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Short communication

# Integration of stable isotope and trace contaminant concentration for enhanced forensic acetone discrimination ☆

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ABSTRACT

We analyzed 21 neat acetone samples from 15 different suppliers to demonstrate the utility of a coupled stable isotope and trace contaminant strategy for distinguishing forensically-relevant samples. By combining these two pieces of orthogonal data we could discriminate all of the acetones that were produced by the 15 different suppliers. Using stable isotope ratios alone, we were able to distinguish 8 acetone samples, while the remaining 13 fell into four clusters with highly similar signatures. Adding trace chemical contaminant information enhanced discrimination to 13 individual acetones with three residual clusters. The acetones within each cluster shared a common manufacturer and might, therefore, not be expected to be resolved. The data presented here demonstrates the power of combining orthogonal data sets to enhance sample fingerprinting and highlights the role disparate data could play in future forensic investigations.



## 1. Introduction

Worldwide acetone production hit 5.1 million metric tons in 2009 [1] with the majority being used as either an industrial feedstock or solvent. While modern society benefits from synthesis products enabled by acetone, its high production rates coupled with its near unregulated accessibility in many sections of the world also make acetone available for malicious-intentioned activities. For instance, acetone is a key intermediary in the production of triacetone triperoxide (TATP), an explosive used in such highly publicized events as the 2005 London subway attacks [2], the attempted 2001 "shoe-bomber" incident on American Airlines flight 63 [3], and the attempted 2009 bombing of Northwest Flight 253 [4].

Benson et al. [5] demonstrated isotopic variability in explosives such as TATP but they were unable to draw conclusions about what caused the isotopic shifts in the samples they synthesized. Lock and Meier-Augenstein [6] demonstrated a link between the isotopic content of starting materials and final explosive when they synthesized the explosive RDX. Similar isotopic behavior in the synthesis of TATP may link isotope values of starting acetone to that of final TATP and provide a forensic link between precursor and final synthetic agent. Partridge et al. [7] demonstrated that chemical impurities in the precursors for explosives including TATP could persist through the complete synthesis and in some cases even be observed in residue following an initiation event. Similar results were achieved in a forensic study of multiple chemical nerve agents [8]. Combining information from both the stable isotope and trace contaminant analysis of TATP or other agents could provide valuable forensic assistance in linking either specific TATP batches or linking a batch of TATP to its precursors. Forensic investigations of TATP highlight one potential use of such a hierarchical approach.

In addition, acetone is often used in the purification of ricin toxin from castor beans [9]. Ricin is a select agent known for its role in many cases, including perhaps most notably the assassination of Bulgarian defector Georgi Markov in 1978 [10]. More recently, in 2001 a supply of ricin was found in the possession of and presumably isolated by Kenneth Olsen in Spokane, Washington [11]. Additionally, in 2003 two letters containing ricin, one of which was mailed to the White House, were identified in the United States [12]; the origin of these letters remains unknown.

With acetone's widespread availability and use for creating multiple weapon agents, forensic methods are needed to enable linking of agents found at multiple event sites or associating precursor chemicals with final products. Stable isotope analysis



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is a promising strategy for discriminating different acetone sources because different synthesis methods, feedstock suppliers, and purity levels exist and are likely implemented across different manufacturers. These differences should result in a range of stable isotope ratios in acetones produced by different manufacturers or in different lots. Stable isotope profiling provides a means for source attribution of various types of forensic evidence [13,14] (e.g. explosive residues, illicit drugs, microbial biomass) and intramolecular carbon isotopes [15] have been used to characterize different synthetic pathways of acetone. However, no published study to date has systematically examined isotopic variability of acetone from an extensive set of manufacturers. Trace constituent analysis is another potential mechanism for discriminating acetones and previous work shows large variations in contaminant concentrations in different samples [16].

In this study we combine the fingerprinting capabilities of stable isotope analysis ($\delta^{13}C$ and $\delta^{2}H$) with trace constituent analysis of four common contaminant compounds (diacetone alcohol, mesityl oxide, phorone, phenol) to permit enhanced discrimination of 21 acetones. It should be noted that these samples were collected from various sources without purification or control of previous use and storage. We used a series of neat acetones for these analyses, but the same methodology could be applied to acetone found in a complex mixture, as may be the case in a forensic context.

## 2. Methods

We obtained acetone from a variety of distributers and augmented this collection with aliquots from existing chemical inventories at Pacific Northwest National Laboratory. All told we collected 21 acetone samples (numbered one through 11 and 19 through 28) distributed by 15 different suppliers (Table 1) with purities ranging from acetone purchased at a hardware store to high purity chemical solvent grade. These acetones were utilized as received with no effort to preserve or adjust for aging or decomposition as may be the case in a real world forensic situation.

### 2.1. Isotope analysis

For stable isotope ratio analysis, we used a Thermo Trace GC Ultra gas chromatograph coupled to a Thermo Delta V Plus isotope ratio mass spectrometer (IRMS, Thermo Scientific, Bremen, Germany). For $\delta^{13}C$ analysis we used a Rtx-1 ms GC column, 60 m length, 0.25 mm ID, 25 μm film thickness (Restek, Bellefonte, PA) for acetone separation. We used a constant flow of 1.5 mL per minute and followed a temperature program of initial temperature at 40 °C for four minutes followed by a ramp at 20 °C per minute to 200 °C for two minutes. We injected the acetone as a 5% solution in trimethyl pentane. We used a combustion reactor (with copper, nickel, and platinum catalysts maintained at 940 °C) to quantitatively convert all carbon in eluting acetone to $CO_2$ which was passed through a Thermo Scientific GCC low flow sample introduction system, through a water trap, and into the IRMS for $\delta^{13}C$ measurement. We used two ethanol isotope standards (Ethanol from C4 origin with $\delta^{13}C$ of $-10.98‰$ VPDB and Ethanol from C3 origin with $\delta^{13}C$ of $-27.53‰$ VPDB) available from the University of Indiana Stable Isotope Reference Materials service to calibrate a house acetone standard which we used daily to ensure accurate isotope determinations. All $\delta^{13}C$ measurements were performed at a minimum of n=4 on a minimum of two different days with all standard deviations at or below 0.17‰.

For $\delta^{2}H$ isotope determination we used a Rtx-5amine GC column, 30 m length, 0.25 mmID, 0.5 mm film thickness (Restek, Bellefonte, PA) for acetone separation. We used a constant carrier flow (1.5 mL per minute) and a temperature program of 65 °C for three minutes followed by a 20 °C per minute ramp to 180 °C for one minute. We used a high temperature conversion reactor (ceramic tube with excess graphite maintained at 1450 °C) to completely convert hydrogen in the acetone to $H_2$ which was passed through the GCC low flow sample introduction and water trap and into the IRMS. We measured $H_3^+$ factors multiple times daily. We used nicotine #5 ($\delta^{2}H=-161.3‰$) from the University of Indiana Stable isotope Reference Materials service to calibrate a house acetone standard which we used as a daily check on instrument accuracy. We performed all $\delta^{2}H$ measurements at a minimum of $n=5$ on

**Table 1**
Stable isotope and trace contaminant data.
We present stable isotope ($\delta^{13}C$ and $\delta^{2}H$) and scaled trace contaminant data for each of the 21 acetone samples analyzed. Shading and boxes highlight three sample sets containing acetones that were indistinguishable from each other using the methods presented here. In all cases, indistinguishable samples share the same manufacturer and in one case share the same lot number.

| Sample | Supplier | Lot number | Isotope Ananlysis | | Normalized contaminant abundance | | | |
|---|---|---|---|---|---|---|---|---|
| | | | $\delta^{13}C$ (‰) | $\delta^{2}H$ (‰) | Diacetone Alcohol | Mesityl Oxide | Phorone | Phenol |
| 1 | A | n/a | -28.14 | -150.6 | 0.30 | 0.02 | 0.01 | 0.02 |
| 2 | B | 501 | -29.68 | -139.7 | 0.03 | 0.02 | 0.03 | 1.00 |
| 3 | C | 59242 | -28.81 | -139.1 | 0.07 | 0.00 | 0.01 | 0.02 |
| 4 | D | 801405 | -28.57 | -156.9 | 0.31 | 0.03 | 0.03 | 0.01 |
| 5 | E | 008-102-1 | -27.93 | -130.2 | 0.05 | 0.08 | 0.02 | 0.03 |
| 6 | E | 008-212-3 | -29.74 | -145.3 | 0.01 | 0.01 | 0.01 | 0.01 |
| 7 | F | 1350701 | -24.15 | -108.1 | 0.44 | 0.07 | 0.03 | 0.02 |
| 8 | G | 82653 | -28.50 | -156.4 | 0.40 | 0.34 | 0.25 | 0.01 |
| 9 | H | VR0732 | -25.54 | -139.0 | 0.73 | 1.00 | 0.84 | 0.04 |
| 10 | H | PW0122 | -25.54 | -139.4 | 0.79 | 0.82 | 0.80 | 0.11 |
| 11 | I | J830220 | -28.63 | -143.8 | 0.50 | 0.33 | 0.19 | 0.05 |
| 19 | J | 2437 KDTP | -25.88 | -167.9 | 0.55 | 0.46 | 0.17 | 0.50 |
| 20 | K | 2261A | -26.98 | -145.4 | 0.81 | 0.61 | 1.00 | 0.05 |
| 21 | F | 02449JE | -28.39 | -138.2 | 0.51 | 0.16 | 0.04 | 0.02 |
| 22 | L | A0902 | -27.32 | -136.9 | 0.90 | 0.08 | 0.01 | 0.03 |
| 23 | M | T30312 | -27.57 | -148.6 | 0.97 | 0.15 | 0.16 | 0.02 |
| 24 | M | T30312 | -27.61 | -148.5 | 1.00 | 0.16 | 0.15 | 0.02 |
| 25 | M | T30312 | -27.57 | -148.0 | 0.94 | 0.19 | 0.16 | 0.02 |
| 26 | N | n/a | -28.90 | -147.1 | 0.57 | 0.16 | 0.09 | 0.05 |
| 27 | I | J831200 | -28.64 | -142.4 | 0.63 | 0.37 | 0.23 | 0.05 |
| 28 | I | J824690 | -28.79 | -143.3 | 0.42 | 0.29 | 0.15 | 0.04 |

different days with the standard deviations for one sample being at 3.6‰ but all others at or below 2.5‰.

### 2.2. Contaminant analysis, GCxGC

We performed trace chemical analysis using a Leco Pegasus 4D GCxGC-MS system (LECO, St. Joseph, MI, USA) equipped with a Gerstel cooled injection system (CIS4) and multipurpose sampler (MPS2) (Gerstel, Baltimore, MD, USA). The data we utilized in this study was previously described with the analytical run conditions [16]. Briefly, we used a 5 μL sample injection on to a −30 °C injector which was heated at 12 °C/s to 300 °C after loading. We used ultra-high purity helium set at a constant flow of 1 mL/min as the carrier gas for GC separation. Sample sequentially passed through a polar (SolGel-Wax, SGE, Inc., Austin, TX, USA) then a moderate polarity (007-1701, Quadrex Corp., Woodbridge, CT, USA) GC column before mass spectrometer analysis. We analyzed all samples in either duplicate or triplicate.

### 2.3. Data transformation and analysis

For contaminant data, we integrated peak volumes using Leco's ChromaTOF software. Because each contaminant was measured independently and peak volumes for different contaminants sometimes varied by several orders of magnitude, we scaled data from each contaminant variable (e.g. phorone, mesityl oxide, and diacetone alcohol) as follows: the minimum peak volume was subtracted from each sample then divided by the difference between the observed minimum and maximum peak volumes. The resulting relative abundance values ranged from zero to one with roughly equivalent standard deviations (~0.15). We did not apply any transformation to the stable isotope values. We generated all plots using Sigmaplot 11.0.

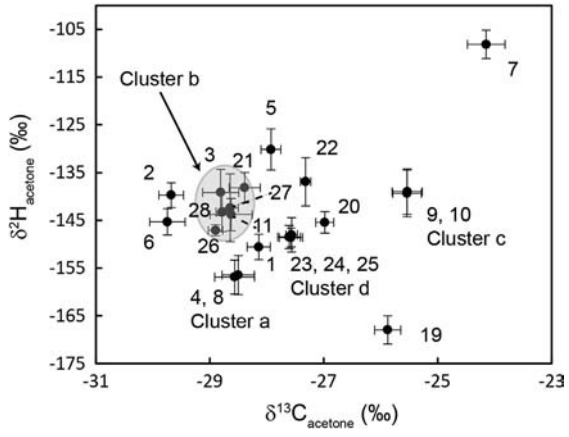


**Fig. 1.** Stable isotope sample differentiation. Comparison of acetones on the basis of carbon ($\delta^{13}C$) and hydrogen ($\delta^{2}H$) stable isotope composition allows many of the samples to be distinguished (errors shown are one standard deviation of replicate measurements for both isotopes). Indistinguishable samples fell into four clusters (a, b, c, and d) that were subjected to trace contaminant analysis as a second differentiating technique.

## 3. Results/discussion

We observed a 4.14‰ and 59.8‰ range in the respective $\delta^{13}C$ and $\delta^{2}H$ stable isotope ratio of the neat acetones indicating sufficient isotopic variation for distinguishing samples. We assessed sample differentiation based on variable space using stable isotope data as variables; acetones were defined as resolved when isotope values for two acetones plus or minus twice their measured standard deviations were non-overlapping. In cases where two acetones could not be distinguished solely by their stable isotope ratios, we compared trace contaminant profiles graphically from the sample distribution. The scaling procedures used on contaminant data prevented estimations of standard deviation/analytical ranges for each acetone source. Based solely on stable isotope analysis we successfully resolved all of the acetones from each other except those that fell into four clusters (Fig. 1 clusters a, b, c, d). We used trace contaminant analysis examining diacetone alcohol, mesityl oxide, phorone, and phenol to attempt to distinguish the acetones within these clusters.

For two of the acetone clusters separation (Fig. 2a) or partial separation (Fig. 2b) of the different acetones is possible with comparison of the relative abundance of phorone and mesityl oxide. For example, acetones 4 and 8 in cluster 'a' (Fig. 1) were indistinguishable by their isotope ratios. However, clear differences in the relative concentrations of phorone and mesityl oxide differentiated these acetone samples (Table 1, Fig. 2a). We observed similar trends for acetones 3, 21, and 26 in cluster 'b' (Fig. 1); comparable carbon and hydrogen isotope values that defied discrimination. Again, trace contaminant analysis added a dimension of resolution (i.e. distinct ranges of phorone and mesityl oxide concentrations) between these samples (Table 1, Fig. 2b).

Some acetone groups remained indistinguishable after isotope and contaminant comparisons. For example, acetones 11-27-28 in cluster 'b', 9-10 in cluster 'd', and 23-24-25 in cluster 'c' could not be further distinguished within a cluster by the relative abundance of any of the four contaminants (Table 1). This is not surprising because these clusters include acetones from the same supplier (11, 27, 28 and 9, 10) or even the same product lot (23, 24, 25), ( Table 1: acetone from supplier 'H' has a solid outline, that from supplied 'I' is shaded, and that from supplier 'M' with the same lot has a dashed outline). The chemical similarity of acetones from the

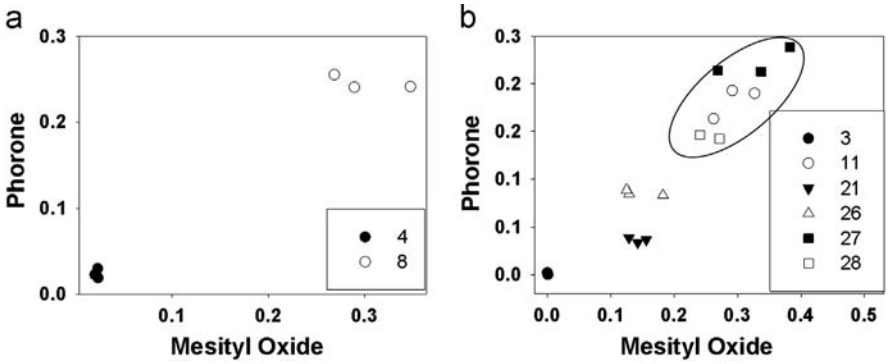


**Fig. 2.** Trace contaminant differentiation of acetone samples. Trace contaminant analysis of samples in clusters a and b (Fig. 1) successfully differentiated acetones that were not distinguishable on isotope content alone. For example, trace analysis enabled differentiation of acetones 4 and 8 (a) and 3 and 26 (b). Samples 11, 27, and 28 (circled) were not differentiated by this approach but share a common manufacturer and thus may not be expected to be distinct.

same manufacturer lot is not surprising. Being from the same lot, these acetones share common feedstock and synthesis and purification steps so we may expect them to be indistinguishable. The fact that we observed similarity in these samples suggests that any handling subsequent to their manufacturer did not alter their isotope or contaminant profiles. It is possible that sample handling could manipulate the isotopic content of volatile compounds such as acetone if significant evaporation occurred in the sample history. Similarly, trace contaminant concentrations could be altered by handling methods. It is important to note that that while the fingerprinting methods described here successfully differentiated every acetone that did not share a common manufacturer, the method is being described as a fingerprinting approach and not as a tool for identifying manufacturer or sample origin. Significant handling could impart its own signatures to the samples and we would expect the methods presented here to differentiate samples based on their current content which in some cases could result from both manufacturing and sample handling processes.

In general, these results demonstrate combining carbon and hydrogen isotopic variation with the relative concentrations of phorone, mesityl oxide, and diacetone alcohol is a useful method for discriminating acetones derived from different sources. However, integrating isotopic variation with contaminant concentration values into a single discrimination measure is a challenge owing to the differences in analytical sensitivities, replicate precision, and variable scaling across these two methods. To compensate for these factors, we implemented a hierarchical approach for comparing acetones. In this scheme, variation in carbon and hydrogen isotopes is examined first. Then, if acetones are not differentiated by isotope values alone the concentrations for each of the four contaminants are examined. We suggest that a similar approach could be used for the attribution of acetone recovered as evidence: isotopic profiles are determined first and compared to a database of acetone reference samples. If the isotopic composition of the unknown sample is closely related to several reference samples (e.g. falls within cluster 'b'), the concentration of contaminants is determined and another set of acetone reference data. Similar statistical schemes have been used successfully in several forensic applications with sample matching [17,18] but has not been widely applied to isotopic profiling studies.

## 4. Conclusion

We tested a suite of neat acetones using orthogonal analytical techniques and found that combining isotope profiles with contaminant concentrations in a hierarchical manner allowed greater differentiation of samples compared to isotopes measurements alone. In fact, this approach permitted complete discrimination of all acetones except those produced by the same manufacturer. While this study was specific for acetone, the approach could be applied to a wide variety of applications including explosive (i.e. TATP) and select agent attribution.

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