

The Separation of Cocaine and Phenyltetrahydroimidazothiazole Mixtures

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ABSTRACT: Phenyltetrahydroimidazothiazole (i.e., levamisole, dexamisole, or tetramisole) has been increasingly utilized as a cutting agent by South American illicit cocaine laboratories for the past eight years, and is now the most predominant adulterant in cocaine produced in Colombia. The salt form of illicit cocaine must be determined when possible for sentencing purposes; this is typically done via infrared spectroscopy. Several separation techniques for cocaine/phenyltetrahydroimidazothiazole mixtures are presented to assist the determination of cocaine salt form during forensic analyses. Additionally, a purification technique is presented for isolation of cocaine for isotope ratio mass spectrometric analysis, a critical component for source determination. Mixtures of cocaine (hydrochloride and base) and phenyltetrahydroimidazothiazole (85:15, 70:30, and 50:50) were prepared and separated with liquid/liquid extractions, ion-pair chromatography, and high pressure liquid chromatography. Recovered cocaine was subsequently analyzed via infrared spectroscopy, gas chromatography-flame ionization detection, and isotope ratio mass spectrometry.

KEYWORDS: Forensic chemistry, cocaine, phenyltetrahydroimidazothiazole, liquid/liquid separations, IRMS

The purity of illicit cocaine seized in the United States has steadily decreased over the past eight years. This trend is primarily due to the addition of adulterants such as phenyltetrahydroimidazothiazole (PTHIT), diltiazem, and hydroxyzine, which began around 2005 [1]. Currently, PTHIT is routinely identified in illicit cocaine samples as levamisole, tetramisole, or a non-racemic mixture of levamisole and dexamisole (Figure 1) in approximately 70% of all domestic cocaine seizures. Traditionally, PTHIT was widely used as an anti-worm medication (anthelmintic) for both humans and animals, but it is no longer approved for use in the United States or Canada due to its toxicity. The proposed explanation for the presence of PTHIT in cocaine samples is to prolong the drug's effects [2].

In the United States, for the purposes of federal prosecution and sentencing, it is necessary to determine the salt form (base or hydrochloride) when analyzing cocaine. Chiral chromatographic methods have been developed for the separation and isomer identification of PTHIT [3-4]; however, few methods have been presented to provide a non-destructive purification of cocaine for the purpose of identifying salt forms. In addition to salt form identification, pure cocaine is needed in elemental analysis-isotope ratio mass spectrometric analyses (EA-IRMS) for the purposes of origin determination [5].

Experimental

Materials

Celite 545 and all chemicals and solvents used were reagent grade or better and were obtained from Sigma-Aldrich (St. Louis, MO). Cocaine hydrochloride (HCl) and tetramisole HCl were obtained from this laboratory's reference materials collection.

The glass chromatographic columns used for ion-pair separations were products of Lurex (Vineland, NJ), and were 260 mm \times 22 mm i.d. with a stem length of 50 mm. Columns were prepared identically to conditions previously reported [6].

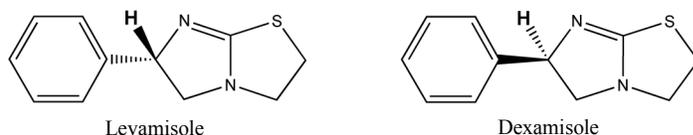


Figure 1 - Structures of levamisole and dexamisole.

Fourier Transform Infrared Spectroscopy (FTIR)

Infrared spectra were obtained on a Thermo-Nicolet Nexus 670 FTIR (Pittsburgh, PA) equipped with a single bounce attenuated total reflectance (ATR) accessory. Instrument parameters were: resolution = 4 cm^{-1} ; gain = 8; optical velocity = 0.4747; aperture = 150; and scans/sample = 16.

Gas Chromatography/Flame Ionization Detection (GC/FID)

GC/FID analyses were performed with an Agilent (Palo Alto, CA) Model 6890N Gas Chromatograph. The sample preparation and GC/FID conditions used have been previously reported [7]. Isopropylcocaine was utilized as the internal standard.

High Performance Liquid Chromatography (HPLC)

HPLC analyses were performed with an Agilent 1200 Series Preparative Liquid Chromatograph using an autosampler equipped with a 1000 μL loop, preparative pump, thermostatted column compartment, and fraction collector. The injection volume was 430 μL . The stationary phase consisted of a silica column, Agilent Zorbax RX-SIL 4.6 mm i.d. \times 100 mm, with a 1.8 μm particle size. The mobile phase consisted of the following elution gradient: An isocratic hold using 96% ammoniated hexane/4% isopropanol for 10 min, followed by a linear gradient for five min to 65% ammoniated hexane/35% isopropanol, hold for 2 min, then a linear gradient for 3 min from 65% ammoniated hexane/35% isopropanol back to the starting mobile phase, for a total run-time of 20 min. The column temperature

was set at 20°C and the flow rate was set to 2 mL/min. Three fractions were collected from 0.5 min to 4.0 min, 4.0 min to 5.0 min, and 5.0 min to 6.0 min. The elution of cocaine and PTHIT was monitored using diode-array detection (DAD) at 254 nm, and the appropriate fractions were combined to collect the entire cocaine peak. The collected fractions were dried at 75°C under a stream of air and transferred to a 4 mL glass vial using a minimal amount of diethyl ether, which was then evaporated to obtain cocaine base powder.

Elemental Analysis-Isotope Ratio Mass Spectrometry (EA-IRMS)

Cocaine samples were analyzed to determine carbon isotopes using a Delta Plus XP isotope ratio mass spectrometer (Thermo Fisher Scientific; Pittsburgh, PA) in continuous flow mode with a ECS 4010 elemental analyzer (Costech Analytical; Valencia, CA). The elemental analyzer reactor tubes were comprised of two quartz glass tubes filled with chromium(III) oxide/silvered cobaltous oxide and reduced copper. The tubes were held at 1040°C and 640°C for combustion and reduction, respectively. Water was removed from the generated combustion gases by a trap filled with magnesium perchlorate. Nitrogen and carbon dioxide were separated with a post-reactor GC column maintained at 65°C.

Liquid/liquid Separations

Two grams of cocaine HCl and tetramisole HCl (85:15) were prepared by weighing each compound separately and mixing thoroughly. Nineteen solvent combinations of chloroform/hexane and chloroform/ether were prepared (10:0, 9:1, 8:2, and 10% increments to 1:9). Approximately 50 mg portions of the cocaine HCl/tetramisole HCl mixture were combined with 4 mL of a selected solvent combination in 15 mL glass round-bottom centrifuge tubes. The tubes were vigorously shaken and centrifuged. The solvent was removed and filtered through a micro-filter. Insoluble material was allowed to dry overnight prior to analysis. This procedure was repeated with heating the samples at 75°C for 10 min upon addition of solvent. The samples were allowed to cool for 1 hour before filtering. The filtered solvent was then evaporated to dryness. Soluble and insoluble materials were analyzed via FTIR and GC/FID.

In addition to the chloroform/hexane and chloroform/ether combinations, aqueous/organic solvent combinations were utilized in order to separate mixtures of cocaine HCl and tetramisole HCl (85:15, 70:30, 50:50) as listed in Table 1. Fifty mg of cocaine HCl/tetramisole HCl were dissolved in an aqueous solution (Solution 1) in a 15 mL glass round-bottom centrifuge tube. Five mL of chloroform was then added, and the tube was shaken vigorously, then centrifuged for approximately 2 min. The chloroform layer was removed, evaporated to dryness, and examined via FTIR/ATR and GC/FID.

Each of the cocaine HCl/tetramisole HCl mixtures (85:15, 70:30, 50:50) were converted to the base form by dissolving the mixture in boiling water and adding dilute ammonium hydroxide until the solution was basic and precipitation occurred. The mixture was allowed to cool, and the water was removed. The remaining base mixture was allowed to dry overnight. Ten 50 mg portions were combined with 5 mL of hexane or ether in 10 separate 15 mL glass round-bottom centrifuge tubes (five

Table 1 - Liquid/liquid separations of cocaine HCl and tetramisole HCl with 5 mL chloroform.

Solution 1	% Cocaine HCl		
	85:15	70:30	50:50
0.250 mL H ₂ O	96.5	94.2	90.0
1.0 mL H ₂ O	98.4	95.3	89.3
0.250 mL 1 N HCl/2 M NaCl	92.4	95.9	91.4

Table 2 - Liquid/liquid separations of cocaine base and tetramisole base with hexane/H₂O (5 mL/5 mL).

Water Washes	% Cocaine Base		
	Cocaine/PTHIT (85:15)	Cocaine/PTHIT (70:30)	Cocaine/PTHIT (50:50)
1	94.4	84.3	77.0
2	97.9	93.5	88.2
3	99.2	97.3	95.8
4	99.7	99.1	98.5
5	100.0	100.0	99.4

Table 3 - Ion pair column separations of cocaine HCl and tetramisole HCl.

Column	% Cocaine HCl		
	85:15	70:30	50:50
1 ^a	100.0 ^d	100.0 ^d	100.0 ^d
2 ^b	100.0 ^d	99.1	99.4
3 ^c	100.0 ^d	98.9	97.6

^a 4 g Celite 545 and 2 mL 1 N HCl/2 M NaCl

^b 2 g Celite 545 and 1 mL 1 N HCl/2 M NaCl

^c 4 g Celite 545 and 2 mL H₂O

^d No PTHIT detected in eluent via GC/FID

Table 4 - IRMS results from cocaine HCl and tetramisole HCl^a mixtures after LC-Prep cleanup procedure.^b

% Cocaine	Corrected $\delta^{15}\text{N}$	Corrected $d^{13}\text{C}$
100%	-9.3 ± 0.06	-29.3 ± 0.00
85%	-9.3 ± 0.05	-29.2 ± 0.05
70%	-9.2 ± 0.00	-29.2 ± 0.00
50%	-9.4 ± 0.10	-29.2 ± 0.05

^a Known values for tetramisole HCl: $\delta^{15}\text{N} = -3.4$; $\delta^{13}\text{C} = -26.9$

^b Triplicate analysis

test tubes per solvent). All samples were heated at 75°C for approximately 5 min. Once the solutions were cooled, 5 mL of water were added to each test tube. The samples were shaken vigorously and centrifuged for 2 min. The solvent layer was removed and washed again with water. The washing process was repeated up to four times (Table 2). The washed solvent was evaporated to dryness and examined via FTIR and GC/FID.

Ion-Pair Chromatography

Three ion-pair columns were prepared to separate mixtures of cocaine HCl and tetramisole HCl (85:15, 70:30, and 50:50). Fifty mg of cocaine HCl/tetramisole HCl were dissolved in 250 μ L of water, combined with 0.5 g of Celite 545, and mixed well. The resulting mixture was transferred to a column packed with a portion of Celite 545 and 1-2 mL of ion pair solution as specified in Table 3. Cocaine was eluted with 35 mL of water-saturated chloroform. Five 5 mL fractions were collected and analyzed via GC/FID for the amount of cocaine present. Appropriate fractions were combined, evaporated to dryness, and examined via FTIR.

HPLC Sample Preparation

Cocaine HCl samples adulterated with tetramisole HCl were purified by preparative liquid chromatography. Using a 25 mg equivalent of cocaine, samples were first dissolved in 1 mL of water in a 15 mL glass round-bottom centrifuge tube. A basic extraction was performed using two drops of concentrated ammonium hydroxide and 5 mL of diethyl ether. The tube was capped, shaken, and centrifuged for approximately five minutes. The ether layer was transferred to a new test tube and

dried under a stream of air at 75°C. The dried sample was then analytically transferred using small aliquots of ether to a 1.5 mL glass autosampler vial. The sample was then evaporated at 75°C to remove all ether. When dry, the sample was diluted with 200 μ L of isopropanol and 600 μ L of hexane, capped and heated for approximately five minutes at 75°C to ensure the sample was completely dissolved in injection solvent.

Results and Discussion

Liquid/liquid Separations

After washing the cocaine HCl/tetramisole HCl mixture (85:15) with several combinations of chloroform/hexane and chloroform/ether, insoluble material and soluble material were both analyzed via FTIR and GC/FID. The cocaine purity of the insoluble material and soluble material never exceeded 85% cocaine. The separation of cocaine HCl and tetramisole HCl did not improve after heating the samples upon addition of solvent. Therefore, this separation scheme was determined to be ineffective and was not further pursued.

Aqueous/organic solvent combinations were then utilized for the separation of the three cocaine HCl/tetramisole HCl mixtures. The starting mixture was initially dissolved in aque-

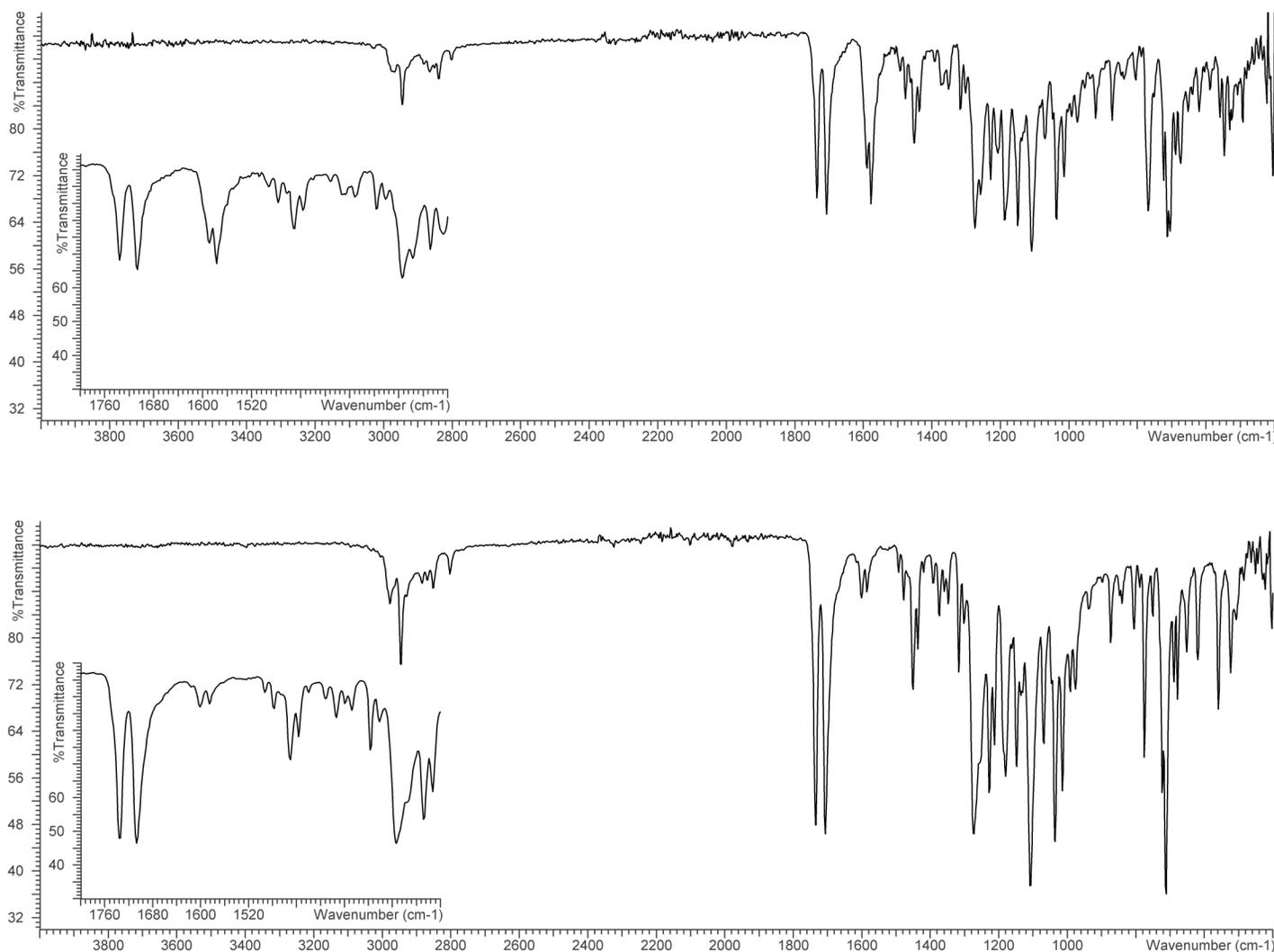


Figure 2 - FTIR spectra of cocaine base/tetramisole base (50:50) mixture (above) and cocaine base after liquid/liquid extraction (below). Inserts of the interfering regions are included for comparison.

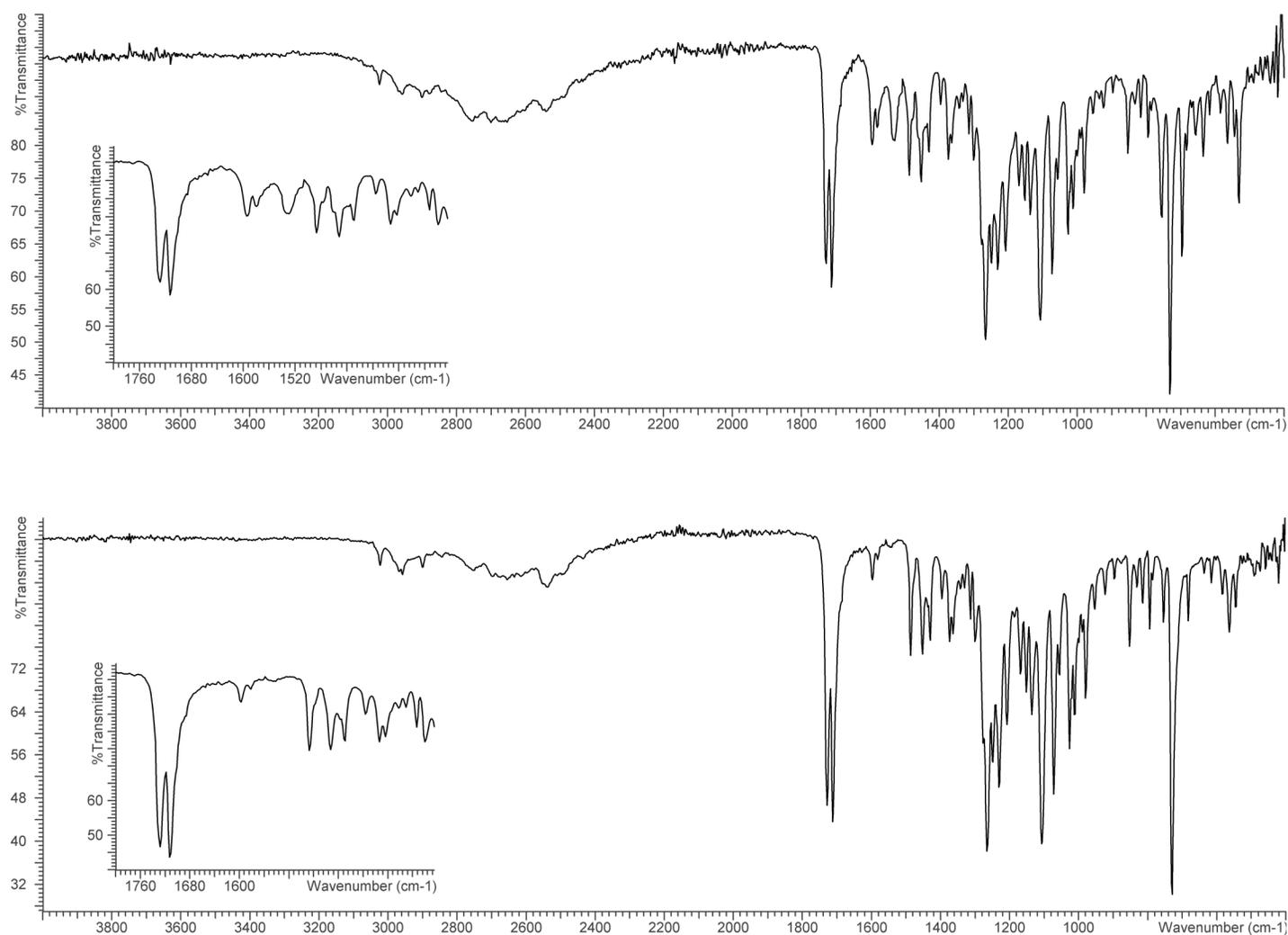


Figure 3 - FTIR spectra of cocaine HCl/tetramisole HCl (50:50) mixture (above) and cocaine HCl after separation via ion pair chromatography (below). Inserts of interfering regions are included for comparison

ous solution, and the cocaine HCl was then extracted with chloroform. The purity of cocaine isolated from these extractions was $\geq 90\%$, on average, between all three HCl mixtures (Table 1). The best separation was achieved using 1.0 mL of water and 5 mL of chloroform.

The three mixtures of cocaine base/tetramisole base (85:15, 70:30, 50:50) were dissolved in hexane and washed with water repeatedly. As illustrated in Table 2, tetramisole base in the 85:15 and 70:30 mixtures was completely removed from the hexane after washing with water five times due to its preferential solubility in water versus cocaine. After five washes of the 50:50 mixture, 99+% pure cocaine was obtained. FTIR spectra of the 50:50 mixture before and after the water purification are shown in Figure 2. The major areas of interference are found between $1800\text{--}1200\text{ cm}^{-1}$ (Figure 2, upper). After five water washes, the mentioned interferences were removed from the IR spectrum (lower Figure 2). This procedure was also performed with ether (instead of hexane); however, it was unsuccessful.

Ion-Pair Separations

Ion-pair chromatography has previously been utilized to separate various mixtures of cocaine and other adulterants such as lidocaine and procaine [8-11]. Based on previous success

with ion-pair chromatographic separations, the same methodology was attempted with cocaine HCl and tetramisole HCl. Three separate columns were prepared with different stationary phase preparations. As shown in Table 3, Column 1 provided the best separation with pure cocaine fractions (PTHIT free) for all three mixtures tested (85, 70 and 50% cocaine HCl). FTIR spectra of the 50:50 mixture before and after purification are shown in Figure 3. Areas of interference are included to illustrate differences in the spectra before and after an ion-pair chromatographic separation. The most significant spectral interferences are shown between $960\text{--}480\text{ cm}^{-1}$. Pure cocaine fractions were also collected with Columns 2 and 3 for the 85:15 cocaine HCl/tetramisole HCl mixture, however, very small amounts of PTHIT were detected with the 70:30 and 50:50 mixtures.

For separations implementing an ion-pair solution, it is important to determine the salt form of cocaine in the starting material. As illustrated in Figures 2 and 3, the salt form can be determined when the purity is only 50%. Knowledge of the salt form prior to the use of a separation technique (liquid/liquid or ion-pair chromatography) will prevent an analyst from inadvertently altering the salt form of cocaine during the removal of phenyltetrahydroimidazothiazole.

Prep-HPLC Separations

The three mixtures of cocaine HCl/tetramisole HCl were separated via preparative-HPLC. Separations were performed in triplicate, and appropriate fractions were combined and prepared as previously described. Once the purified cocaine base was obtained and dried, samples were examined via EA-IRMS. The carbon and nitrogen isotope data were consistent among all samples analyzed, indicating the cocaine was no longer adulterated with tetramisole (Table 4). Due to the minimal variation in the isotope ratios observed in comparison to the known values of the cocaine HCl standard, it was noted the HPLC separation caused little fractionation during the purification.

Conclusions

Several techniques were utilized for the separation of cocaine and tetramisole mixtures. GC/FID and FTIR data were obtained to effectively determine the purity and salt form of the recovered cocaine. For mixtures of cocaine base/tetramisole base, the best method to purify the cocaine was a liquid/liquid separation employing hexane and water; five water washes successfully removed tetramisole base from the cocaine. The most successful technique for separation of cocaine HCl/tetramisole HCl utilized an ion-pair chromatographic column packed with 4 g of Celite 545 and 2 mL 1 N HCl/2 M NaCl. However, chloroform/H₂O extractions did remove a significant amount of the tetramisole for FTIR. The described HPLC methodology isolated the cocaine with minimal fractionation occurring for IRMS analyses of cocaine samples.

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