



TATP isotope ratios as influenced by worldwide acetone variation

John D. Howa*, Janet E. Barnette, Lesley A. Chesson, Michael J. Lott, James R. Ehleringer¹

IsoForensics, Inc., 421 Wakara Way, Suite 100, Salt Lake City, UT 84108, United States



ARTICLE INFO

Keywords:
Isotope ratio
TATP
Forensic
Explosive
Acetone
Terrorism

ABSTRACT

Isotope ratio analysis has been shown to discriminate samples of forensic interest and to link many synthesized and natural materials to their precursors when traditional chemical and physical analyses cannot. Successful application of stable isotope analysis to chemicals of interest requires a background of likely variations in stable isotope ratios; often, this background population can be generated from analysis of possible precursors and the relationships of stable isotopes of precursor(s) to product(s), which may depend on synthesis techniques. Here we measured the carbon ($^{13}\text{C}/^{12}\text{C}$) and hydrogen ($^2\text{H}/^1\text{H}$) isotope ratios of the oft-illicitly manufactured explosive triacetone triperoxide, TATP, and one of its precursors, acetone. As acetone is the sole source of carbon and hydrogen to TATP, a survey of acetone from 12 countries was conducted to explore the breadth of $^{13}\text{C}/^{12}\text{C}$ and $^2\text{H}/^1\text{H}$ variation in the precursor, and therefore, its product. Carbon and hydrogen isotope ratios were measured using continuous flow isotope ratio mass spectrometry (IRMS) techniques. We observed greater ranges in both C and H isotope ratios of acetone than previously published; we also found that country-of-purchase was a large contributing factor to the observed variation, larger than acetone grade and brand. Following clandestine production methods, we observed that the stable isotope ratios of TATP retained the stable isotope signatures of acetone used in synthesis. We confirmed the robustness of TATP carbon isotope ratios to both recrystallization and time-dependent sublimation, important considerations when faced with the task of practical sampling of potential unexploded TATP from a crime scene.

1. Introduction

The powerful high explosive triacetone triperoxide (TATP) can be made from household chemicals—acetone, hydrogen peroxide, and strong acids—easily obtained at hardware and beauty supply stores. Nicknamed “the mother of Satan,” this primary explosive is shock-sensitive, sublimates, and is potentially unstable, making it unsuitable for legitimate uses [1], but perfect for the terrorist's toolbox. Its use in improvised explosive devices, alongside other peroxide-based explosives such as hexamethylene triperoxide diamine (HMTD), is becoming more prevalent, as seen in recent high-profile incidents such as the “shoe” and “underwear” bombers, the July 2005 London transit bombings, and the 2016 Brussels airport attack [2]. As the use of homemade explosives, such as TATP, in improvised explosive devices becomes more prevalent, the ability of law enforcement to link a precursor, such as acetone, to a clandestine lab or possibly to the explosive itself is of forensic interest.

When examined as a pure crystalline material, traditional chemical analyses cannot unequivocally link a particular batch of TATP to a bomb or cache of precursors; however, stable isotope ratio analysis

provides forensic chemists a reliable technique to distinguish among chemically identical materials by their stable isotope ratios [3,4], and has been shown to be useful for discriminating among explosives, including TATP [5,6]. In many circumstances, the precursors of manufactured explosives, even those made using improvised methods, can be related to the final product by their isotope ratios [7–10].

By the reaction mechanism to produce TATP [6], all oxygen atoms originate from the hydrogen peroxide, and all carbon and hydrogen atoms originate from acetone. The oxygen atoms in TATP are likely related to the peroxide oxygen in hydrogen peroxide solutions, which have been surveyed extensively by Barnette et al. [11] and, on a smaller scale, by Bezemer et al. [6]. The carbon and hydrogen isotope ratios of TATP are strongly related to that of the acetone precursor, as shown by Bezemer et al. [6] over different reaction conditions. When TATP yield is high with respect to acetone, we would expect the carbon and hydrogen isotope ratios of the precursors to match the product closely, as demonstrated previously for the explosive pentaerythritol tetranitrate (PETN) made using high-yield methods [10]. Thus, a survey of acetone may be a good proxy for the expected variation of isotope ratios in TATP in the course of investigations.

* Corresponding author.

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

¹ Present address: Dept. of Biology, University of Utah, Salt Lake City, UT 84112, United States.

As discussed in recent literature [4,12,13], interpretation of forensic data beyond “intelligence gathering” requires a sufficient characterization of the background variation within and between “sources,” no matter the definition of “source” used for the interpretation. Methods for analyzing isotope ratios of acetone have been published alongside results from samples found in laboratories [14] and encountered in casework [6], but no systematic survey of acetone has been conducted to date.

Here we present an isotopic survey of acetone of several different grades from 12 countries, with a major focus on the United States. The relationship between carbon and hydrogen isotope ratios was examined, as they were expected to co-vary in a fashion similar to their petrochemical sources. Various factors such as acetone grade (“hardware”, “nail”, “scientific”, and “unspecified”), brand, and country of purchase were examined to determine their influence on the overall variation of carbon and hydrogen isotope ratios. Isotope ratios of TATP synthesized from several of these acetone samples were compared to that of the acetone. To confirm the robustness of practical collection methods as it applies to isotope ratio analysis of TATP and its precursors, we examined the effects of evaporation of acetone as well as the effects of sublimation and recrystallization of TATP on their respective isotope ratios.

2. Materials and methods

2.1. Sample collection

Acetone samples were collected from 13 different locations in 11 countries outside the US as well as from 63 locations within 27 states of the US (see Fig. 1). The entire survey consisted of 96 acetone samples in different grades categorized as “scientific,” “hardware,” and “nail,” with a fourth “unspecified” grade, and were obtained from hardware, grocery, beauty, and scientific suppliers. Brand name, e.g., “Ace,” “J. T. Baker,” etc. was also recorded (31 different brands). Acetone samples collected by the authors were stored at room temperature in their original container. Other samples were obtained from colleagues by mail. Shipped samples were subsampled to 1.5 mL, stored in 4-mL borosilicate glass vials, and kept at 4 °C after receipt. The glass vials were sealed with caps including PTFE-lined septa, and the caps secured with Parafilm.

TATP was synthesized in six batches from four different acetones that had measured $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values. *Warning: TATP is a primary explosive sensitive to friction and impact. The storage, handling, and synthesis of TATP are hazardous activities that require safety considerations for handling high explosives.* The particular synthesis method used was chosen as it was of practical and popular use in the clandestine “literature.” Samples were synthesized at two separate locations. Samples from batches “E” through “I” were received in crystalline form; three additional samples of TATP from these syntheses were received following recrystallization from organic solvents. Four additional synthesized TATP samples (batches “A” through “D”) were synthesized



Fig. 1. Map of locations from the worldwide survey of acetone in this study. Following the country label are two numbers (a, b) corresponding to: (a) the number of locations from which samples were collected in that country, and (b) the total number of samples collected in that country.

Table 1

Key for matching product TATP to acetone and hydrogen peroxide IDs in syntheses. Sample IDs refer to samples and analyses given in Supplementary Information. Synthesis batches refer to data points in Fig. 3; IDs 71–74 were used in the sublimation experiment (Fig. 4).

Synthesis batch	Raw TATP ID	Recrystallized TATP ID	Acetone ID	Peroxide ID	Peroxide conc. (w/w%)
A	–	71	–	–	–
B	–	72	–	–	–
C	–	73	–	–	–
D	–	74	–	–	–
E	88	89	104	–	50
F	90	91	104	–	35
G	92	93	104	–	20
H	94	–	100	–	–
I	95	–	102	–	–
J	254	255	18	12	35

from acetone that was not collected for isotope ratio analysis, and used to examine heterogeneity and the effects of sublimation; these samples were also received in crystalline form (see Table 1).

Synthesis was conducted by slowly adding hydrogen peroxide to chilled (5 °C) acetone while stirring with a magnetic stir bar, keeping the reaction temperature below 11.5 °C. Hydrogen peroxide was of concentrations between 20% and 50%. The amount of acetone used was adjusted to keep a constant acetone: peroxide molar ratio, maintaining acetone as a limiting reagent. After 30 min, sulfuric acid (50%) was slowly added to the reaction vessel, keeping reaction temperature below 13 °C. After crystals formed and the solution thickened, the solution was allowed to crystallize overnight at 4 °C. (*Warning: at this point, the material is at risk for explosion.*) Water was used to wash the crystals, transfer the slurry onto filter paper, and filter. After the crystals were allowed to air dry, they were transferred to a storage vessel. Raw TATP was stored at room temperature in 1-dram borosilicate glass vials sealed with Parafilm.

2.2. Sublimation/evaporation experiments

To examine the effect of sublimation on the carbon isotope ratios of TATP, samples from each of synthesis batches “A” through “D” were loaded in duplicate at a target weight of 1.2 mg into tin capsules used for EA-IRMS (see below) and allowed to sublimate on a laboratory benchtop for 0, 3, 7, and 14 days. The material remaining in capsules was analyzed immediately following the designated elapsed time. To examine the effect of evaporation on carbon isotope ratios of acetone, four vials of a single stock acetone (ID 17; see Supporting Information) were weighed to ~15 g in 20-mL scintillation vials and capped with a permeable nylon mesh to avoid contamination with airborne dust. Vials were allowed to evaporate in a fume hood at ambient temperature (~21 °C) while weighing and subsampling each vial (~0.3 mg) at 0, 5.5,

22, 30, and 52 h. Subsamples were immediately capped to stop further evaporation. To examine the effect of evaporation on hydrogen isotope ratios of acetone, nine vials of a single stock acetone (ID 14) were force-evaporated to targets amounts of 90%, 80%, ... 10% of the original mass using dry, low-flow air via blowdown apparatus and heat block set to slightly above ambient lab temperature. Samples were capped when near the target amounts, and the weight of remaining acetone recorded. The maximum time for evaporation was 27 min (for vial containing 10% of the original mass). An additional vial of acetone was sampled without force-evaporation as a control.

2.3. Sample purity

Of the 96 acetone samples collected in the survey, 57 were analyzed by gas chromatography/mass spectrometry (GCMS) to confirm chemical purity. Acetone samples (0.2 μL) were injected into a Thermo Trace GC (Thermo Fisher Scientific, Hampton, NH, USA) via PAL autosampler (CTC Analytics, Zwingen, Switzerland). The split/splitless injector was held at 280 °C with a 400:1 split ratio. Compounds were separated using a J&W DB5 column (30 m \times 0.25 mm, 0.25 mm film thickness; Agilent, CA, USA) using the following temperature program: initial temperature 40 °C with a 3-min hold time, ramp at 40 °C/min to 150 °C with a 2-min hold time. Acetone eluted at 1.22 min, water at 1.50 min, and methanol at 1.54 min. Signal was generated using the quadrupole MS detector set to EI^+ ionization with the source held at 200 °C and the GC interface held at 280 °C; peaks formed by total ion count (TIC) signal were identified by fragmentation pattern and retention time, then quantified by peak height relative to the total height of peaks.

2.4. Isotope ratio analysis

Samples were analyzed via isotope ratio mass spectrometry (IRMS) at SIRFER (Stable Isotope Ratio Facility for Environmental Research; University of Utah, Salt Lake City, UT, USA). For $^{13}\text{C}/^{12}\text{C}$, TATP samples were loaded at a size of 1 mg into tin capsules and introduced into a Carlo Erba 1108 elemental analyzer (EA) via a zero-blank autosampler (Costech, CA, USA). Gases formed from combustion were carried from the EA to Finnigan MAT Delta S isotope ratio mass spectrometer connected via a ConFlo interface. TATP samples were analyzed in sequence alongside capsules of a previously calibrated cellulose reference material (RM) (SIRFER Cellulose, “Cell”). Due to potential sample loss through sublimation, all TATP samples were loaded and analyzed within the same day except where noted below. Acetone samples (0.5 μL) were manually injected directly onto the oxidation reactor of the EA via 10- μL syringe (SGE Analytical Science, TX, USA) and manual injection port. Acetone samples were analyzed in pairs in sequence alongside an octane RM with a known $^{13}\text{C}/^{12}\text{C}$ isotope ratio.

For $^2\text{H}/^1\text{H}$ isotope ratios, TATP samples were loaded at a size of 0.15 mg into silver capsules and introduced into a ThermoFinnigan Delta + XL isotope ratio mass spectrometer via a ConFlo interface after pyrolysis at 1400 °C in a Thermo high temperature conversion elemental analyzer (TC/EA) equipped with a glassy carbon reactor, which was configured for “reversed-flow” carrier after Gehre et al. [15]. CO and H_2 gases were separated by a 1-m molecular sieve GC column. TATP sample sequences were prepared as above, with the addition of two isotopically distinct cellulose RMs (H1 and L6) to correct for scale compression or expansion. Acetone samples were introduced to the TC/EA via PAL autosampler in 0.2 μL injections; GC vials for the autosampler were loaded with 0.4 mL of acetone and capped with PTFE-lined septa. Three additional vials of water RMs of known $^2\text{H}/^1\text{H}$ (DI, EV, and ZE, provided by SIRFER) were sampled periodically during a sequence.

$^{13}\text{C}/^{12}\text{C}$ isotope ratios are expressed in parts per thousand (‰) relative to the standard VPDB in δ -notation [16]; $^2\text{H}/^1\text{H}$ isotope ratios are likewise expressed relative to the standard VSMOW. Acetone $\delta^{13}\text{C}$

values within a sequence were normalized such that the octane RM was -30.2‰ . The octane RM was previously calibrated via traditional offline combustion and pure gas isotope ratio analysis (dual inlet) using international RMs IAEA-CH-7 (formerly PEF-1, NIST 8540; $\delta^{13}\text{C} = -32.15\text{‰}$) and NBS 22 (NIST 8539, $\delta^{13}\text{C} = -30.03$). We note that current good practice guidelines for IRMS [17] suggest all measurements should be normalized with at least two RMs to correct for scale expansion/contraction. The effect of scale expansion/contraction was expected to be small for $^{13}\text{C}/^{12}\text{C}$ measurements in the studied range (-35‰ to -20‰), supported by estimated between-sequence uncertainty (see statistics section, below) for acetone measurements (see Results) roughly equivalent to a baseline uncertainty of 0.2‰. Acetone $\delta^2\text{H}$ values were “two-point” normalized such that mean DI and ZE $\delta^2\text{H}$ values were -123.0‰ and -0.1‰ , respectively. DI and ZE were previously calibrated offline against VSMOW ($\delta^2\text{H} = 0\text{‰}$) and SLAP ($\delta^2\text{H} = -428\text{‰}$).

For TATP samples, carbon isotope ratio data were normalized such that the mean $\delta^{13}\text{C}$ value of the RM Cell within an EA-IRMS sequence was its known value of -24.4‰ . Too few TATP samples were re-analyzed to calculate within/between sequence $\delta^{13}\text{C}$ uncertainties, though within-sequence standard deviations for duplicate analyses of two samples in the sublimation experiment were less than 0.3‰. TATP hydrogen isotope ratio data were normalized with laboratory cellulose RMs H1 and L6, whose $\delta^2\text{H}$ values (-95.2‰ and $+65.6\text{‰}$, respectively) were calibrated using *n*-alkane RMs with known $\delta^2\text{H}$ values relative to VSMOW. These RM $\delta^2\text{H}$ values were monitored for stability relative to the alkane RMs over a period of several years. We acknowledge that the cellulose RMs H1 and L6 contain exchangeable hydrogen, though the effect of laboratory moisture on RMs and samples should be minimal. This was supported by the low between-sequence uncertainty for $\delta^2\text{H}$ measurements of TATP of 2.2‰, less than the within-sequence uncertainty of 2.7‰. To verify the accuracy of the TATP $\delta^2\text{H}$ values, four vials of TATP were reanalyzed alongside the alkane RMs as well as IAEA-CH-7, with known $\delta^2\text{H}$ value of $-100.3\text{‰} \pm 2.0\text{‰}$ [16]. TATP in three of the vials (two vials of sample ID 254 and one of sample ID 255; batch J) had likely sublimated during storage over the last ~ 8 years, as large rectangular (3–4 mm) crystals were observed at the necks of the vials. The $\delta^2\text{H}$ values of the newer analyses (normalized using the *n*-alkane RMs, a tetracosane and octacosane) were significantly more positive (difference = 11.4‰, $T_{39} = 9.371$, $p < 0.001$) for the sublimated samples. However, the samples that had not apparently sublimated and recrystallized (IDs 90 and 94) had $\delta^2\text{H}$ values only slightly more negative than their previous values normalized using the RMs H1 and L6 (difference = -1.3‰ , $T_{17} = -2.375$, $p = 0.0296$). These tests were conducted using mixed-effects models (see statistics section, below) where normalization scheme (H1/L6 vs. *n*- C_{24}/n - C_{28}) was a fixed effect, and sample ID was a random effect. The results of this check suggest the original measurements of TATP relative to cellulose reference materials were indeed accurate on the VSMOW isotope scale.

2.5. Statistical analysis

Data used in the preparation of this article are available as [Supplemental information](#) in “csv” (comma-delimited) format. Statistical analysis was conducted using the programming language R version 3.4.2 [18]. All tests for statistical significance had a Type I error (α) set at 5%. As the number of isotope analyses per sample within a sequence varied, as well as the number of repeated analyses between sequences (unbalanced design), decomposition of variance for C and H isotope ratio measurements was conducted through random effects models using the package lme4 [19] to extract between-sequence and within-sequence variances. These approximations for sources of uncertainty were combined for use in a Deming regression model (conducted using the package mcr [20]) to calculate a slope and intercept for the linear relationship between $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values. To examine the extent of

correlation between C and H isotope ratios, two-sided tests (using the base function `cor.test`) were conducted using Pearson's product moment correlation coefficient (r).

Tests for significant differences in C or H isotope ratios between different grades of acetone, and between acetone purchased in different countries, were conducted using mixed-effects models with `lme4`; the `lmerTest` package [21] was used to calculate p -values for differences in least square means via the Satterthwaite approximation. Fixed effects were assigned as grade and country-of-purchase; sequence number and sample ID were assigned as random effects to take into account within-sample and between-sample variation. Few samples were collected from each sampled country outside the United States, so differences in acetone carbon and hydrogen isotope ratios between countries were only tested relative to the United States. To compare the variance in acetone isotope ratios attributable to the different observed factors (country-of-purchase, grade, brand, ID, and sequence number), separate random effects models were fit using maximum likelihood and tested against simpler models using ANOVA. Chi-square tests were used to calculate p -values. Summary tables of statistical tests, as well as other results from linear mixed-effects and random-effects models, are included in [Supplementary material](#).

Separate mixed-effects models (one for C isotopes and one for H isotopes) were used to compare the isotope ratios of TATP to those of acetone used in the synthesis; in these, “compound” (acetone vs. TATP) was considered a fixed effect while sample ID nested within synthesis batch were considered random effects. Likewise, we examined the effect of recrystallization on C and H isotopes with recrystallization (true or false) as a fixed effect and synthesis batch as a random effect. The effect of recrystallization on heterogeneity was tested by two-sided F test (using the base function `var.test`) comparing variance of recrystallized TATP to “raw” TATP. The effect of evaporation of acetone on C and H isotopes was tested by linear models of log-transformed fraction of acetone remaining in the form $\delta \sim \log_e(1 - f_{\text{evaporated}})$. $f_{\text{evaporated}}$ was calculated by w/w. The effect of time of sublimation of TATP on $\delta^{13}\text{C}$ values and C content (w/w% remaining) was modeled using day as a fixed effect and sample ID as a random effect.

3. Results

[Fig. 2](#) is a dual isotope plot of mean $\delta^{13}\text{C}$ (vs. VPDB) and $\delta^2\text{H}$ (vs. VSMOW) values of 93 acetone samples in the worldwide survey;

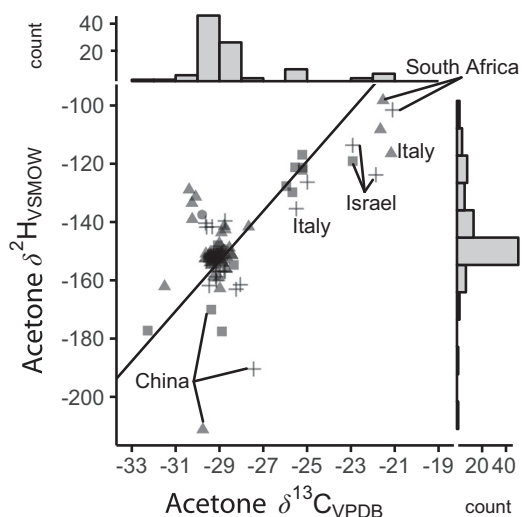


Fig. 2. Dual isotope plot of mean $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values of 93 acetone samples in the worldwide survey. Marginal histograms describe counts of samples measured. Triangles indicate “hardware” grade, squares “nail” grade, crosses “scientific” grade, and circles “unspecified” grade. Line describes relationship between $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values by Deming regression ($x:y$ ratio = 0.033).

excluded are three samples determined to be less than 96% acetone by GCMS. These mean $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values were significantly correlated ($r = 0.73$, $df = 90$, $p < 0.001$). We observed large between-sample range relative to uncertainty, with a range of 11.09‰ in $\delta^{13}\text{C}$ means and estimated standard deviations of 0.19‰ and 0.07‰ for between-sequence and within-sequence uncertainty, respectively; and a range in $\delta^2\text{H}$ means of 112.6‰ and estimated standard deviations of 0.9‰ and 0.7‰ for between-sequence and within-sequence uncertainty, respectively. The variances from these two factors were combined for estimated combined standard deviations of 0.21‰ for $\delta^{13}\text{C}$ and 1.13‰ for $\delta^2\text{H}$, then used to calculate a variance ratio for $\delta^{13}\text{C}:\delta^2\text{H}$ of 0.033, which was used in the Deming regression model $\delta^2\text{H} \sim \delta^{13}\text{C}$. The slope and intercept were both significant, with 95% confidence intervals of 6.5–13.0 for the slope and 37‰ to 222‰ for the intercept.

The majority of the acetone samples (see [Fig. 2](#)) had $\delta^{13}\text{C}$ values near -29 ‰ and $\delta^2\text{H}$ values near -151 ‰, typical of petroleum sources such as coal and crude oil [22]. The “hardware” grade of acetone had $\delta^{13}\text{C}$ values significantly more negative than the “scientific” grade (LMM, $d = -1.2$ ‰, $T_{90} = -2.25$, $p = 0.03$) and “nail” grade (LMM, $d = -1.5$ ‰, $T_{87} = -2.40$, $p = 0.02$); other comparisons within grade were not significant (see [Supplementary material for tables of differences](#)). Grade had no significant effect on $\delta^2\text{H}$ values.

Isotope ratios of acetone were found to vary significantly by country of purchase. Samples from China, Israel, Italy, and South Africa had $\delta^{13}\text{C}$ or $\delta^2\text{H}$ mean values significantly different from the USA means; sample means from these countries are labeled in [Fig. 2](#). Austria, Brazil, England, Greece, Mexico, Portugal, and Sweden did not have mean $\delta^{13}\text{C}$ or $\delta^2\text{H}$ values significantly different from those of the USA (see [Supplementary material for tables of differences from USA means](#)). In random effects models where country-of-purchase and brand were considered as random factors alongside sources from measurement uncertainty, “country” was considered the significantly greater source of variation, explaining more than 4 times the amount of variance as brand for $\delta^{13}\text{C}$ ($\chi^2 = 45$, $p < 0.001$). In a separate model, as there was no variation of grade within brand, country-of-purchase was found to be the source of more than 26 times the variance as grade ($\chi^2 = 44$, $p < 0.001$). Similar patterns were found for variation in $\delta^2\text{H}$ values, with country-of-purchase explaining more than 3 times the amount of variance as brand ($\chi^2 = 54$, $p < 0.001$) and more than 20 times the variance as grade ($\chi^2 = 35$, $p < 0.001$).

Where available, the $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values of the synthesized TATP were compared to the acetone precursor (see [Fig. 3](#)). The means of the precursor and product isotope ratios were significantly correlated in $\delta^{13}\text{C}$ ($r = 0.96$, $df = 4$, $p = 0.002$) but not quite in $\delta^2\text{H}$ values, likely because of the small sample size ($r = 0.81$, $df = 4$, $p = 0.053$). The linear models predicting TATP $\delta^{13}\text{C}$ and TATP $\delta^2\text{H}$ values based on those of the acetone (using means of each compound per synthesis batch) are illustrated by the dotted lines in [Fig. 3](#); the slopes were not significantly different from zero for either isotope, and intercepts were not significantly different from zero for either isotope. A mixed-effects model showed a significant, albeit small difference between paired TATP and acetone $\delta^{13}\text{C}$ values (LMM, $T_7 = -3.11$, $p = 0.0171$; predicted TATP $\delta^{13}\text{C}$ values were more negative than the acetone by 0.25‰). Conversely, TATP $\delta^2\text{H}$ values were more positive than the acetone by 6.7‰, though the difference was not quite significant (LMM, $T_{5.5} = 2.5$, $p = 0.0508$).

Effects of sublimation over time on TATP $\delta^{13}\text{C}$ values were examined using samples from four different TATP synthesis batches (A–D, see [Table 1](#) and [Fig. 4](#)). We found no significant effect of sublimation time on $\delta^{13}\text{C}$ values (-0.004 ‰ per day, $df = 10$, $p = 0.618$) even though the effect on the amount of material remaining (determined as w/w% C) was significant (-0.74 ‰ per day, $df = 10$, $p = 0.032$).

Tests for the effects of evaporation on acetone were conducted in two different studies, as outlined in Materials and Methods – (1) one acetone sample was evaporated under ambient conditions and periodically sampled for measurement of carbon isotope ratios, while (2) another acetone sample was force-evaporated to a series of target

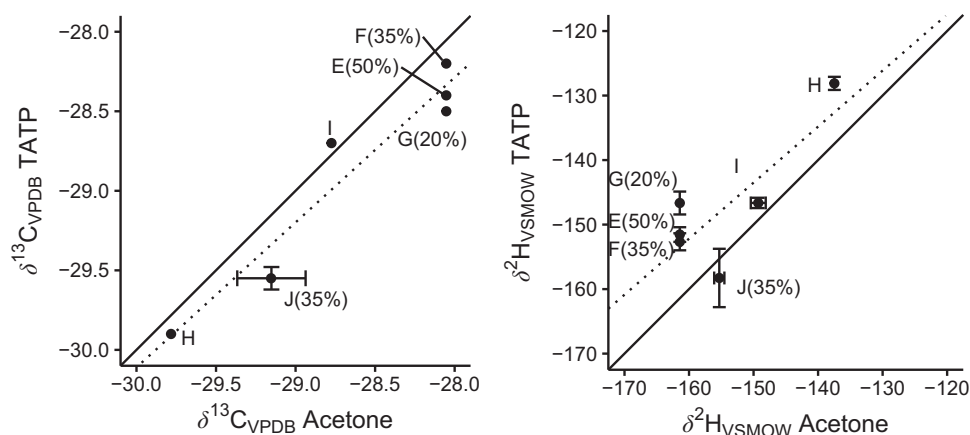


Fig. 3. Precursor acetone vs. (left) TATP $\delta^{13}\text{C}$ values and (right) TATP $\delta^2\text{H}$ values for six different TATP syntheses. Where available, concentration of hydrogen peroxide reactant is indicated. Error bars show standard uncertainty (1σ) of a future measurement based on repeated measurements of the products and reactants, where available. Dotted lines indicate predicted TATP $\delta^{13}\text{C}$ or $\delta^2\text{H}$ values from those of the acetone precursor using ordinary least squares. Solid lines illustrate a theoretical 1:1 relationship between precursor and product δ -values.

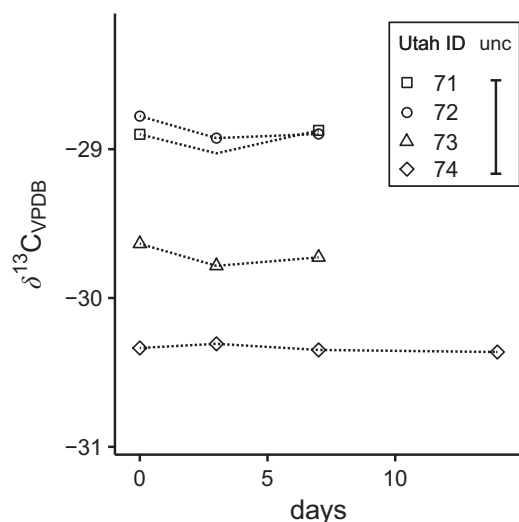


Fig. 4. Carbon isotope ratio ($\delta^{13}\text{C}$ values) for four different TATP samples allowed to sublimate over 0–14 days. Error bars (unc) indicate standard uncertainty (1σ) of a future measurement based on a small number of repeated TATP measurements (maximum $\text{sd} = 0.27\text{‰}$). Dotted lines connect samples of the same ID.

remaining masses for measurement of hydrogen isotope ratios. For the passive ambient evaporation study, carbon isotope ratios of four different vials of the same acetone sample increased at first slightly, then dramatically, for a maximum predicted shift of $+8\text{‰}$ for acetone that has been almost completely evaporated (see Fig. 5). The linear model in the form $\delta \sim \log_e(1 - f_{\text{evaporated}})$ had a significant slope of -1.49 ($p < 0.001$; multiple $r^2 = 0.75$). In the forced-evaporation study,

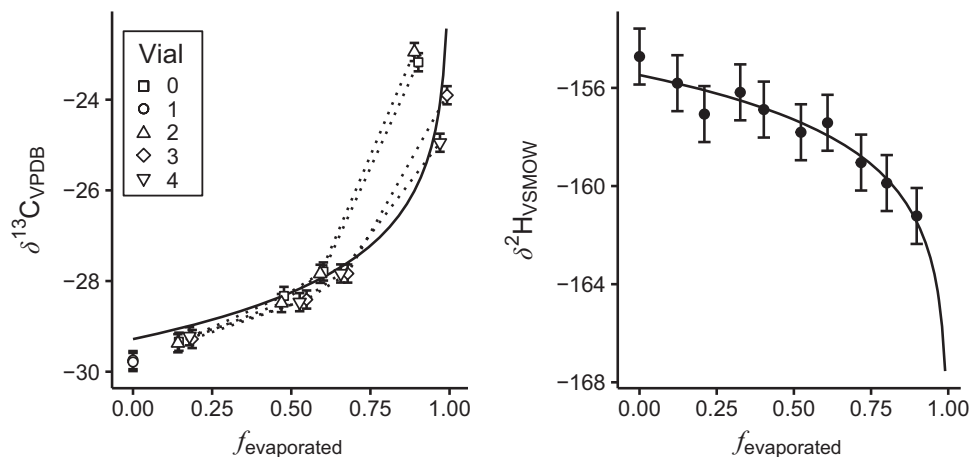


Fig. 5. Carbon isotope ratios ($\delta^{13}\text{C}$ values, left) and hydrogen isotope ratios ($\delta^2\text{H}$ values, right) of acetone, as influenced by evaporation. Four separate vials of one acetone sample (ID 17) were evaporated under ambient conditions and periodically sampled for measurement of carbon isotope ratios (left). Dotted lines connect vials that were sequentially sampled. Nine separate vials of one acetone sample (ID 14) were force-evaporated to a target remaining mass under dry air and slightly elevated temperatures, then measured for hydrogen isotope ratios (right). Solid lines indicate predicted values for the linear model $\delta \sim \log_e(1 - f_{\text{evaporated}})$ for each study. Error bars indicate standard uncertainty (1σ) of a future measurement based on the linear combination of between- and within-sequence uncertainties for acetone isotope measurements (see Results).

hydrogen isotope ratios of different vials of the same acetone sample decreased with evaporation, but only slightly, for a predicted maximum shift of -12‰ (see Fig. 5). Though the effect was small, the linear model for $\delta^2\text{H}$ values in the same form as that for $\delta^{13}\text{C}$ values had a significant slope of 2.62 ($p < 0.001$, multiple $r^2 = 0.94$).

TATP subsamples of several synthesis batches were sampled prior and posterior to recrystallization and analyzed to determine if there was any effect on $\delta^{13}\text{C}$ or $\delta^2\text{H}$ values. Among synthesis batches, there was no significant effect of recrystallization on the $\delta^{13}\text{C}$ values ($T_{15.4} = 1.7$, $p = 0.533$) or $\delta^2\text{H}$ values ($T_{97.4} = -1.6$, $p = 0.124$). Likewise, recrystallization had no statistically significant effect on heterogeneity as modeled by variance ratios of raw to recrystallized TATP ($F_{6, 18} = 0.59$, $p = 0.266$ for $\delta^{13}\text{C}$; $F_{55, 49} = 1.47$, $p = 0.175$ for $\delta^2\text{H}$). It should be noted that synthesis batches A-D were not subsampled “raw,” as seen in Table 1).

4. Discussion

The ranges of $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values of this worldwide acetone survey were larger than in previous studies based on casework [6] and laboratory samples [14], highlighting the importance of a thorough survey of explosive precursors, such as acetone, to generate background data for source attribution. In comparison to the previous acetone surveys of Bezemer et al. [6] and Moran et al. [14], the isotopic variation we observed in this study was roughly twice as large for both $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values. An analysis of the relationships between acetones at the time of synthesis, distribution, and/or packaging and the available consumer product was outside the scope of this project. However, we hypothesize that the large ranges we observed in $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values of acetone available to consumers worldwide were likely due to

the inhomogeneity of the global chemical and petroleum trades. More thorough knowledge of these relationships, and how they are affected by time and space, may allow for inference of the region-of-origin of acetone samples.

One important cause of isotopic variation may be the source of carbon and hydrogen in benzene and propylene, which form the precursor to acetone in the “cumene” production method [23] widely used in the USA. As these precursors can be produced from several different petrochemical sources that have very different carbon and hydrogen isotope ratios [22], or produced from different methods such as steam cracking or distillation [24], the resultant acetone (as well as the final product TATP) is likely to retain most of that isotopic variation. The correlation between $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values of acetone was not unexpected, as carbon and hydrogen isotopes in petroleum (and petrochemical products) are subject to similar fractionation mechanisms, especially in small molecules [25]. As country-of-purchase was found to be the largest factor influencing variation in acetone $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values, we may expect a geographical signal – nonetheless influenced by trade patterns and market factors – to emerge with an even more comprehensive survey of acetone. Within and between country variations in the acetone supply chains as well as distributions of factories producing acetone were unknown to us, but could have been the basis for country-level variations in acetone stable isotope ratios.

Though we found little evidence for anything but a 1:1 correlation between $\delta^{13}\text{C}$ of precursor acetone and of its product TATP, as well as between $\delta^2\text{H}$ of precursor and product, we acknowledge that the observations published by Bezemer et al. [6] are likely related to a low yield with respect to acetone. The authors' $\delta^{13}\text{C}$ observations (product more depleted in ^{13}C than precursor) fit the classic kinetic fractionation theory whereby the precursors with the lighter isotopes are consumed faster; however, the $\delta^2\text{H}$ observations (product more enriched in ^2H than precursor) suggest a different fractionation mechanism. One suggestion is that crystallization of TATP during synthesis may have an effect on TATP stable isotope ratio values, as the heavier isotope will concentrate in well-ordered structures [26], leaving the lighter isotope in solution, and thereby subject to evaporation or dissociation. This theorized mechanism is the equilibrium isotope effect, potentially modulated by kinetic effects, which would entail a primary kinetic effect for hydrogen but only a secondary effect for carbon.

Concerns about the robustness of the isotope ratio analysis technique for TATP are valid due to its known volatility and potential instability. The results from our recrystallization experiments show that recrystallized TATP was not significantly more or less isotopically homogenous than the TATP originally precipitated from synthesis solution, and that the isotope ratios were conserved. The ability to dissolve TATP for transport or removal from an improvised explosive device, while preserving its isotope signature, has obvious practical utility for forensic investigators. The robustness of the carbon and hydrogen isotope ratios in TATP following sublimation also puts to rest concerns that the timing of extraction of a forensic sample from a crime scene may have a negative effect of the ability to source TATP using isotope ratio analysis. This non-effect is likely due to the mass of the TATP molecule relative to the weight differences of a substitution in carbon isotopes. A similar study should be conducted to examine the robustness of the hydrogen (and potentially oxygen) isotope ratio during sublimation.

Due to the lighter mass of acetone, its evaporation over significant time frames may be of concern to the forensic investigator that wants to compare isotope ratios of potential acetone precursors to a TATP seizure. An acetone sample's isotope ratio may not be representative of the acetone used in a TATP synthesis if it had been subject to isotopic fractionation. Though the fractionation factor associated with evaporation is likely to be dependent on several factors, including container size and shape, temperature, and flux of air into the surroundings, we found that ambient evaporation up to 25% of the original mass has little-to-no significant effect on open acetone samples in the lab

with respect to carbon isotope ratio, and forced evaporation had little effect on hydrogen isotope ratios. However, investigators should still be wary when comparing acetone samples that may have evaporated, and only collect samples from original containers when conducting background population studies. More research should be done to understand the variation of acetone fractionation factors under evaporation in different environments expected in casework.

5. Conclusions

In this worldwide survey of acetone of three grades from 12 countries, we confirmed the utility of isotope ratio analysis to discriminate acetone samples, and approximately doubled the ranges of background data previously available in the literature for both carbon and hydrogen isotope ratios. Though many of the samples we collected were concentrated at $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values of -29‰ and -50‰ , respectively, country-of-purchase was by far the greatest contributor to variation within the dataset, suggesting the possibility of a geographical correlation to isotope ratios of acetone. Carbon isotope ratios of “hardware” grade acetone were only slightly depleted relative to “scientific” and “nail” grade acetone samples, and hydrogen isotope ratios were roughly the same across all grades, suggesting that grade has little predictive power over the isotope ratios of the material. Petrochemical sources, which may relate to country-of-origin, are probably much more predictive of the isotope ratios of acetone than either grade or brand.

We found that TATP carbon and hydrogen isotope ratios closely matched the carbon and hydrogen isotope ratios of precursor acetone, making acetone a good proxy for generating a background dataset of potential isotope ratios of TATP; this would be important for evaluation of evidence in a legal setting. Though acetone C and H isotopes may be affected in samples that have been allowed to evaporate extensively, the extent of evaporation and known fractionation factors can be used to estimate the stable isotope ratios of the original material. TATP isotope ratios were robust to both recrystallization (C and H isotopes) and sublimation (in C isotopes), which is encouraging for the utility of the isotope ratio analysis method in practical recovery of bomb scene materials.

Acknowledgements

We thank James Ruff for assistance in statistical analyses and Richard Strobel (ATF) for providing TATP samples and synthesis information. We also thank our colleagues worldwide who submitted acetone samples for the survey.

Funding

Original research was supported by the U.S. Bureau of Alcohol, Tobacco, Firearms, and Explosives (ATF) and sponsored by Technical Support Working Group (TSWG) contract W91CRB-07-C-0071. IsoForensics, Inc. provided support for production of this manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.talanta.2018.01.001>.

References

- [1] J. Koehler, R. Meyer, *Explosives*, VCH, Weinheim, Germany, 1993.
- [2] C.J. Chivers, Quantity of Explosive Found in Belgium Surprises Officials, N. Y. Times. <<https://www.nytimes.com/2016/03/24/world/europe/brussels-explosives-tatp.html>> (Accessed 8 September 2017), 2016.
- [3] J.R. Ehleringer, T.E. Cerling, J.B. West, Forensic science applications of stable isotope ratio analysis, in: R.D. Blackledge (Ed.), *Forensics Anal Cut Edge New Methods Trace Evid Anal*, John Wiley & Sons, Inc, San Diego, California, USA, 2007, pp. 399–422.

- [4] 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).
- [5] 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.
- [6] K.D. Bezemer, M. Koeberg, A.E. Heijden, C.A. Driel, C. Blaga, J. Bruinsma, A.C. Asten, The potential of isotope ratio mass spectrometry (IRMS) and gas chromatography-IRMS analysis of triacetone triperoxide in forensic explosives investigations, *J. Forensic Sci.* 61 (2016) 1198–1207.
- [7] 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>.
- [8] C.M. Lock, M. van Breukelen, M. Koeberg, Forensic investigation of isotopic linkages between hexamine and the peroxide explosive HMTD, in: *Proceedings of the 4th Forensic Isot. Ratio Mass Spectrometer FIRMS Netw. Conference*, 2009.
- [9] 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.
- [10] 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.
- [11] J.E. Barnette, M.J. Lott, J.D. Howa, D.W. Podlesak, J.R. Ehleringer, Hydrogen and oxygen isotope values in hydrogen peroxide, *Rapid Commun. Mass Spectrom.* 25 (2011) 1422–1428.
- [12] C.G. Aitken, F. Taroni, J. Wiley, *Statistics and the Evaluation of Evidence for Forensic Scientists*, Wiley Online Library, 2004.
- [13] I. Alberink, A. Bolck, S. Menges, Posterior likelihood ratios for evaluation of forensic trace evidence given a two-level model on the data, *J. Appl. Stat.* 40 (2013) 2579–2600.
- [14] J.J. Moran, C.J. Ehrhardt, J.H. Wahl, H.W. Kreuzer, K.L. Wahl, Integration of stable isotope and trace contaminant concentration for enhanced forensic acetone discrimination, *Talanta* 116 (2013) 866–869.
- [15] M. Gehre, H. Geilmann, J. Richter, R. Werner, W. Brand, Continuous flow 2H/1H and 18O/16O analysis of water samples with dual inlet precision, *Rapid Commun. Mass Spectrom.* 18 (2004) 2650–2660.
- [16] W.A. Brand, T.B. Coplen, J. Vogl, M. Rosner, T. Prohaska, Assessment of international reference materials for isotope-ratio analysis (IUPAC Technical Report), *Pure Appl. Chem.* 86 (2014) 425–467.
- [17] J.F. Carter, V.J. Barwick, Good practice guide for isotope ratio mass spectrometry, FIRMS (2011), (<http://www.forensic-isotopes.org/gpg.html>).
- [18] R. Core Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2015 (<https://www.R-project.org/>).
- [19] D. Bates, M. Mächler, B. Bolker, S. Walker, Fitting linear mixed-effects models using lme4, *J. Stat. Softw.* 67 (2015) 1–48, <http://dx.doi.org/10.18637/jss.v067.i01>.
- [20] E. Manuilova, A. Schuetzenmeister, F. Model, mcr: Method Comparison Regression. R package version 1.2. 1 [cited 2015 June 16], 2015.
- [21] A. Kuznetsova, P.B. Brockhoff, R.H.B. Christensen, Package 'lmerTest', R Package Version 2 (2015) 0–33.
- [22] J. Meija, T.B. Coplen, M. Berglund, W.A. Brand, P. De Bièvre, M. Gröning, N.E. Holden, J. Irrgeher, R.D. Loss, T. Walczyk, Atomic weights of the elements 2013 (IUPAC Technical Report), *Pure Appl. Chem.* 88 (2016) 265–291.
- [23] V. Zakoshansky, The cumene process for phenol-acetone production, *Pet. Chem.* 47 (2007) 273–284.
- [24] D.-S. Huang, S.-H. Wu, C.-Y. Huang, C.-Y. Lin, An exploration of intramolecular carbon isotopic distributions of commercial acetone and isopropanol, *Org. Geochem.* 30 (1999) 667–674.
- [25] Y. Tang, Y. Huang, G.S. Ellis, Y. Wang, P.G. Kralert, B. Gillaizeau, Q. Ma, R. Hwang, A kinetic model for thermally induced hydrogen and carbon isotope fractionation of individual n-alkanes in crude oil, *Geochim. Cosmochim. Acta* 69 (2005) 4505–4520.
- [26] J. Hoefs, *Stable Isotope Geochemistry*, Springer Verlag, 2009.