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PAPER

CRIMINALISTICS

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Multiple Stable Isotope Characterization as a Forensic Tool to Distinguish Acid Scavenger Samples*

ABSTRACT: Acid scavengers are frequently used as stabilizer compounds in a variety of applications. When used to stabilize volatile compounds such as nerve agents, the lower volatility and higher stability of acid scavengers make them more persistent in a post-event forensic setting. Compound-specific isotope analysis of carbon, nitrogen, and hydrogen in three acid-scavenging compounds (*N*,*N*-diethylaniline, tributylamine, and triethylamine) were used as a tool for distinguishing between different samples. Combined analysis of multiple isotopes improved sample resolution, for instance differentiation between triethylamine samples improved from 80% based on carbon alone to 96% when combining with additional isotope data. The compound-specific methods developed here can be applied to instances where these compounds are not pure, such as when mixed with an agent or when found as a residue. Effective sample matching can be crucial for linking compounds at multiple event sites or linking a supply inventory to an event.

KEYWORDS: forensic science, compound-specific isotope analysis, stable isotope, acid scavenger, nerve agent, sarin, triethylamine, *N*,*N*-diethylaniline, tributylamine

The lethality and relatively straightforward synthetic routes of some nerve agents make them a potent terrorist threat. For instance, sarin, propoxy-(2)-methyl phosphoryl fluoride, is a highly toxic acetylcholinesterase inhibitor used in a chemical terrorist attack by the Aum Shinrikyo cult (1,2). The attack involved a synchronized release of sarin in crowded subway lines in Tokyo and resulted in 12 deaths and numerous injuries (1,3). Investigation of the attack identified diethylaniline as a major species in the sarin-containing solution released at the event sites (1,2,4). Tertiary amines, including diethylaniline, are frequently included in sarin and other nerve agent inventories to act as stabilizers, preventing the buildup of acidic conditions that can lead to hydrolytic loss of agent toxicity (5). Subsequent police raids on Aum Shinrikyo compounds after the Tokyo attack revealed stockpiles of multiple chemicals used for sarin synthesis, including diethylaniline (1,4). Further investigation revealed that diethylaniline was utilized by the group in every synthetic step in sarin production.

In this case, the presence of raw materials combined with additional evidence from the Aum Shinrikyo compound provided a link between an agent collected at an event site and the source of that agent. However, additional tools for linking nerve agents such as sarin released at one event site with either precursor chemicals found off site or an agent released at a different site may provide crucial information in cases where ancillary connections are not forthcoming.

The stable isotope content of molecules has been used as a forensic signature to link substrates to products, natural products to growth environments, and manufactured substances to factories (6,7). Because the stable isotopic composition of different samples or manufacturing batches of the same substance can vary, stable isotope content provides another dimension for sample matching that goes beyond simple chemical identity. Stable isotope analysis of the carbon, nitrogen, and hydrogen components of either agents themselves or other compounds found with the agents may provide matching information between different samples or between an agent and reagent stockpiles.

Stable isotope analysis of chemical agents may be useful for sample matching and event linking. However, nerve gases can be difficult to investigate as they are frequently prone to decomposition in the environment and are highly volatile (1), so direct stable isotope analysis may be thwarted by low sample recovery and stability. Acid-scavenging additives, however, can be less labile and less volatile than the nerve agents they are intended to preserve and may thereby be more likely present in sufficient quantities for stable isotope analysis following an event. Further, stabilizers need to be included with most nerve agents to prevent their decomposition (5), making the stabilizers a prime target for stable isotope analysis.

We collected a total of 33 neat tertiary amine acid scavenger samples (*N*,*N*-diethylaniline, tributylamine, and triethylamine) and analyzed each sample for its carbon, nitrogen, and hydrogen stable isotope ratio. Both bulk and compound-specific techniques could be employed for the stable isotope analysis of these compounds. Bulk isotopic analysis is a higher-throughput technique, but requires analysis of pure compounds, while compound-specific isotope analysis

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(CSIA) is able to select specific analytes from a more complex matrix. CSIA has demonstrated utility in multiple forensic applications including tracking environmental contaminants (8–10), tracing the origin of illicit drugs (11), and investigating instances of inappropriate drug use (see discussion in [12]). We chose to develop compound-specific techniques (13,14) for these isotope measurements to ensure method utility when samples are not found at high purity, such as when they are added as a stabilizer to a chemical agent preparation or recovered as a residue after an agent dispersal event.

Materials and Methodology

Samples

We purchased five *N,N*-diethylaniline, four tributylamine, and seven triethylamine samples from three different chemical suppliers (Sigma-Aldrich, St. Louis, MO; Thermo Fisher Scientific, Waltham, MA; Spectrum Chemicals, New Brunswick, NJ). We augmented the sample set by collecting 17 additional triethylamine samples from chemical inventories at other laboratories within Pacific Northwest National Laboratory. In some cases, original supplier/manufacturer information was not available for these additional samples.

Isotope Analysis

We used a Thermo Delta V Plus isotope ratio mass spectrometer (IRMS; Thermo Fisher Scientific, Bremen, Germany) for all isotope ratio determinations. To permit CSIA, we introduced all samples to the IRMS via a Thermo Trace GC Ultra gas chromatograph (GC). Following chromatographic separation from solvent and any contaminants, we converted the eluted samples to simple gases and then routed them through a Thermo GCC low-flow sample introduction system directly into the IRMS. We report measured isotope ratio values ($^{13}C/^{12}C$, $^{15}N/^{14}N$, or D/H) in delta (δ) notation where $\delta = (R_{Sa}/R_{Std} - 1) \times 1000\%$, and R_{Sa} and R_{Std} are the isotope ratio of the sample and an internationally recognized reference material respectively: Vienna Pee Dee Belemnite for carbon, air for nitrogen, and Vienna Standard Mean Ocean Water for hydrogen (15).

Carbon and Nitrogen Analysis

Carbon and nitrogen sample analysis followed similar methods. We diluted samples in either 1-octanol (for triethylamine) or hexanes (for *N,N*-diethylaniline and tributylamine) prior to sample injection. We used an Rtx1MS (Restek, Bellefonte, PA), 50 m, 0.25 mm ID column for analyte separation. The GC oven method for triethylamine samples started with 1 min at 40°C followed by a 20°C/min ramp to 300°C and at temperature for 1 min. We ran *N,N*-diethylaniline and tributylamine samples using an oven program with an initial 1 min at 100°C followed by a 20°C/min ramp to 280°C and at temperature for 1 min.

Upon elution, a helium flow passed the analytes through a microcombustion reactor (940°C) followed by a reduction reactor (650°C), resulting in complete conversion of all organic material to CO_2, N_2, and H_2O. For carbon analysis, we passed this mixture through a water trap and then directly into the IRMS for isotope analysis. For nitrogen analysis, we passed the sample flow through a liquid nitrogen trap to remove any CO_2 from the sample prior to its introduction to the IRMS. The presence of CO_2 in an N_2 sample interferes with the accurate isotopic analysis of N_2, because some CO_2 molecules break down into CO, which has the same mass as N_2, in the mass spectrometer. In both cases, we included isotope reference gas pulses into the IRMS before and after each analyte measurement. We calibrated reference gases (CO_2 and N_2) using glutamic acid standards USGS 40 and USGS 41 (National Institute of Standards and Technology, Gaithersburg, MD). Finally, we used a nicotine standard (nicotine #1 available from the University of Indiana Stable Isotope Reference Materials service; Bloomington, IN) to ensure proper calibration of our GC-IRMS.

Hydrogen Analysis

We performed hydrogen analysis using an Rtx5-amine column (Restek), 30 m, 0.25 mm ID. We diluted samples into hexanes for *N,N*-diethylaniline and tributylamine and into acetone for triethylamine prior to analysis. For *N,N*-diethylaniline and tributylamine samples, we used a GC oven method with 2 min at 50°C followed by a 20°C/min ramp to 250°C and at temperature for 3 min. For triethylamine samples, we used an initial GC temperature of 45°C for 5.5 min followed by a 20°C/min ramp to 200°C and at temperature for 3 min.

Upon elution from the GC, a helium flow carried the analytes through a pyrolysis microreactor maintained at 1450°C. We added octane to each sample, so the early-eluting octane peak would ensure the pyrolysis reactor contained excess carbon to maintain reducing conditions (14). The pyrolysis reactor converted all hydrogen in the analytes to H_2 which we introduced to the IRMS for isotopic measure. A minimum of once a day we also characterized the H_3^+ factor for the IRMS to minimize isotope measurement drift with differing peak size (16). Additionally, we adjusted sample injection to maintain peak sizes between 5 and 15 V*s in total peak area measured. We included H_2 reference pulses prior to and subsequent to sample elution in each sample run. We previously calibrated δD of the reference gas using multiple H_2 reference standards provided by Oztech Trading Corporation (Safford, AZ). We calibrated a house nicotine standard following two approaches. First, we performed bulk analysis of the nicotine sample using a thermal conversion elemental analyzer (TCEA) with sample referencing to a bulk methanol sample obtained from the University of Indiana Stable Isotope Reference Materials service. Second, we calibrated the house nicotine standard against nicotine #5 (described earlier) using our compound-specific, GC-IRMS method. The average value of 12 measurements by TCEA-IRMS was −173.6%, and the average value of 16 measurements by GC-IRMS was −173.9%. The difference between these average values is less than the instrument precision for H isotope ratio measurements by TCEA-IRMS (2%), and the means from both sets of analyses were indistinguishable based on a statistical *t*-test. We made daily measurements of a house nicotine standard and corrected sample data based on the offset between observed and accepted δD values.

Results and Discussion

We measured $\delta^{13}C$, $\delta^{15}N$, and δD on 33 acid scavenger samples (five *N,N*-diethylaniline, four tributylamine, and 24 triethylamine) (Table 1). We measured each isotope ratio of each sample a minimum of three times and most ratios a minimum of six times over at least 2 days. We observed an average standard deviation (SD) of the carbon isotope ratio measurements of 0.25%, 0.4% for nitrogen, and 4.0% for hydrogen. We included blind duplicate samples consisting of separate bottles of each acid scavenger purchased from the same supplier and with the same lot number (D3 and D4, TB3 and TB4, and TE3 and TE4) in all isotopic analyses. Measurement

TABLE 1—*Measured isotope values from acid scavengers.*

	δ^{13}C (‰)	SD (‰)	*n*	δ^{15}N (‰)	SD (‰)	*n*	δD (‰)	SD (‰)	*n*
N,*N*-Diethylaniline									
D1	−22.6	0.1	10	−1.7	0.1	13	−175.6	2.1	14
D3	−27.6	0.1	10	−2.3	0.1	13	−135.1	3.5	15
D4	−27.5	0.2	10	−2.3	0.2	13	−136.8	2.5	12
D6	−21.9	0.1	10	−2.0	0.2	13	−187.8	3.6	13
D7	−30.3	0.2	11	−1.5	0.2	13	−114.4	2.9	14
Tributylamine									
TB1	−27.4	0.2	10	5.3	0.6	10	−115.6	3.5	14
TB3	−28.8	0.2	10	3.4	0.5	13	−130.6	3.2	14
TB4	−28.8	0.2	10	3.3	0.3	11	−129.6	3.5	13
TB5	−29.7	0.2	10	2.6	0.3	11	−129.8	1.1	13
Triethylamine									
TE1	−17.6	0.3	9	1.3	0.4	6	−155.0	3.0	7
TE2	−17.7	0.4	7	1.4	0.2	6	−155.4	5.3	7
TE3	−13.2	0.4	7	4.9	0.3	6	−222.7	5.2	7
TE4	−13.2	0.3	7	5.0	0.2	5	−222.6	5.1	6
TE6	−12.1	0.2	7	6.1	0.3	7	−214.4	5.3	6
TE8	−13.2	0.3	7	5.4	0.4	6	−227.4	3.8	6
TE9	−13.0	0.2	7	5.2	0.4	6	−227.7	5.8	6
TE13	−29.9	0.2	7	0.0	0.4	6	−110.5	3.3	6
TE14	−10.9	0.2	7	1.7	0.3	6	−188.4	3.7	6
TE15	−18.4	0.2	7	4.5	0.3	6	−229.0	2.7	6
TE17	−27.3	0.3	7	1.8	0.2	6	−162.5	2.8	6
TE18	−17.5	0.1	7	3.5	0.3	6	−147.4	2.8	6
TE19	−27.8	0.3	7	0.3	0.4	6	−152.0	3.9	6
TE20	−28.1	0.2	7	0.1	0.3	6	−149.6	2.4	6
TE21	−12.9	0.2	7	4.7	0.3	6	−222.4	3.6	6
TE23	−29.3	0.1	7	4.7	0.6	8	−178.5	3.9	6
TE24	−18.8	0.3	7	0.0	0.3	7	−168.3	6.3	6
TE25	−12.1	0.2	7	5.7	0.4	8	−212.0	3.0	6
TE26	−12.5	0.1	7	3.8	0.6	8	−222.5	2.6	6
TE27	−13.6	0.1	6	5.0	0.9	8	−261.6	5.2	6
TE29	−17.4	0.2	7	0.9	0.3	3	−170.6	6.4	6
TE30	−10.4	0.2	7	2.1	0.3	3	−196.1	2.6	6
TE31	−27.9	0.2	7	−5.3	0.5	3	−167.6	4.4	5
TE32	−18.5	0.2	7	1.2	0.4	3	−198.3	5.1	6

agreement in each isotope between the blind duplicate samples indicates both isotopic homogeneity within a manufacturing lot and measurement reproducibility for each of the isotopes measured.

We observed wide ranges in measured isotope values. For instance, the 24 triethylamine samples display over a range of 20% in carbon isotopes, 11% in nitrogen isotopes, and 150% in hydrogen isotopes. Ideally these wide isotope ranges could be used to distinguish between different samples and provide a basis for sample matching as well as for linking potential synthetic precursors to materials collected at an event site.

To test whether isotope ratios alone could distinguish between different triethylamine samples, we performed a series of comparisons between different samples. For each comparison, we determined whether the measured isotope ratio for one sample ± the average SD (listed earlier) rested within the interval defined by the measured isotope ratio of any other sample ± the average SD. We considered two samples to match if these intervals overlapped to any degree, and we considered the samples differentiated if the intervals did not overlap (Table 2). This test was repeated using a matching interval of ± twice the average SD. There were a total of 253 comparisons for the triethylamine samples comparing each sample but omitting one member of the blind duplicate pair.

TABLE 2—*Comparisons based on isotope values.*

	One SD		Two SD	
	Matches (#)	Differentiated (%)	Matches (#)	Differentiated (%)
^{15}N	52	79.4	95	62.5
^{2}H	36	85.8	64	74.7
^{13}C	24	90.5	44	82.6
^{13}C and ^{15}N	13	94.9	29	88.5
^{13}C and ^{2}H	11	95.7	28	88.9
^{13}C, ^{15}N, and ^{2}H	9	96.4	21	91.7

Table 2 shows that over 90% of the triethylamine samples could be differentiated on the basis of their carbon isotope ratio alone. Adding the isotope ratio data from nitrogen and hydrogen allowed us to differentiate 96% of the comparisons between samples. When an interval of 2 SD was used, over 82% of the samples could be differentiated based on their carbon isotope ratios. As in the previous case, expanding the comparison to also include the nitrogen and hydrogen isotope data reduced the number of matches between samples, thereby increasing the percentage of samples that we differentiated based on isotopes to over 91%.

Conclusions

We performed compound-specific stable isotope analysis on 33 pure acid scavenger samples using gas chromatography coupled to isotope ratio mass spectrometry and report their carbon, nitrogen, and hydrogen isotope content. We chose to explore these compounds as they are known stabilizers in nerve agents and other potential threat compounds. Stable isotope analysis provided a basis for distinguishing between different acid scavengers and could therefore be used for sample matching between an inventory of these compounds and those in a threat agent or to link agents used at different events. Using compound-specific techniques would permit isotope ratio

measurement of acid scavengers found as a minor constituent within a matrix, such as a sample likely to be recovered in a forensic setting, or as a residual synthetic precursor in a chemical agent.

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