Full length articles

Isolation of components of plastic explosives for isotope ratio mass spectrometry

John D. Howa [a],*, Michael J. Lott [a], Lesley A. Chesson [a], James R. Ehleringer [b]

[a] IsoForensics Inc., 421 Wakara Way, Suite 100, Salt Lake City, UT 84108, United States
[b] Department of Biology, University of Utah, Salt Lake City, UT 84112, United States

ARTICLE INFO

ABSTRACT

An improved method for the separation of the components of plastic explosives is described with the goal of stable isotope ratio analysis of each isolated component. The binder and plasticizer of a plastic explosive were extracted into cyclohexane, leaving the explosive components and other insoluble material. The plasticizers, oils, and additives were next extracted from the dried cyclohexane-soluble fraction into acetone, leaving the polymeric binder. Plasticizers, oils, and additives were identified by GC–MS and individual plasticizers, oils, dyes, and antioxidants were isolated by GC and/or flash chromatography free of interfering materials. The explosive components were extracted from the cyclohexane-insoluble fraction with acetone, and then quantitatively analyzed for individual explosive compounds via HPLC-UV/Vis spectroscopy. Individual explosive compounds were isolated via preparative HPLC. The separation process was validated by gravimetric, HPLC-UV/Vis, EA, GC–MS, TC/EA, and FTIR results consistent with the expected formulation of the plastic explosives. The isolated components were analyzed for component-specific stable isotope ratios. Control mixtures of previously characterized components of two common plastic explosive formulations were also separated and analyzed. These isolated components retained the isotope ratios prior to mixture, demonstrating the robustness and reliability of the technique. Two example C-4 explosives from different sources, with indistinguishable chemical composition and raw explosive $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotope ratios, were extracted and analyzed. The resultant binder and plasticizer $^{2}H/^{1}H$ and $^{13}C/^{12}C$ results were used to discriminate between the two C-4 samples that were otherwise apparently identical.

© 2016 Elsevier B.V. All rights reserved.

Introduction

Analytical chemists conduct a variety of analyses on explosives for intelligence and investigation purposes. The chemical makeup of an explosive or explosive residue is often a vital piece of information, and can differentiate sources of explosive material, whether that source is an environmental contaminant site, a stockpile diverted from a legitimate source, or a clandestine manufacturing facility [1]. Ideally, an explosive would have a characteristic, identifying "signature" that allows law enforcement and intelligence agencies to unambiguously identify its source – e.g., a particular block, package, batch, lot, or manufacturer.

The chemical properties of explosives and their residues are often the most characteristic features of a bomb scene, notwithstanding the physical construction of the bomb itself. Though there are a near-infinite variety of formulations to produce chemical explosives, the similarities between different manufacturers' formulas, combined with the inherent variability in a single manufacturer's formula, may lead an investigator to falsely equate two explosives from different sources that have similar chemical properties – i.e., a "false match" [3,4]. These false matches may not be due to poor analytical practices, but inherent variability in bulk measurements of a composite material, like an explosive.

Isotope ratio analysis of explosives—and more powerfully, isotope ratio analysis of individual explosive components—provides for discrimination that is not possible through chemical analysis alone [5–15]. This is especially true when extraneous contaminants may obscure the chemical profile of an explosive component [9,16–18], individual components from explosive residue extraction require compound-specific sourcing data [19–21], or links between an explosive precursor and product are part of the investigation [22–25]. In this paper, we demonstrate and validate methods in a framework described separately [26] that can be used to isolate multiple components from plastic explosives. We show that isolated components from mock C-4 and Semtex mixtures are

* Corresponding author.
   E-mail address: howa@isoforensics.com (J.D. Howa).

sufficiently pure to produce isotope ratios that reflect those of the original components. We also show how component-specific isotope ratio analysis of nonexplosive materials of a mixture can help discriminate explosive samples that were otherwise indistinguishable based on chemical composition analysis, isotope ratio analysis of the raw explosive, or analysis of the most abundant compound in C-4 (RDX).

Analysis of multiple components of an explosive is an example of using multiple orthogonal techniques, where significantly different approaches for matching and discriminating materials arrive at similar conclusions [18]. This approach supplements and validates explosive isotopic evidence in the criminal justice system, which has increasingly higher standards of data and method validation [27,28]. A statistical basis for evaluating component-specific evidence with relation to equating sources requires a background population dataset for the separate components, including a well-defined source level [29,30]. These statistical methods have been discussed elsewhere [31], and are applicable for explosives, as well [4]. Though providing background data are outside the scope of this paper, we demonstrate the first step to this goal—consistently good discrimination—by reliably and robustly separating and analyzing individual plastic explosive components.

Materials and methods

Chemicals

Optima-grade acetone, cyclohexane, and dichloromethane were obtained from Fisher Scientific (Tustin, CA, USA). HPLC-grade methanol, water, and acetonitrile were purchased from VWR Scientific Products (South Plainfield, NJ, USA). Standard explosive solutions of HMX, RDX, and PETN for the creation of a UV spectral library and calibration of HPLC working standards were purchased from AccuStandard, Inc. (New Haven, CT, USA). IsoForensics, Inc. provided the C-4 explosive samples (labeled "1", "4", and "1261") and pure explosive material used for control mixtures and method development. RDX (identifier 522), HMX (identifier 291), and PETN (identifier 544) reference materials for elemental analysis, extraction controls, and IRMS quality control were characterized according to a previously published method [32]. C18 resin (BAKERBOND® Octadecyl 40 μm Prep LC packing) for flash chromatography was obtained from VWR (cat #JT7025-00, lot J25087). Mineral oil for method development was obtained from Malinckrodt (cat #6358, lot 6358 A08638). Other commercially available chemicals used as reference materials were obtained from Sigma–Aldrich (Saint Louis, MO, USA); these were styrene-butadiene rubber (branched copolymer, 21 w/w% styrene, catalog #432474-100G, lot 01708BJ), polyisobutylene (cat #181455-100G, lot MKBK8213V), bis(2-ethylhexyl) adipate (cat #02140, lot 1319382 @0107326), N-phenyl-2-naphthaleneamine (cat #178055-25G, lot 05609HJV), and Sudan I (cat #103624-25G, lot S50809). Alkanes used for retention time indices and isotope ratio normalization included docosane (cat #134457-100G, lot 07404TA), dodecane (D221104-100ML, lot 17320TA), dotriacontane (cat #D223107-5G, lot 08220AE), eicosane (cat #219274-5G, lot 02812EE), hexadecane (cat #296317-100ML, lot 00742BC), hexatriacontane (cat #H12552-25G, lot 10818PC), octacosane (cat #O504-25G, lot 06725AD), octadecane (cat #O652-25G, lot 17119MD), tetracosane (cat #T8752-25G, lot 18101EE), and tetradecane (cat #172456-100G, lot100118HB). All drying and manipulation of volatile organic substances took place in a fume hood or properly vented area.

Control mixtures

Control mixtures, mimicking the compositions typical of C-4 and Semtex, were produced to demonstrate the robustness and reliability of the fraction separation techniques. The relevant isotope ratios and elemental compositions of individual "source" components used in the mixtures were previously measured (Table 1), then compared to those of the same components after they were isolated from the control mixtures. The relative amounts of each component in the mixtures are also listed in Table 1. To prepare the mixtures, the listed amounts of each explosive component (HMX and RDX for C-4, PETN and RDX for Semtex) were added in crystalline form to the vials, mixtures of the nonexplosive components dissolved in cyclohexanes were added, and the completed mixtures dried under an oil-free air stream. The nonexplosive mixtures were composed of bis(2-ethylhexyl) adipate, mineral oil, and polyisobutylene for C-4; mineral oil, N-phenyl-2-naphthaleneamine, Sudan I, and styrene-butadiene rubber for Semtex. Four separate aliquots of each control mixture were prepared. Additional aliquots of each nonexplosive mixture were processed alongside the explosive mixtures as controls.

Overview of explosive fraction separation scheme

The goal of the fraction separations described in this paper is the quantitative isolation of individual components of plastic explosives in sufficient purity for isotope ratio analysis of each component. A scheme for the complete separation is shown in Fig. 1. In summary, the binder and plasticizer of a plastic explosive were extracted into cyclohexane, separating them from explosive components and other insoluble material. The plasticizers, oils, and additives were then extracted with acetone from the dried cyclohexane-soluble fraction, leaving the polymeric binder. The explosive components were extracted from the cyclohexane-insoluble fraction with acetone. Dyes and antioxidants were separated from other aliphatic material in the cyclohexane-soluble, acetone-soluble fraction using a column of non-endcapped C18 resin. Preparative HPLC was used to isolate the explosive compounds according to a previously published method [33]. Purified explosive compounds, polymeric binders, and bulk oil fractions were analyzed using EA-IRMS for carbon and nitrogen isotope ratios; the binder and oil fractions were analyzed using TC/EA-IRMS for hydrogen isotope ratios. GC-IRMS methods provided hydrogen and carbon compound-specific isotope ratios of the plasticizers, dyes, and the antioxidant.

Cyclohexane-soluble fractions

Plastic explosive material (50–60 mg) was weighed into an ashed, labeled, and pre-weighed 1-dram glass vial. The weight of the explosive was recorded to 0.001 mg (for gravimetric analysis). Optima-grade cyclohexane (2 mL) was added and the vial shaken for 16–24 h on a benchtop shaker. If the shape of the sample was not disrupted overnight, the sample was placed back on the shaker for several more hours. The vial was then spun using a low-speed, tabletop centrifuge to clarify the solution of any suspended insoluble particles. The supernatant was transferred via Pasteur pipet to another ashed, labeled, and pre-weighed 1-dram vial. This is the "cyclohexane-soluble" fraction. An additional 2-mL aliquot of cyclohexane was used to extract the remainder of oils, additives, plasticizers, and binders from the original sample vial (shaken for 1 h) and added to the cyclohexane-soluble fraction. Both cyclohexane-soluble and cyclohexane-insoluble fractions were dried under an oil-free air stream and weighed.

A 2-mL aliquot of Optima grade acetone was added to the cyclohexane-soluble fraction. In samples containing 1–2% binder (typical of C-4-like compositions) the insoluble binder was released from the side of the vial and was visible as a colorless film suspended in the solvent. The vial was shaken for 1 h on the benchtop shaker, and then shaken on a Vortex mixer for 10 s. The insoluble binder coalesced into a small (∼1–2 mm) ball. The acetone-soluble fraction was removed via Pasteur pipet and transferred to another ashed, labeled, pre-weighed 1-dram glass vial. The acetone extraction was repeated (2 mL) on the cyclohexane-soluble fraction and the supernatant combined with the previously acquired acetone-soluble fraction (the "oil" fraction). Both fractions were dried and weighed. The cyclohexane-soluble, acetone-insoluble fraction containing the polymeric binder (the "binder" fraction) was then ready for analysis by FTIR, EA-IRMS, and TC/EA-IRMS.

The cyclohexane-soluble, acetone-soluble fraction was prepared for GC–MS for compound identification and quantification, and then processed if necessary to remove interfering compounds prior to GC-IRMS. Samples were dissolved into 0.5–1.8 mL hexanes to a nominal concentration of 200 μg/mL per resolved compound. A retention time standard, consisting of normal-alkanes of carbon length 14, 16, 18, 20, 22, 24, 28, 32, and 36 was also prepared in advance for calculation of Kovats indices, normalization of isotope ratio data, and assessment of quality control. Compounds were identified by Kovats index and comparison of fragmentation patterns to the mass spectral library supplied with the Excalibur software. Samples containing resolved peaks (antioxidant, dyes, plasticizers) were analyzed for $^2H/^1H$ by GC-IRMS at 20 ng hydrogen per resolved compound per injection. Samples were diluted to produce 20 ng carbon per resolved compound per injection for $^{13}C/^{12}C$ analysis by GC-IRMS.

For the control mixture mimicking a Semtex sample, separation of the antioxidant and dye from the relatively large amounts of oil was necessary, as the oil coeluted from the GC column in the form of an unresolved complex mixture. This flash chromatography separation step was conducted using a short pipet column containing 0.2 g of C18 resin (described above) loaded with hexanes. The column was conditioned with 2 mL of dichloromethane, followed by 2 mL hexanes. The sample, dissolved in 0.5 mL of hexanes, was loaded onto the column and followed by a 0.5-mL rinse of the sample vial (also hexanes). The remainder of the first fraction, containing aliphatic hydrocarbons, was eluted with an additional 0.5 mL of hexanes. The second fraction, containing the antioxidant and dye, was eluted using three 0.5-mL aliquots of dichloromethane. A final third fraction was eluted using 0.5 mL of acetone. Fractions were dried under an oil-free air stream prior to dissolution in hexanes for GC (as above). No significant differences between the original oil and the oil prepared via flash chromatography were found in $^2H/^1H$ or $^{13}C/^{12}C$ isotope ratios.

Cyclohexane-insoluble fractions

The cyclohexane-insoluble fractions were treated in a fashion similar to the hexane-insoluble fractions from a previously described method [33]. Briefly, two 2-mL aliquots of acetone were used to extract the explosive components from

Table 1

Gravimetric, HPLC-UV/Vis, δ^{13}C, δ^2H, and δ^{15}N data for components isolated from control mixtures of C-4 and Semtex compared to theoretical component composition and known isotope ratios of the individual components prior to mixture. Analysis of true C-4 sample 1261 is shown for comparison. Delta (δ) values are in "permil" (‰), or parts per thousand. Values in italics are calculated from the sum of total components measured. Four of each type of control mixture were prepared. Gravimetric data were aggregated by mixture type (C-4 or Semtex). Relative error for gravimetric measurements was ~1–3% except for the oil fraction of Semtex, which was ~ 25%. Relative error for HPLC data was ~4–10%. Isolated components from each of the 8 mixture vials were measured for isotope ratios 1–2 times each where possible; listed values for each component are aggregated as mean ± SD for that mixture type (C-4 or Semtex).

| Sample	Fraction/component	Target amount added (mg)	Theor. w/w%	δ^{13}C, source	δ^2H, source	δ^{15}N, source	Component isolated	w/w% (gravimetric)	w/w% (HPLC)	δ^{13}C, isolated	δ^2H, isolated	δ^{15}N, isolated
Control mix C-4	*Explosive*		90.0%					88.4%	85.8%			
n = 4	RDX	40.900	81.8%	−29.70 ± 0.04		−16.16 ± 0.03	*RDX		77.8%	−29.70 ± 0.05		−16.20 ± 0.05
	HMX	4.100	8.2%	**−51.03 ± 0.09**		**−1.93 ± 0.18**	*HMX		8.0%	**−50.82 ± 0.08**		**−2.13 ± 0.06**
	Binder (PIB)	1.300	2.6%	**−25.83 ± 0.04**	−72.5 ± 2.5		Binder	2.7%		**−25.88 ± 0.03**	−72.5 ± 0.9	
	Oil		7.4%						6.5%			
	BEHA	2.800	5.6%	−30.65 ± 0.07	−159.9 ± 2.9		**BEHA			−30.61 ± 0.07	−159.7 ± 1.6	
	Mineral oil	0.900	1.8%				Mineral oil					
Control mix Semtex	*Explosive*		83.1%					78.4%	71.2%			
n = 4	RDX	20.775	41.6%	−37.43 ± 0.35		−16.62 ± 0.07	*RDX		35.5%	−37.43 ± 0.12		−16.66 ± 0.05
	PETN	20.775	41.6%	−46.53 ± 0.44		−10.96 ± 0.28	*PETN		35.7%	−46.49 ± 0.17		−10.87 ± 0.1
	Binder (SBR)	4.500	9.0%	−26.61 ± 0.11	−33.1 ± 3.1		Binder	8.6%		−26.60 ± 0.03	−33.8 ± 1.9	
	*Oil***		7.9%				**Hexane fraction	9.8%				
	Mineral Oil	3.450	6.9%	−27.97 ± 0.01	−136.4 ± 1.1					−27.95 ± 0.09	−136.7 ± 2.2	
	Dye	0.250	0.5%	−27.41 ± 0.13	**−147.0 ± 1.2**		**Sudan I			−27.33 ± 0.21	**−150.8 ± 1.8**	
	Antioxidant	0.250	0.5%	−26.99 ± 0.05	−186.1 ± 5.8		**2NPA			−27.00 ± 0.09	−183.6 ± 1.4	
True C-4 (1261)	*Explosive*		90.0%					88.9%	86.3%			
n = 8	RDX		81.8%				*RDX		77.7%	−46.69 ± 0.08		−2.39 ± 0.58
	HMX		8.2%				*HMX		8.7%	−49.08 ± 0.24		−3.01 ± 0.46
	Binder (PIB)		2.6%				PIB	2.6%		−28.25 ± 0.12	−87.2 ± 1.0	
	Oil		7.4%						6.7%			
	BEHA		5.6%				**BEHA			−29.14 ± 0.08	−122.6 ± 1.6	
	Process oil		1.8%				Process oil					

Bold: Significantly different at the α = 0.05 level.

Dye is Sudan I; antioxidant is 2-naphthylphenylamine.

* By HPLC.

** By GC-IsoLink or GC–MS.

*** Only 3 of this fraction was further processed.
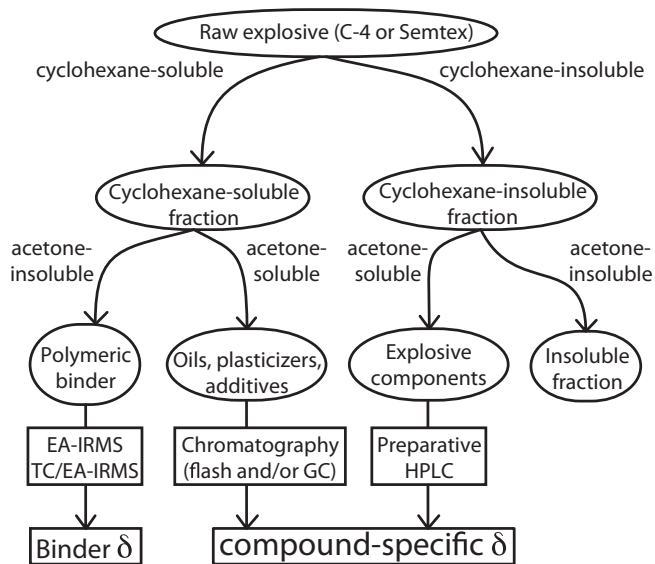
Fig. 1. Scheme for fraction separation of components of plastic explosives for isotope ratio mass spectrometry of the individual components.

the cyclohexane-insoluble fraction, dried and weighed, then prepared for HPLC (the "explosive" fraction). Any cyclohexane-insoluble, acetone-insoluble material (the "insoluble" fraction – typically a very small amount for C-4) was also dried and weighed. Preparative HPLC was used to isolate the individual explosive compounds HMX, RDX, and PETN from the cyclohexane-insoluble, acetone-soluble fraction.

Instrumentation and instrument methods

HPLC-UV/Vis analysis and isolation of individual explosive compounds from the explosive fraction was conducted according to a previous publication [33]. $^{13}C/^{12}C$ and $^{15}N/^{14}N$ analysis of RDX, HMX, and PETN was conducted using a modification of EA-IRMS (thermal decomposition) according to a previously published method [32] on a Costech ECS 4010 EA (elemental analyzer, Costech, CA, USA) attached to a Thermo Finnigan MAT 253 via a ConFlo IV interface. Both raw, unpurified explosive compounds as well as HPLC-purified materials were used to calculate the $\delta^{13}C$ and $\delta^{15}N$ values of the source material. Explosive component $\delta^{13}C$ values were corrected for blank as determined by excess carbon.

$^{13}C/^{12}C$ analysis of the binder samples, raw explosive, and RDX of the example C-4 (samples 1 and 4) was conducted using conventional EA methods (including oxygen pulse). Oil samples were loaded into 3.5×9 mm tin capsules via hexanes and dried prior to folding the capsules. Isotope ratios were "stretch-shift" corrected using two previously characterized urea reference materials [32]. Quality control was verified using polyisobutylene, RDX, and styrene-butadiene rubber reference materials. A Thermo TC/EA (high-temperature conversion elemental analyzer) was used to convert binder and bulk oil samples into H_2 gas for $^2H/^1H$ measurements at 1400 °C in a glassy carbon reactor. Samples and reference materials (0.08 mg) were loaded into 3.2×4 mm silver capsules and dropped into the reactor via zero-blank autosampler. Oil samples were loaded into 3.2×4 mm silver boats via hexanes and allowed to dry prior to analysis. Isotope ratios were corrected using previously calibrated tetracosane and octacosane reference materials [34], and verified using polyisobutylene and styrene-butadiene rubber.

FTIR analyses were performed on Nicolet iS10 FT-IR spectrometer collecting wavelengths between 400 and 4000 cm^{-1}. Samples were loaded directly onto the diamond ATR (attenuated total reflectance) plate. Gravimetric analyses were conducted on a Sartorius MC-5 microbalance (resolution ± 1 μg) in pre-weighed 1-dram vials. Due to drift and environmental factors, the estimated propagated error from duplicate measurements of 80 vials for a gravimetric measurement (gross wt. − tare wt.) was ±46 μg.

Gas chromatography-mass spectrometry (GC–MS) was conducted on a Thermo Trace GC 2000 coupled to a Thermo Finnigan Trace quadrupole MS. Samples (1 μL) were injected into a split/splitless injector held at 280 °C. The injector was in "split" mode with a split flow of 12 mL/min for a split ratio of 10:1. The column and temperature program were similar to those in the isotope ratio method (see below). The MS detector was in electron impact (EI+) mode with a source held at 200 °C and the GC interface held at 280 °C. Mass spectra were collected as total ion count over 33–425 amu with 10 scans/peak.

Isotope ratios for plasticizer, dye, and antioxidant components of source material as well as control mixtures were determined using compound-specific techniques. Gas chromatography-combustion-isotope ratio mass spectrometry (GC–C-IRMS) and gas chromatography-high-temperature conversion-isotope ratio

mass spectrometry (GC-TC-IRMS) were conducted on a Thermo Trace GC Ultra gas chromatograph coupled to a Thermo Finnigan MAT 253 via a Thermo Scientific GC IsoLink interface. Samples (1 μL) were injected into a split/splitless injector held at 280 °C in "splitless with surge" mode. The surge was at 23.4 psi and held for 30 s. The helium carrier was held at a constant flow of 1.3 mL/min after the injection surge. Compounds were separated using a J&W DB-5 fused silica column (30 m × 0.25 mm, 0.25 mm film thickness, Agilent, California, USA) using the following temperature program: initial temperature 102 °C, hold time 4 min, ramp to 300 °C at 18 °C/min, hold for 8 min, ramp to 320 °C at 20 °C/min, hold for 2 min (total run time of 26 min). The IsoLink interface was in backflush between 0–350 s and 1580–1680 s. For combustion into carbon dioxide, the combustion reactor was maintained at 1030 °C; for high-temperature conversion into hydrogen gas, the high-temperature conversion reactor was maintained at 1400 °C. Chromatographic peaks from combustion or high-temperature conversion of bis(2-ethylhexyl) adipate, N-phenyl-2-naphthaleneamene, and Sudan I were detected at their corresponding Kovats indices relative to n-alkane reference materials. The alkanes used for normalization of GC-based compound-specific $^{13}C/^{12}C$ data were octadecane (n-C18, $\delta^{13}C_{VPDB} = -33.33 \pm 0.14‰$) and octacosane (n-C28, $\delta^{13}C_{VPDB} = -28.99 \pm 0.04‰$). We used tetracosane (n-C24, $\delta^2H_{VSMOW} = -36 \pm 4‰$) and the same octacosane (n-C28, $\delta^2H_{VSMOW} = -250 \pm 5‰$) for $^2H/^1H$ data.

Stable isotope ratios are expressed in this paper as the difference between rare and abundant isotope ratios of a sample and an internationally accepted isotopic standard relative to the standard; e.g., $\delta^{13}C_{VPDB} = (R_s - R_{VPDB})/R_{VPDB}$, where R_s and R_{VDB} are the $^{13}C/^{12}C$ ratios of the sample and standard, respectively. Delta (δ) values are presented in "permil" (‰), or parts per thousand. For $^{13}C/^{12}C$, the standard is Vienna Pee Dee belemnite; the other isotope ratios used in this work are $^2H/^1H$ (VSMOW scale) and $^{15}N/^{14}N$ (AIR scale).

Hydrogen isotope ratios of n-alkane standards used for normalization of sample $^2H/^1H$ data were previously calibrated to the VSMOW scale using the defined δ^2H values of VSMOW (0‰, exactly) and SLAP (−428‰, exactly), with an error of 3‰ [34]. The values of the $^{13}C/^{12}C$ normalization reference materials were likewise calibrated to the VPDB scale using the internationally available isotopic reference materials NBS22 (−30.03 ± 0.04‰), PEF-1 (−32.15 ± 0.05‰), USGS40 (−26.39 ± 0.04‰), and USGS41 (37.63 ± 0.05‰). Calibration of the urea reference materials for EA-IRMS was described previously [32]. The use of δ-notation and reference materials calibrated to international isotope scales complies with current IUPAC recommendations [35] as well as best practices adopted by the Forensic Isotope Ratio Mass Spectrometry (FIRMS) Network [36].

Statistical analysis was performed using the open-source statistical programming language R, available via the Internet at http://cran.r-project.org. Tests for significant differences between two groups were conducted using 2-sample Student's t-test with Welch's correction, and had a Type I error (α) set at 5%. Long-term expanded uncertainty for δ-values was defined as the combined standard uncertainty of a single measurement (estimated by the standard deviation of multiple analyses of a reference material in conditions of reproducibility) times a coverage factor [37]. The coverage factor was chosen to provide an interval corresponding to a 95% confidence level. These values are available as Supplemental Data (Table S1).

Results and discussion

Validation of separation method using control mixtures

Table 1 contains the gravimetric analysis, HPLC-UV/Vis analysis, and $\delta^{13}C$, δ^2H, and $\delta^{15}N$ results of the two control mixtures made to mimic plastic explosives typical of C-4 and Semtex. Also listed is the analysis of the repeated processing (n = 8) of a true C-4 sample. The columns on the left list the theoretical amount of each fraction (i.e., "explosive," "binder," and "oil") and component, as well as the isotope ratios of the materials prior to mixing and subsequent isolation. No significant differences were found between the $\delta^{13}C$ and $\delta^{15}N$ results of isolated explosive components and those of the original material, except for HMX, which was slightly different from the original material (−0.21‰ in $\delta^{13}C$, p = 0.005;+0.20‰ in $\delta^{15}N$, p = 0.044). This demonstrates that the process of isolating each explosive component was complete (i.e., there was no contamination). Few significant differences between the source material and the isolated components were shown in the nonexplosive materials. The polyisobutylene $\delta^{13}C$ value was very slightly more negative than the original material (0.06‰, p = 0.02). The dye δ^2H values were slightly more negative in the extracted material than in the original source (3.7‰, p = 0.04). These differences in both explosive and nonexplosive components were small relative to long-term analytical uncertainty (at the level of reproducibility).
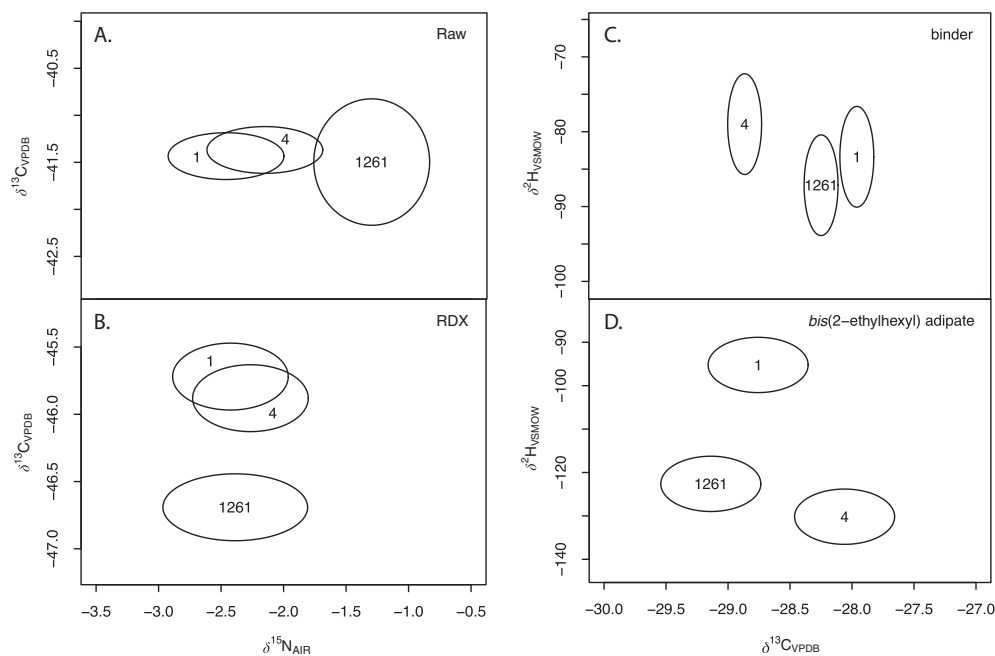
Fig. 2. Dual isotope plots (δ^{15}N and δ^{13}C; δ^{13}C and δ^2H) of example C-4 samples and components isolated from those samples. Plots are (A) "raw," prior to component separation; (B) RDX; (C) isolated binder component; and (D) *bis*(2-ethylhexyl) adipate. The height and width of each oval are, for each represented isotope, the greater of sample standard deviation or the long-term expanded uncertainty of repeated measurements of an equivalent reference material (C-4, RDX, binder and *bis*(2-ethylhexyl) adipate; see Supplemental Data, Table 1).

Table 2
Gravimetric and HPLC data for samples 1, 4, and 1261. Sample 1261 was analyzed 8 separate times. Using the analytical uncertainty of sample 1261 as a guide, Samples 1 and 4 are indistinguishable by gravimetric and HPLC analysis, and are very similar in composition to Sample 1261. Fractions measured are the binder fraction, oil fraction, explosive fraction (xf), and insoluble fraction; the explosive fraction was further analyzed for the components RDX and HMX by HPLC. Weights (wt) are in mg.

ID	Subsample/ statistic	Raw wt	Binder wt	Oil wt	xf wt	Insoluble wt	Binder w/w %	Oil w/w %	xf w/w %	Insoluble w/w %	RDX by HPLC	HMX by HPLC
1		55.393	1.417	3.524	49.739	0.154	2.6%	6.4%	89.8%	0.3%	83.6%	9.1%
4		51.297	1.194	3.651	45.713	0.191	2.3%	7.1%	89.1%	0.4%	82.0%	9.5%
1261	0	46.100	1.162	3.308	40.567	-0.100	2.5%	7.2%	88.0%	*	*** 80.5%	*** 9.1%
	1	55.600	1.361	3.712	45.018	0.100	2.4%	6.7%	81.0%	0.2%	73.3%	8.1%
	2	53.900	1.287	3.924	48.071	0.255	2.4%	7.3%	89.2%	0.5%	81.0%	8.9%
	3	44.757	1.221	3.178	38.611	0.095	2.7%	7.1%	86.3%	0.2%	76.2%	8.3%
	4	48.956	1.212	3.317	43.639	0.014	2.5%	6.8%	89.1%	*	**	**
	6	52.334	1.375	3.266	46.442	0.178	2.6%	6.2%	88.7%	0.3%	75.1%	8.4%
	7	48.893	1.363	3.279	43.783	0.182	2.8%	6.7%	89.5%	0.4%	81.6%	9.3%
	8	46.933	1.333	3.232	41.510	0.173	2.8%	6.9%	88.4%	0.4%	76.4%	8.3%
	Mean						2.6%	6.9%	87.5%	0.3%	77.7%	8.6%
	sd						0.2%	0.3%	2.8%	0.1%	3.3%	0.5%

* Below detection limit.
** Not determined.
*** Mean of 3 measurements.

The relatively small amount of error in each gravimetric, HPLC-UV/Vis, and isotope ratio measurement demonstrates the reliability of the measurements over several distinct samples ($n = 4$ for each control mixture, $n = 3$ for the real C-4 sample).

For the most part, the gravimetric results of the control mixtures confirmed the amount of material originally added. A discrepancy in the amount of explosive material recovered in the control Semtex mixture (78% gravimetric, 71% HPLC versus the theoretical amount of 83%), combined with a larger-than-expected amount of material in the oil fraction (9.8% gravimetric versus the theoretical amount of 7.9%) suggests that some of the explosive material was co-extracted into the oil fraction. However, the isolated explosive compounds (after preparative HPLC) were not isotopically different from the original materials, demonstrat-

ing that no measurable isotopic fractionation took place during co-extraction.

Examples using true C-4

The gravimetric results of the true C-4 sample confirmed the theoretical amount of material in each fraction, based on previous measurements as well as results from the literature [38–40]. The carbon, hydrogen, and nitrogen elemental analysis of the isolated components also confirmed their purity and the accuracy of the technique for separating components of plastic explosives like C-4 and Semtex. The identity and purity of individual compounds that were analyzed by GC–MS and HPLC-UV/Vis were also confirmed by retention time indices (see Supplemental Data,

Table S2 and Figs. S1–S7), UV–Vis spectra (see Supplemental Data, Figs. S1–S3), and EI-mass spectra (see Supplemental Data, Figs. S8–S10) by comparison to reference materials. The identity and purity of the polyisobutylene binder isolated from C-4 was confirmed by comparison to reference material via FTIR spectroscopy (see Supplemental Data, Fig. S11).

As an example of the utility of this fraction separation approach to add valuable forensic data to the analysis of an explosive sample, we present the results from two C-4 samples from different sources; these explosives were separated in manufacturing date by 11 years. These samples are indistinguishable by the δ^{13}C and δ^{15}N values of the raw (pre-separation) material (see Fig. 2A), as well as gravimetric and HPLC-UV/Vis analysis (see Table 2). These two additional examples also have very similar δ^{13}C and δ^{15}N values in their RDX component, precluding their discrimination by isolation of this component alone (see Fig. 2B). However, δ^{13}C and δ^2H analysis of the binder components and *bis*(2-ethylhexyl) adipate (Fig. 2C and D) clearly demonstrated that the samples can be discriminated by analysis of these minor components. The combination of multiple isotope ratio analyses of multiple components provides further information on explosive samples of forensic interest, and also discriminates samples of similar composition, even in cases where isotope ratios of the largest component are indistinguishable.

Conclusions

The method for separation of components of plastic explosives described here successfully partitions the components into 3–4 fractions based on their solubility: binder, oil, explosive, and insoluble. These fractions can be further separated into components using HPLC and/or GC to produce isotope ratios of individual compounds, such as RDX and HMX for the explosive fraction, and Sudan I and N-phenyl-2-naphthaleneamine for the oil fraction. The addition of isotopic analysis of nonexplosive materials (e.g., binder) allows for discrimination between chemically indistinguishable C-4 samples that cannot be differentiated by δ^{13}C and δ^{15}N analysis of raw material or only the RDX component. Using these separation techniques, the isolated components are sufficiently pure to produce isotope ratios that are both consistent and accurately reflect the isotope ratios of the components prior to fraction separation. Though the additional evidence gained from component-specific analysis clearly can help distinguish explosives with similar characteristics, the problem of evaluating the evidence given source propositions, such as an explicit link between a known and a recovered explosive, requires additional background data. We propose that surveys examining the δ^{13}C and δ^2H signatures of binders and plasticizers from plastic explosives be undertaken to provide background population data so that investigations into explosive source can be conducted in a consistent manner acceptable to the forensic community and the judicial system. These kind of data would also be invaluable to intelligence as a way to track shifting supplies or suppliers of plastic explosives.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.forc.2016.07.003.

References

[1] J.T. Thurman, Practical bomb scene investigation, CRC Press, Boca Raton, FL, 2006.
[3] F. Taroni, C. Aitken, P. Garbolino, A. Biedermann, DNA evidence, in: Bayesian Network and Probabilistic Inference Forensic Science, John Wiley & Sons Ltd, 2006, pp. 131–182, http://dx.doi.org/10.1002/0470091754.ch5.
[4] G. Pierrini, S. Doyle, C. Champod, F. Taroni, D. Wakelin, C. Lock, Evaluation of preliminary isotopic analysis (^{13}C and ^{15}N) of explosives: a likelihood ratio approach to assess the links between Semtex samples, Forensic Sci. Int. 167 (2007) 43–48.
[5] A. Nissenbaum, The distribution of natural stable isotopes of carbon as a possible tool for the differentiation of samples of TNT, J. Forensic Sci. 20 (1975) 455.
[6] R.B. Coffin, P.H. Miyares, C.A. Kelley, L.A. Cifuentes, C.M. Reynolds, Stable carbon and nitrogen isotope analysis of TNT: two dimensional source identification, Environ. Toxicol. Chem. 20 (2001) 2676–2680.
[7] M.J. Lott, J.D. Howa, J.R. Ehleringer, Locating the origins of explosives through stable isotope ratio analysis, in: 1st Forensic Isot. Ratio Mass Spectrometer FIRMS Netw. Conf., 2002. http://www.forensic-isotopes.org/conference/conf2002/conf2002.html.
[8] S. Benson, C. Lennard, P. Maynard, C. Roux, Forensic applications of isotope ratio mass spectrometry – a review, Forensic Sci. Int. 157 (2006) 1–22.
[9] J.R. Ehleringer, T.E. Cerling, J.B. West, in: R.D. Blackledge (Ed.), Forensic Anal. Cut. Edge New Methods Trace Evid. Anal., Wiley-Interscience, Hoboken, New Jersey, 2007, pp. 399–422.
[10] S.J. Benson, C.J. Lennard, P. Maynard, D.M. Hill, A.S. Andrew, C. Roux, Forensic analysis of explosives using isotope ratio mass spectrometry (IRMS)–discrimination of ammonium nitrate sources, Sci. Justice 49 (2009) 73–80.
[11] S.J. Benson, C.J. Lennard, P. Maynard, D.M. Hill, A.S. Andrew, C. Roux, Forensic analysis of explosives using isotope ratio mass spectrometry (IRMS): preliminary study on TATP and PETN, Sci. Justice 49 (2009) 81–86.
[12] S.J. Benson, C.J. Lennard, D.M. Hill, P. Maynard, C. Roux, Forensic analysis of explosives using isotope ratio mass spectrometry (IRMS). Part 1: instrument validation of the DELTA$_{plus}$XP IRMS for bulk nitrogen isotope ratio measurements, J. Forensic Sci. 55 (2010) 193–204.
[13] S.J. Benson, C.J. Lennard, P. Maynard, D.M. Hill, A.S. Andrew, K. Neal, H. Stuart-Williams, J. Hope, G. Stewart Walker, C. Roux, Forensic analysis of explosives using isotope ratio mass spectrometry (IRMS), Part 2: forensic inter-laboratory trial: bulk carbon and nitrogen stable isotopes in a range of chemical compounds (Australia and New Zealand), J. Forensic Sci. 55 (2010) 205–212.
[14] D. Widory, J.J. Minet, M. Barbe-Leborgne, Sourcing explosives: a multi-isotope approach, Sci. Justice 49 (2009) 62–72.
[15] H. Brust, M. Koeberg, A. van der Heijden, W. Wiarda, I. Mügler, M. Schrader, G. Vivo-Truyols, P. Schoenmakers, A. van Asten, Isotopic and elemental profiling of ammonium nitrate in forensic explosives investigations, Forensic Sci. Int. (2014).
[16] Analysis of Semtex Explosives, in: Adv. Anal. Detect. Explos., 1992, pp. 409–427.
[17] J.D. Howa, M.J. Lott, J.R. Ehleringer, The relationship between HMX and RDX stable isotopes can reveal production and subsequent mixing information in explosive seizures, 4th Forensic Isot. Ratio Mass Spectrometer FIRMS Netw. Conf., 2010.
[18] J.C. Oxley, M. Marshall, S.L. Lancaster, Principles and issues in forensic analysis of explosives, Forensic Chem. Handb. (2011) 23.
[19] R.R. McGuire, C.A. Velsko, C.G. Lee, E. Raber, The use of post detonation analysis of stable isotope ratios to determine the type and production process of the explosive involved, 1993. http://www.osti.gov/scitech/biblio/10177768 (accessed 19.01.2016).
[20] R. McGuire, C. Lee, C. Velsko, E. Raber, Application of stable isotope ratios to the analysis of explosive residues, in: Proc. Fifth Int. Symp. Anal. Detect. Explos. Wash. DC, 1995, pp. 4–8.
[21] A. Bernstein, Z. Ronen, E. Adar, R. Nativ, H. Lowag, W. Stichler, R.U. Meckenstock, Compound-specific isotope analysis of RDX and stable isotope fractionation during aerobic and anaerobic biodegradation, Environ. Sci. Technol. 42 (2008) 7772–7777.
[22] C.M. Lock, W. Meier-Augenstein, Investigation of isotopic linkage between precursor and product in the synthesis of a high explosive, Forensic Sci. Int. 179 (2008) 157–162, http://dx.doi.org/10.1016/j.forsciint.2008.05.015.
[23] R. Aranda, L.A. Stern, M.C. McCormick, R.F. Mothershead, J.A. Barrow, Isotopic Analysis of the Explosive Urea Nitrate and Its Component Ions for Forensic Applications, in: 2008: p. 0805.
[24] C.M. Lock, H. Brust, M. van Breukelen, J. Dalmolen, M. Koeberg, D.A. Stoker, Investigation of isotopic linkages between precursor materials and the improved high explosive product hexamethylene triperoxide diamine, Anal. Chem. 84 (2012) 4984–4992, http://dx.doi.org/10.1021/ac300642c.
[25] J.D. Howa, M.J. Lott, J.R. Ehleringer, Observations and sources of carbon and nitrogen isotope ratio variation of pentaerythritol tetranitrate (PETN), Forensic Sci. Int. 244 (2014) 152–157.
[26] L.A. Chesson, J.D. Howa, M.J. Lott, J.R. Ehleringer, Short communication: development of a methodological framework for applying isotope ratio mass spectrometry to explosive components, Forensic Chem., 2016 (submitted for publication).
[27] J.R. Ehleringer, CSI or isogap? Forensic potential of isotopes, investigative needs, and judicial requirement, Plenary Talk, 4th Forensic Isot. Ratio Mass Spectrometer FIRMS Netw. Conf., 2010.
[28] J.R. Ehleringer, S.M. Matheson Jr, Stable isotopes and courts, Utah Law Rev. 2010 (2010) 385–442.
[29] N. Gentile, R.T. Siegwolf, P. Esseiva, S. Doyle, K. Zollinger, O. Delémont, Isotope ratio mass spectrometry as a tool for source inference in forensic science. a critical review, Forensic Sci. Int. (2015).
[30] C. Champod, I.W. Evett, G. Jackson, Establishing the most appropriate databases for addressing source level propositions, Sci. Justice J. Forensic Sci. Soc. 44 (2004) 153.

[31] C.G. Aitken, F. Taroni, J. Wiley, Statistics and the evaluation of evidence for forensic scientists, Wiley Online Library, 2004.

[32] M.J. Lott, J.D. Howa, L.A. Chesson, J.R. Ehleringer, Improved accuracy and precision in $\delta^{15}N_{AIR}$ measurements of explosives, urea, and inorganic nitrates by elemental analyzer/isotope ratio mass spectrometry using thermal decomposition, Rapid Commun. Mass Spectrom. 29 (2015) 1381–1388, http://dx.doi.org/10.1002/rcm.7229.

[33] J.D. Howa, M.J. Lott, L.A. Chesson, J.R. Ehleringer, Carbon and nitrogen isotope ratios of factory-produced RDX and HMX, Forensic Sci. Int. 240 (2014) 80–87.

[34] B.J. Tipple, M.A. Berke, C.E. Doman, S. Khachaturyan, J.R. Ehleringer, Leaf-wax n-alkanes record the plant–water environment at leaf flush, Proc. Natl. Acad. Sci. 110 (2013) 2659–2664.

[35] Willi.A. Brand, Tyler.B. Coplen, Vogl Jochen, Rosner Martin, Prohaska Thomas, Assessment of international reference materials for isotope-ratio analysis (IUPAC Technical Report), Pure Appl. Chem. 86 (2014) 425, http://dx.doi.org/10.1515/pac-2013-1023.

[36] J.F. Carter, V.J. Barwick, Good practice guide for isotope ratio mass spectrometry, FIRMS (2011). http://www.forensic-isotopes.org/gpg.html.

[37] JCGM/WG 1, Evaluation of measurement data — Guide to the expression of uncertainty in measurement, (2008).

[38] R. Keto, Improved method for the analysis of the military explosive composition C-4, J. Forensic Sci. 31 (1986) 241.

[39] C. Midkiff, W. Washington, Systematic approach to detection of explosive residues. 4. Military explosives, J. Assoc. Off. Anal. Chem. 59 (1976) 1357–1374.

[40] K.L. Steele, Forensic analysis of C-4 and commercial blasting agents for possible discrimination, University of Central Florida Orlando, Florida, 2007.