obtained with 8 scans using a 1.0 second delay, 45^O pulse, and a 2.99 second acquisition time. The scan width was 6410 Hz [Figure 5]. Note that etodolac cannot be analyzed in D_2O, because it is insoluble in water.

Results and Discussion

Although the levels of adulteration have widely varied, etodolac is typically present in heroin at approximately 1% or below. With the exception of the GC/FID chromatography, the presented data is unremarkable. When etodolac is analyzed by GC/FID, four peaks are present due to the thermal breakdown of the compound (the breakdown products were not identified). The presented data will assist in the identification of etodolac.

References

1. http://www.mayoclinic.com/health/drug-information/DR602209 (last accessed Dec 6, 2008).

2. Merck Index. 13th Ed. Whitehouse Station, NJ: Merck Research Laboratories, 2001; 685.

3. http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.Overview&DrugName=ETODOLAC (last accessed Dec 6, 2008).

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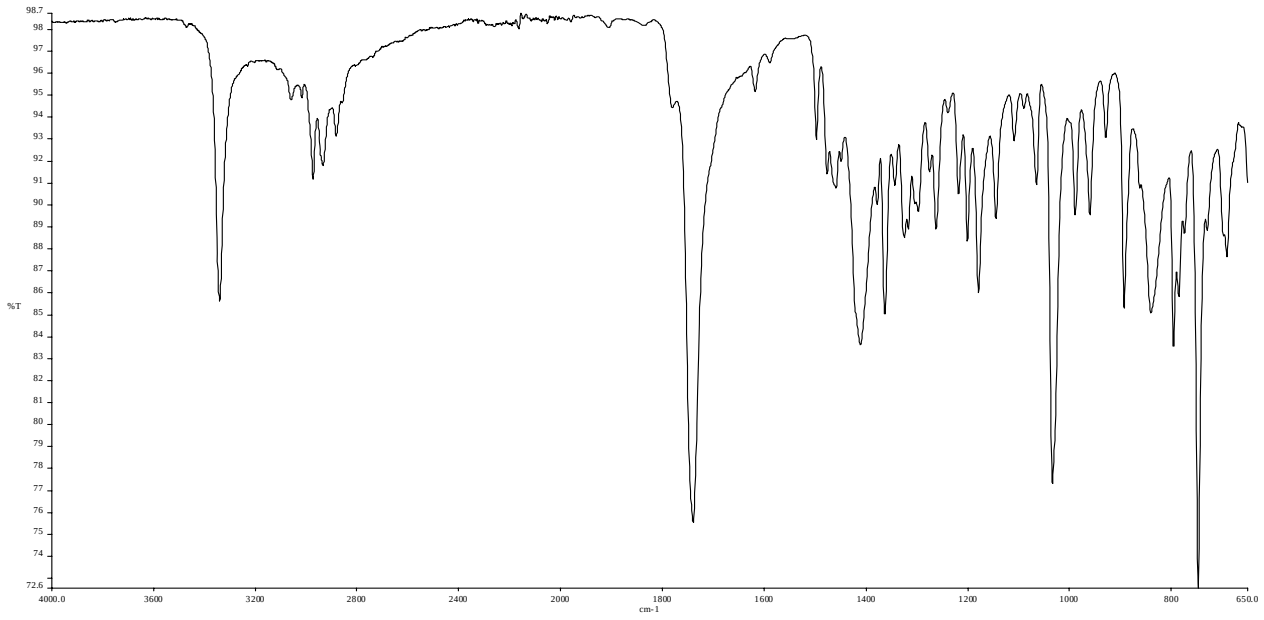

Figure 2. FTIR/ATR Spectrum of Etodolac.

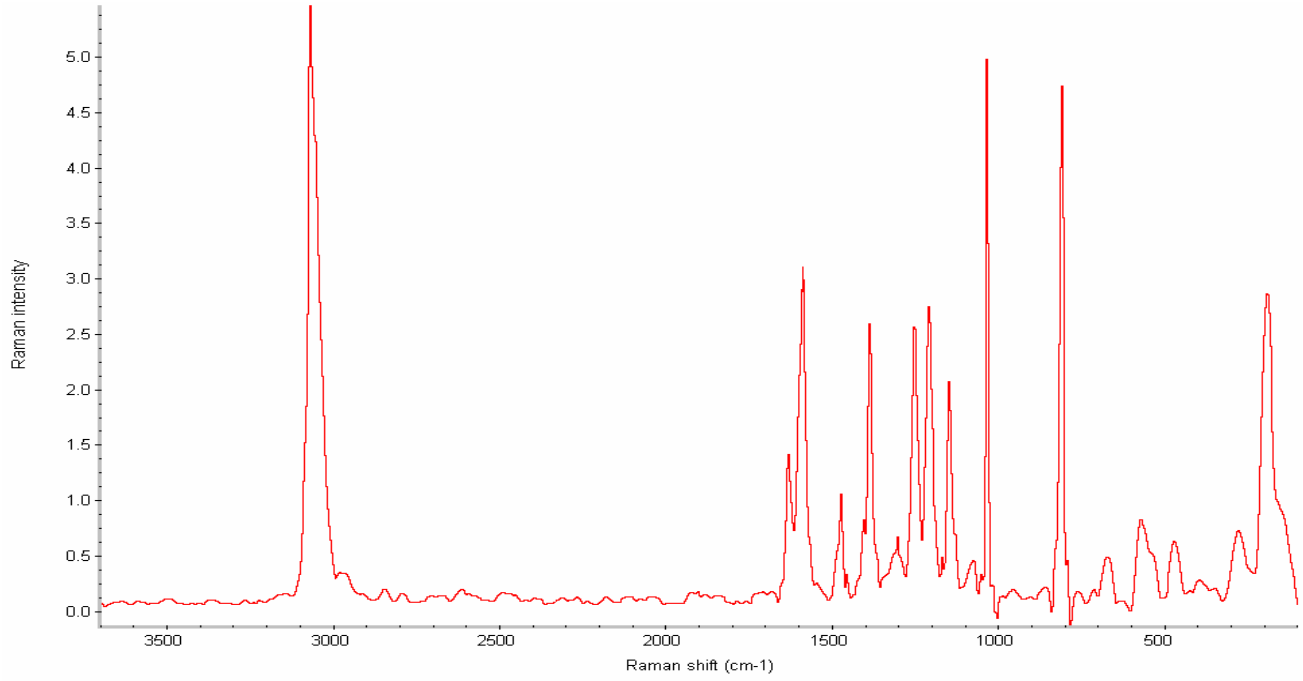

Figure 3. FT Raman Spectrum of Etodolac.

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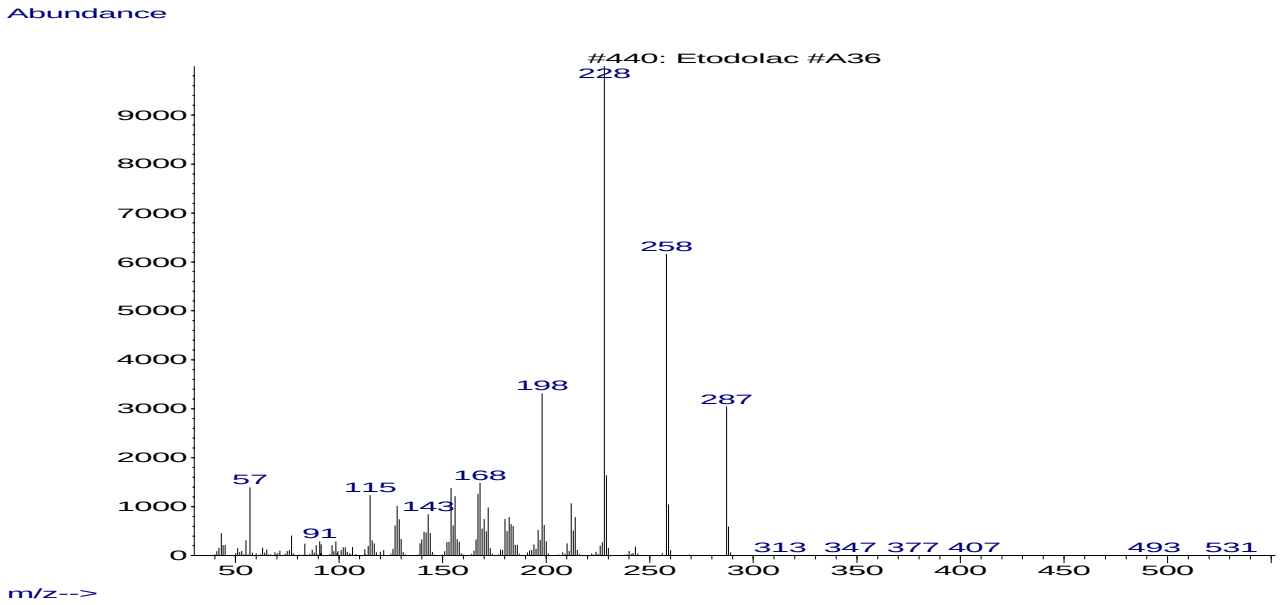

Figure 4. Electron Impact Mass Spectrum of Etodolac.

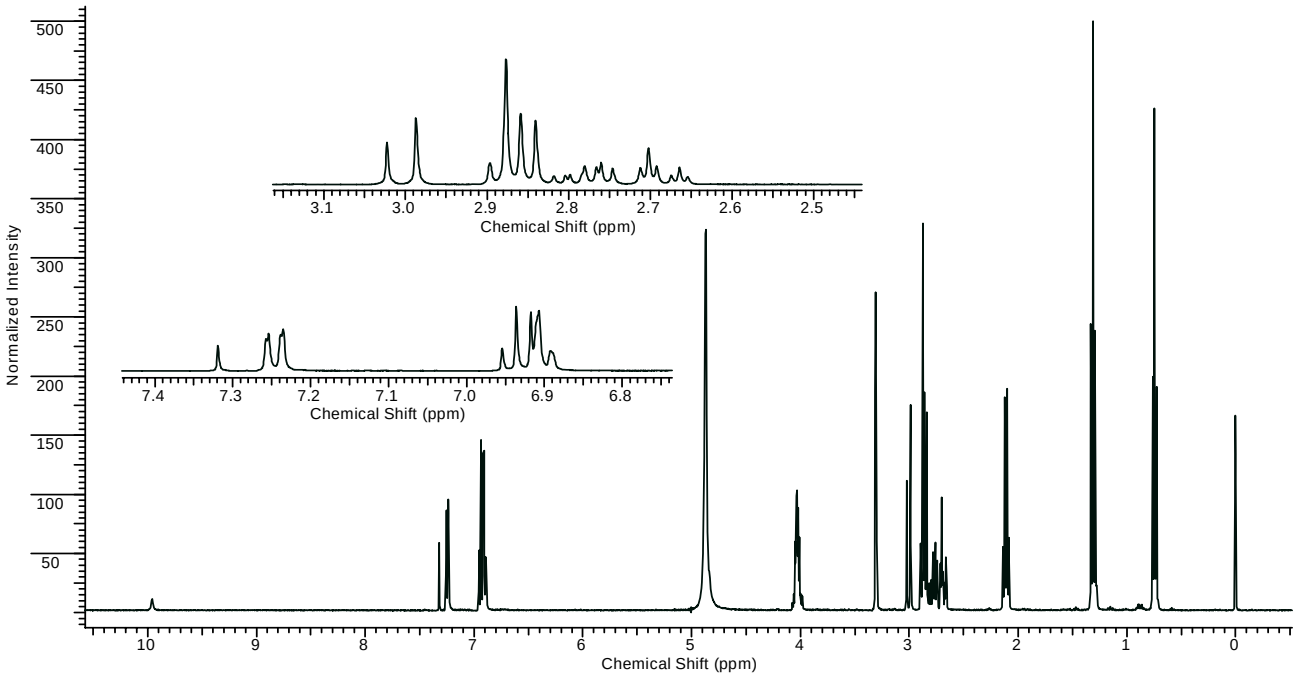

Figure 5. 400 MHz ^{1}H-NMR Spectrum of Etodolac in CD_3OD.

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Technical Note

Determination of Cocaine in Various South American Commercial Coca Products

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ABSTRACT: Cocaine content is provided for several coca products including coca tea, medicinal tonics and rubs, and alcohol. Although these products are legal in most of South America, they are considered controlled substances in the United States and in most other countries. The cocaine was separated from complex matrices utilizing trap column chromatography. Gas chromatography / mass spectrometry / selective ion monitoring was used for cocaine identification and quantitation. The amount of cocaine in for these products ranges from 0.00 - 0.65 μg/mg.

KEYWORDS: Cocaine, Coca Products, Quantitation, Mass Spectrometry, Selective Ion Monitoring, Forensic Chemistry

Introduction

Although cocaine is controlled virtually worldwide, coca is legitimately cultivated in the South American countries of Peru and Bolivia. While it is illegal to extract the cocaine from the coca leaf in all of these counties, the coca leaf and various coca leaf extracts have long been legally used to relieve fatigue, hunger, and provide nutritional value. Along these lines, Duke *et al.* determined that ingesting coca leaves met the recommended dietary allowance for calcium, iron, phosphorous, vitamin A, vitamin B, and vitamin E [1]. Traditional South American medicine also uses coca leaf to alleviate headaches, rheumatism, abrasions, malaria, ulcers, asthma, and parasites, and studies have shown that several of these traditional treatments are valid [2].

However, in the United States and in many other countries, it is illegal to obtain, possess, or use coca products. The U.S. Code of Federal Regulations (CFR) lists coca leaves and any derivative or preparation of coca leaves as Schedule II substances [3]. The CFR excludes substances that contain de-cocainized coca leaves and leaf extracts that do not contain cocaine and ecgonine [3]. Numerous studies have quantitated the amount of cocaine and alkaloids found in coca leaf [4-7]. Studies have also determined the percent cocaine in coca tea and how it is metabolized in the body [8-9].

Although the analyses of coca leaf, coca extracts, and illicit cocaine exhibits are routine, analyses of food, medicinal, and beauty products that contain small amounts of coca leaf or coca extracts can be challenging due to the variety and complexity of the matrices. This investigation determined the amounts of cocaine in various matrices, including coca tea, medicinal tonics, rubs, alcohol, beauty products, and food products. Cocaine was isolated from the matrices via trap column chromatography, and identified and quantitated via gas chromatography / mass spectrometry / selective ion monitoring (GC/MS/SIM).

Experimental

Materials: Chloroform was a product of Burdick and Jackson Laboratories (Muskegon, MI). Diethylamine (DEA) and acid-washed Celite 545 were products of Sigma-Aldrich Chemical (Milwaukee, WI). Isopropylcocaine (used as an internal standard, ISTD) was synthesized in-house [10]. All standard solutions were prepared in 50 mL acid-washed glass volumetric vials. Trap column chromatography was performed using Lab Glass columns (260 mm x 22 mm). All the commercial coca products that were analyzed in this study were obtained from open markets in La Paz, Bolivia.

Gas Chromatography / Mass Spectrometry / Selective Ion Monitoring (GC/MS/SIM): 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 was fitted with a 30 m x 0.25 mm ID fused silica capillary column coated with 0.25 μm DB-1 (J&W Scientific, Rancho Cordova, CA). The oven temperature was programmed as follows: Initial temperature 100^{O}C; initial hold 0.0 min; program rate 6.0^{O}C/min; final temperature 300^{O}C; final hold 5.67 min. The injector was operated in the split mode (21.5 : 1) at 280^{O}C. The MSD was operated in selective ion monitoring (SIM) mode. The fragment ions 82.1, 182.1, and 303.2 Daltons were monitored with a 500 millisecond dwell time for cocaine. The fragment ions 82.1, 210.2, and 331.2 Daltons were monitored with a 500 millisecond dwell time for isopropylcocaine. The auxiliary transfer line to the MSD and the source were maintained at 280^{O}C and 230^{O}C, respectively.

Standard Solutions for Quantitative Determination of Cocaine: Individual $CHCl_3$ solutions each containing 22.50 μg/mL of isopropylcocaine and 9.90, 20.62, 25.57, 30.51, 35.47, and 41.24 μg/mL of cocaine base, respectively, were prepared. Linearity was confirmed over the concentration ranges; linear regression analysis determined the correlation coefficient (R^2) as exceeding 0.996.

Sample Preparation and Extractions: Cocaine was isolated from wax-like, aqueous, and candy-like coca products utilizing a slight modification of the trap column chromatography utilized by Moore *et al.* [5].

Wax-Like Samples: Between 500 - 1000 mg of wax-like samples were dissolved in 1 mL of $CHCl_3$ containing 22.50 μg/mL of isopropylcocaine and 1 mL of water-saturated $CHCl_3$ (hereafter WSC). This solution was vortexed and then placed onto a glass chromatographic column containing 4 g of Celite 545 mixed with 2 mL of 0.36 N sulfuric acid. The column was eluted with 50 mL of WSC (discarded) followed by 50 mL WSC containing 500 μL DEA (collected and evaporated *in vacuo* to a residue). The residue was reconstituted in approximately 1 mL of $CHCl_3$, dried over anhydrous sodium sulfate, filtered, and examined via GC/MS/SIM.

Aqueous Samples: Between 2 - 10 mL of liquid samples were evaporated *in vacuo* to a residue. The residue was reconstituted in a mixture of 1 mL of $CHCl_3$, 1 mL of the $CHCl_3$ containing 22.50 μg isopropylcocaine, and 3 drops of water. This solution was placed onto a glass chromatographic column containing 4 g of Celite 545 mixed with 2 mL of 0.36 N sulfuric acid. The cocaine from these samples was isolated and analyzed in the same manner as detailed above under "Wax-Like Samples."

Candy Samples: Two pieces (approximately 8 g) of candy were ground and dissolved in water (approximately 2 mL). The solution was basified with saturated NaOH until pH 8 - 9, and the cocaine was extracted with 1 mL of $CHCl_3$ containing 22.50 μg isopropylcocaine and placed onto a glass chromatographic column containing 4 g of Celite 545 mixed with 2 mL of 0.36 N sulfuric acid. The cocaine from these samples was isolated and analyzed in the same manner as detailed above under "Wax-Like Samples."

Leaf, Vitamin, and Alcohol Samples: Cocaine was isolated from coca tea and the coca vitamin by weighing approximately 2 mg directly into a GC vial containing 1 mL of $CHCl_3$ containing 22.50 μg of isopropylcocaine and 250 μL of DEA. The samples were examined via GC/MS/SIM. A 250 μL aliquot of the alcohol sample was added to 1 mL of $CHCl_3$ containing 22.50 μg of isopropylcocaine spiked with 50 μL of DEA.

Results and Discussion

Two typical GC/MS/SIM chromatograms are shown in Figure 1. Figure 1a (Vitamins) illustrates a relatively low concentration of cocaine, while Figure 1b (Medicinal Tonic) illustrates a significant quantity of cocaine. Collectively, the analyses indicated that the products contained from 0.00 - 0.65 μg/mg cocaine (Table 1). The cocaine (if any) in the shampoo could not be determined due to the difficulty of isolating cocaine from this matrix. In addition, one of the alcohol products did not contain cocaine (see below). The appearance and manufacturer of all of the aqueous medicinal tonics was the same; their cocaine content ranged from 0.01 - 0.38 μg/mg, indicating that there were significant differences in the amount of coca leaf added to each product. All of the medicinal rubs had the same waxy appearance and a strong odor of coca leaf; their cocaine content ranged from 0.01 - 0.10 μg/mg (relatively low). Visual inspection of these latter products confirmed that only small amounts of particles were distributed (unevenly) throughout the waxy matrix. The cocaine content in the teas and the ground leaf samples ranged from 0.46 - 0.65 μg/mg. The cocaine content of the ground leaf and tea samples were consistent with coca leaf [4-7]. Both candy samples were brown, sticky substances that smelled similar to coca leaf; one had the consistency of chewing gum, while the other was more like a hard candy. They were determined to contain 0.003 - 0.01 μg/mg cocaine. The Ajayu de Coca Pachamama (liquor) was a clear liquid with a distinct alcohol smell. Despite its suggestive name, this product did not contain cocaine. The Ron Fernando Ron De Coca (liquor) was a green liquid that smelled similar to coca leaf and alcohol; it contained 0.22 μg/mg of cocaine.

Conclusions

Trap column chromatography can be utilized to isolate cocaine from complex bulk matrices. The utilization of GC/MS/SIM, coupled with a structurally related internal standard, gave excellent sensitivity and linearity, and could determine cocaine content down to the microgram per gram level. Utilizing the described methodology, cocaine was readily detected and determined in all the commercial products except for a shampoo and one alcoholic liquor. In the case of coca leaf, the described method was able to determine cocaine content from as little as 2 milligrams of sample.

References

1. Duke JA, Aulik D, Plowman T. Nutritional value of coca. Botanical Museum Leaflets Harvard University 1975;24(6):113-8.

2. Weil AT. The therapeutic value of coca in contemporary medicine. Journal of Ethnopharmacology 1981;3(2-3):367-76.

3. Code of Federal Regulations 2002;21(1308.12):85.

4. Holmstedt B, Jaatmaa E, Leander K, Plowman T. Determination of cocaine in some South American species of Erythroxylum using mass fragmentation. Phytochemistry 1977;16:1753-5.

5. Moore JM, Casale JF, Klein RFX, Cooper DA, Lydon J. Determination and in-depth chromatographic analyses of alkaloids in South America and greenhouse cultivated coca leaves. Journal of Chromatography A 1994;659:163-75.

6. Plowman T, Rivier L. Cocaine and cinnamoylcocaine content of Erythroxylum species. Annals of Botany 1983;51:641-59.

7. Rivier L. Analysis of alkaloids in leaves of cultivated Erythroxylum and characterization of alkaline substances used during coca chewing. Journal of Ethnopharmacology 1981;3:313-35.

8. Jenkins AJ, Llosa T, Montoya I, Cone, E. Identification and quantitation of alkaloids in coca tea. Forensic Science International 1996;77:179-89.

9. Engelke BF, Gentner WA. Determination of cocaine in "Mate de Coca" herbal tea. Journal of Pharmaceutical Sciences 1991;80(1):96.

10. Piñero EL, Casale JF. Quantitation of cocaine by gas chromatography - flame ionization detection utilizing isopropylcocaine as a structurally related internal standard. Microgram Journal 2006;4(1-4):47-53.

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Table 1. Cocaine Content of Selected Coca Products.

Product	Treatment	Matrix	µg/mg Cocaine
Ron Fernando Ron De Coca	liquor	Alcohol	0.22
Ajayu de Coca Pachamama	liquor	Alcohol	Not detected
Adelgazante	Diet	Aqueous	0.39
Anti Diabetico	Diabetes	Aqueous	0.15
Tos Asma	Asthma	Aqueous	0.29
Prostata	Prostate Problems	Aqueous	0.38
Parasitos	Parasites	Aqueous	0.33
Ulceras	Ulcers	Aqueous	0.01
Tonico	Tonic	Aqueous	0.26
Pomada Natural de Coca Contra Dolores: Reumaticos, Musculares, Varices Y Huesos	rheumatic, muscular, veins, and bones	Wax	0.07
Pomada Natural de Coca Para: Artritis Y Gota	Cream for arthritis and foot pain	Wax	0.10
Pomada Natural de Coca Para: Hemorroides	Cream for hemorrhoids	Wax	0.04
Pomada de Molle	Cream for moles	Wax	0.01
Chicle Cocaplus	Chewing gum	Candy	0.01
Caramelos Y ElixirEnergizante de Altura	Caramel candy for energy booster	Candy	0.003
Mate Windsor	Tea	Tea	0.59
Kokasana	Tea	Tea	0.46
Harina De Coca	Nutritional Supplement	Ground Leaf	0.65
Coca Premium	Nutritional Supplement	Ground Leaf	0.59
Shampoo	Beauty Product	Liquid	N/A

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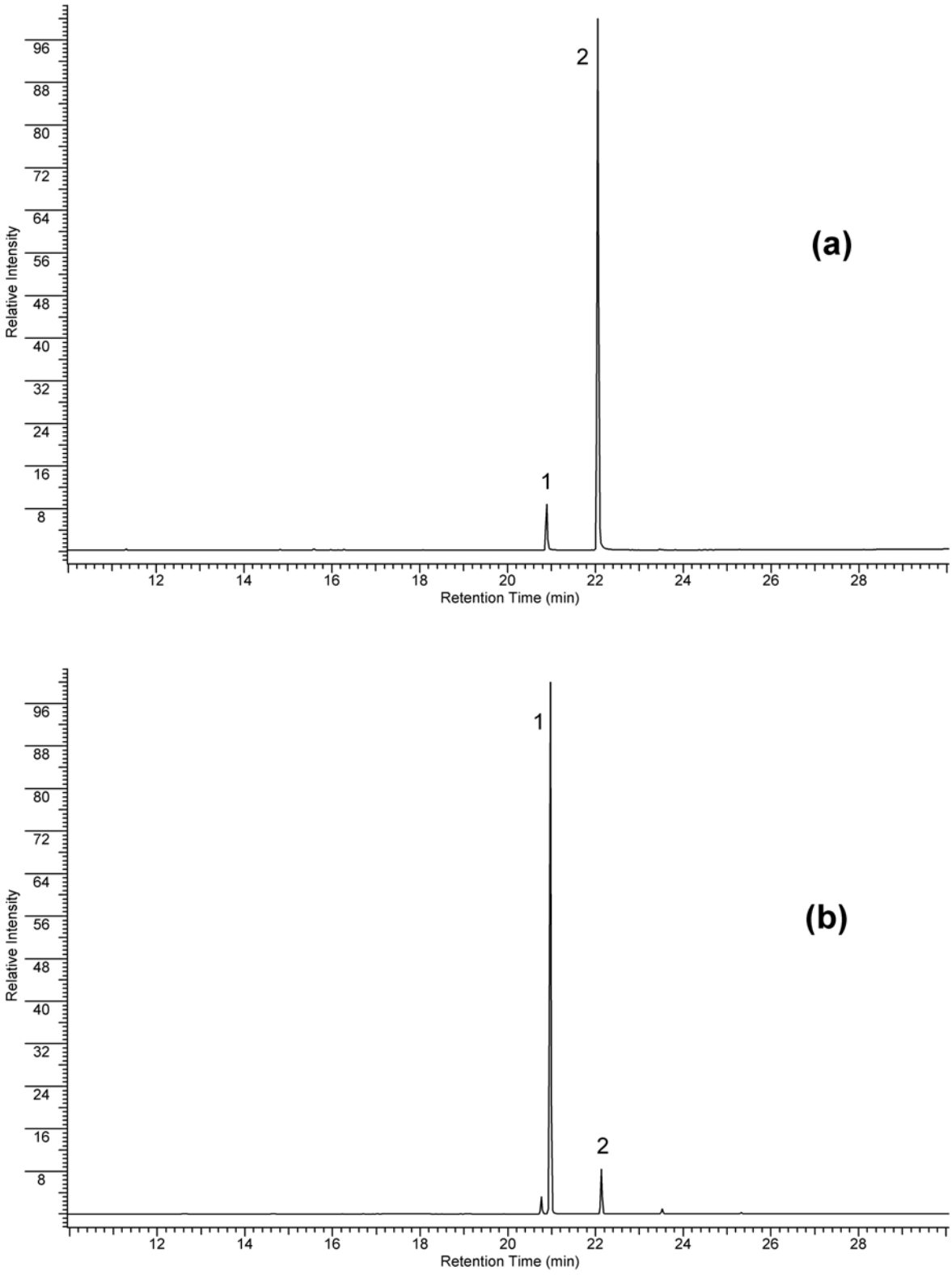

Figure 1. Partial Reconstructed Selected Ion Chromatograms of: (A) 2.73 mg of Coca Premium Vitamins Containing 0.593 µg/mg Cocaine; and (B) 5.00 g of Adelgazante Medicinal Tonic Containing 0.385 µg/mg Cocaine. Peak Identification: 1 = Cocaine; and 2 = Isopropylcocaine (ISTD).

"Crack" Cocaine: A Study of Stability over Time and Temperature

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ABSTRACT: Changes in the appearance, weights, purity levels, and alkaloidal profiles of 146 laboratory-prepared "crack" cocaine exhibits stored under different temperatures and packaging types, were studied over a one year period. An accelerated aging study (elevated temperature, one month) was also performed with 2 "crack" cocaine exhibits, to simulate very long-term or higher temperature storage. The results indicate that higher purity "crack" that was prepared by the classic method is reasonably stable over 12 months if stored at or below 20^{O}C, irrespective of incidental moisture and/or packaging type. However, extended storage times and/or elevated temperatures can result in weight loss and/or degradation, especially for samples sealed in plastic bags or heat-sealed evidence envelopes.

KEYWORDS: Cocaine, "Crack," Stability, Storage, Degradation, Weight Loss, Forensic Chemistry

Introduction

Cocaine base, commonly referred to as "crack," is a major drug of abuse. Forensic drug analysts routinely analyze "crack" exhibits and present their findings in court. Over the past 20 years, many instances of weight loss and degradation of stored "crack" exhibits have been noted, especially for exhibits stored for long time frames or under non-ideal conditions. Such changes can be an issue for forensic chemists when testifying at trial, particularly if the original results are not in agreement with reanalyses that were conducted within the same laboratory, at a different forensic laboratory, or independently by chemists employed by the defense.

Weight changes in cocaine hydrochloride exhibits have been previously studied, with research concluding that weight gain is often due to water absorption associated with packaging [1]. Cocaine hydrochloride degradation processes have also been previously studied, and the resulting products have been characterized [2-5]. The stability of cocaine hydrochloride in aqueous solutions has also been extensively researched [6-8]. Minor alkaloids present in illicit cocaine exhibits, such as the truxillines, have also been examined for stability over time, with the conclusion that a direct relationship exists between the sample age and the increased levels of the truxilline degradation products, i.e., the truxillic and truxinic acids [9].

However, a search of the literature found no studies on the stability of "crack" cocaine, or the products resulting from the degradation of "crack." In this study, the stability of "crack" was monitored in two independent experiments. In the first, 146 prepared samples were stored for one year at three different temperatures (room temperature (20^{O}C), refrigerator (5^{O}C), and freezer (-5^{O}C)) and two different types of packaging (standard zip-lock plastic bags and Heat Sealed Evidence Envelopes (HSEEs)). In this case, the exhibits were examined for weight loss, changes in purity, and degradation on a monthly basis. In the second experiment, two prepared samples were stored for one month at 65^{O}C, one unsealed and one sealed in a zip-lock plastic bag. The elevated temperature was used to simulate very long term storage. In the latter case, the exhibits were analyzed at the start and finish only.

For the one year study, the "crack" was prepared via the "classical" technique. In this method, cocaine hydrochloride is dissolved in water, and an alkaline substance such as sodium bicarbonate or sodium carbonate is added to precipitate cocaine base. The solution is brought to a boil, and the cocaine base melts and forms an oil that pools on the bottom of the container. The solution is cooled, and the oil solidifies, allowing the water layer to be poured off. For the one month (high temperature) study, the "crack" was prepared via the production technique known by the street term "whipping." In this method, water is "whipped" into the molten cocaine base prior to its solidification, to increase its bulk. The samples used for the one year study were processed using the "classical" method because it gives a reasonably uniform product (homogeneity is necessary for valid sample comparisons). The samples used for the one month study were processed using the "whipping" method because it maximizes the amount of water in the sample, and water can be considered to be the key factor in sample degradation during extended or higher temperature storage.

Other types of "crack" production techniques, including the "microwave" method, were not employed in this study because they give inhomogeneous products that contain extensive sodium bicarbonate and other processing impurities.

Figure 1 illustrates the products resulting from the degradation of cocaine and the cinnamoylcocaines in "crack." The amounts of benzoylecgonine and the cinnamoylecgonines were tracked to determine the extent of cocaine and cinnamoylcocaine degradation, respectively (ecgonine methyl ester and ecgonine were also quantitated, but are not reported here because they result from the degradation of both cocaine and the cinnamoylcocaines). The amounts of tropacocaine and trimethoxycocaine present in "crack" were also tracked, because these alkaloids are key "marker" compounds that are used in many cocaine profiling (signature) programs [2,4,5,10].

Experimental

Materials: "Crack" cocaine was produced in-house as described below, starting with uncut, illicitly prepared cocaine hydrochloride from the laboratory inventory. Pharmaceutical cocaine base (used as the quantitation standard for all GC analyses) was obtained from Merck Chemical (Rahway, NJ). Chloroform was a distilled-in-glass product of Burdick and Jackson Laboratories (Muskegon, MI). Reagent grade diethylamine was obtained from Sigma-Aldrich Chemical Company (Milwaukee, WI). N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was a product of Pierce Chemical (Rockford, IL). *para*-Fluorococaine and isopropylcocaine (both used as internal standards (ISTDs)) were synthesized in-house.

Gas Chromatograph / Flame Ionization Detection (GC/FID): Quantitative analyses of cocaine were performed using isopropylcocaine as a structurally related ISTD [11]. An Agilent (Palo Alto, CA) Model 6890N gas chromatograph fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 µm DB-1 (J&W Scientific, Rancho Cordova, CA) was used for cocaine quantitation. An isothermal oven temperature of 250^{O}C was used for 7.00 min. Hydrogen (99.999 percent UHP) was the carrier gas at a flow rate of 1.1 mL/min. The injection port and detector were maintained at 280^{O}C. Samples (2 µL) were injected in the split mode (25 : 1) by an Agilent 7683 Series Auto Injector. Nitrogen was used as the auxiliary make-up gas for the detector. For all quantitations, a minimum of triplicate analyses (N = 3) were performed and results are reported as the average.

Quantitative analyses of cocaine alkaloids and their degradation products were performed using a previously detailed chromatographic impurity signature profile analysis method [2]. Analyses were conducted using an Agilent Model 6890N gas chromatograph fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 µm DB-1701 (J&W Scientific). The oven temperature was programmed as follows: Initial temperature 170^{O}C; 1 min hold; program rate 4^{O}C/min to 200^{O}C; program rate 6^{O}C/min to a final temperature of 275^{O}C; 9 min hold. Samples (1 µL) were injected in the split mode (21.3 : 1) by an Agilent 7683 Series Auto Injector. The injection port and flame ionization detector were maintained at 230^{O}C and 300^{O}C, respectively. Hydrogen (99.999 percent UHP) was the carrier gas at a flow rate of 1.1 mL/min. Nitrogen was used as the auxiliary

make-up gas for the detector. For all quantitations, a minimum of triplicate analyses (N = 3) were performed and results are reported as the average.

Sample Preparation for Cocaine Quantitation: About 16 - 20 mg of cocaine base were weighed (to the nearest 0.01 mg) into a 50 mL Erlenmeyer flask. Samples and standards were diluted with 20 mL of chloroform containing 50 µL of DEA and 5.0 mL of the isopropylcocaine ISTD solution (0.9 mg/mL) [11].

Sample Preparation for Signature Analysis: Approximately 4 - 5 mg of cocaine base was weighed (to the nearest 0.01 mg) into an autosampler vial. The samples were diluted using 250 µL of the *para*-fluorococaine ISTD solution (0.20 mg/mL), 250 µL of MSTFA was added, and the resulting solution was heated for 30 min at 75^{O}C. Samples were allowed to cool to room temperature (approximately 30 min) prior to injection into the GC [2].

Production of "Crack" Cocaine for the One Year Stability Study: Approximately 2 kg of uncut cocaine hydrochloride were dissolved in 10 L of water, and saturated sodium bicarbonate added until pH 8 was obtained. The solution was brought to a boil, and the cocaine base melted and settled to the bottom. The solution was then allowed to cool, the water was poured off, and the solidified "crack" cocaine was removed and separated into two batches. The first batch was immediately placed into plastic bags while still wet (hereafter referred to as "fresh"), while the second was allowed to "dry out" for two hours before being placed into bags (hereafter referred to as "dry"). Although packaged wet, the fresh batch did not in fact contain a significant amount of water. Each batch was divided equally into zip-lock plastic bags and HSEEs, with approximately 4 grams of cocaine base in each bag. The samples were weighed (to the nearest 0.1 g) for initial net weights. Samples were stored at: 20^{O}C, 5^{O}C, and -5^{O}C. Approximately every 30 days, one sample from each temperature condition/package type was analyzed for cocaine purity, weight change, and cocaine degradation.

Production of "Crack" Cocaine for the One Month (Accelerated Aging) Stability Study: Two samples were prepared: *Sample 1* - Saturated sodium bicarbonate (100 mL) was added to approximately 110 grams of illicit cocaine base in a beaker and heated. After the cocaine melted, the mixture was "whipped" to a nearly homogeneous composition, then allowed to cool and solidify. The net weight of the resulting "whipped crack" was determined, and small samples were removed to establish the starting cocaine purity and level of degradation; the bulk sample was stored in an oven at 65^{O}C, unsealed. After one month, the net weight, purity, and degradation of the resulting product were determined. *Sample 2* - The experiment was repeated with approximately 105 grams of illicit cocaine base and 60 mL of water (not containing sodium bicarbonate); however, in this latter case the resulting "whipped crack" was sealed in a zip-lock plastic bag before being stored in the oven.

Results and Discussion

12 Month Study of "Classically"-Prepared Fresh and Dry "Crack" Samples

Because the "crack" samples that were prepared for the 12 month experiments were uniform, of reasonably high purity, and contained little/no sodium bicarbonate and only incidental moisture, this study gives a "best case" scenario for stability and weight loss. The large number of samples (N = 146) allow for valid comparisons. Use of other preparative methods, or the presence of excess water, excess sodium bicarbonate, or other impurities, would have resulted in non-uniform samples and invalid comparisons (Note: Illicitly prepared "crack" samples typically vary widely in their composition, and therefore are unsuited for a study of this type).

Moisture content did not have a significant effect on purity, weight change, and/or degradation of the samples (i.e., the fresh and dry samples did not differ significantly versus each other after 12 months). Similarly, packaging type also did not play a significant role on sample stability - samples stored in either zip-lock plastic bags or HSEEs displayed similar trends when comparing purity, weight change, and degradation.

Cocaine purity results were very similar across all temperatures and packaging types, with an average purity range of 86.0 - 86.9% for HSEE stored samples, and 85.7 - 86.8% for zip-lock plastic bag stored samples. Tables 1 and 2 show the cocaine purity results for HSEE and zip-lock plastic bag stored samples, respectively.

Weight losses were highest for samples stored at room temperature, with a 2 - 5% loss after 12 months. There was a 2 - 3.5% loss at refrigerator temperature, and from a 0.5% loss to a 1.5% gain at freezer temperatures. Not surprisingly, fresh samples displayed slightly greater losses than dry samples. Tables 3 and 4 show the percent weight change over 12 months for samples stored in HSEE and zip-lock plastic bags, respectively.

Various other cocaine alkaloids were also monitored. Many of these compounds are co-extracted with cocaine from coca leaf, and are observed in most illicit cocaine exhibits [3]; others may result from the degradation of cocaine [2]. Understanding and monitoring changes in these trace alkaloids are critical for laboratories that utilize signature methodologies for comparative analyses of exhibits [4] or for intelligence-deriving purposes, because even minor changes can impact cocaine classifications. Figure 2 contains the chromatographic profiles of "crack" cocaine stored at room temperature in HSEEs from start (Figure 2a) to finish (Figure 2b). Increases in ecgonine (peak #3), benzoylecgonine (peak #7), and *cis*- and *trans*-cinnamoylecgonine (peaks #9 and #11), are evident, as is a small decrease in trimethoxycocaine (peak #12).

Benzoylecgonine results from the hydrolysis of cocaine [2]. From start to finish, benzoylecgonine concentration changes for frozen samples were 0.05 - 0.10% (by weight), and for refrigerated samples 0.05 - 0.66% (by weight). Samples stored at room temperature, however, had more significant changes, 0.05 - 1.62% (by weight). Fresh samples showed a slightly higher rate of benzoylecgonine formation versus dry samples under the same conditions; however, this rate difference was minimal compared to the effect of storage temperature. Figure 3 illustrates the benzoylecgonine content in HSEEs over the 12 month study.

Cis- and *trans*-cinnamoylecgonine methyl esters, commonly referred to as the cinnamoylcocaines, are naturally occurring alkaloids that are co-extracted with cocaine from coca leaf. Degradation (hydrolysis) of the cinnamoylcocaines results in formation of *cis*- and *trans*-cinnamic acid and *cis*- and *trans*-cinnamoylecgonine. The results from this study indicate that there was a small increase in the cinnamoylecgonines. Again, the samples stored at freezer temperatures showed the smallest increases, while those stored at room temperature showed the highest increases (however, the relative change was small regardless of storage temperature). Figures 4 and 5 illustrate the total cinnamoylcocaine and total cinnamoylecgonine contents of "crack" stored over time in HSEEs, respectively.

Tropacocaine and trimethoxycocaine are also naturally occurring alkaloids that are co-extracted with cocaine from coca leaf [3]. These compounds are key components in classifying the origin of cocaine exhibits [10]; therefore, understanding their long-term stability is of great importance. Somewhat surprisingly, tropacocaine showed virtually no changes during the study, regardless of storage conditions and temperatures. Table 5 illustrates tropacocaine results (% by weight) for "crack" stored in HSEEs. However, trimethoxycocaine did degrade similarly to cocaine and the cinnamoylcocaines; i.e., samples stored at freezer temperatures showed very little degradation versus those stored at room temperatures. Figure 6 illustrates the trimethoxycocaine results (% by weight) for samples stored in HSEEs.

Accelerated Aging Study of "Whipped Crack" Samples

The accelerated aging (elevated temperature) study was performed to simulate both extended room temperature storage [12] and short term storage at elevated temperatures (for example, in a law enforcement officer's vehicle in summertime heat). As detailed in the Experimental section, two "whipped crack" exhibits were prepared and stored in an oven at 65^{O}C, one unsealed (Sample 1) and the other sealed in a zip-lock plastic bag (Sample 2). After one month, the samples were reanalyzed for purity, weight change, and degradation. Surprisingly, Sample 1 did not undergo significant degradation, with changes in alkaloid concentration very similar to the "Classical

Crack" samples monitored in the 12-month study. Cocaine purity actually increased almost 14% from the initial quantitation, but this increase was due to the evaporative loss of the water that was "whipped" into the sample prior to the oven storage (the overall net weight decreased by 62.95 g, or 37.3% of the initial weight). The resulting cocaine base remained crystalline and showed no noticeable change in color. Figures 7a and 7b show the chromatographic profiles for the initial and final analyses, respectively. The sample did not undergo significant cocaine degradation to benzoylecgonine (peak #7); however, increased amounts of benzoic acid (peak #1) and ecgonine (peak #3) were observed.

In contrast to Sample 1, however, Sample 2 degraded significantly, giving a thick, dark brown, molasses-like liquid. A similar result was reported by LeBelle *et al.* [4], who stored a sample of cocaine hydrochloride at 60^{O}C and at high humidity for 13 days (liquefaction was noted after the first day). Figures 8a and 8b show the chromatographic profiles for the initial and final analyses, respectively. The cocaine decreased dramatically (57.3% to less than 1%, peak #6), while the benzoylecgonine increased equally dramatically (0.06% to 44.4%, peak #7). The chromatograms also show increased concentrations of benzoic acid (peak #1), ecgonine methyl ester (peak #2), ecgonine (peak #3), and *cis*- and *trans*-cinnamoylecgonine (peaks #9 and #11). The cinnamoylcocaines also degraded similarly to cocaine, resulting in elevated cinnamoylecgonines. The amount of trimethoxycocaine was also much lower, decreasing from 0.26% to 0.06%. Tropacocaine was the only alkaloid that did not undergo significant degradation, with results similar to the initial analysis. Table 6 compares the results between Samples 1 and 2. Interestingly, this cocaine exhibit experienced a net weight loss similar to the unsealed exhibit, decreasing by 52.6 g or 32.5% of the initial weight (Note: Zip-lock plastic bags are not impermeable to water vapor).

Conclusions

To prevent degradation and weight loss, "crack" is best stored at -5^{O}C or below. However, for virtually all forensic laboratories or law enforcement agencies, such storage is not practical. Fortunately, this study indicates that relatively dry "crack" cocaine samples stored at room temperature may not undergo significant degradation within one year. However, this study used laboratory-prepared, reasonably pure samples with minimal moisture and/or sodium bicarbonate content. Retail (street) level samples that are highly adulterated or that contain excess water or sodium bicarbonate would be expected to degrade at a faster rate. Long-term storage of "crack" in sealed packaging (i.e., zip-lock plastic bags or HSEEs) may result in extensive cocaine degradation and significant weight loss. Similarly, even short-term storage of "crack" in sealed packaging at very elevated temperatures, such as within the trunk of an officer's vehicle during summer months, can result in rapid degradation and weight loss. Finally, "crack" that contains large amounts of occluded water (e.g., "whipped crack" or similar) may undergo significant weight loss if stored unsealed, due to the evaporative loss of water.

References

1. Santos NA. Moisture absorption problems associated with the packaging of cocaine hydrochloride. Microgram 1992;25(2):28-32.*

2. Casale JF, Waggoner RW. A chromatographic impurity signature profile analysis for cocaine using capillary gas chromatography. Journal of Forensic Sciences 1991;36(5):1312-1330.

3. Moore JM, Casale JF. In-depth chromatographic analyses of illicit cocaine and its precursors, coca leaves. Journal of Chromatography A 1994;674:165-205.

4. LeBelle M, Callahan S, Latham D, Lauriault G, Savard C. Comparison of illicit cocaine by determination of minor components. Journal of Forensic Sciences 1991;36(4):1102-1120.

5. Ensing JG, Racamy C, de Zeeuw RA. A rapid gas chromatographic method for the fingerprinting of illicit cocaine samples. Journal of Forensic Sciences 1992;37(2):446-459.

6. Casale JF, Meyers RP. The stability of cocaine in Agua Rica/Agua Madre. Microgram 1996;29(7):175-178.*

7. Murray JB, Al-Shora HI. Stability of cocaine in aqueous solution. Journal of Clinical Pharmacy 1978;3(1):1-6.

8. Alexander GL. Acid hydrolysis of cocaine. Microgram 1984;17(3):41-45.*

9. Moore JM, Casale JF, Cooper DA. Comparative determination of total isomeric truxillines in illicit, refined, South American cocaine hydrochloride using capillary gas chromatography - electron capture detection. Journal of Chromatography A 1996;756:193-201.

10. Ehleringer JR, Casale JF, Lott MJ, Ford VL. Tracing the geographic origin of cocaine. Nature 2000;408:311-312.

11. Pinero EL, Casale JF. Quantitation of cocaine by gas chromatography - flame ionization detection utilizing isopropylcocaine as a structurally related internal standard. Microgram Journal 2006;4(1-4);47-53.

12. Moore JM, Casale JF. The discoloration of illicit drug samples. Microgram Journal 2008;6(3-4):128-145 (this issue).

* Law Enforcement Restricted Issue.

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Table 1. Cocaine Base Purity of Heat Sealed Evidence Envelope (HSEE) Stored Samples.

Month	20°C Dry	20°C Fresh	5°C Dry	5°C Fresh	(-5°C) Dry	(-5°C) Fresh
0	88.8	86.3	88.8	86.3	88.8	86.3
1	85.6	87.5	87.1	86.6	87.1	86.9
2	87.1	88.0	87.6	88.2	87.2	86.9
3	85.5	85.3	86.4	86.6	86.4	85.9
4	86.1	86.2	86.9	87.7	86.8	88.1
5	85.7	85.7	85.7	85.8	87.0	86.0
6	84.9	85.5	86.1	86.0	85.4	86.7
7	86.3	87.0	85.7	86.3	87.0	87.1
8	87.6	86.7	86.8	87.5	87.8	87.6
9	82.2	83.3	83.3	83.0	83.4	83.8
10	86.6	85.3	87.0	86.8	87.8	87.6
11	87.2	85.6	87.0	87.4	87.2	87.2
12	85.6	85.9	86.0	86.3	87.5	88.3

Table 2. Cocaine Base Purity of Plastic Bag Stored Samples.

Month	20°C Dry	20°C Fresh	5°C Dry	5°C Fresh	(-5°C) Dry	(-5°C) Fresh
0	88.8	86.3	88.8	86.3	88.8	86.3
1	86.1	86.2	86.7	86.9	86.3	87.7
2	86.2	86.4	87.2	87.0	86.8	87.0
3	86.0	84.9	86.4	86.6	86.6	86.6
4	86.2	86.1	86.8	87.1	87.1	87.9
5	84.5	83.4	86.0	85.4	85.7	85.6
6	86.4	86.2	86.6	87.0	87.6	87.5
7	85.4	86.4	86.3	86.4	86.3	86.0
8	86.7	86.0	86.0	86.1	85.9	85.5
9	83.8	84.3	84.1	85.1	84.7	84.9
10	86.5	85.2	85.7	86.7	87.1	87.5
11	88.0	87.7	88.2	88.7	89.6	89.5
12	87.4	85.0	85.4	86.0	85.6	86.2

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Table 3. Percent Weight Change of HSEE Stored Samples.

Month	20°C Dry	20°C Fresh	5°C Dry	5°C Fresh	(-5°C) Dry	(-5°C) Fresh
1	-0.2	-3.0	1.0	-2.0	1.0	0.7
2	-1.4	-1.1	0.0	-3.1	0.2	0.9
3	-2.3	-2.6	0.5	-2.6	1.1	0.7
4	-2.4	-4.7	-1.0	-2.8	0.2	-0.5
5	-1.0	-3.8	-0.5	-2.5	-0.4	-0.5
6	-1.1	-4.7	-0.5	-3.3	-0.2	-1.1
7	-2.5	-4.2	-0.6	-2.1	0.4	0.2
8	-3.2	-4.6	0.2	-2.3	0.7	-0.8
9	-2.0	-4.3	-2.4	-4.7	-2.7	-3.5
10	-2.2	-3.3	0.0	-2.5	0.4	0.0
11	-2.7	-3.9	-0.8	-2.6	0.5	-0.3
12	-2.0	-3.3	-1.6	-2.1	1.3	1.6

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Table 4. Percent Weight Change of Plastic Bag Stored Samples.

Month	20°C Dry	20°C Fresh	5°C Dry	5°C Fresh	(-5°C) Dry	(-5°C) Fresh
1	-1.8	-2.2	-0.5	-1.3	0.2	-0.3
2	-2.6	-3.3	-1.0	-2.8	0.0	-0.2
3	-2.5	-2.6	-1.0	-2.6	0.3	-0.5
4	-3.0	-3.8	-1.2	-2.1	-0.8	-1.0
5	-2.8	-4.0	-1.2	-2.9	0.0	-0.3
6	-3.2	-4.6	-1.2	-2.5	-0.2	-1.1
7	-2.7	-3.7	-1.4	-2.9	0.0	-0.7
8	-2.8	-4.2	-1.7	-2.7	0.0	-0.7
9	-3.0	-4.4	-1.9	-1.7	0.2	-2.3
10	-2.8	-4.2	-1.6	-2.9	-0.5	-1.3
11	-2.9	-4.4	-1.8	-1.9	-0.2	-0.3
12	-2.7	-4.7	-2.1	-3.5	-0.5	1.6

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Table 5. Tropacocaine Percent in HSEE Stored Samples.

Month	20°C Dry	20°C Fresh	5°C Dry	5°C Fresh	(-5°C) Dry	(-5°C) Fresh
0	**0.10**	**0.09**	**0.10**	**0.09**	**0.10**	**0.09**
1	0.11	0.10	0.11	0.11	0.11	0.11
2	0.10	0.10	0.11	0.11	0.10	0.10
3	0.10	0.09	0.10	0.10	0.10	0.10
4	0.09	0.09	0.10	0.10	0.10	0.10
5	0.09	0.09	0.10	0.10	0.10	0.10
6	0.09	0.10	0.10	0.10	0.09	0.10
7	0.09	0.10	0.10	0.10	0.10	0.10
8	0.09	0.08	0.09	0.10	0.10	0.10
9	0.09	0.08	0.09	0.09	0.10	0.09
10	0.09	0.09	0.09	0.10	0.10	0.10
11	0.09	0.08	0.09	0.09	0.10	0.10
12	0.09	0.09	0.09	0.09	0.10	0.09

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Table 6. Comparison of Sealed and Unsealed "Crack" Cocaine Stored at 65ºC (Summary of Results from Accelerated Study).

Accelerated Results (%) by weight	Unsealed Time = 0	Unsealed Time = 1 Month	Sealed Time = 0	Sealed Time = 1 Month
Weight Loss	N/A	37.3% loss	N/A	32.5% loss
Cocaine Purity	64.3%	78.1%	57.3%	< 1%
Benzoylecgonine	0.12%	0.10%	0.06%	44.4%
Cinnamoylcocaines	4.41%	5.47%	3.67%	0.11%
Cinnamoylecgonines	0.05%	0.01%	0.01%	1.98%
Tropacocaine	0.07%	0.09%	0.06%	0.08%
Trimethoxycocaine	0.31%	0.39%	0.26%	0.06%

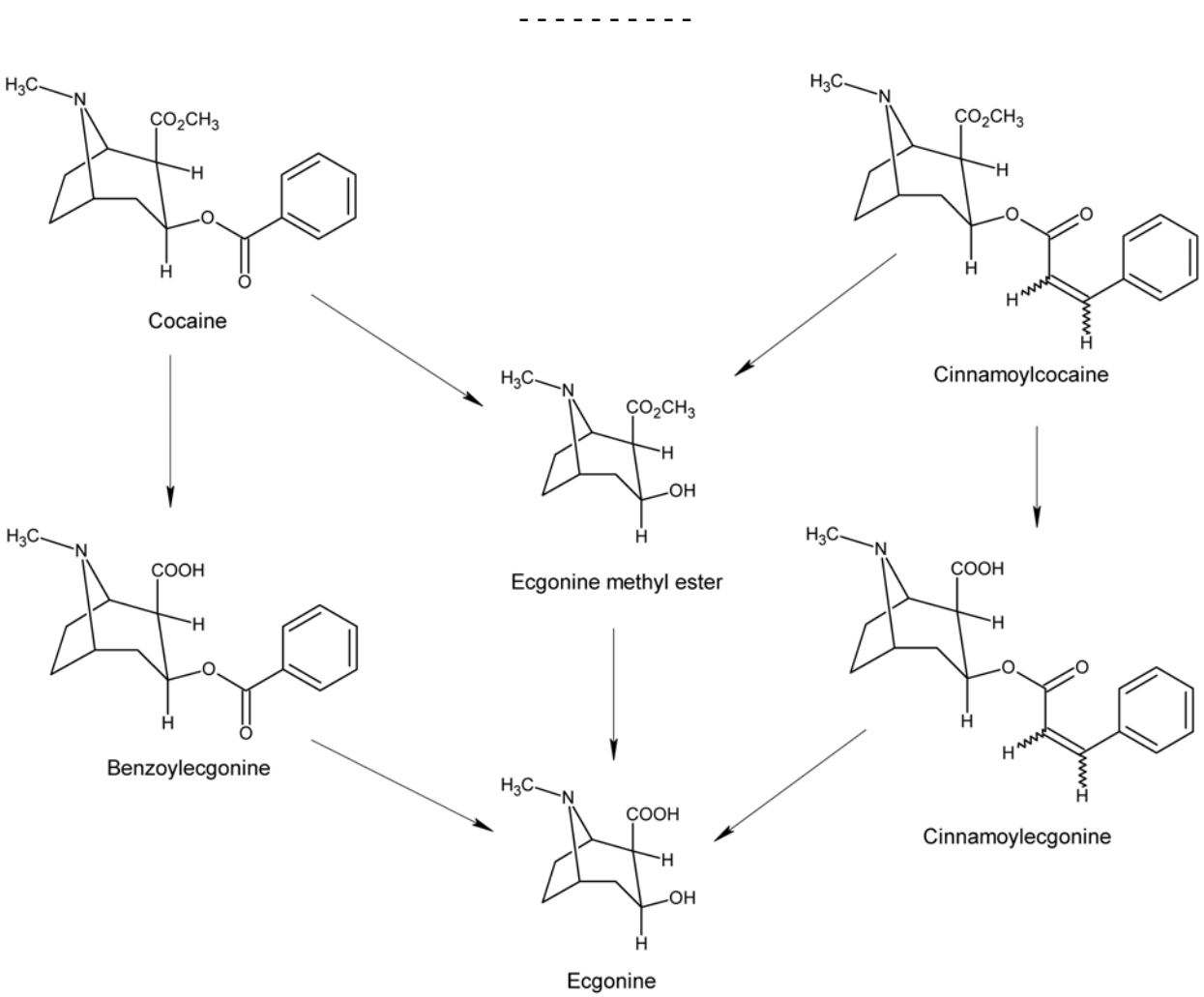

Figure 1. Cocaine and Cinnamoylcocaine Degradation Products.

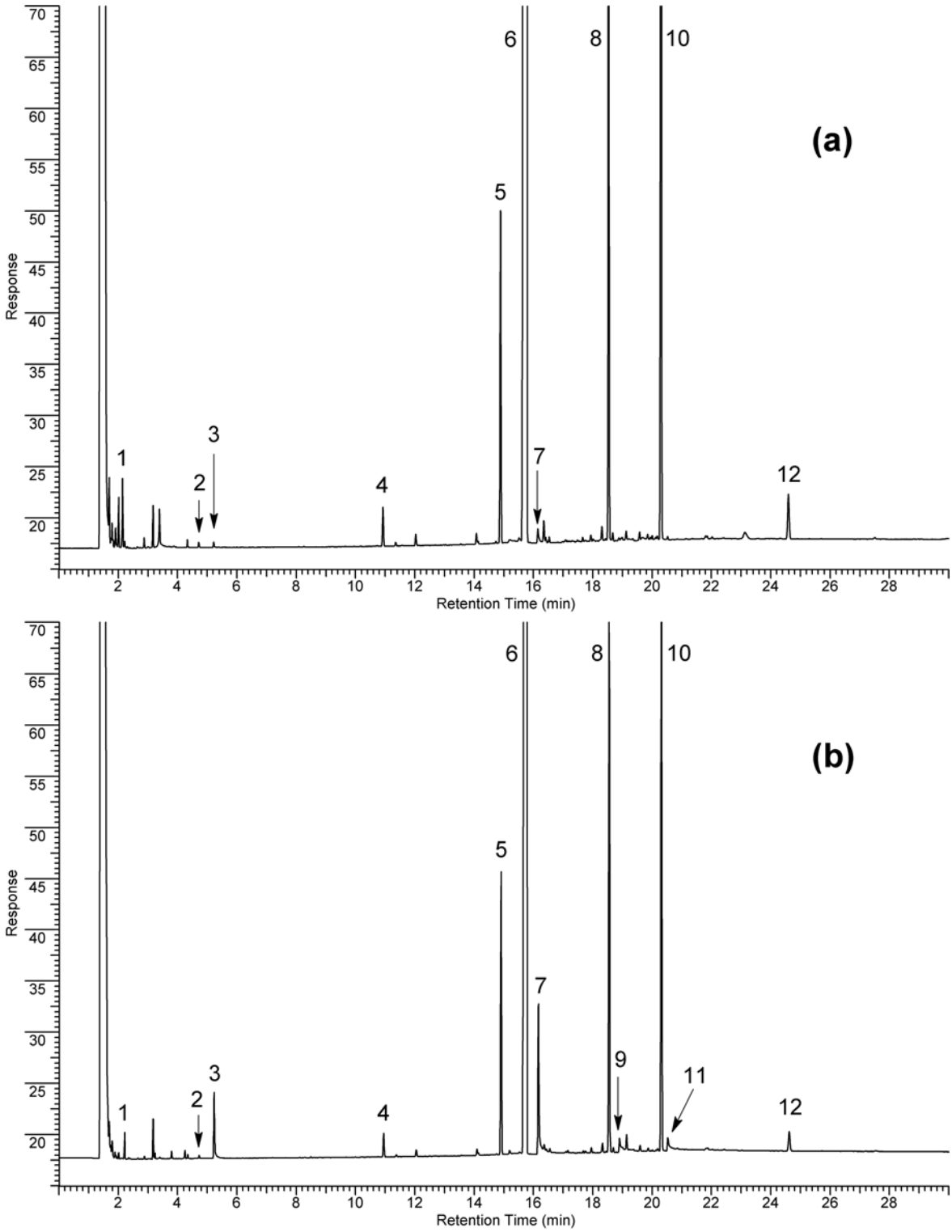

Figure 2. Chromatographic Profiles of "Crack" Cocaine Stored at Room Temperature in HSEE: (A) Time = 0; and (B) Time = 12 Months. Peak Identification: 1 = Benzoic Acid-TMS; 2 = Ecgonine Methyl Ester-TMS; 3 = Ecgonine-di-TMS; 4 = Tropacocaine; 5 = *para*-Fluorococaine (ISTD); 6 = Cocaine; 7 = Benzoylecgonine-TMS; 8 = *cis*-Cinnamoylcocaine; 9 = *cis*-Cinnamoylecgonine-TMS; 10 = *trans*-Cinnamoylcocaine; 11 = *trans*-Cinnamoylecgonine-TMS; and 12 = Trimethoxycocaine.

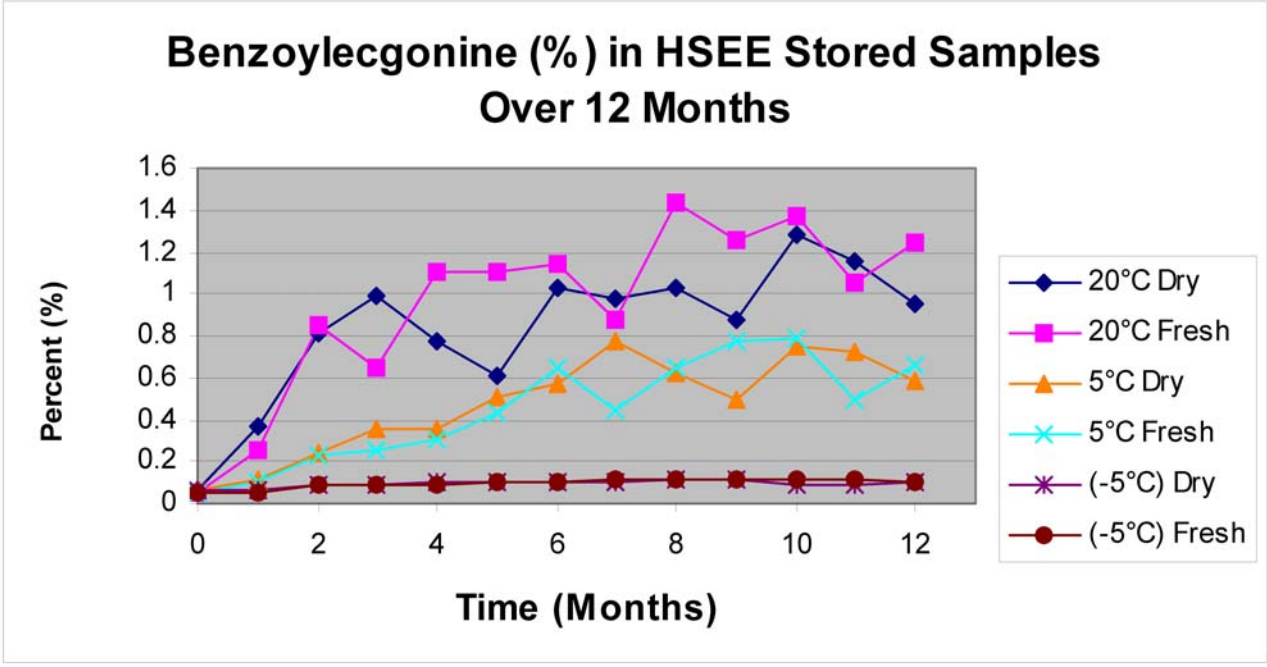

Figure 3. Benzoylecgonine Percent in HSEE Stored Samples.

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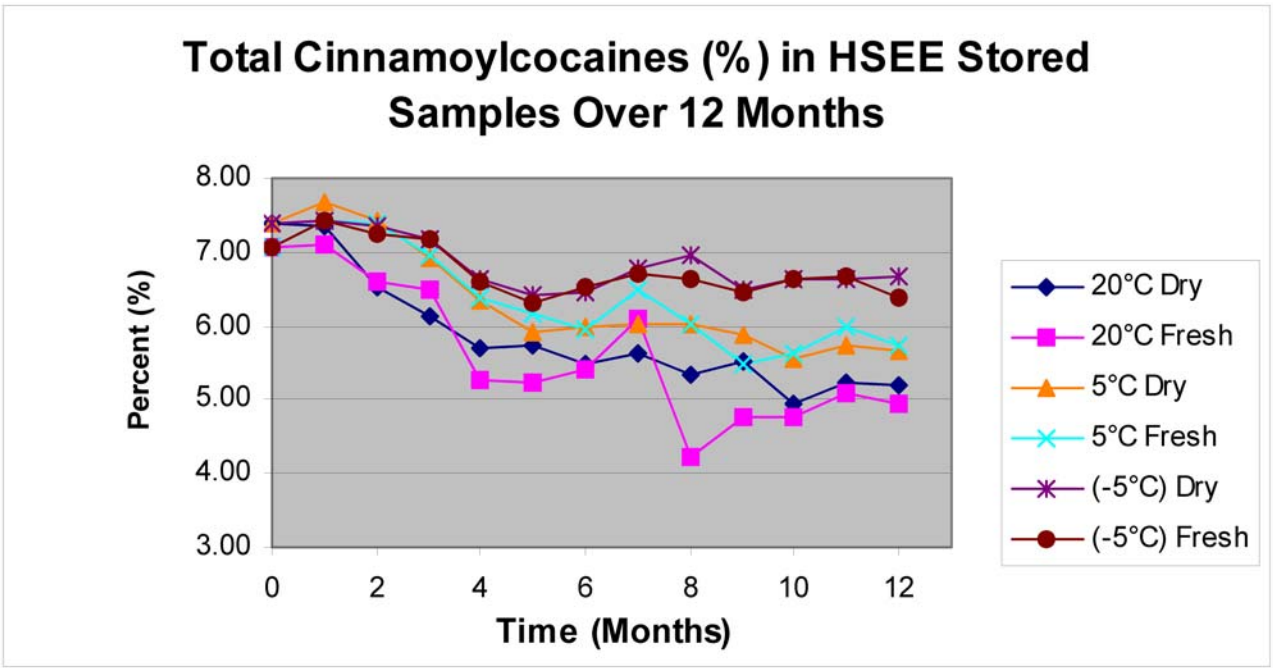

Figure 4. Total Cinnamoylcocaines Percent in HSEE Stored Samples.

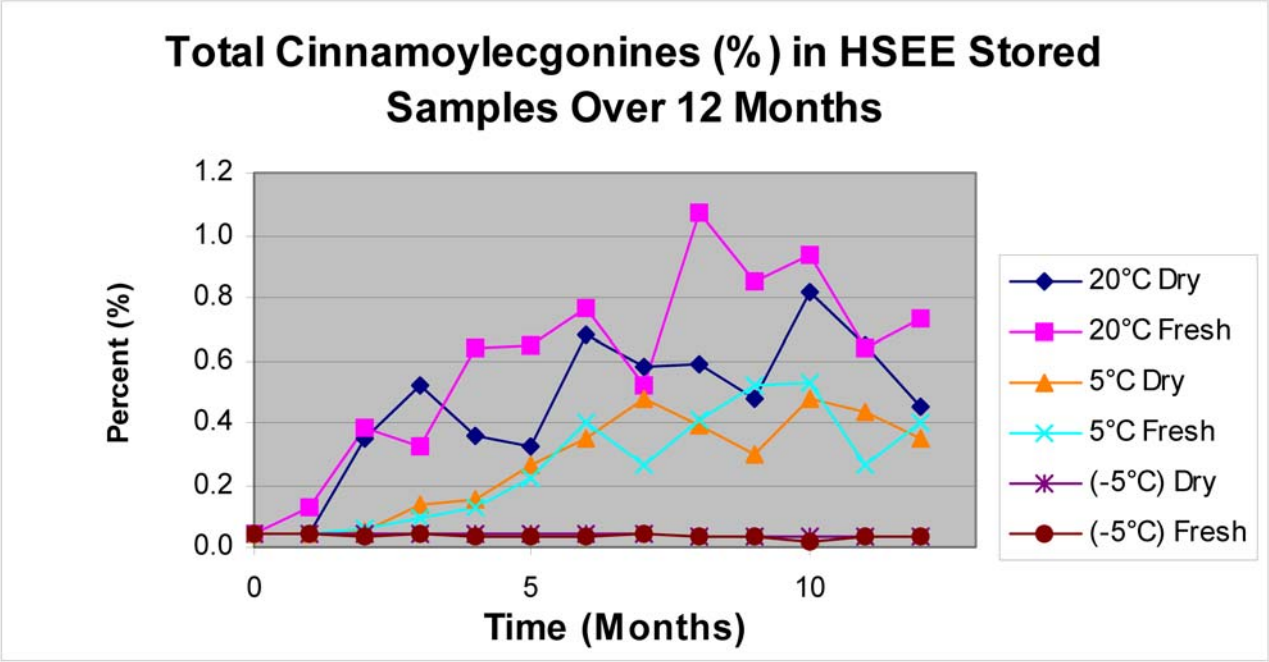

Figure 5. Total Cinnamoylecgonines Percent in HSEE Stored Samples.

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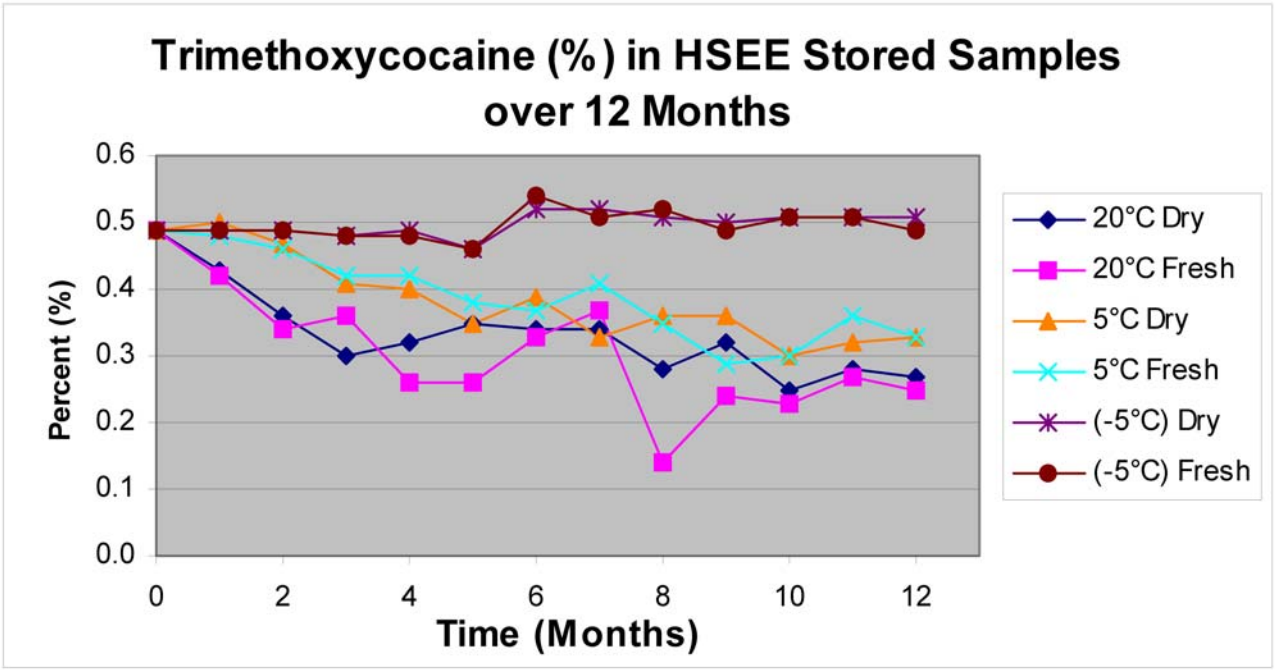

Figure 6. Trimethoxycocaine in HSEE Stored Samples.

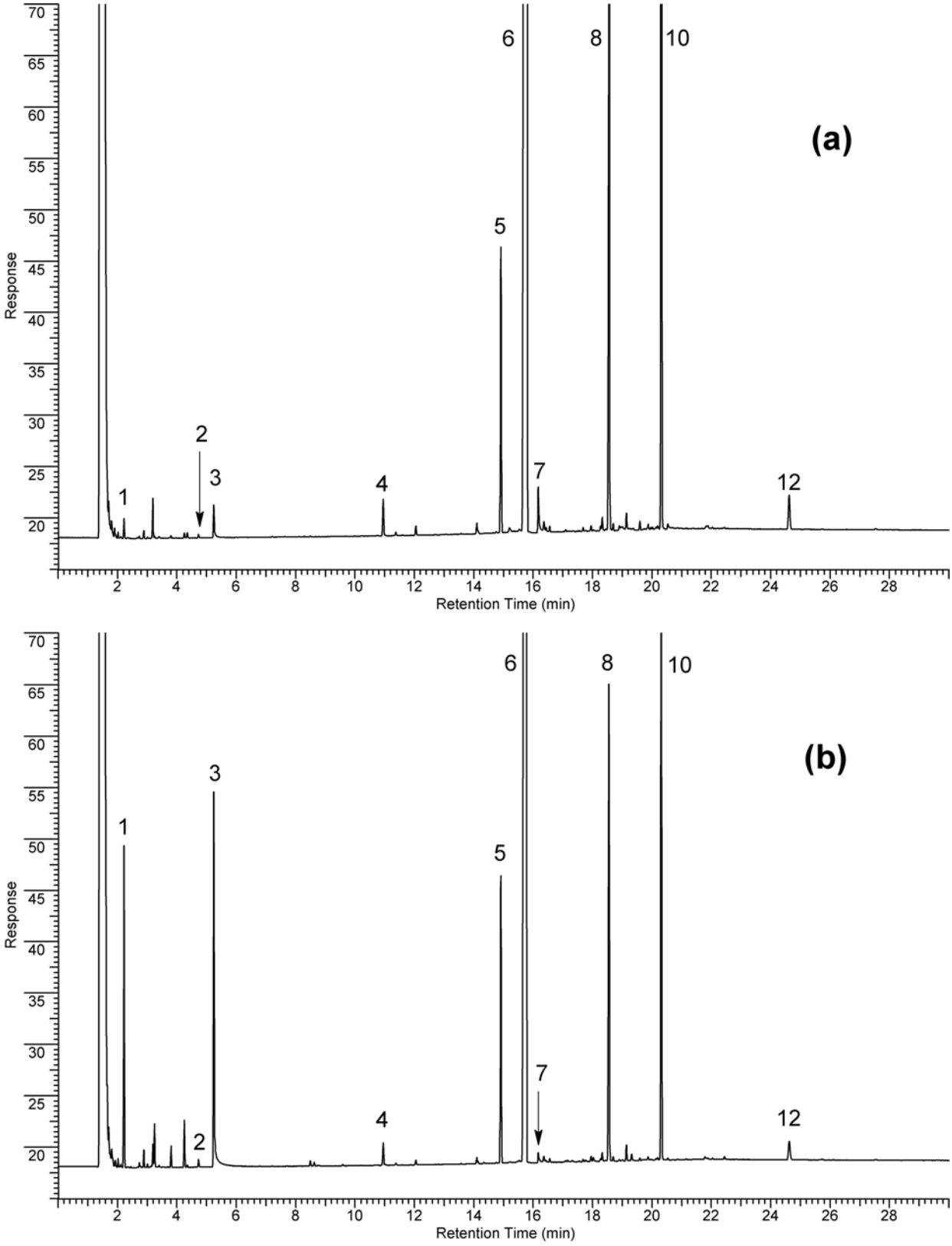

Figure 7. Chromatographic Profiles of "Crack" Cocaine Stored Unsealed in the Accelerated Study: (A) Time = 0; and (B) Time = 1 Month. Peak Identification: See Figure 2.

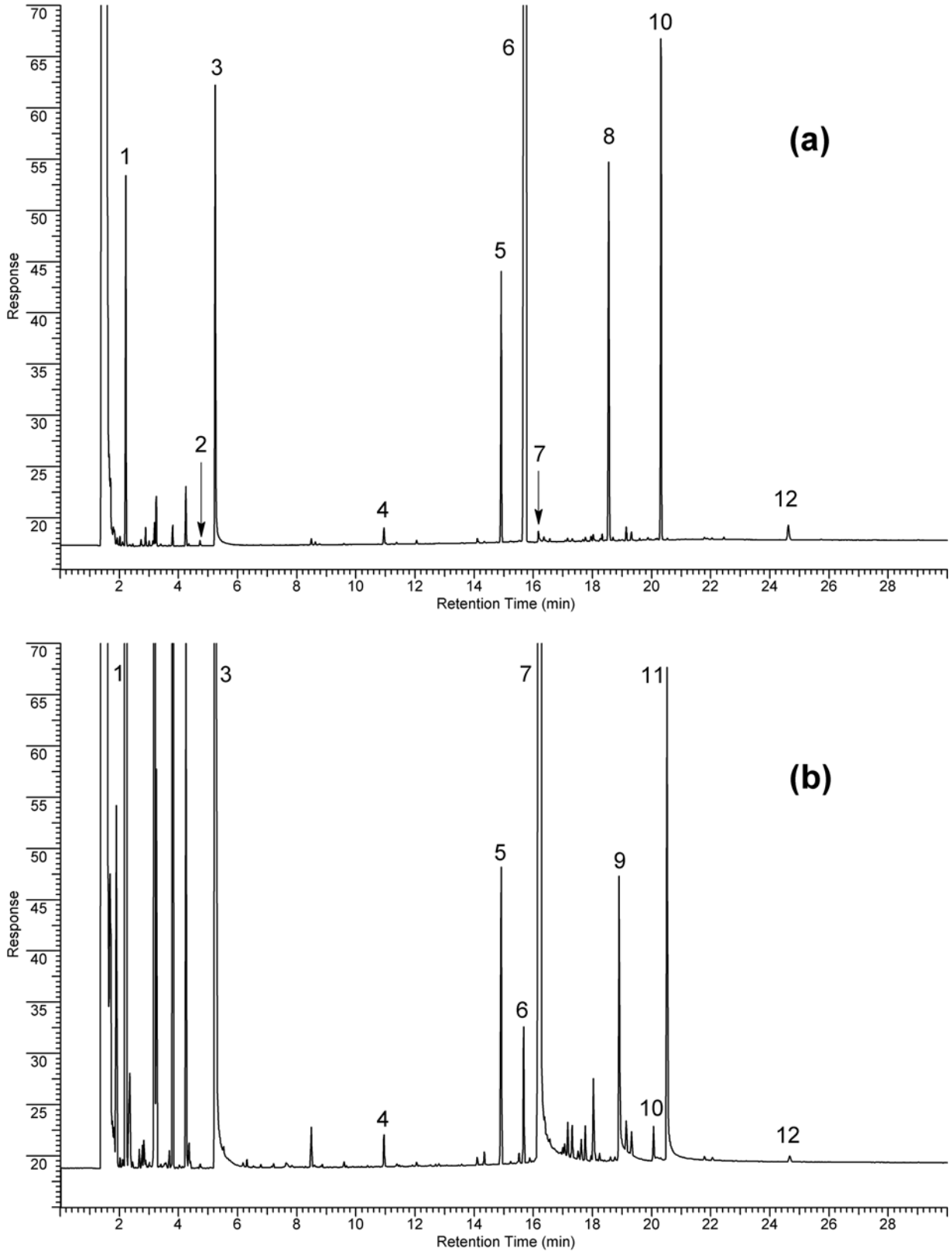

Figure 8. Chromatographic Profiles of "Crack" Cocaine Stored Sealed in Accelerated Study: (A) Time = 0; and (B) Time = 1 Month. Peak Identification: See Figure 2.

“The Lost Manuscript” - A Posthumous Publication of:

The Discoloration of Illicit Drug Samples

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ABSTRACT: Discoloration (browning) of illicit cocaine exhibits during long-term storage is a common but not universal phenomenon. In order to gain a better understanding of the discoloration process(es), already discolored seized samples, and a wide variety of authentic drug mixtures that similarly discolored under long-term ambient and accelerated temperature - humidity studies, were subjected to acid base workup, column chromatography, and in-depth analyses of the pertinent fractions by EI and CI GC/MS, UV/Vis, and IR. The discolored samples were all found to contain a primary aromatic amine (either procaine or benzocaine), a sugar (either lactose or dextrose), and an acid (such as cocaine hydrochloride, boric acid, benzoic acid, etc.) The rate of discoloration of the drug mixtures was both pH and temperature dependant, i.e., the rate of sample browning increased with lower pH and/or higher temperature. All discolored samples that contained procaine or benzocaine also contained N-formylprocaine or N-formylbenzocaine, respectively, and these are therefore *bona fide* “marker” compounds for the browning of illicit cocaine. These derivatives are believed to be formed following the degradation of lactose or dextrose to 5-hydroxymethylfurfural, which in turn degraded to formic and levulinic acids; subsequent formylation of procaine or benzocaine gave the respective “marker” compounds. A number of highly colored compounds (yellow, blue, purple, and pink) were observed in column and thin-layer chromatography of the discolored samples, and are responsible for the sample discoloration. These compounds were not identified, but are believed to derive from condensation reactions between 5-hydroxymethylfurfural with the various amines in the samples.

KEYWORDS: Cocaine Hydrochloride, Discoloration, 5-Hydroxymethylfurfural, Procaine, Benzocaine, N-Formylprocaine, N-Formylbenzocaine, Lactose, Dextrose, Forensic Chemistry

[Foreword by the Corresponding Author and the *Microgram* Editor: This manuscript was authored in 1974 by then Senior Forensic Chemist Jim Moore; it was intended and formatted for publication in *Microgram*, but apparently was never submitted. It was re-discovered on July 14, 2008 by the co-author, Senior Research Chemist John Casale. It is published here verbatim (including 1970’s formatting and scientific abbreviations), except that some experiments were repeated by the corresponding author to re-create legible figures, the above Abstract and Keyword set were provided by the Editor, and the layout was slightly reformatted by the Editor for improved readability.]

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† Original author (FDA 1963-68; BNDD 1968-73; and DEA 1973-98); deceased February 24th, 1999 (see: Microgram 1999;32(4):133 (Note: Law Enforcement Restricted issue)).

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Introduction

This paper reports the results of a two-year investigation studying the discoloration of illicit drug samples. The study was initiated as a result of several forensic laboratories reporting problems associated with the discoloration, or "browning," of illicit cocaine samples over a prolonged period of time. This "browning" phenomenon was of forensic interest in that there were discrepancies in the chemist's description of the sample prior to analysis and later, upon identifying the sample during court testimony. In most cases, the samples were white when first examined but subsequently acquired a brown coloration by the time the samples were reopened for court proceedings. This change in coloration caused significant problems for the chemists in that it could be assumed that a sample mix-up had occurred, and the disposition of the case would be in doubt based upon this reasoning. Dugar, *et al.* [1] have investigated this discoloration phenomenon in contraband cocaine.

The study in this paper satisfactorily characterizes the "browning" phenomenon occurring in certain illicit samples. This characterization is based upon: (A) a thorough literature review that describes the discoloration of legitimate pharmaceutical preparations, (B) in-depth analyses of illicit samples known to have undergone the discoloration process, (C) ambient and accelerated temperature-humidity studies conducted on authentic drug mixtures, and (D) the isolation and characterization of signature compounds associated with the discoloration process in illicit and authentic samples.

A. *Literature Review*

There has been considerable study of the discoloration associated with legitimate pharmaceutical products. Blaug and Huang [2,3] have described the discoloration of amphetamine sulfate - spray dried lactose and amphetamine sulfate - dextrose mixtures when subjected to elevated temperatures. In these studies, a product of sugar decomposition, namely 5-hydroxymethylfurfural (5-HMF), was suggested to be present. Dugar *et al.* [1] also reported the presence of 5-HMF in sugar-containing illicit samples that underwent discoloration. Castello and Mattocks [4] and Duvall *et al.* [5,6] reported interaction of various primary amines with lactose and dextrose and the subsequent discoloration of such samples. In these studies, the rate of "browning" was found to be dependant upon temperature and pH. Several investigators had studied the role of 5-HMF and related substances in the discoloration of amine - sugar mixtures [7-11]. Brownley and Lachman [12] studied the formation of 5-HMF in spray-dried lactose as well as conventionally-processed lactose.

The above-referenced studies clearly demonstrated that when mixtures of primary amines (amphetamine was most often studied) and certain sugars (lactose and dextrose) were subjected to elevated temperature and humidity conditions, significant discoloration occurred. Several authors reported a relationship between the rate of discoloration and pH of the sample. Finally, most of the studies reported a relationship between drug mixtures that had discolored and the presence of 5-HMF.

B. *In-Depth Analyses of Illicit Samples*

A number of illicit drug samples known to have undergone the discoloration process were obtained from various forensic laboratories. All samples were subjected to in-depth analyses for drug constituents as well as diluents. These analyses revealed that the samples had the following elements in common: (1) all samples contained cocaine hydrochloride, (2) all samples had undergone a white-to-brown color transition after prolonged storage under unspecified conditions, (3) all samples contained either lactose or dextrose, (4) all samples contained a primary aromatic amine, such as benzocaine, and (5) all samples were acidic in nature; this acidity was due, in part, to the presence of substances such as cocaine HCl, boric acid, etc. The results of the in-depth analyses indicated a positive correlation between sample composition and the discoloration process described by other investigators.

C. *Ambient and Accelerated Stability Studies of Authentic Samples*

A large number of authentic samples were prepared for stability studies (see Table I). The composition of these samples was based upon elements believed responsible, in part, for the discoloration process. The authentic samples were studied under ambient conditions for about two years and under accelerated conditions for about two months.

The primary amines used in this study were benzocaine and procaine HCl. These are two widely-used adulterants associated with illicit cocaine and heroin samples, respectively. The sugars used were lactose and dextrose. Preliminary studies indicated that mannitol and sucrose did not contribute significantly to the "browning" process. Since the literature review suggested a difference in the "browning" rates of spray-dried and conventionally-processed lactose, both were used in this study. The pH of the samples was controlled over an acid range by the introduction of substances of varying acidity. In order of increasing pKa, these substances were: oxalic acid, citric acid, benzoic acid, cocaine HCl, boric acid, heroin HCl, and procaine HCl. The concentration of all compounds was varied over a wide range.

In the ambient study, the authentic samples listed in Table I were placed in glass vials with screw-on plastic caps and stored in the dark. At the end of a two-year period, the samples were examined for discoloration. Table II lists those samples that exhibited significant discoloration. The samples listed in Table I, and not included in Table II, did not exhibit significant discoloration.

A review of Table II reveals that all samples that discolored contained either lactose or dextrose, benzocaine or procaine HCl, and were distinctly acidic. It should be noted that the concentration of the acidic component did not influence the discoloration significantly. On the other hand, the majority of the samples that did discolor contained the more acidic components, (i.e., oxalic, citric, and benzoic acids). Though the cocaine HCl - benzocaine - dextrose mixtures were of higher pH values, these samples also discolored. Though the explanation for this is not clear, it may be due to decomposition of cocaine with subsequent formation of benzoic acid resulting in a decrease of pH. It is also apparent from Table II that for a sample of given acidity, benzocaine - dextrose mixtures discolored more rapidly than benzocaine - lactose mixtures. Such a distinction is not apparent for procaine-containing samples.

The samples listed in Table I were also subjected to accelerated conditions for 61 days. The humidity varied from 50 - 70%, while the temperature was gradually increased from ambient to 48^{O}C. Fig. 1 illustrates the results of the accelerated study. As the samples discolored significantly (brown to dark brown), they were removed from the temperature-humidity chamber and noted in Fig. 1. Due to the large number of samples that discolored, only a representative cross-section are noted in Fig. 1.

The results of the accelerated stability studies can be summarized as were the ambient study described previously. Additionally, there does exist a positive correlation between the rate of discoloration of the authentic samples and increases in temperature. There is no apparent difference in the discoloration rates of samples containing the various grades of lactose, including spray-dried and conventionally-processed.

Some of the samples subjected to the accelerated study showed no significant discoloration. These included most of the two-component samples as well as those three-component mixtures that did not contain either a sugar, a primary amine, or a strong acid. Those samples that apparently contained the necessary components for "browning," but showed no significant discoloration, were generally of a higher pH, and usually contained lactose as the sugar diluent. These included samples containing lactose mixed with heroin HCl - procaine HCl, heroin HCl - benzocaine, boric acid - procaine HCl, boric acid - benzocaine, cocaine HCl - procaine HCl, and cocaine HCl - benzocaine. In general, samples containing dextrose were found to discolor at a significantly faster rate than those containing lactose.

Table I - Composition of Authentic Samples Subjected to Ambient and Accelerated Studies [a]

1. 2% Ox - 49% Bnzn - 49% Lac
2. 25% Ox - 37% Bnzn - 37% Lac
3. 70% Ox - 15% Bnzn - 15% Lac
4. 2% Ox - 49% Proc - 49% Dex
5. 25% Ox - 37% Proc - 37% Dex
6. 70% Ox - 15% Proc - 15% Dex
7. 2% Ox - 49% Bnzn - 49% Dex
8. 25% Ox - 37% Bnzn - 37% Dex
9. 70% Ox - 15% Bnzn - 15% Dex
10. 25% Ox - 37% Proc - 37% Lac
11. 2% Ox - 49% Proc - 49% Lac
12. 70% Ox - 15% Proc - 15% Lac
13. 2% Cit - 49% Proc - 49% Dex
14. 25% Cit - 37% Proc - 37% Dex
15. 70% Cit - 15% Proc - 15% Dex
16. 2% Cit - 49% Bnzn - 49% Dex
17. 25% Cit - 37% Bnzn - 37% Dex
18. 70% Cit - 15% Bnzn - 15% Dex
19. 2% Cit - 49% Proc - 49% Lac
20. 25% Cit - 37% Proc - 37% Lac
21. 70% Cit - 15% Proc - 15% Lac
22. 2% Cit - 49% Bnzn - 49% Lac
23. 25% Cit - 37% Bnzn - 37% Lac
24. 70% Cit - 15% Bnzn - 15% Lac
25. 2% Bzoc - 49% Proc - 49% Dex
26. 25% Bzoc - 37% Proc - 37% Dex
27. 70% Bzoc - 15% Proc - 15% Dex
28. 2% Bzoc - 49% Bnzn - 49% Dex
29. 25% Bzoc - 37% Bnzn - 37% Dex
30. 70% Bzoc - 15% Bnzn - 15% Dex
31. 2% Bzoc - 49% Proc - 49% Lac
32. 25% Bzoc - 37% Proc - 37% Lac
33. 70% Bzoc - 15% Proc - 15% Lac
34. 2% Bzoc - 49% Bnzn - 49% Lac
35. 25% Bzoc - 37% Bnzn - 37% Lac
36. 70% Bzoc - 15% Bnzn - 15% Lac
37. 2% Coc - 49% Proc - 49% Dex
38. 25% Coc - 37% Proc - 37% Dex
39. 70% Coc - 15% Proc - 15% Dex
40. 2% Coc - 49% Bnzn - 49% Dex
41. 25% Coc - 37% Bnzn - 37% Dex
42. 70% Coc - 15% Bnzn - 15% Dex
43. 2% Coc - 49% Proc - 49% Lac
44. 25% Coc - 37% Proc - 37% Lac
45. 70% Coc - 15% Proc - 15% Lac
46. 2% Coc - 49% Bnzn - 49% Lac
47. 25% Coc - 37% Bnzn - 37% Lac
48. 70% Coc - 15% Bnzn - 15% Lac
49. 2% Bor - 49% Proc - 49% Dex
50. 25% Bor - 37% Proc - 37% Dex
51. 70% Bor - 15% Proc - 15% Dex
52. 2% Bor - 49% Bnzn - 49% Dex
53. 25% Bor - 37% Bnzn - 37% Dex
54. 70% Bor - 15% Bnzn - 15% Dex
55. 2% Bor - 49% Proc - 49% Lac
56. 25% Bor - 37% Proc - 37% Lac
57. 70% Bor - 15% Proc - 15% Lac
58. 2% Bor - 49% Bnzn - 49% Lac
59. 25% Bor - 37% Bnzn - 37% Lac
60. 70% Bor - 15% Bnzn - 15% Lac
61. 2% Her - 49% Proc - 49% Lac
62. 25% Her - 37% Proc - 37% Lac
63. 70% Her - 15% Proc - 15% Lac
64. 2% Her - 49% Bnzn - 49% Lac
65. 25% Her - 37% Bnzn - 37% Lac
66. 70% Her - 15% Bnzn - 15% Lac
67. 2% Her - 49% Proc - 49% Dex
68. 25% Her - 37% Proc - 37% Dex
69. 70% Her - 15% Proc - 15% Dex
70. 2% Her - 49% Bnzn - 49% Dex
71. 25% Her - 37% Bnzn - 37% Dex
72. 70% Her - 15% Bnzn - 15% Dex
73. 25% Coc - 25% Bnzn - 50% Ox
74. 25% Coc - 25% Proc - 50% Ox
75. 25% Her - 25% Proc - 50% Ox
76. 50% Proc - 50% Ox
77. 50% Bnzn - 50% Ox
78. 50% Dex - 50% Ox
79. 50% Lac - 50% Ox
80. 50% Coc - 50% Ox
81. 50% Her - 50% Ox

- -

[a] Key to Abbreviations: Ox = oxalic acid, Cit = citric acid, Bzoc = benzoic acid, Coc = cocaine HCl, Bor = boric acid, Her = heroin HCl, Proc = procaine HCl, Bnzn = benzocaine, Dex = dextrose, Lac = lactose. Various grades of lactose, including spray-dried and conventionally processed were used.

Table II - Authentic Samples that Exhibited Discoloration after a Two-Year Study under Ambient Conditions [a]

Sample Number [b]	Sample Appearance
4, 5, 6, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 20, 21, 27, 28, 29, 30, 41, 42	Uniform dark brown to black
1, 2, 3, 7, 8, 9, 23, 24, 25, 26, 32, 33, 40 49, 50, 52, 53	Uniform light to medium brown
16, 22, 23	Dark specks in white powder

- -

[a] Ambient conditions: avg. room temperature = 24 - 26^{O}C; humidity range = 60 - 80% RH.
[b] Refer to Table I for sample composition.

* * * * *

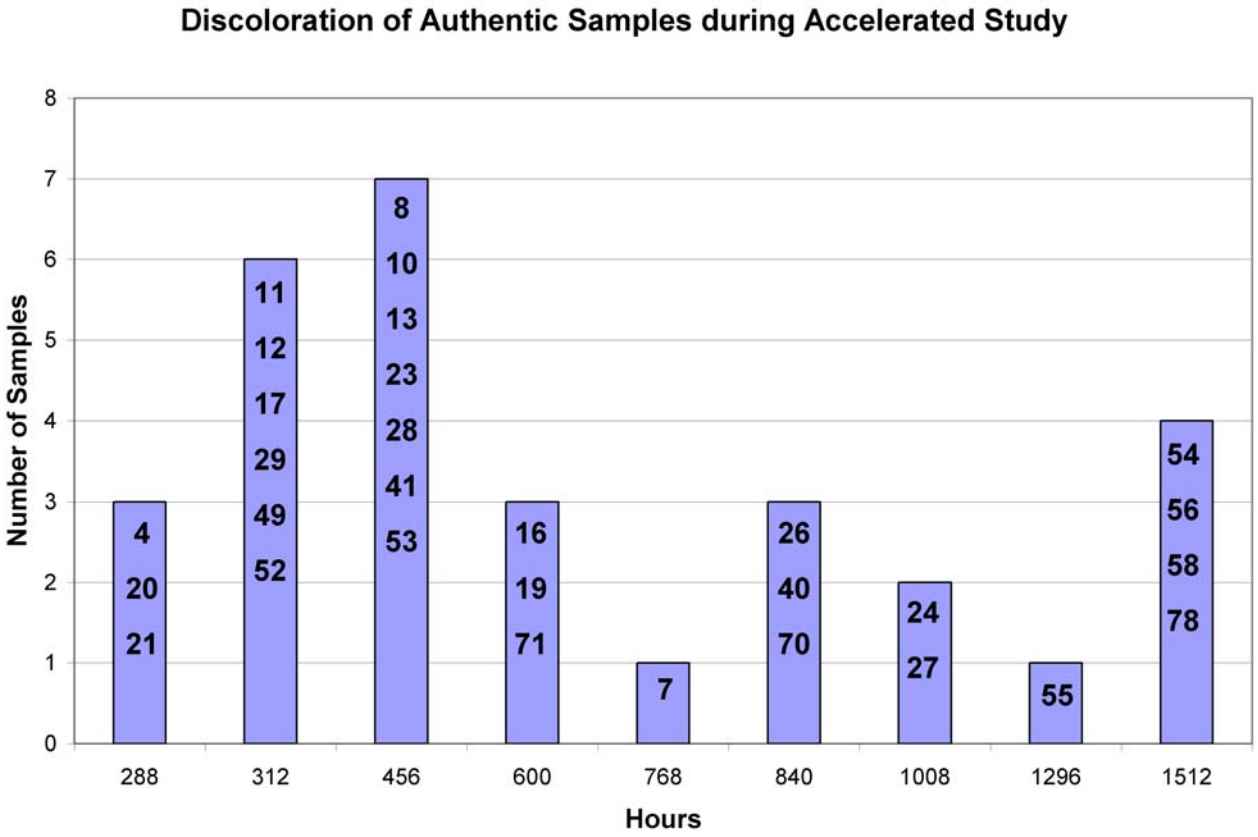


Figure 1: Graph illustrating discoloration of authentic samples subjected to accelerated conditions. As samples discolored, they were removed from temperature-humidity chamber and noted on graph. Refer to Table I for sample composition.

In summary, the results of the ambient and accelerated studies have established a clear relationship between the discoloration of illicit samples and their composition and storage conditions. Despite this positive correlation, full characterization of this "browning" phenomena would not be complete unless signature compounds that resulted as by-products of the discoloration process were isolated and identified. This work is described below.

D. *Isolation and Characterization of Signature Compounds*

1. Chemicals and Solvents

   The formic acid used in this study was 88% analytical reagent grade and obtained from Mallinkrodt Chemical Works (St. Louis, Missouri). The deuterated formic acid (DCOOH) was 99% atom % D and was supplied by Merck and Co., Inc. (St. Louis, Missouri). All other chemicals and solvents used were of high quality and obtained from the usual commercial sources.

2. Drug Standards

   All drug materials were provided by the Special Testing and Research Laboratory, Drug Enforcement Administration.

3. Chromatographic Materials

   (a) The chromatographic partitioning columns were 250 mm in length x 22 mm i.d., and obtained from Kontes Glass Co. (Vineland, New Jersey).

   (b) The diatomaceous earth used in the partitioning work was Celite 545 acid-washed (AW), and obtained from Johns-Manville Co. (Inglewood Cliff, New Jersey).

   (c) All thin layer chromatography was done on glass plates coated with silica gel GF (250 or 2000μ thickness). These plates were obtained from Analtech, Inc. (Newark, Delaware).

   (d) All gas chromatographic columns were obtained from Applied Science Laboratories (State College, Pennsylvania) (see body of paper for dimensions). The various column packings were also obtained from Applied Science Laboratories. These included 3% OV-1 and 3% OV-25, all on Chromosorb WHP (100-120M). All internal standards were also obtained from Applied Science Laboratories.

4. Instrumentation

   (a) Gas Chromatography - The gas chromatograph work was done on a Packard 7400 gas chromatograph equipped with a flame ionization detector (FID) (see body of paper for other GLC parameters).

   (b) Ultraviolet Spectrometry (UV) - All UV spectra were recorded on a Cary 14 spectrophotometer.

   (c) Infrared Spectrometry (IR) - All IR spectra were recorded on a Perkin-Elmer 457 spectrophotometer.

   (d) Gas Chromatography - Mass Spectrometry (GC-MS) - A Finnigan 4000 mass spectrometer was used in this study. It was interfaced with a Finnigan 9610 GC and Finnigan 6110 data system. The gas chromatograph was equipped with a 6 ft. x 2 mm i.d. glass column packed with 3% OV-1 and Gas Chrom Q (80-100M).

   All electron impact (EI) spectra were acquired under the following conditions: emission current - 0.35 mA, amplifier sensitivity - 10 - 8 A/V, electron energy - 70 eV, and electron multiplier - 1600 V. The carrier gas was Helium and maintained at a flow rate of about 20 cc/min. The column temperature was programmed between 150 and 250^{O}C, while the ionizer, separator, and transfer line temperatures were maintained at 250, 260, and 260^{O}C, respectively.

All chemical ionization (CI) spectra were obtained under the following conditions: Helium was the carrier gas and methane was used as the reactant gas; the ionizer was maintained at a pressure of about 0.40 torr and a temperature of 200^{O}C; all other parameters are the same as for the EI study.

5. Isolation of Signature Compounds

During the in-depth analyses of the discolored, illicit samples described previously, trace amounts of unidentified impurities were detected. The methodology described below was developed in order to isolate these impurities in sufficiently pure form for spectroscopic characterization.

(a) *Isolation of Signature Compounds in Illicit and Authentic, Brown Samples Containing Benzocaine*

About 0.5 cc of 0.1N H_2SO_4 is mixed with 1 gm Celite 545 AW and packed moderately in a chromatographic partitioning column. An appropriate quantity of sample is dissolved in 2 cc of 0.1N H_2SO_4 and 3 gm of Celite 545 AW are added; after mixing, the sample is packed moderately above the bottom layer of the column. The column is eluted with about 50 - 75 cc of water-saturated ethyl ether. This eluate contains benzocaine and the signature compound. Cocaine and other basic drugs are retained by the column. The ether eluate is evaporated gently to dryness. Depending upon sample composition, this fraction may be sufficiently pure for spectroscopic characterization.

If additional "cleanup" is necessary, the following chromatographic procedure may be used. The residue obtained above from the ether eluate is triturated with 2 cc of 0.1N $NaHCO_3$; 3 gm of Celite 545 are added and mixed until fluffy; this mixture is packed moderately in a chromatographic partitioning column containing a layer of 0.5 cc 0.1N $NaHCO_3$ mixed with 1 gm of Celite 545 AW. The column is then eluted with 75 - 100 cc of water-saturated petroleum ether. This fraction consists primarily of benzocaine. The column is then eluted with 50 - 75 cc water-saturated ethyl ether. This fraction contains the signature compound and trace amounts of benzocaine. The ether eluate is evaporated carefully to dryness. The residue may be characterized spectroscopically or subjected to further purification using the TLC technique described below.

The residue obtained above is dissolved in a small volume of methylene chloride and spotted or streaked on a silica gel GF plate (250 or 2000μ thickness). The plate is developed with a solvent mixture of ethyl ether : petroleum ether (65:35). After development, the plate is dried and viewed under short wave UV. Both benzocaine and the signature compound appear as dark spots or bands at R_f values of about 0.45 and 0.17, respectively. The spot or band representing the signature compound is removed from the plate and placed in a small vial. Methanol is added to the vial and warmed gently on a steam bath. The vial is centrifuged and the methanol is decanted and evaporated carefully to dryness. The residue may be subjected to spectroscopic characterization.

Isolation of the signature compound may also be accomplished using GLC fraction collection techniques. Table III gives the appropriate GLC parameters and retention data for benzocaine, the signature compound, and internal standards.

The signature compound isolated by one or more of the chromatographic procedures given above is subjected to MS, UV, and IR characterization described later in this paper.

(b) *Isolation of Signature Compounds in Illicit and Authentic Brown Samples Containing Procaine*

An appropriate quantity of sample is dissolved in 2 cc of water; the solution is made basic with $NaHCO_3$ and 3 gm of Celite 545 AW are added; after uniform mixing, the sample is packed in a column containing a layer of 0.5 cc of 0.1N $NaHCO_3$ mixed with 1 gm of Celite 545 AW. The signature compound is

isolated following petroleum and ethyl ether elutions, as described above for samples containing benzocaine (ethyl ether eluate contains the signature compound as well as small quantities of procaine).

If necessary, additional TLC purification may be required as described above for benzocaine-containing samples. The adsorbent used is silica gel GF and the solvent system is ammonia-saturated chloroform : methanol (18:1). The R_f values for the procaine and the signature compound are about 0.8 and 0.6, respectively. (Note: using this solvent system, acetylprocaine and the signature compound have similar R_f values.)

Isolation of the signature compound may also be accomplished using GLC fraction collection techniques. Table IV gives the GLC parameters and retention data for procaine, the signature compound, and internal standard.

* * * * *

Table III - GLC Data for Benzocaine, Internal Standards, and Signature Compound

	Retention Time (Min.)	
	3% OV-1 [a]	3% OV-25 [b]
Compound		
Benzocaine	2.2	1.8
Signature Compound	5.0	5.1
Eicosane	9.9	-
Hexacosane	-	9.2

[a] 6 ft. x ¼ in. i.d. column packed with 3% OV-1 on Chromosorb W HP (100 - 120M), injector temp. = 275^{O}C, column temp. = 190^{O}C, manifold temp. = 250^{O}C, detector temp. = 250^{O}C; N_2 carrier flow = 60 cc/min.

[b] 6 ft. x ¼ in. i.d. column packed with 3% OV-25 on Chromosorb W HP (100 - 120M), injector temp. = 275^{O}C, column temp. = 205^{O}C, manifold temp. = 250^{O}C, detector temp. = 250^{O}C; N_2 carrier flow = 60 cc/min.

* * * * *

Table IV - GLC Data for Procaine, Internal Standard, and Signature Compound

	Retention Time (Min.)	
	3% OV-1 [a]	3% OV-25 [b]
Compound		
Procaine	3.8	2.6
Octacosane	-	4.3
Signature Compound	8.0	6.1

[a] 6 ft. x ¼ in. i.d. column packed with 3% OV-1 on Chromosorb W HP (100 - 120M), injector temp. = 275^{O}C, column temp. = 220^{O}C, manifold temp. = 250^{O}C, detector temp. = 250^{O}C; N_2 carrier flow = 60 cc/min.

[b] 6 ft. x ¼ in. i.d. column packed with 3% OV-25 on Chromosorb W HP (100 - 120M), injector temp. = 275^{O}C, column temp. = 240^{O}C, manifold temp. = 265^{O}C, detector temp. = 265^{O}C; N_2 carrier flow = 60 cc/min.

The signature compound isolated from procaine-containing samples by one or more of the chromatographic procedures given above are subjected to MS, UV, and IR identification outlined below.

E. *Identification*

1. Signature Compound in Benzocaine-Containing Samples

(a) *Mass Spectral Analysis*

The purified residue obtained above from benzocaine-containing samples was introduced into the GC-MS under conditions described earlier.

The EI spectrum of the signature compound was rather simple and quite similar to the EI spectrum of benzocaine (Fig. 2a and 2b). Benzocaine and the signature compound yielded molecular ions at *m/e* 165 and *m/e* 193, respectively. Both compounds exhibited prominent ions at $(M-28)^+$, $(M-45)^+$, and $(M-73)^+$. In both compounds, an ion of moderate intensity was noted at *m/e* 65.

The CI spectra of the signature compound confirmed the molecular weight of 193 obtained from the EI spectrum. This confirmation was supported by the presence in CI of an intense quasi-molecular ion at *m/e* 194 as well as the expected adduct ions at $(M+29)^+$ and $(M+41)^+$.

The EI and CI data obtained above suggested the signature compound to be closely related to benzocaine, but substituted with a functional group of 29 mass units, such as an ethyl or formyl substituent.

(b) *UV Analysis*

In methanol, benzocaine yielded a UV maximum at 292 nm, while the signature compound gave a maximum at 269 nm. This hypsochromic shift of 23 nm would suggest that an electron withdrawing group had been introduced in the phenyl ring of benzocaine, probably *para*- to the carboxyethyl group. Inspection of the benzocaine molecule revealed that substitution on the highly reactive amino function would be a likely occurrence. The substitution of an ethyl group on the amino function would have an inductive effect which would probably increase the wavelength of UV maximum. However, the introduction of a formyl group would decrease the interaction of the lone pair of electrons of nitrogen with the phenyl ring resulting in a decrease in wavelength of UV maximum.

Since MS and UV analyses suggested the signature compound to be N-formyl substituted benzocaine, an infrared analysis was done to support this postulation.

(c) *Infrared Analysis* [12,13]

The infrared spectra (KBr medium) of benzocaine and the signature compound were studied and found to be similar, but not identical. The IR spectrum of the signature compound supported a formyl substituent on the amino function of benzocaine. While benzocaine exhibited three bands between 3500 and 3200 cm^{-1} due to asymmetric, symmetric, and bonded NH_2 stretching vibrations, the signature compound exhibited only one intense band at 3310 cm^{-1}. This was strong evidence that substitution on the nitrogen function had occurred. This was further supported by the absence of the intense NH bending vibrational band found at 1630 cm^{-1} in primary aromatic amines such as benzocaine. The substituent on the nitrogen function was probably carbonyl as supported by an additional carbonyl band at 1700 cm^{-1} and a band at 1530 cm^{-1} due probably to the NH bending vibration found in amides (Amide II band).

The other bands in the spectrum of the signature compound were similar to benzocaine. These intense bands were at 1685 cm^{-1} and 1170 cm^{-1}, due to C-O stretching modes. Intense bands at 1595 cm^{-1} and 1505 cm^{-1}, were due to the phenyl moiety; and absorption bands between 850 cm^{-1} and 750 cm^{-1}, were due to C-H out-of-plane bending in the phenyl ring.

The combined UV, IR, and MS data suggested the signature compound to be N-formylbenzocaine (ethyl-*p*-formamidobenzoate). This structure is confirmed later in this paper by synthesis of the compound and its deuterated analogue and comparing its spectral data with that of the isolated signature compound. Additionally, a mechanistic interpretation of the mass spectral fragmentation of N-formylbenzocaine is presented.

2. Signature Compound in Procaine-Containing Samples

(a) *Mass Spectral Analysis*

The purified residue obtained above from samples containing procaine was introduced into the GC-MS under conditions described earlier.

The EI spectra of the signature compound and procaine were similar (Fig. 3a and 3b). Both spectra yielded ions at *m/e* 58, 65, 71, 86, 92, 99, 120, and 137. Additionally, procaine yielded an ion at *m/e* 164, while the signature compound produced ions at *m/e* 148, 192, and 249. Since procaine did not yield a molecular ion, it was not surprising that the molecular ion was not detected for the signature compound.

The CI spectrum of procaine yielded an intense quasimolecular ion at *m/e* 237 as well as the expected adduct ions at $(M+29)^+$ and $(M+41)^+$. The CI spectrum of the signature compound produced an intense quasimolecular ion at *m/e* 265 as well as the expected adduct ions at $(M+29)^+$ and $(M+41)^+$. These data suggested the molecular weight of the signature compound to be 264 amu.

The EI and CI data suggested the signature compound to be related to procaine, but with an additional substituent of 29 mass units. Since N-formylbenzocaine had been identified above, the substituent in procaine was hypothesized to be a formyl group.

b. *Ultraviolet Analysis*

The UV maxima of procaine and the signature compound in methanol were at 295 and 271 nm, respectively. This supported an assignment of a formyl group as a substituent on the aromatic nitrogen function in procaine (for discussion, refer above to UV analysis of benzocaine-related signature compound).

c. *Infrared Analysis*

The infrared spectra of procaine base (KBr medium) and the signature compound base (salt plates) were studied (signature compound base is an oil). As in benzocaine, procaine base exhibits three intense bands between 3500 and 3200 cm^{-1} due to NH_2 stretching vibrational modes. In the signature compound, only one significant band appeared at 3300 cm^{-1}. However, unlike N-formylbenzocaine, this band was rather broad and of low to moderate intensity. This was probably due to the fact that hydrogen bonding occurred because the spectrum was obtained as an oil. Nonetheless, it was apparent that substitution on the aromatic amine function in procaine had occurred. This assignment was supported by the absence of

(Continued on Page 140)

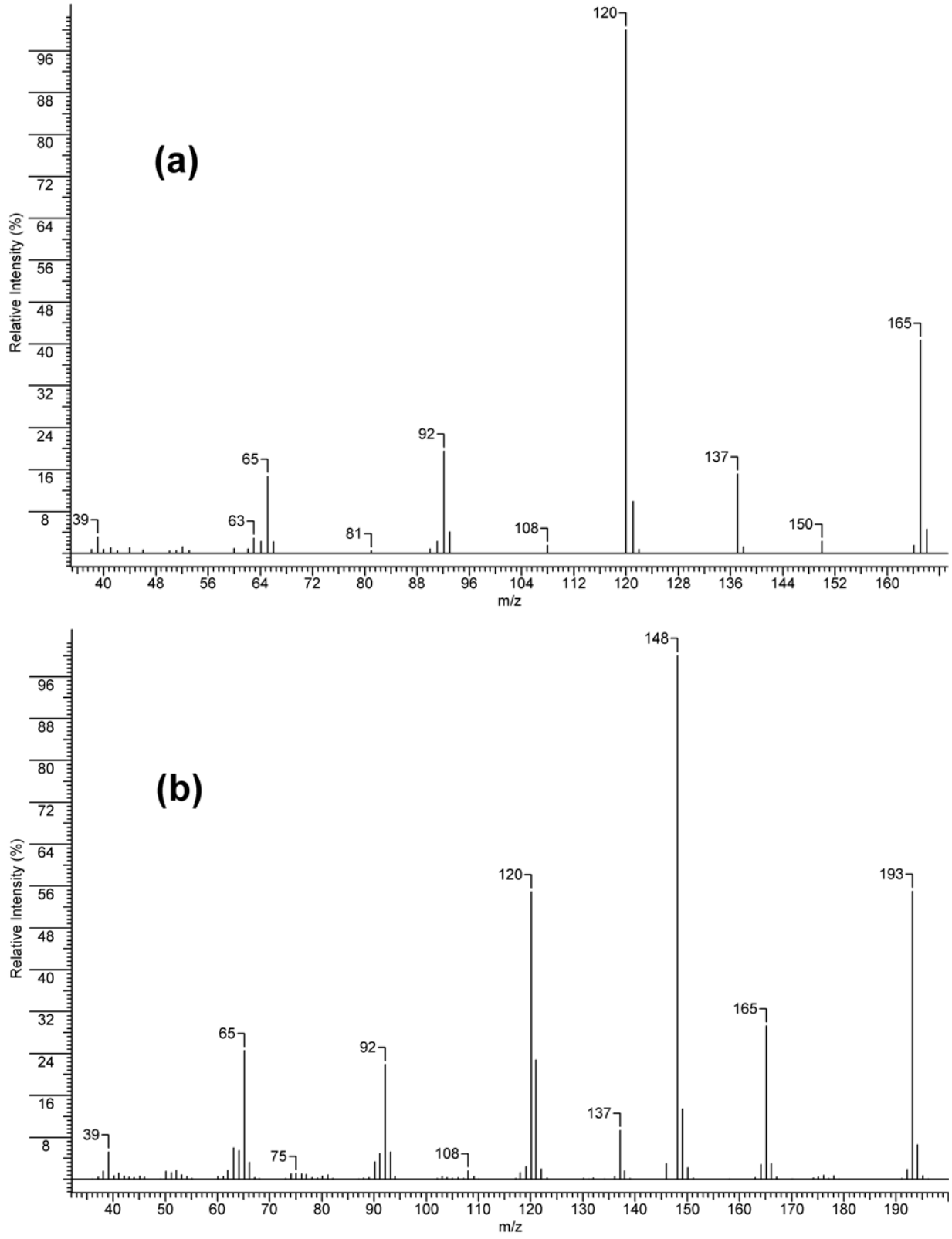


Figure 2: Spectra regenerated on GC-MSD. Conditions utilized were identical to those published in Microgram Journal 2006;4(1-4):47-53. (a) EI mass spectrum of benzocaine. (b) EI mass spectrum of N-formylbenzocaine.

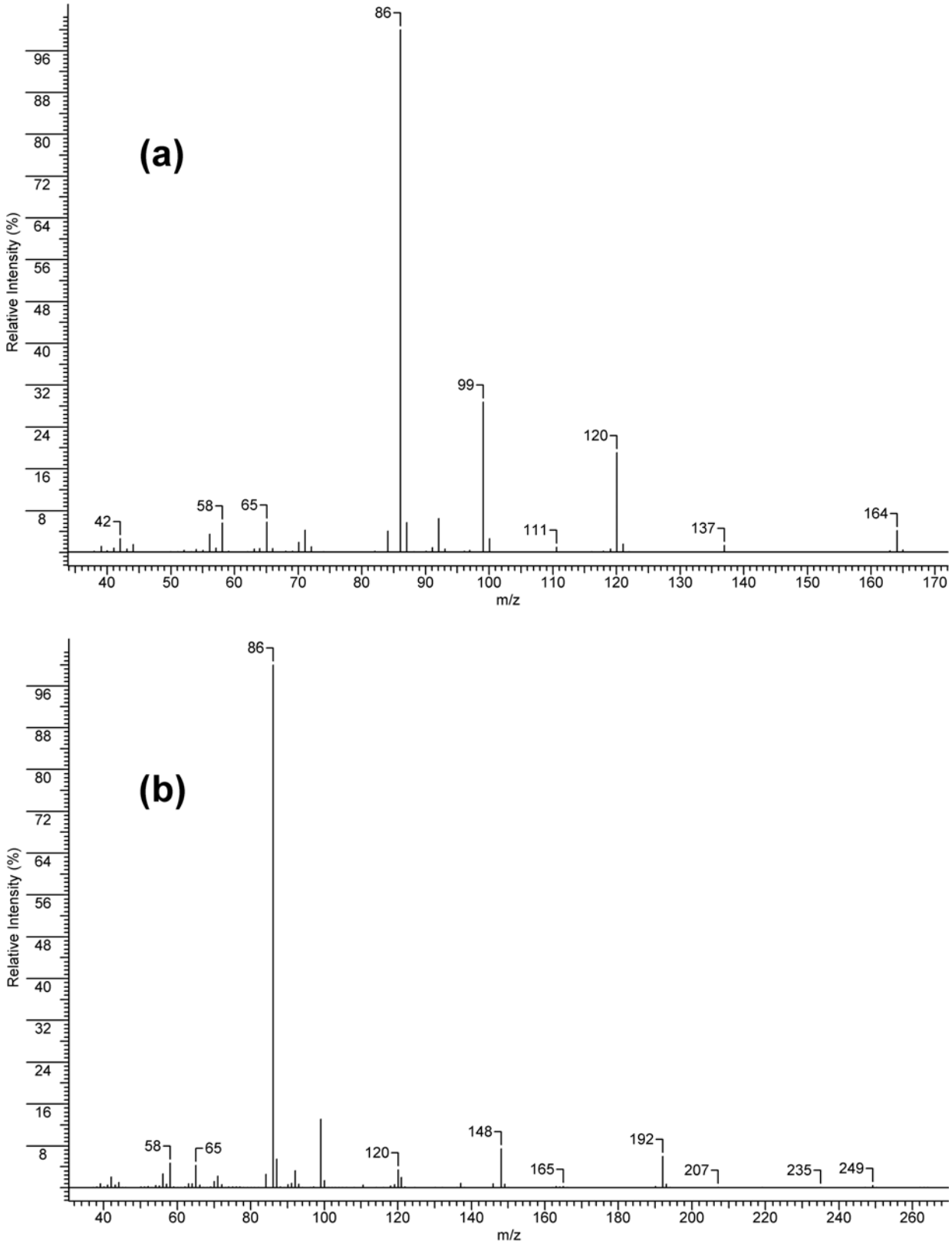


Figure 3: Spectra regenerated on GC-MSD. Conditions utilized were identical to those published in Microgram Journal 2006;4(1-4):47-53. (a) EI mass spectrum of procaine. (b) EI mass spectrum of N-formylprocaine.

the NH bending mode at 1620 cm^{-1} due to the NH_2 group in procaine. The N substituent is probably a carbonyl function, as supported by a very broad and intense band at 1700 cm^{-1}. This band represented a combination of two carbonyl bands, one due to the amido function and the other due to the ester moiety (note: acetylprocaine also exhibits one intense and broad band at about 1700 cm^{-1}). Further support for a carbonyl substituent on the aromatic nitrogen function arises from an intense band at 1530 cm^{-1} due to the NH bending mode found in amides (Amide II band). The other bands in the spectrum of the signature compound were similar to procaine base. These included bands at 1700 cm^{-1} due, in part, to the ester carbonyl stretching mode at 1270 and 1170 cm^{-1} due to the C-O stretching mode, an intense band at 1595 cm^{-1} due to the phenyl moiety, and absorption bands between 850 and 750 cm^{-1} due to C-H out-of-plane bending in the phenyl ring.

The combined UV, IR, and MS data suggested the signature compound, isolated from discolored authentic samples containing procaine, to be N-formylprocaine (2-diethylamino-*p*-formamidobenzoate). This structure was confirmed by the synthesis of this compound and its deuterated analogue and comparing its spectral data with that of the isolated signature compound. Additionally, a mechanistic interpretation of the mass spectral fragmentation of N-formylprocaine is given.

3. Synthesis of N-Formylbenzocaine, N-Formylprocaine, and their Deuterated Analogues

One gram quantities of benzocaine and procaine were dissolved in separate 10 cc portions of formic acid. One gram quantities of both compounds were also dissolved in separate 10 cc portions of deuterated formic acid (DCOOH). After about 20 hours at room temperature, the solutions were diluted with a large volume of ice water and made basic with $NaHCO_3$. The signature compounds and small amounts of unreacted benzocaine and procaine were extracted from the $NaHCO_3$ solution into ethyl ether. The ether extracts were passed through anhydrous sodium sulfate and evaporated to dryness. N-formylbenzocaine is a white solid, and N-formylprocaine is an oil. Both signature compounds were synthesized in greater than 90% yield.

The UV, IR, and MS spectral data for the synthesized standards were virtually identical with the signature compounds isolated from samples, thus confirming their identity as N-formylbenzocaine and N-formylprocaine.

4. Mechanistic Interpretation of Mass Spectral Fragmentation of N-Formylbenzocaine and N-Formylprocaine [14,15]

The N-formyl and deutero-formyl derivatives of benzocaine and procaine were synthesized as described above.

(a) *N-Formylbenzocaine* (Fig. 2b and 4)

The EI mass spectrum of N-formylbenzocaine yields a moderately intense molecular ion at *m/e* 193 (I). As expected, the deuterated analogue produced a molecular ion at *m/e* 194. An ion of moderate intensity at *m/e* 165 is due to expulsion of ethylene from the molecular ion with hydrogen transfer to the fragment ion (II). Though this ion could be rationalized as elimination of the formyl group with hydrogen transfer, the appearance of *m/e* 137 in benzocaine supports the former postulation. This assignment is confirmed by the appearance of an ion at *m/e* 166 in the deuterated species. The base peak at *m/e* 148 is due to elimination of C_2H_5O from the molecular ion with the fragment ion charge on the oxygen (III). A weak ion formed at *m/e* 137 is due to loss of CO and C_2H_4 with a double hydrogen transfer (IV). As expected, a shift of 1 amu occurred upon deuteration. A moderately intense ion at *m/e* 65 is probably due to the

cyclopentadienyl ion, an ion seen frequently in the spectra of aromatic amines (V). An intense ion found at *m/e* 120 (VI) is due to losses of C_2H_5O and the formyl group with the formyl hydrogen transferred to the charged species. Confirmation is obtained by the appearance of an intense ion at *m/e* 121 in the deuterated species. A moderately intense ion at *m/e* 92 is probably due to the amino phenyl moiety and involves hydrogen transfer from the expelled formyl group (VII). An ion at *m/e* 93 in the deuterated species confirms this postulation.

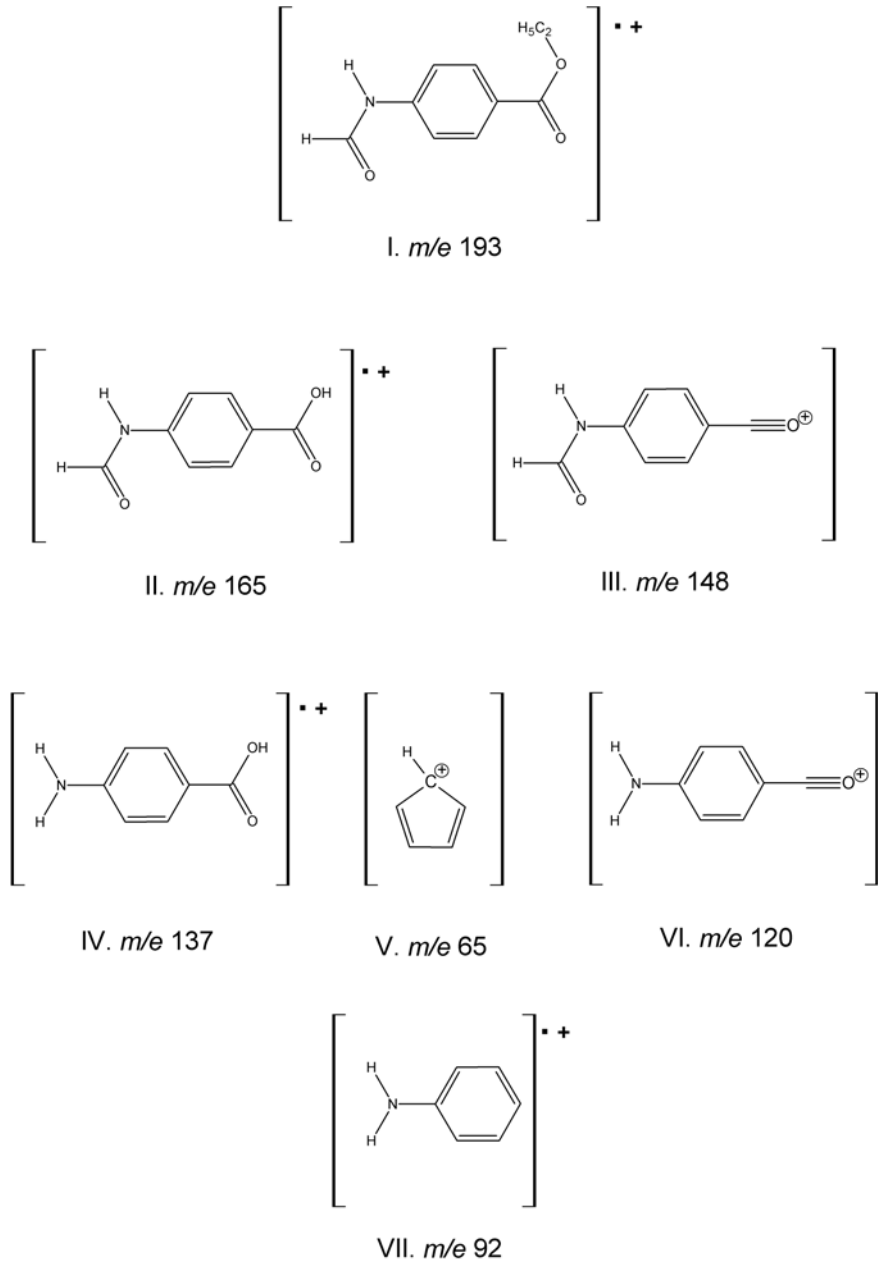
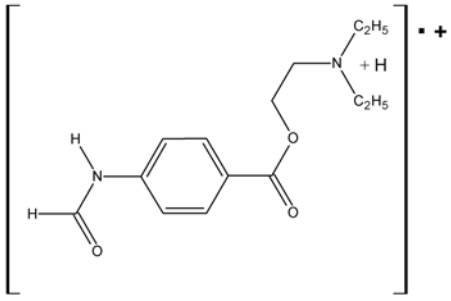


Figure 4: Prominent ions in EI mass spectrum of N-formylbenzocaine.

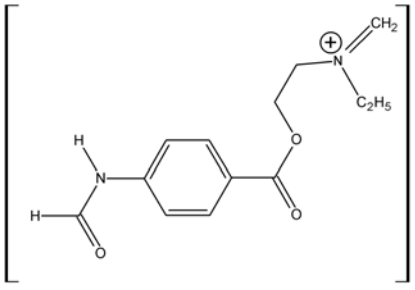
* * * * *

(b) *N-Formylprocaine* (Fig. 3b and 5)

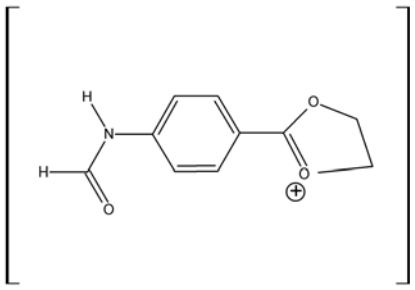
N-Formylprocaine does not yield a detectable molecular ion under EI conditions. However, under CI conditions, an intense quasimolecular ion at *m/e* 265 is present (VIII). Under EI, a weak ion is observed at *m/e* 249 which shifts 1 amu upon deuteration (IX). This ion is due to the elimination of a methyl group from the diethylamino function in the parent molecule. An ion of low intensity occurs at *m/e* 192 and is due to elimination of the diethylamino function from the molecular ion (X). Upon deuteration, an expected shift of 1 amu is observed. An ion of moderate intensity is found at *m/e* 99 and does not shift upon deuteration. It is due to fission of C(1)-O bond with loss of hydrogen from C1 (XI). The most intense ion in the spectrum occurs at *m/e* 86 and is due to fission of the C(1)-C(2) bond with charge retention on the diethylamino moiety (XII). As expected, no shift was observed upon deuteration. Ions at *m/e* 120 and 148 can be rationalized as for N-formylbenzocaine (Fig. 4).



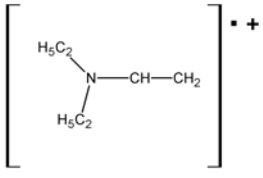
VIII. *m/e* 265 CI



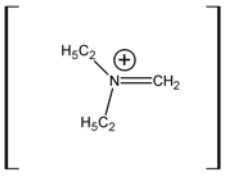
IX. *m/e* 249 EI



X. *m/e* 192 EI



XI. *m/e* 99 EI



XII. *m/e* 86 EI

Figure 5: Prominent ions in the EI and CI mass spectrum of N-formylprocaine.

5. Evaluation of N-Formylbenzocaine and N-Formylprocaine as Signature Compounds

In order to establish that the N-formyl derivatives of benzocaine and procaine were useful as signature compounds, it was necessary to demonstrate their presence in discolored authentic samples and their absence in samples that exhibited no discoloration. A number of authentic samples were analyzed for the presence of N-formylbenzocaine and N-formylprocaine. These authentic samples had been subjected to ambient and accelerated studies and exhibited either no discoloration or marked browning. Table V illustrates these results. It is evident from Table V that the N-formyl derivatives are present in those samples that discolored significantly, yet could not be detected in those samples that did not discolor. These findings supported their value as signature compounds.

* * * * *

Table V - Evaluation of N-Formyl Derivatives of Benzocaine and Procaine as Signature Compounds

Sample Number	Study	Significant Discoloration	Presence of N-Formylbenzocaine	Presence of N-Formylprocaine
19	Accelerated	Yes	-	Yes
10	Accelerated	Yes	-	Yes
49	Accelerated	Yes	-	Yes
54	Accelerated	Yes	-	Yes
18	Accelerated	Yes	-	Yes
28	Accelerated	Yes	-	Yes
54	Ambient	No	No	-
59	Ambient	No	No	-
23	Accelerated	Yes	-	Yes
47	Accelerated	Yes	-	Yes
64	Accelerated	No	No	-
65	Ambient	No	No	-
52	Accelerated	Yes	-	Yes
50	Ambient	Yes	-	Yes
22	Ambient	Yes	-	Yes

* * * * *

In order to further correlate the presence of N-formyl derivatives with the browning process, the following was done. Authentic samples composed of 10% 5-HMF, 10% acid catalyst (boric acid), and 80% of either procaine HCl or benzocaine were prepared and then placed in the dark at room temperature for one day. After this time period, the samples were analyzed for the appropriate signature compounds. The N-formyl derivatives of both benzocaine and procaine were detected in these samples. Additionally, the samples exhibited marked discoloration. These results indicate that 5-HMF plays a role in the acid-catalyzed formation of the N-formyl derivatives. This is not surprising, in that 5-HMF has been associated with sugar decomposition. Furthermore, the subsequent decomposition of 5-HMF to formic acid has been reported [7,9]. Formic acid then reacts readily with either benzocaine or procaine to form the N-formyl derivatives.

Some preliminary work has been done in the isolation of additional signature compounds in an illicit sample known to have undergone discoloration. The sample consisted, in part, of cocaine HCl, boric acid, benzocaine, and dextrose. The N-formylbenzocaine signature was detected in this sample. The additional signature compounds were colored substances and isolated as described below. The discolored sample was placed on a dilute HCl - Celite 545 AW column and eluted with water-saturated ethyl ether

as described earlier for benzocaine-containing samples. After discarding the ether eluate, the column was eluted with water-saturated chloroform. The chloroform was evaporated to less than 1 cc and transferred to the top of a neutral alumina column. The column was eluted initially with diethyl ether followed by ether-chloroform mixtures and then finally chloroform. A series of purple, blue, and pink-colored compounds eluted through the column (note: Rodd's Chemistry of Carbon Compounds states that furfural reacts with primary amines, resulting in a ring opening and condensation with the amine to form a red-colored compound). Analysis of the combined column eluates on silica gel TLC plates revealed the presence of a number of colored substances (blue, purple, pink, and yellow). Though these colored compounds appear promising as signatures, further work must be done for their full characterization.

Summary

The study described in this paper can be summarized as follows:

A. The illicit and authentic samples that underwent discoloration, or browning, contained a primary aromatic amine, namely procaine or benzocaine, and a sugar, either lactose or dextrose; the samples also contained an acid catalyst, such as cocaine HCl, boric acid, benzoic acid, etc.

B. The rate of discoloration was pH dependant, i.e., the rate of sample browning increased as the pH of the sample decreased.

C. The rate of sample discoloration increased as the temperature of the sample increased.

D. N-Formyl derivatives of benzocaine and procaine were established as *bona fide* signature compounds in discolored samples. These derivatives are believed to be formed following the decomposition of 5-HMF to formic acid and the subsequent formylation of the aromatic amine function in benzocaine and procaine.

E. The presence of colored compounds (yellow, blue, purple, and pink) in the discolored samples appear promising as additional signature compounds.

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References

1. Dugar, S., Catalano, T., and Cerrato, R., "Discoloration Effects of Diluents in Contraband Cocaine," Journal of Forensic Sciences, JFSCA, Vol. 19, 1974, pp. 868-72.

2. Blaug, S.M. and Huang, W.-T., "Interaction of Dextroamphetamine Sulfate with Spray-Dried Lactose," Journal of Pharmaceutical Sciences, Vol. 61, Nov. 1972, pp. 1770-75.

3. Blaug, S.M. and Huang, W.-T., "Interaction of D-Amphetamine Sulfate with Dextrose in Solution," Private Communication.

4. Castello, R.A. and Mattocks, A.M., "Discoloration of Tablets Containing Amines and Lactose," Journal of Pharmaceutical Sciences, Vol. 51, Feb. 1962, pp. 106-08.

5. Duvall, R.N., Koshy, K.T., and Pyles, J.W., "Comparison of Reactivity of Amphetamine, Methamphetamine and Dimethylamphetamine with Lactose and Related Compounds," Journal of Pharmaceutical Sciences, Vol. 54, April 1965, pp. 607-11.

6. Duvall, R.N., Koshy, K.T., and Dashiell, R.E., "Comparative Evaluation of Dextrose and Spray-Dried Lactose in Direct Compression Systems," Journal of Pharmaceutical Sciences, Vol. 54, Aug. 1965, pp. 1196-1200.

7. Wolfrom, M.L., Scheutz, R.D., and Cavalieri, L.F., "Chemical Interactions of Amino Compounds and Sugars. IV. Significance of Furan Derivatives in Color Formation," Journal of the American Chemical Society, Vol. 71, Oct. 1949, pp. 3518.

8. Wolfrom, M.L., Scheutz, R.D., and Cavalieri, L.F., "Chemical Interactions of Amino Compounds and Sugars. III. The conversion of D-Glucose to 5-(Hydroxymethyl)-2-furaldehyde," Journal of the American Chemical Society, Vol. 70, 1948, pp. 514-17.

9. Singh, B., Dean, G.R., and Cantor, S.M., "The Role of 5-(Hydroxymethyl)-furfural in the Discoloration of Sugar Solutions," Journal of the American Chemical Society, Vol. 70, 1948, pp. 517-22.

10. Scallet, B.L. and Gardner, J.H., "Formation of 5-Hydroxymethylfurfural from D-Glucose in Aqueous Solutions," Journal of the American Chemical Society, Vol. 67, 1945, pp. 1934-35.

11. Janicki, C.A. and Almond, Jr., H.R., "Reaction of Haloperidol with 5-(Hydroxymethyl)-2-furfuraldehyde, an Impurity in Anhydrous Lactose," Journal of Pharmaceutical Sciences, Vol. 63, Jan. 1974, pp. 41-43.

12. Nakanishi, K., Infrared Absorption Spectroscopy, Holden-Day, Inc., San Francisco, 1963.

13. Rao, C.N.R., Chemical Applications of Infrared Spectroscopy, Academic Press, New York, 1963.

14. Budzikiewicz, H., Djerassi, C., and Williams, D.H., Interpretation of Mass Spectra of Organic Compounds, Holden-Day, Inc., San Francisco, 1965.

15. McLafferty, F.W., Interpretation of Mass Spectra, W.A. Benjamin, Inc., Reading, Massachusetts, 1973.

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