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].

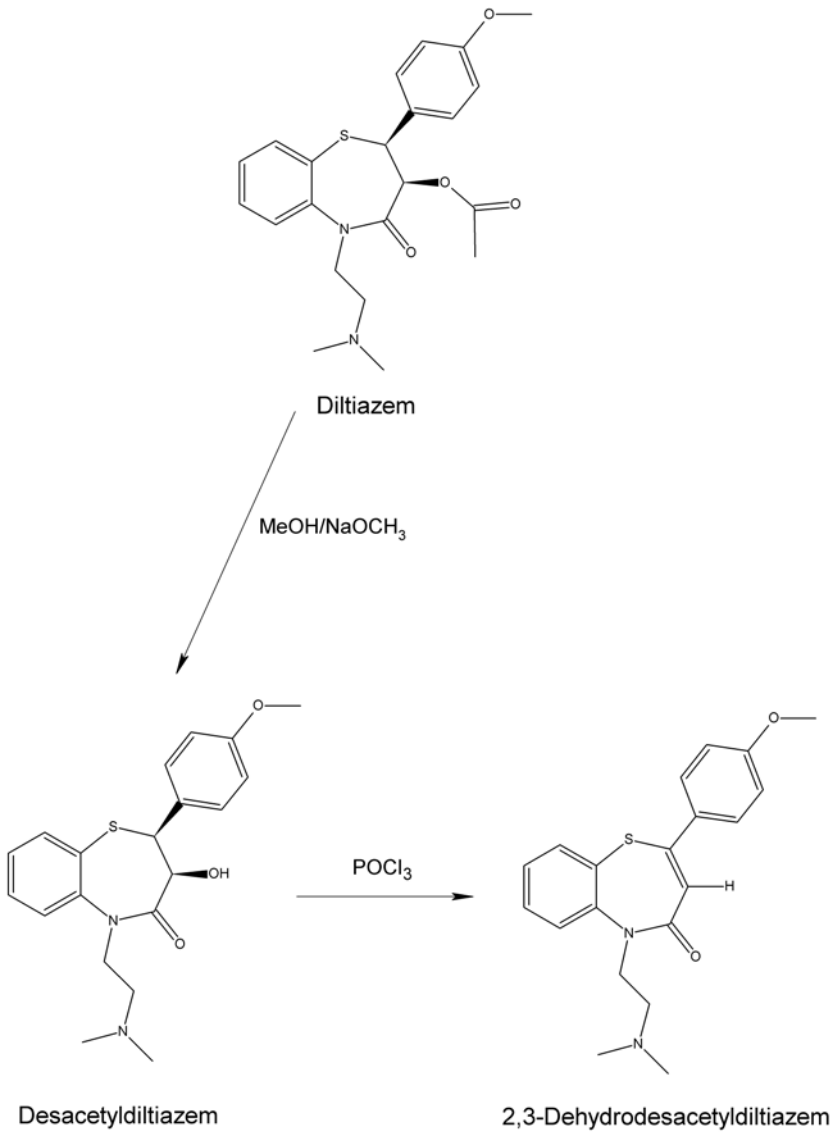

Figure 1. Synthesis and Structural Formulas of Diltiazem and Related Compounds.

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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.

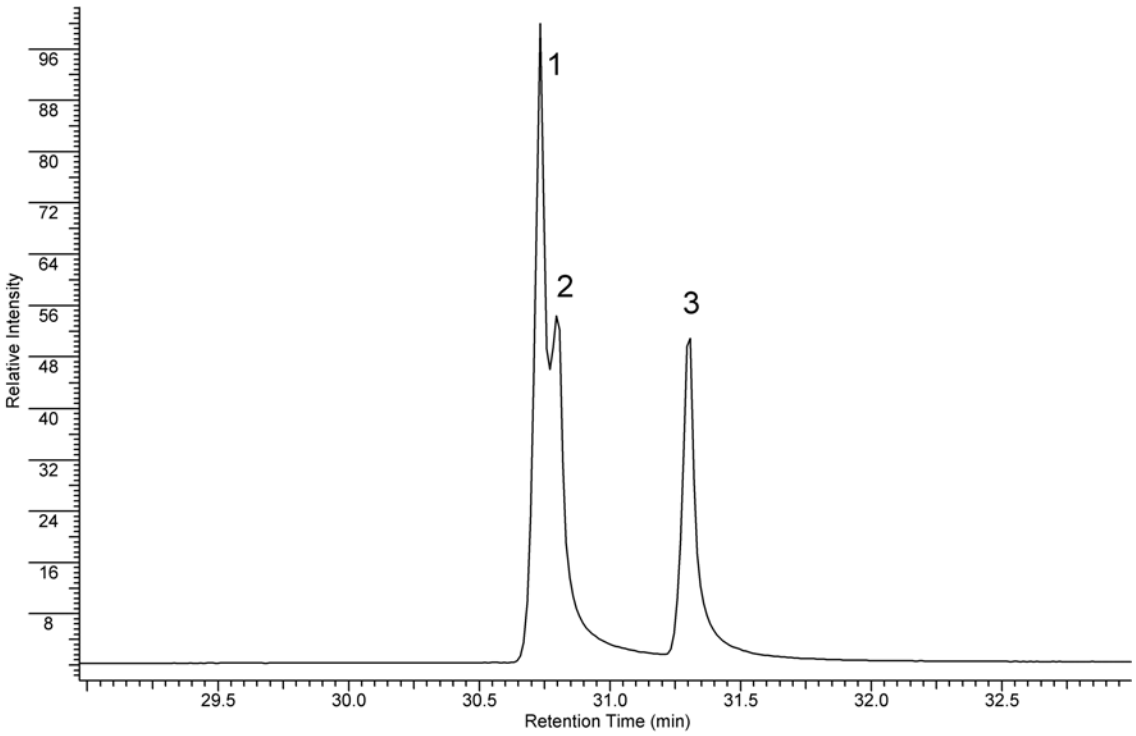

Figure 2. Partial Reconstructed Total Ion Chromatogram Containing Diltiazem and Suspected Impurities/Artifacts. Peak Identification: 1 = Diltiazem, 2 and 3 = Suspected Diltiazem Impurities/Artifacts.

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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.

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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

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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.

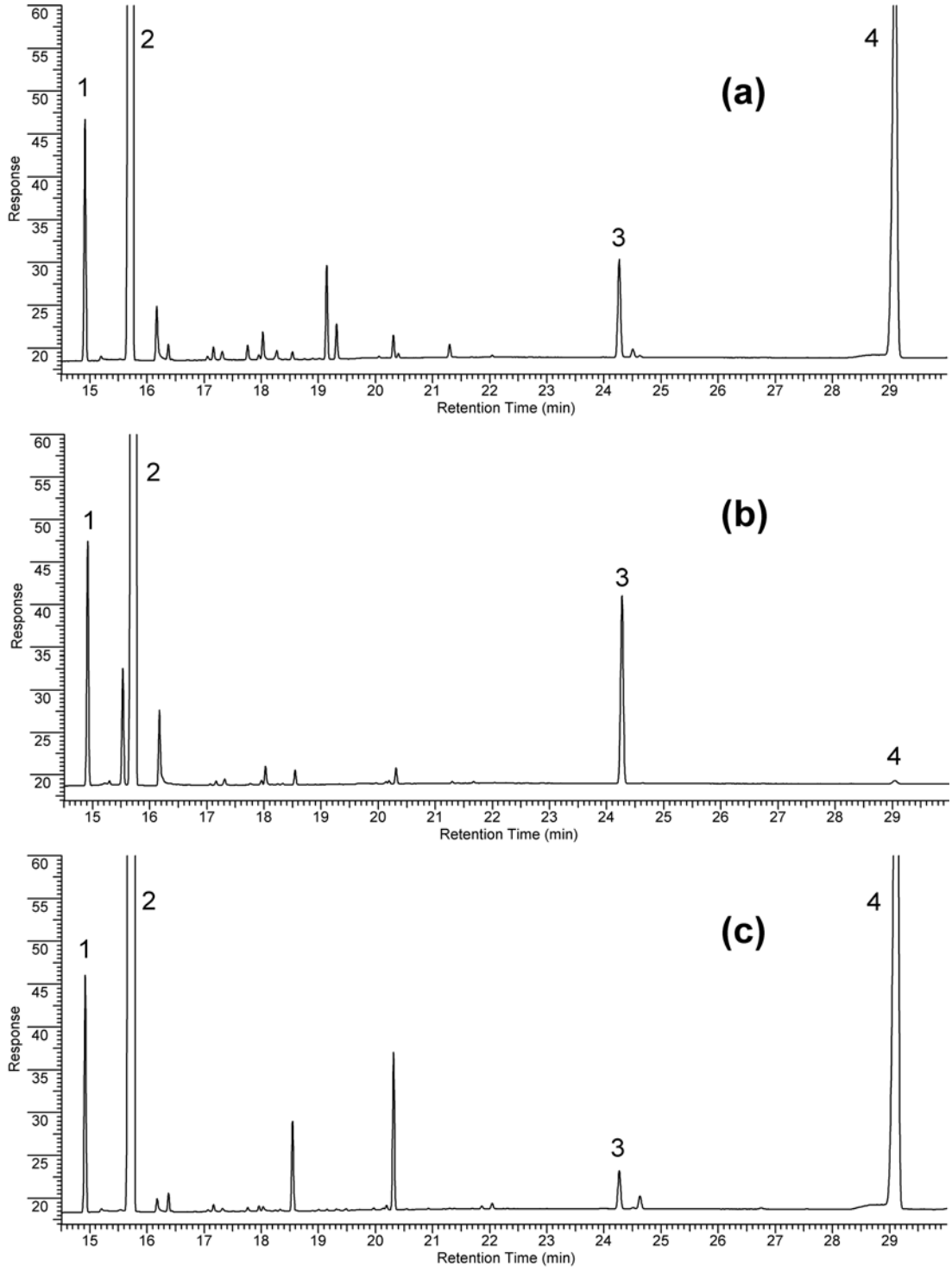

Figure 3. Partial Reconstructed GC/FID Chromatograms of: (A) Cocaine Base Exhibit #1 Containing 44.2% Cocaine, 0.67% Desacetyldiltiazem, and 7.1% Diltiazem; (B) Cocaine Base Exhibit #2 Containing 54.5% Cocaine, 1.3% Desacetyldiltiazem, and Trace Diltiazem; (C) Cocaine Hydrochloride Exhibit Containing 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.

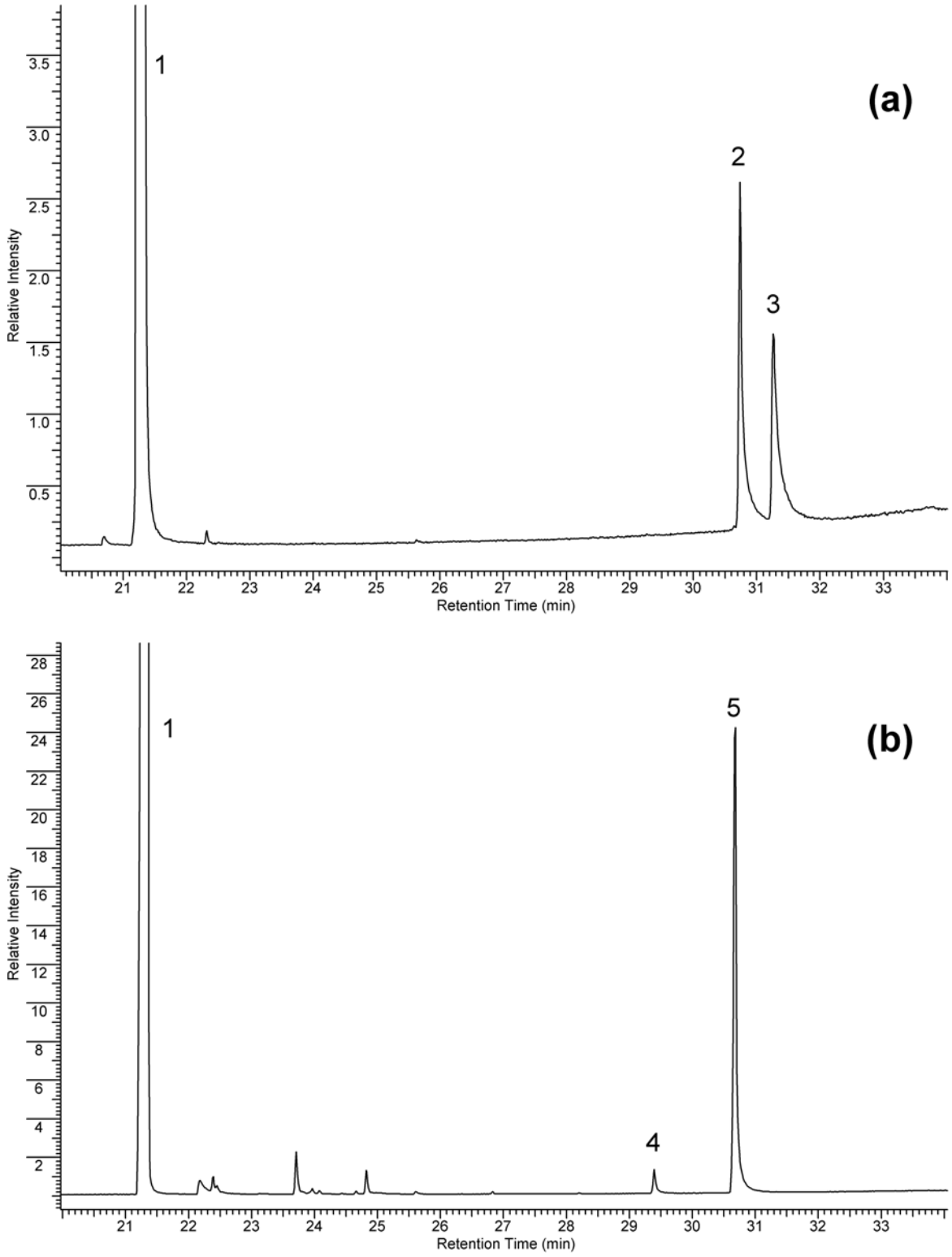

Figure 4. Partial Reconstructed Total Ion Chromatograms of Cocaine Base Exhibit #1 Using: (A) Methanol as Injection Solvent; and (B) $CHCl_3$/MSTFA as Injection Solvent. Peak Identification: 1 = Cocaine, 2 = Desacetyldiltiazem, 3 = Diltiazem Artifact, 4 = Desacetyldiltiazem-TMS, and 5 = Diltiazem.

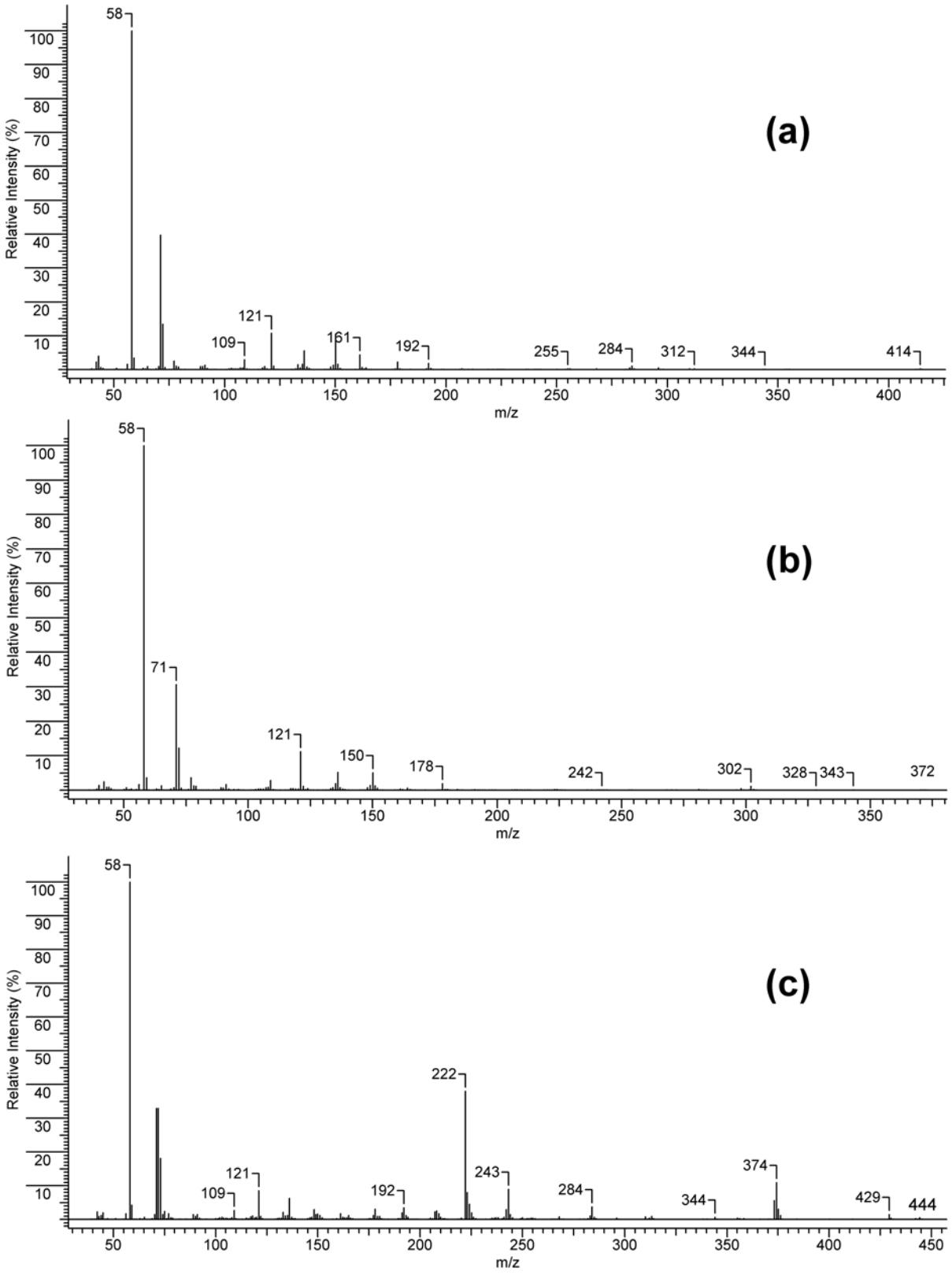

Figure 5. Electron Ionization Mass Spectrum of: (A) Diltiazem; (B) Desacetyldiltiazem; and (C) Desacetyldiltiazem-TMS Derivative.

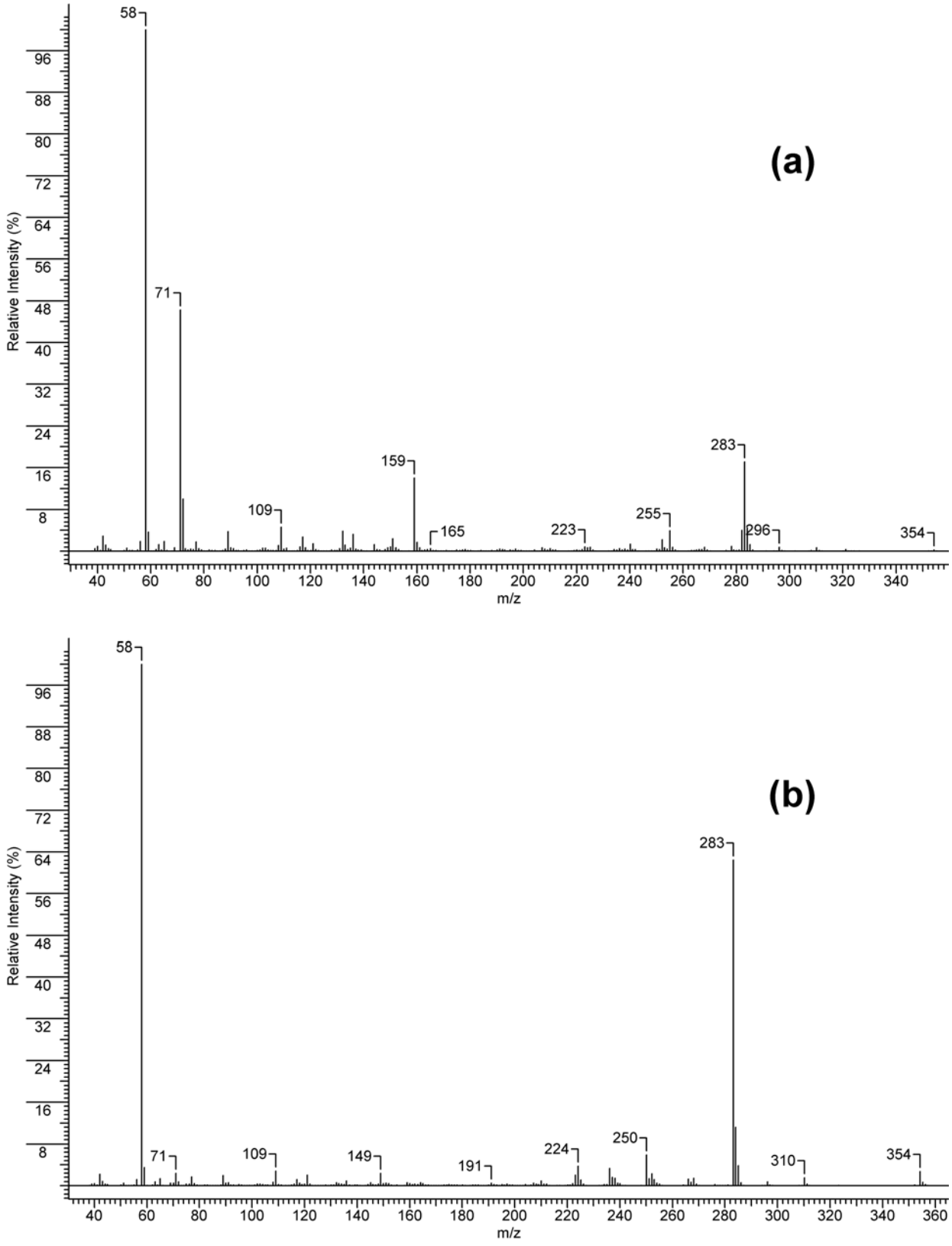

Figure 6. Electron Ionization Mass Spectrum of (A) Diltiazem Artifact; and (B) 2,3-Dehydrodesacetyldiltiazem.

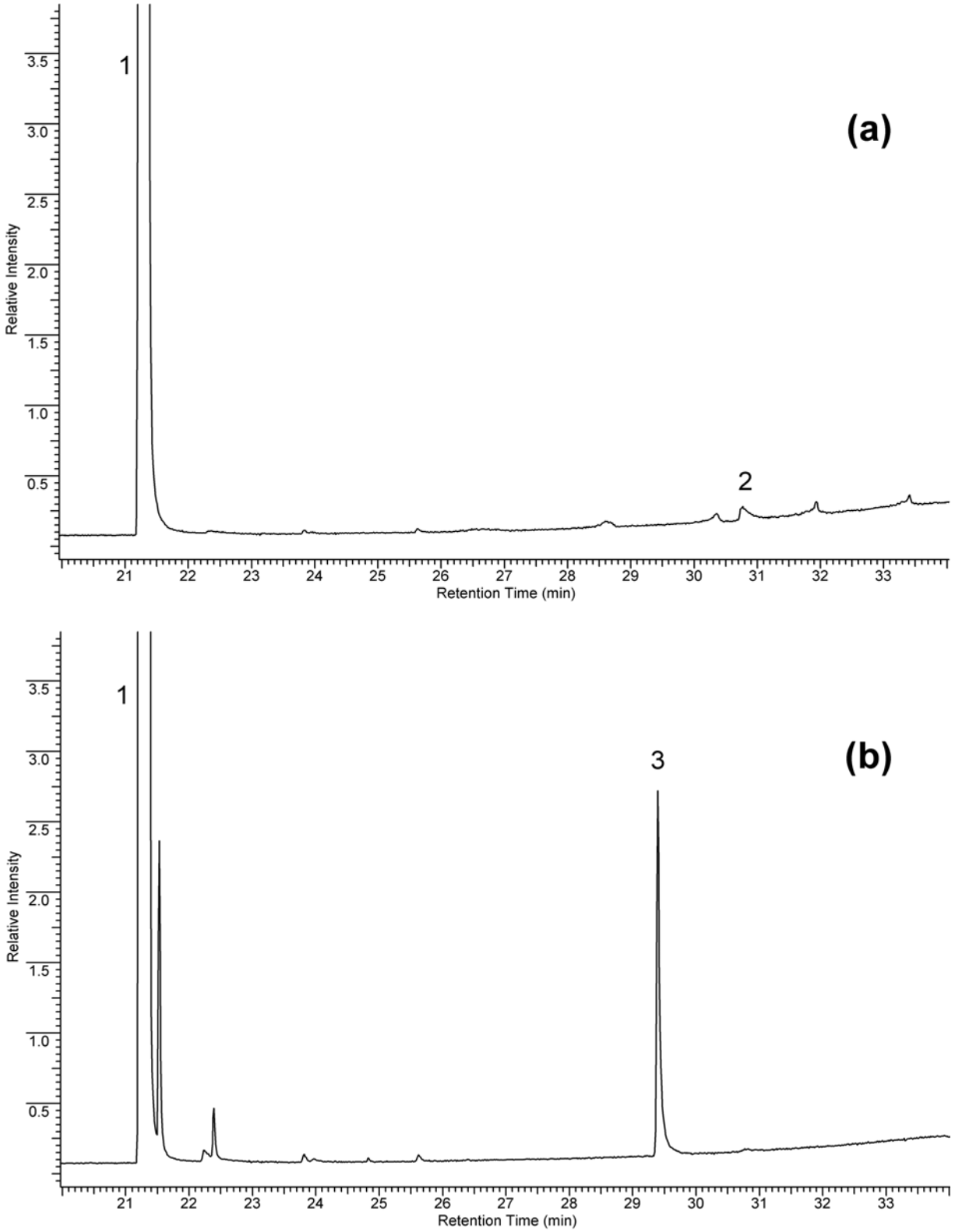

Figure 7. Partial Reconstructed Total Ion Chromatograms of Cocaine Base Exhibit #2 Using: (A) Methanol as Injection Solvent; and (B) $CHCl_3$/MSTFA as Injection Solvent. Peak Identification: 1 = Cocaine; 2 = Desacetyldiltiazem; and 3 = Desacetyldiltiazem-TMS Derivative.

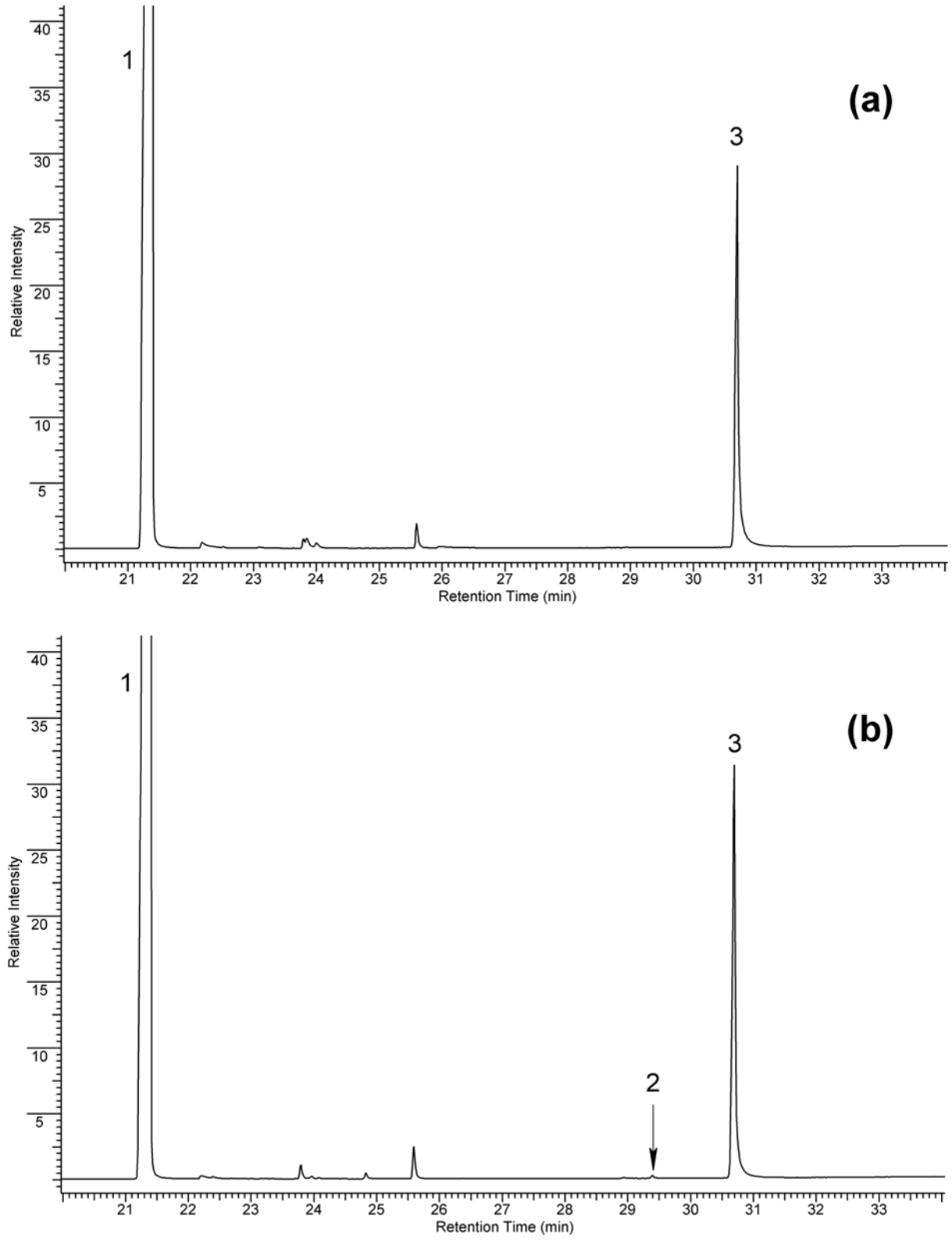

Figure 8. Partial Reconstructed Total Ion Chromatograms of Cocaine Hydrochloride Exhibit Using (A) Methanol as Injection Solvent; and (B) $CHCl_3$/MSTFA as Injection Solvent. Peak Identification: 1 = Cocaine, 2 = Desacetyldiltiazem-TMS, and 3 = Diltiazem.

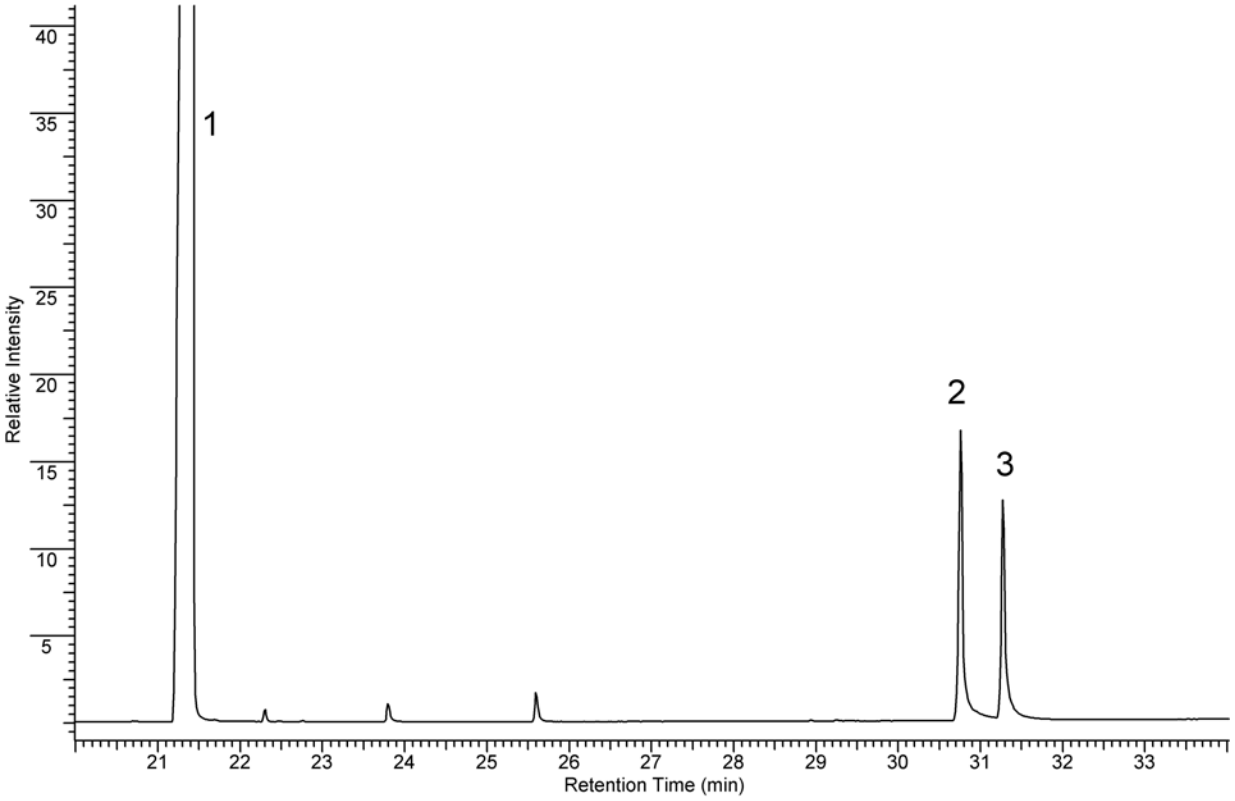

Figure 9. Partial Reconstructed Total Ion Chromatogram of Cocaine Base ("Crack") Produced from the Cocaine Hydrochloride Exhibit. Peak Identification: 1 = Cocaine; 2 = Desacetyldiltiazem; and 3 = Diltiazem Artifact.

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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.

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