JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, January 2011, Vol. 56, No. S1
doi: 10.1111/j.1556-4029.2010.01594.x
Available online at: onlinelibrary.wiley.com

TECHNICAL NOTE

CRIMINALISTICS

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Simultaneous Identification and $\delta^{13}C$ Classification of Drugs Using GC with Concurrent Single Quadrupole and Isotope Ratio Mass Spectrometers*

ABSTRACT: In this study, $\delta^{13}C$ values of six cocaine samples were identified and classified using a single quadrupole mass spectrometer and an isotope ratio mass spectrometry (IRMS) as simultaneous gas chromatography detectors. Our instrument modification is simple to use and is useful (i) when the sample is of limited size or can only be injected once, (ii) to help identify peaks in a complicated IRMS chromatogram, and (iii) to help differentiate very simple systems when impurity profiling is not possible. The EI-MS confirmed the identity of cocaine in each sample. The IRMS data distinguished 12 of the 15 possible pair-wise comparisons at the 95% CL. Three samples could not be differentiated by their $\delta^{13}C$ ratios for cocaine. ANOVA demonstrated that the measurement variance was consistently larger than the sample variance. As the $\delta^{13}C$ values clearly show, this technique enables the exclusion of a potential common source even when two samples have otherwise identical chemical and physical properties.

KEYWORDS: forensic science, forensic chemistry, cocaine detection, isotope ratio mass spectrometry, gas chromatography–isotope ratio mass spectrometry, gas chromatography–mass spectrometry

In 2007, more than 1.8 million drug-related arrests were carried out in the United States by federal, state, and local law enforcement agencies. Intelligence estimates indicate a vast majority of the cocaine available in U.S. drug markets is smuggled by Mexican drug trafficking organizations across the U.S./Mexican border (1). It is therefore crucial for law enforcement officials to monitor vessels or possible trafficking routes being used to bring drugs into the United States. According to US News and World Report, drug smugglers are using handmade submarines to ship up to 12 tons of cocaine into the United States per vessel. The Coast Guard speculated that 32% of all the cocaine being smuggled from Columbia into the United States in 2008 was being brought in using this type of vessel (2). When investigating vessels, the Coast Guard will often come across drugs hidden within containers on board. According to a CBS station WFOR-TV in Miami, dozens of tightly wrapped bricks of cocaine were found carefully packed inside hidden compartments in a fuel tank onboard a ship (3). As a commonly occurring Schedule I drug, cocaine is of major significance to a number of law enforcement agencies. The United States Drug Enforcement Administration has created a cocaine signature program based on the abundance of coca extract impurities in the cocaine to keep track of the specific regions where large-scale crops of coca plants are grown (4). Some other specific signatures that can be used for identification and classification purposes are nitrogen isotope ratios ($^{15}N/^{14}N$) of the compound truxilline and carbon isotope ratios ($^{13}C/^{12}C$) of the compound trimethoxycocaine, both of which are found in relatively high abundance in coca plant matter (5).

As an extension of the Locard exchange principle (6), forensic drug samples have the potential to be transported or transferred between the drug package and any person(s) or object(s) with which it comes into contact. Therefore, drugs found on a suspect's clothing, a shipment container, or any surface of the crime scene could all be sources of trace residues of the drug. Samples of these trace amounts could be collected and tested as a possible source match or could provide exclusionary evidence. The steps that have occurred from the time the coca plant, (*Erythroxylum coca* and *E. novogranatense*), a C3 photosynthesis plant, was harvested to the time of possession by the suspect, are complex and varied. Therefore, the ability to trace a collected sample back to its source would prove very helpful. Stable isotope analyses provide an additional "isotopic fingerprint" that could further characterize forensic evidence, via a sample's stable isotope signature (7). Chemical processing of materials can also result in distinctive stable isotopic ratios (8), as can impurities left behind during production (4). When cocaine is cut, mixed, or converted to crack, the isotopic ratios remain largely preserved, thereby enabling source identification to a specific manufacturer (4,9). Stable isotopes can provide sufficient insights that allow an investigator to trace the manufacturing origins of the material at hand or how many different "cooks" or batches could have contributed to the samples that were seized (10). Researchers have already shown that isotope ratio mass spectrometry (IRMS) can be used to assign the geographic origins of illicit cocaine (5,10–12) and the effects of fractionation during the illicit production of cocaine (13,14).

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*Financial assistance received from the National Science Foundation (0745590).

Received 6 Sep. 2009; and in revised form 23 Nov. 2009; accepted 19 Dec. 2009.

Ehleringer et al. (5) showed that with the use of isotopic analysis, it is possible to pinpoint geographic locations of seized batches of cocaine and such results have already been put into a library. Ehleringer et al. (10) have also shown the current major geographic origins of cocaine and how the cocaine could be identified on the basis of natural variations in isotope ratios related to their variations in environmental habitat parameters. Janzen et al. (15) used Euclidian distance as a tool to identify matching cocaine profiles to establish links between samples. Further research in these areas could, in time, result in a database that would allow the chemical tracking of selective drug routes (15).

The confirmatory identification of controlled substances is one of the most common and important tasks of a forensic drug analyst (16). The hyphenated technique of gas chromatography and mass spectrometry (GC/MS) has long been the gold standard in forensic science and serves as a category A, or confirmatory method according to SWGDRUG guidelines (17). Single quadrupole mass spectrometers are a staple in most crime laboratories because of their ease of use, modest cost, robustness, and uniform fragmentation patterns observed between vendors and models. Here, a single quadrupole mass spectrometer provides the retention time data and characteristic fragmentation patterns used to confirm the presence of cocaine and any impurities in the samples. Simultaneously, the IRMS determines the carbon isotopic abundance ratios of the cocaine or impurity peaks. To our knowledge, the simultaneous use of these two detectors has only been demonstrated on a few occasions and never before for forensic applications.

Single quadrupole mass spectrometers were coupled to GC-IRMS systems and tested for precision and accuracy of the coupled detectors using n-alkanes and polycyclic aromatic hydrocarbons both in soil (18) and to analyze sediments left by fire to determine the impact on climate biosphere interactions (19). In another application, a single quadrupole mass spectrometer coupled to a GC-IRMS was used to analyze a marine halogenated natural product within skua samples and whale blubber samples by Vetter et al. (20) and to take measurement of carbon isotopes of chloromethane emission flux in the atmosphere compared to that originating from various sources (21). A more extensive look at halomethanes was measured in the same manner by Kalin et al. (22) for future hope in understanding the stratospheric ozone destruction. Ion trap mass spectrometers were coupled to GC-IRMS and tested for precision and accuracy of the coupled detectors (23) using a standard consisting of four alkanes of different concentrations (24), and specific positions in organic compounds separated from complex mixtures were analyzed to demonstrate the absence of rearrangement during activation and fragmentation (25). This approach eliminates the need for two separate sample injections into two different GC ovens with two different GC columns. Without this instrument modification, identification and compound-specific $^{13}C/^{12}C$ determination typically require two independent GC systems (26). Very careful characterization is required to validate retention times between the two instruments to ensure the identification of each peak in the IRMS profile is correct.

The benefits of this single injection method include the lowering of sample preparation time and reduced sample amounts, a reduction in the risk of error due to having to prepare two samples, along with the shear simplicity of using one autosampler and getting two results. This approach could be important for samples of limited size, or that can only be injected once, such as through solid phase microextraction (SPME) or pyrolysis. This is a proof of concept manuscript to show that both detectors can work effectively with a once-injected sample. From a forensic perspective, the IRMS is considered to be secondary in importance to the single quadrupole mass spectrometer because the primary goal of GC/MS is confirmation of identification of a scheduled drug through GC/EIMS; the secondary goal—achieved simultaneously in this work—is the establishment of potential common sources through GC/C/IRMS.

Materials and Methods

Chemicals

The samples of cocaine utilized in our research include two commercially available cocaine free base standards (A and B), a commercially available cocaine HCl standard (C), and three street crack cocaine samples (D, E, and F); details are listed in Table 1. Cocaine HCl and cocaine free base were both purchased from Sigma Aldrich (St. Louis, MO), and all other cocaine samples were obtained through local law enforcement agencies. All samples (A, B, C, D, E, and F) were prepared by dissolving 2 mg aliquots of each cocaine sample in 1 mL of high performance liquid chromatography grade methanol (Sigma Aldrich). Three aliquots of each sample and a blank sample were analyzed four times each, in random order.

An IRMS standard (Chiron International Standards, Laramie, Wyoming) consisting of n-undecane (C_{11}) with a $\delta^{13}C$ value of $-26.11‰$, n-pentadecane (C_{15}) with a $\delta^{13}C$ value of $-30.22‰$, and n-eicosane (C_{20}) with a $\delta^{13}C$ value of $-33.06‰$, each at 0.15 mg/mL in cyclohexane was used in 1 μL injections to calibrate the reference CO_2 similar in nature to an n-alkane mixture used by Merritt et al. (27). Calibration of the reference CO_2 used the same injection, separation, and detection arrangement as the sample runs. Each IRMS chromatogram starts and finishes with three and one 20 sec burst of reference CO_2, respectively (28). The C_{11} peak in the isotope standard mixture was used to calibrate the reference cylinder of CO_2 to a user-defined value of $-11.56‰$ ($R_{standard}$) immediately prior to running all the samples. The isotope ratios are measured relative to universal standards and reported in the delta notation, δ:

$$\delta = 1000(R_{sample} - R_{standard})/R_{standard} \quad (1)$$

The value R_{sample} is the abundance ratio of the minor, heavier isotope of the element to the major, lighter isotope (e.g., $^{13}C/^{12}C$) (29). Our method was set up to run three standard gas peaks before the sample run and one at the end of the sample run to ensure instrument stability. In all the sample runs, the second reference peak shown in Fig. 3 was selected as the $R_{standard}$ value to be used for Eq. (1) when determining a δ value for the sample of cocaine.

Gas Chromatography–Mass Spectrometry/Isotope Ratio Mass Spectrometry

GC/MS analyses were performed using a single quadrupole mass spectrometer (HP5970B; Hewlett Packard [now Agilent], Santa Clara, CA) and an isotope ratio mass spectrometer (Delta

TABLE 1—*Details of the cocaine samples used in this study.*

Cocaine Sample	Company	Name	Lot No.
A	Sigma Aldrich, St. Louis, MO	Cocaine Free Base	97H1018
B	Sigma Aldrich, St. Louis, MO	Cocaine Free Base	44H0207
C	Sigma Aldrich, St. Louis, MO	Cocaine HCL	025K1024
D	Ohio Law Enforcement	Street Cocaine	–
E	Ohio Law Enforcement	Street Cocaine	–
F	Ohio Law Enforcement	Street Cocaine	–

Plus Advantage; Thermo Finnigan, Waltham, MA). These instruments were then used in combination with a gas chromatograph (Trace GC; Thermo Finnigan) equipped with an autosampler (AS3000; Thermo Finnigan). A typical cycle time for the GC-IRMS was 25–30 min, and samples were placed on a 100 sample wheel which allowed for unattended sample analysis. The GC column was a 5% diphenyl-polydimethylsiloxane (DB-5) (60 m × 0.25 mm × 0.25 μm; J & W Scientific, Folsom, CA). The effluent was split using a low-dead-volume X-connector (Valco Instruments Co., Inc., Houston, TX) so that ~10% of the effluent flowed to the single quadrupole mass spectrometer for structural elucidation and ~90% flowed to the IRMS for isotopic analysis. Because the IRMS and the single quadrupole mass analyzer differ in sensitivity, the flow rates to each were split in a 90:10 ratio, respectively, to obtain similar detection limits of both instruments. The transfer line to the HP single quadrupole instrument was a deactivated fused silica (1.0 m × 0.1 mm) capillary (Agilent). The transfer line from the oxidation tube to the GC Combustion III interface was a 1.0 m × 0.2 mm I.D. deactivated fused silica capillary (Thermo Finnigan). Split flows were measured manually, outputted to 1 atm, using a universal flow meter (ADM2000; Agilent).

The GC oven temperature was programmed as follows: initial temperature 170°C (hold 9.5 min); program rate, 16°C/min final temperature 280°C (hold 2 min). The injector temperature was set at 280°C and the helium carrier gas flow rate was 2.5 mL/min. A volume of 1 μL was injected in splitless mode using the aforementioned autosampler.

The temperature of the auxiliary transfer line to the single quadrupole was set at 280°C. The majority of the transfer line was heated using the heater and thermocouple, which were originally supplied for a typical set up of a Trace GC controlled by the software provided, Isodat 2.0 (Thermo Finnigan). The transfer line that was attached to the single quadrupole mass spectrometer had to be installed on the right side, unlike typical left-sided setups. This required an extension to the original transfer line to allow for passage through the outer shell, circuit board, and oven wall to reach the inside of the oven, as shown in Fig. 1*a*. The GC is configured to allow a transfer line through this wall, but needs to be drilled out. A 5 cm diameter hole was drilled in the outer wall on the right side of the GC oven containing the electrical circuit board and the outside panel. A 2 cm hole was drilled into the right side of the metal GC oven siding. A metal guide sleeve (7.5 cm × 0.12 cm I.D.) with a (3.2 cm) collar was placed into the 2 cm hole. The extension (10.0 cm × 0.75 mm I.D.) was added onto the end of the transfer line so that the transfer line extended into the inside of the oven. This portion of the transfer line was heated using a BIH heavy insulated heating tape (BriskHeat Corporation, Columbus, OH) that was controlled by a manual temperature controller (Staco Energy Products Co., Dayton, OH). An OMEGA DP20 (OMEGA Engineering, Inc., Stamford, CT) external thermocouple was used to ensure that the heater was accurately heating the extended transfer line thereby maintaining a specific temperature. Total ion chromatograms were recorded from *m/z* 50–550 using HP Chemstation B.02.05 (Agilent). The trigger wire from the single quadrupole was connected to the generic handshake port in the back of the Trace GC so that the injection trigger from the autosampler would be recognized, as seen in Fig. 1*b*. The data were exported as a text file for comparison with the National Institute of Standards and Technology (NIST) mass spectral library (US Secretary of Commerce, USA) to confirm the identity of the compounds of interest.

For isotope ratio measurements, the isotope ratio mass spectrometer (Delta Plus Advantage; Thermo Finnigan) was used via a

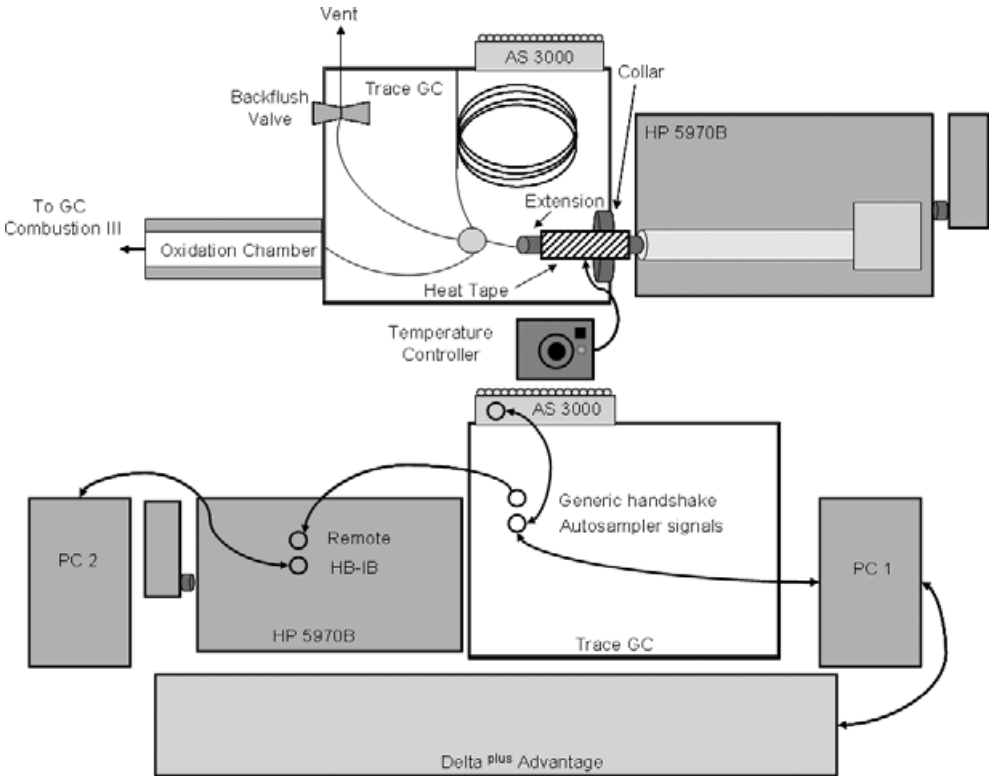


FIG. 1—*Schematic of (a) our GC-MS set up with a transfer line extension and heat tape configuration and (b) GC-MS-IRMS wiring to control the autosampler signal to both PCs indicating that the sample injection has been made and the run should begin. GC, gas chromatography; MS, mass spectrometry; IRMS, isotope ratio mass spectrometry.*

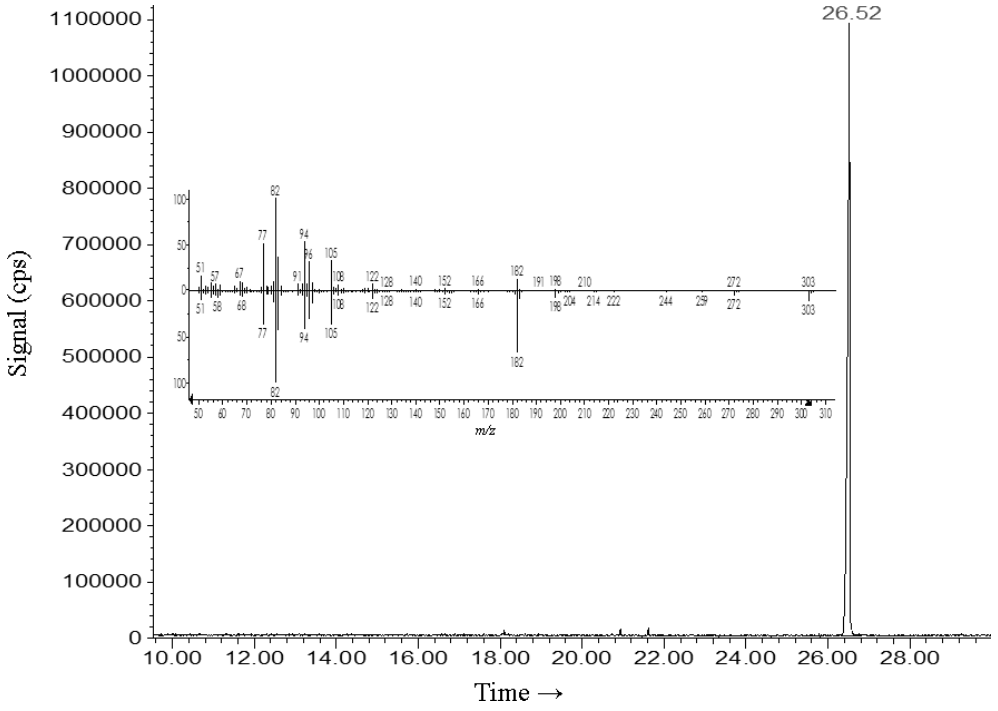


FIG. 2—*TIC chromatogram and mass spectrum of the peak at 26.52 min. The retention index and mass spectrum of this sample C confirm the identity of the sample as cocaine. The National Institute of Standards and Technology head to tail output confirms the presence of cocaine with a 77.1% probability score.*

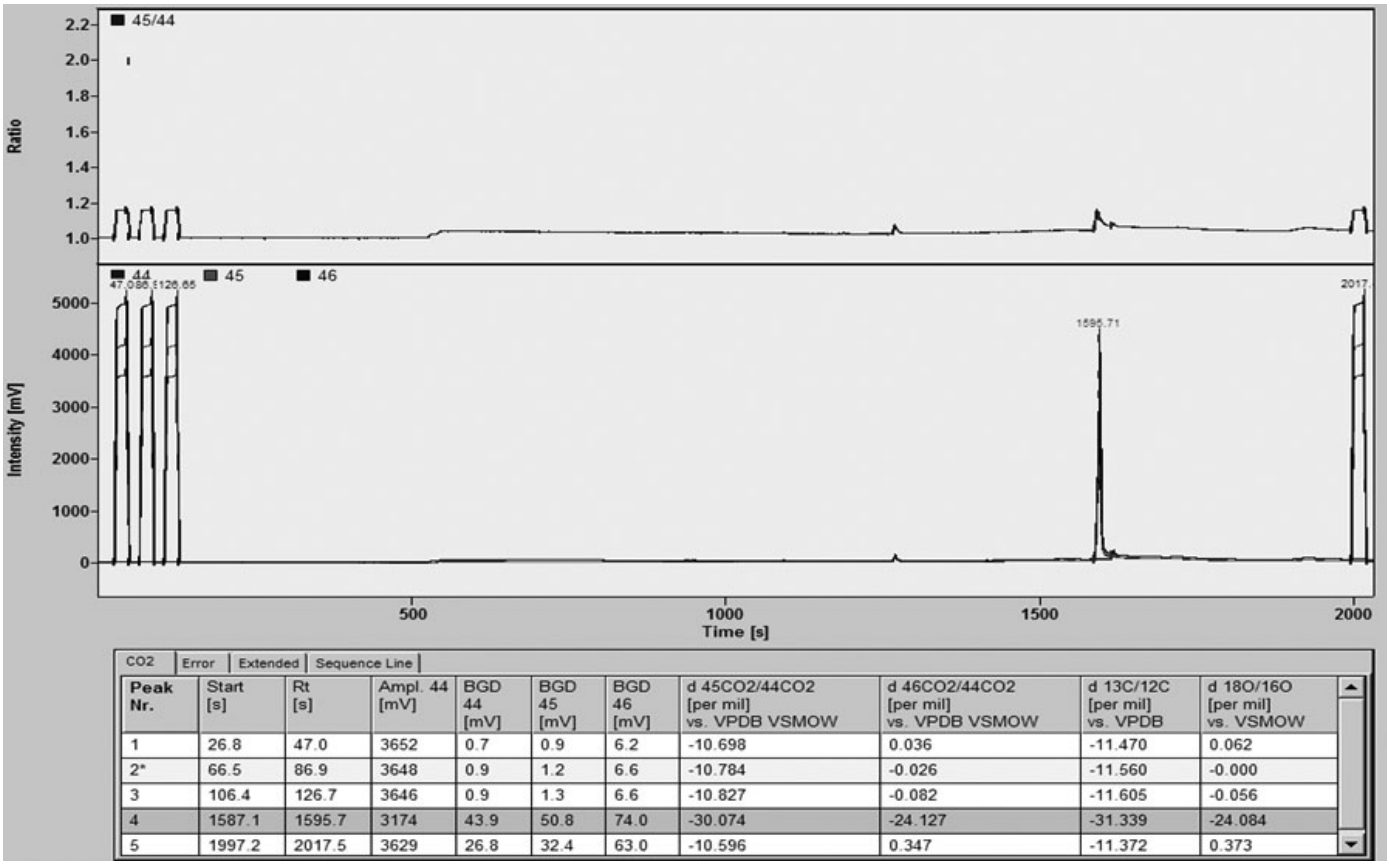
combustion interface (GC Combustion III; Thermo Finnigan). The combustion oven temperature was held at 940°C, and the reduction oven temperature was held at 650°C. Data acquisition was carried out using Isodat 2.0 Software (Thermo Finnigan). For carbon GC-IRMS, ion chromatograms of three isotopic peaks of CO_2 were collected by three individual ion collectors with different specific sensitivities, i.e., ions at *m/z* 45 ($^{13}C^{16}O^{16}O$) and ($^{12}C^{16}O^{17}O$) were recorded with 100-fold sensitivity relative to *m/z* 44 and ions at *m/z* 46 ($^{12}C^{16}O^{18}O$) were recorded with 300-fold sensitivity relative to *m/z* 44 (27,30). We began each run with three injections of the high purity (99.997%) CO_2 reference gas (Airgas, Great Lakes, Independence, OH), of which the second CO_2 peak, seen in Fig. 3, is designated as the standard to be used for calculating the δ ($^{13}C/^{12}C$) values by the instrument acquisition software. The isotope standard and samples were injected, separated, and analyzed under identical conditions, thereby adhering as closely as possible to the identical treatment principle (31,32).

To improve the quality of the measurement and eliminate any error arising from misbalance, each sample should ideally be adjusted so that the amplitude of the sample signal is as close to the CO_2 reference gas peak as possible. When standard and sample are run at significantly different signal sizes, the linearity of the instrument results is small, but significant, absolute differences in the measured $\delta^{13}C$ values (33).

Results and Discussion

Cocaine was used as an example drug sample to demonstrate the quality and effectiveness of the single injection/dual detection system. A chromatogram and mass spectrum obtained on the single quadrupole mass spectrometer are shown in Fig. 2. The retention index and fragmentation pattern of sample C both confirm the identity of the sample as cocaine. The spectrum shown in Fig. 2 clearly displays the fragmentation pattern peaks that are used to commonly identify cocaine by peaks 82, 182, and 303, (16) providing a class A, confirmative analysis (34). The NIST head to tail output confirming the presence of cocaine can also be seen in Fig. 2, showing both the library and the actual spectrum. The probability score from the NIST library for all samples ranged between 52.9% and 77.1%. In all cases, cocaine was the highest ranked match. The mass spectra from each cocaine sample look virtually identical because they have the same chemical composition. This is true in cases where impurity peaks are not present. This further highlights the importance of a secondary analysis technique to establish the possibility or exclusion of a common source. Figure 3 shows the concurrent IRMS output acquisition for the same injection of cocaine sample C. The carbon isotopic ratios can be used to exclude the possibility that two samples of drugs are from the same source. Our primary interest was to establish the sources of variation in $\delta^{13}C$ values for cocaine and to determine which samples could have come from the same source. To establish the variation of $\delta^{13}C$ values in each sample (sampling variance), three aliquots of each sample were analyzed in quadruplicate samplings.

Pooled standard deviations for each sample in the measured carbon isotope ratios varied between 0.17–0.31‰ ($N = 12$). The plot shown in Fig. 4 enables the reader to visualize the separation between the different batches of cocaine. The error bars show the 95% confidence intervals for each aliquot. Using ANOVA, sampling error could be separated from the measurement error. Sampling standard deviations varied from 0.009‰ to 0.076‰ (average = 0.03‰) for the six samples, whereas measurement standard deviations varied from 0.17‰ to 0.30‰ (average = 0.23‰). The sampling standard deviations were on average 11 times smaller than the measurement standard deviations, thereby demonstrating that the samples were all relatively homogenous and that most of the error in the results stems from the IRMS instrument itself.



Peak Nr.	Start [s]	Rt [s]	Ampl. 44 [mV]	BGD 44 [mV]	BGD 45 [mV]	BGD 46 [mV]	d 45CO2/44CO2 [per mil] vs. VPDB VSMOW	d 46CO2/44CO2 [per mil] vs. VPDB VSMOW	d 13C/12C [per mil] vs. VPDB	d 18O/16O [per mil] vs. VSMOW
1	26.8	47.0	3652	0.7	0.9	6.2	-10.698	0.036	-11.470	0.062
2*	66.5	86.9	3648	0.9	1.2	6.6	-10.784	-0.026	-11.560	-0.000
3	106.4	126.7	3646	0.9	1.3	6.6	-10.827	-0.082	-11.605	-0.056
4	1587.1	1595.7	3174	43.9	50.8	74.0	-30.074	-24.127	-31.339	-24.084
5	1997.2	2017.5	3629	26.8	32.4	63.0	-10.596	0.347	-11.372	0.373

FIG. 3—*Example of a concurrent isotope ratio mass spectrometry output acquisition for the single injection of cocaine sample C.*

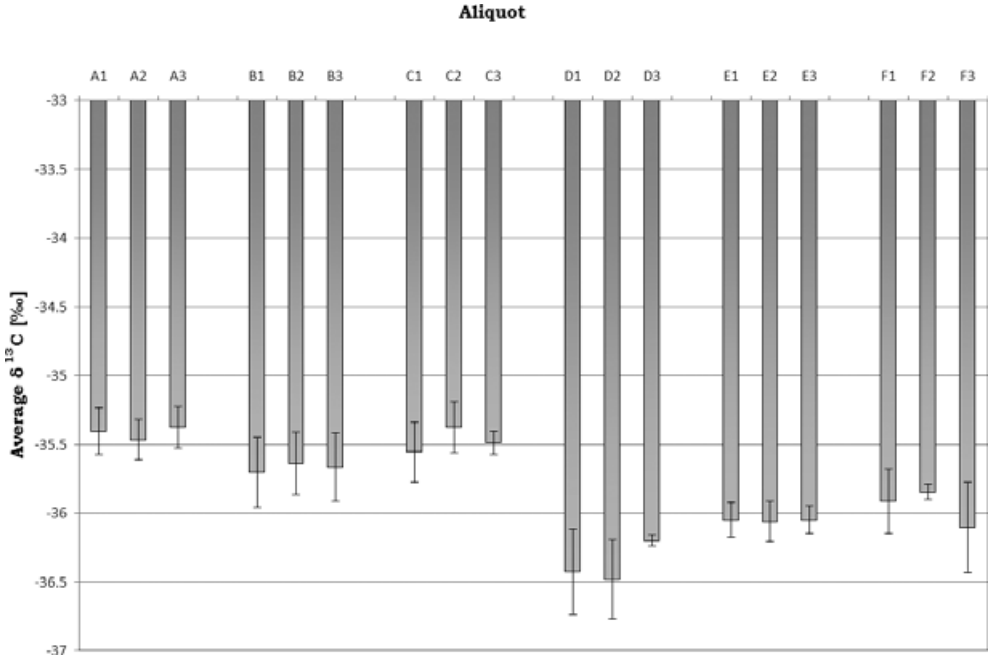


FIG. 4—*Average $\delta^{13}C$ values of six cocaine samples. Results represent three aliquots of each sample with quadruplicate sampling and error bars indicate 95% confidence interval.*

Each cocaine sample was tested for unique $\delta^{13}C$ values using monometric statistics. When comparing the F-calculated results in Table 2 with the F-critical values, our results show that the sample mean for sample A can be distinguished at the 95% confidence level (CL) from samples B, D, E, and F. Table 3 shows the probabilities or *p*-values calculated from the F values in Table 2. Similarly, the samples B, D, and E can all be distinguished at the 95% CL. The following samples could not be distinguished based on the

TABLE 2—*F-calculated values for six cocaine samples: cocaine free base standard (samples A and B), cocaine HCl standard (sample C), and three street crack cocaine samples (samples D, E, and F). Cells with a white background are significantly different at the 95% CL. Cells with a gray background are not significantly different.*

	F-calculated					
	A	B	C	D	E	F
A	–	12.45	0.39	123.24	90.06	23.69
B	–	–	6.69	49.01	18.33	3.97
C	–	–	–	90.62	54.55	16.71
D	–	–	–	–	15.85	15.03
E	–	–	–	–	–	1.20
F	–	–	–	–	–	–

TABLE 3—p-*values for six cocaine samples: cocaine free base standard (samples A and B), cocaine HCl standard (sample C), and three street crack cocaine samples (samples D, E, and F). Cells with a white background are significantly different at the 95% CL. Cells with a gray background are not significantly different.*

	p-value					
	A	B	C	D	E	F
A	–	1.9×10^{-3}	0.54	1.8×10^{-10}	3.1×10^{-9}	7.3×10^{-5}
B	–	–	1.7×10^{-2}	5.0×10^{-7}	3.0×10^{-4}	5.9×10^{-2}
C	–	–	–	2.9×10^{-9}	2.2×10^{-7}	4.9×10^{-4}
D	–	–	–	–	6.3×10^{-4}	8.2×10^{-4}
E	–	–	–	–	–	0.28
F	–	–	–	–	–	–

carbon isotope ratios of cocaine: A and C, B and F, and E and F. Although these three pairs cannot be excluded as having a potential common source from one another, this does not exclude the possibility that they do in fact come from different sources—an important legal point. It is very possible that samples E and F come from a common source. Previous studies have shown that street cocaine typically varies between −25.3‰ and −35.22‰ (35), a significantly broader range than the samples presented here. One might expect that the analysis of a larger number of cocaine samples would therefore occupy a broader range of delta value than we obtained with our limited sample size.

Statistical validation and confidence reporting are two very important criteria recommended by the National Academy of Science report to Congress in their recommendations for the forensic science community in the United States (36). Results involving samples with significantly different mean $\delta^{13}C$ values clearly fall within the guidelines of statistical and confidence reporting. However, a significantly larger database of the frequency of $\delta^{13}C$ values needs to be generated to determine the probability of two random samples having indistinguishable sample means.

Conclusion

IRMS has shown the ability to be a reliable and accurate method that can be used concurrently with a single quadrupole mass spectrometer to provide confirmatory identification of a scheduled drug (or impurities), with an additional level of discriminatory power. The use of IRMS for the comparison of questioned versus questioned samples or questioned versus known samples of drugs has virtually unlimited applications. The dual detector approach would be necessary with very limited sample sizes or with samples that can only be injected one time (such as SPME). This type of instrument modification would also be beneficial when dealing with extremely complicated samples matrices where peak identification is difficult; i.e., where the IRMS is of primary interest and the single quadrupole is used to assist the interpretation. Through a second injection of each sample, it would be possible to obtain N, H, or O isotope analysis, which could add a greater degree of discriminating power in establishing or excluding potential common sources of drugs. In the future, when combining more then one isotope ratio, IRMS analyses will be an asset in providing additional information as to the geographic locations of crops being grown and harvested, and for providing statistical measures of differences between samples, as already shown by Ehleringer et al.

Conflict of interest: The authors have no relevant conflicts of interest to declare.

Acknowledgments

The authors thank Roger Husted from Thermo Finnigan for technical support and Bascom French for machining the transfer line extension. We also thank NSF for funding through grant number 0745590.

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