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Measurement of Stable Isotope Ratios in Methylamphetamine: A Link to its Precursor Source.

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Abstract

The illicit drug methylamphetamine is often prepared from the precursor ephedrine or pseudoephedrine, which in turn are obtained by three processes: extraction from the *Ephedra* plant ('natural'), via fermentation of sugars ('semi-synthetic'), and by a 'fully-synthetic' route from propiophenone. We report the first method to differentiate between the three industrial routes used to produce the precursors ephedrine and pseudoephedrine by measurement of stable isotope ratios of nitrogen ($\delta^{15}N$), hydrogen ($\delta^{2}H$) and carbon ($\delta^{13}C$). Analysis of 782 samples of seized methylamphetamine allowed classification into three groups using k-means clustering or the expectation-maximization algorithm applied to a Gaussian mixture model. By preparing 30 samples of ephedrine by the 'fully-synthetic' industrial process, and measuring their $\delta^{15}N$, $\delta^{2}H$ and $\delta^{13}C$ values, we observed that ^{15}N becomes significantly depleted compared to the methylamine starting material. Conversion of ten ephedrine samples to methylamphetamine showed this depletion is maintained in the final drug product, of which the $\delta^{15}N$, $\delta^{13}C$ and $\delta^{2}H$ values were distinct from those of ephedrine and methylamphetamine samples of a 'semi-synthetic' (fermentation pathway) origin. Combining modeling analysis with the new experiments and published information on the values of $\delta^{2}H$ gave a definitive assignment of the three model groups, and equations to obtain probabilities for the precursor origin of any new sample. A simple rule of thumb is also presented. Making an assignment using delta values is particularly useful when no other chemical profiling information is available.

Introduction

Methylamphetamine is the α-methyl derivative of β-phenylethylamine and possesses a chiral center at the carbon atom bearing the amino group. It may exist as either *d*-methylamphetamine or *l*-methylamphetamine and the enantiomeric form with the greatest central nervous system activity is *d*-methylamphetamine.[1] Many synthetic approaches may be used to prepare methylamphetamine including a number of clandestine production methods that rely on *l*-ephedrine or *d*-pseudoephedrine as precursors. This is mainly because the chemical reactions employed, i.e. reduction of the benzylic hydroxyl group is easy to perform on a large scale and gives high yields of only the *d*-methylamphetamine.[2] Ephedrine is a legitimate industrial chemical manufactured by industry using one of the synthetic procedures shown in Figure 1 and most pseudoephedrine is produced by the simple acid isomerization of ephedrine.[3] However, methods employing P2P as the precursor have seen a resurgence recently particularly in the United States.

Profiling of methylamphetamine

Chemical profiling of methylamphetamine has as its strategic goal the determination of the synthetic route and the identity of precursor chemicals.[2,4] This information may assist in monitoring the diversion of legitimate industrial chemicals for illegitimate purposes. In Australia the National Measurement Institute (NMI) undertakes conventional chemical profiling of many illicit drugs to provide forensic intelligence to the Australian Federal Police (AFP). AFP Intelligence Analysts use this information to disrupt drug trafficking rings. Methods used to profile the drug methylamphetamine include: (i) determination of organic impurity profile, (ii) chiral analysis (iii) elemental analysis and (iv) identification of adulterants and diluents. A

spin-off from this is that the same chemical information may be used to provide operational intelligence, i.e. assist law enforcement in linking different seizures.[4b] The organic impurity profile is often used to establish such links although great care should be exercised before any inference is made. Increasingly forensic scientists are appreciating the value in the measurement of the light element stable isotopes as a complementary procedure to conventional chemical profiling.[5] Stable isotope profiling is useful both to establish synthetic pathways and identify source commonality.[6]

(i) *Ephedra*

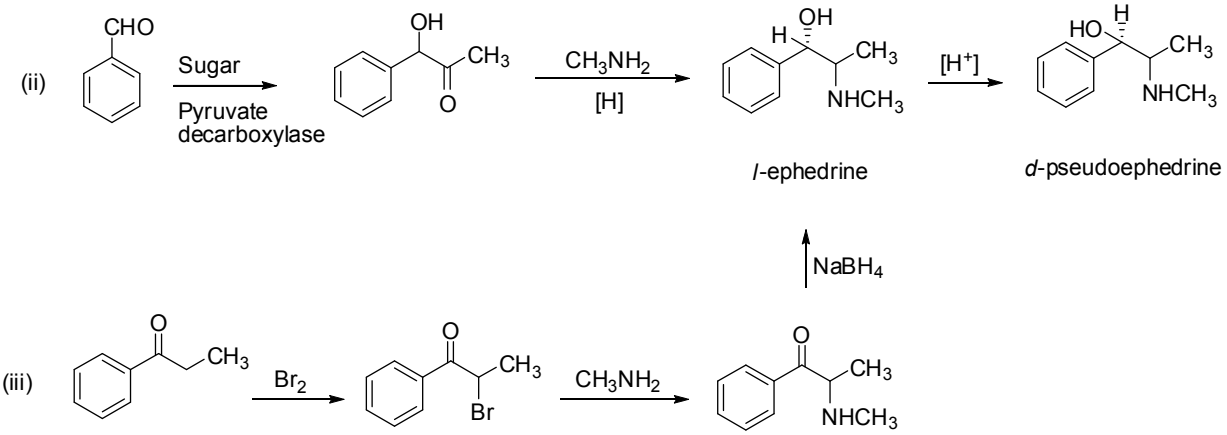

Figure 1: The three major industrial methods for production of ephedrine (pseudoephedrine): (i) extraction of *ephedra* ('natural' route); (ii) the 'semi-synthetic' preparation by fermentation of a sugar source with benzaldehyde and (iii) the 'fully-synthetic' preparation commencing with bromination of propiophenone.

Previous work in these laboratories[5d] and by Kurashima et al.[7] and Makino et al.[8] established a relationship between δ^2H and $\delta^{13}C$ values of methylamphetamine and its chemical precursors[9]. These studies showed that ephedrine or pseudoephedrine, here after referred to as ephedrine (pseudoephedrine), made industrially using the fermentation or 'semi-synthetic' process (Figure 1) had increased δ^2H values. Most benzaldehyde used in the fermentation process is highly enriched in deuterium because most industrial benzaldehyde is produced by the catalytic oxidation of toluene and results in benzaldehyde with δ^2H_{VSMOW} values up to +700 ‰. As a result methylamphetamine made using this type of ephedrine (pseudoephedrine), also had increased δ^2H values as demonstrated by the authors through synthesis. To distinguish methylamphetamine prepared using the remaining two ('fully-synthetic', 'natural') routes to ephedrine (pseudoephedrine) Makino et al.[8] suggested that $\delta^{15}N$ values may be valuable.[3] Several samples of ephedrine and pseudoephedrine having a 'fully-synthetic' provenance, i.e. prepared from propiophenone (Figure 1.iii) were shown to have decreased $\delta^{15}N$ values compared to the 'natural' and 'semi-synthetic' forms[3]. To test the hypothesis that the 'fully-synthetic' procedure results in depletion of the nitrogen stable isotope values, 30 samples of ephedrine were prepared by brominating propiophenone and reacting the resulting 2-bromopropiophenone with various methylamine sources, and ten of these were converted to methylamphetamine.

Evidence of identity, or matching data from one sample with another, is increasingly being given in probabilistic terms using likelihood ratios, albeit with some controversy[10]. Information is given to courts as how many more times the evidence is likely to have been found under the prosecution hypothesis, than under the defense hypothesis. By calculating the probability that the delta values of a sample are from a synthetic method for ephedrine (pseudoephedrine) by one route or another we can offer the advice, for example, that it is "1000 times more likely to have found the delta values of the sample if the ephedrine were prepared by the fully synthetic route than if it were prepared by the natural route, and 5000 times more likely than if it were prepared by the semi-synthetic route". A numerical scale thus allows judgments to be made about the strength of evidence.

Classification algorithms

It is known that there are three major routes to ephedrine (pseudoephedrine), but there is rarely hard information on the precursor route given only a seized methylamphetamine sample. Our data were delta values $\delta^{15}N$, $\delta^{2}H$ and $\delta^{13}C$ of a set of 782 methylamphetamine samples seized by the Australian Federal Police over a two-year period, plus ten samples prepared in our laboratory from ephedrine synthesized from the 'fully-synthetic' route.

Two 'unsupervised' classification methods were chosen to cluster the data, namely 'k-means clustering' and the 'expectation-maximization (EM) algorithm' applied to a Gaussian mixture model, asking only that samples fall into one of three, initially unspecified, groups. At this stage the mathematical analysis does not identify which preparative method should be assigned to which cluster. The location of the data from the laboratory-synthesized samples (assuming they all fall in the same group) allows assignment of the 'fully-synthetic' group. Other information from the literature can be

used to assign the group comprising the samples from the 'semi-synthetic' route. The 'natural' group can then be assigned to the remaining cluster.

Once the three groups identified by a clustering algorithm (k-means or EM, hopefully in agreement) have been associated with one of the three routes to ephedrine (pseudoephedrine), the data is effectively classified. Using these results, simple 'rules of thumb' can then be derived that classify the majority of methylamphetamine samples. These 'rules-of-thumb' are useful for quickly assessing isotopic data before probabilities of routes are calculated by more sophisticated methods.

A brief description and rationale for choosing the classification algorithms is given here, and a mathematical description of each is given in the Experimental section. k-means clustering[11] is a simple classification algorithm in which a specified number of groups are created that minimize the sum of distances between each sample and the nearest centroid of a group. 'Distance' between two locations in the present system is the Euclidean distance based on the coordinates of $\delta^{15}N$, $\delta^{2}H$ and $\delta^{13}C$. Once such a group is established it is possible to draw planes that bisect the line between pairs of centroids and create rules for classifying samples. The advantages of k-means clustering are its simplicity of implementation, the transparency of the algorithm, and that no assumption about the distribution of each group is required. However, although the basic method classifies a point, it does not indicate the strength of the classification. In illicit drug analysis, it is desirable to have some confidence about an assignment, particularly to know when a particular assignment only marginally favors a class. As the second classification method we turned to a Bayesian classifier in which groups are described by weighted Gaussian probability density functions (PDFs) and assignments are made using the EM algorithm[12], which finds the mean,

covariance matrix and weight for each group that maximizes the likelihood of the data. The output of the algorithm is an equation that calculates the probabilities of a new sample being in each of the three classes.

In this paper we report the measurement of stable isotope delta values $\delta^{15}N$, $\delta^{13}C$ and $\delta^{2}H$ of 782 seized samples of methylamphetamine and, together with measured delta values of samples of ephedrine and methylamphetamine synthesized in our laboratory, present classification algorithms for the precursor synthesis route for new samples based on only measured delta values.

Experimental Section

Reference Materials

All reference materials, internal standards and surrogate standards used in the chemical profiling of methylamphetamine were obtained from the reference collection of the National Measurement Institute Australia.

Synthesis

Synthesis of 2-bromopropiophenone

2-Bromopropiophenone was prepared by a modification of the method described by Schmidt.[13]

Synthesis of 2-(N-methylamino)propiophenone

2-(N-methylamino)propiophenone was prepared from 2-bromopropiophenone using a modification of the method described by DeRuiter et al.[14]

Synthesis of ephedrine

Ephedrine was prepared by reducing 2-(N-methylamino)propiophenone using sodium borohydride. The method used was a modification of the method described by Salouros et al.[15]

Synthesis of methylamphetamine

Methylamphetamine was prepared via a modification of the method described by Salouros et al.[15]

Sample Identification

The identity of the methylamphetamine was verified by gas chromatography / mass spectrometry using the method of Andersson et al.[16] The methylamphetamine purity was determined using gas chromatography with flame ionization detection, as outlined in our previous work.[5d]

Stable Isotope Measurement

Sample preparation

Approximately 0.8 mg of substance was weighed into tinfoil capsules (3.3 mm x 5 mm, IVA Analysentechnik, Meerbusch Germany) for $\delta^{13}C/\delta^{15}N$ analysis and approximately 0.15 mg to 0.20 mg into silver foil capsules for $\delta^{2}H$ analysis.

Isotopic calibration and quality control of EA/TC-IRMS measurements

A Flash Elemental Analyser (EA) 1112, with dual combustion and thermal conversion (TC) capabilities, connected to a ConFlo IV interface and Delta V Plus Mass Spectrometer (ThermoScientific, Bremen, Germany) was used to determine $\delta^{15}N$, $\delta^{13}C$ and $\delta^{2}H$ values by continuous flow for samples synthesized in this laboratory. Data was acquired using ISODAT 2.5 (ThermoScientific, Bremen, Germany). Prior to sequence acquisitions, zero enrichments were performed using each of the three reference gases. The standard deviation of nine 20-second gas pulses was determined to be less than 0.1 ‰ for N_2 and CO_2, and 0.5 ‰ for H_2.

Sample sequences for $\delta^{15}N$ and $\delta^{13}C$ analysis were bracketed by triplicates of acetanilide reference standard ($\delta^{15}N_{Air} = +1.2 \pm 0.1$ ‰, $\delta^{13}C_{VPDB} = -29.5 \pm 0.1$ ‰) purchased from Indiana University. System performance was verified by analysis of a

high purity (94.9% as HCl form) methylamphetamine quality control every three samples. Measured $\delta^{13}C$ values, reported as per mille (‰) differences from the Vienna Pee Dee Belemnite (VPDB) international standard, were determined relative to high purity (> 99.5 %) CO_2 gas (BOC gases, Sydney, Australia; $\delta^{13}C$ = –6.8 ‰) that was calibrated against NBS 19 (Environmental Isotopes Pty. Ltd, Sydney, Australia). Ultra high purity (> 99.99 %) N_2 gas (BOC gases, Sydney, Australia) was calibrated internally using *l*-alanine ($\delta^{15}N_{Air}$ = –1.1 ‰) provided by Dr Yukiko Makino at the University of Tokyo and verified using the acetanilide standard. The precision of $\delta^{15}N$ and $\delta^{13}C$ measurements was determined to be 0.4 ‰ and 0.2 ‰ respectively.

Measured $\delta^{2}H$ values, reported as per mille (‰) differences from the Vienna Standard Mean Ocean Water (VSMOW) international standard, were determined relative to ultra high purity (> 99.99 %) H_2 gas (BOC gases, Sydney, Australia; $\delta^{2}H$ = –340 ‰) calibrated against reference materials, namely; C36 *n*-alkane (hexatriacontane; $\delta^{2}H_{VSMOW}$ = –247 ± 1 ‰), phenanthrene ($\delta^{2}H_{VSMOW}$ = –84 ± 1 ‰) and icosanoic acid methyl ester ($\delta^{2}H_{VSMOW}$ = +76 ± 1 ‰) obtained from Indiana University. Subsequent repeat analysis (*n* = 650) of a high purity methylamphetamine check sample assigned the $\delta^{2}H$ value of this laboratory quality control, analyzed every three samples within each sequence, to be –191 ± 4 ‰. The H_3^+ factor (3.39 ‰/nA) was determined daily from reference H_2 gas pulses with signal size linearly incremented. Precision of $\delta^{2}H$ measurements as monitored by standards and laboratory controls was determined to be 4 ‰.

Measurement uncertainties of delta values were determined by combining bias and precision contributions in quadrature according to the GUM uncertainty framework[17] and as described in our earlier work.[5d] For a 95 % confidence interval (*k* = 2) an

expanded uncertainty (U) for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ measurements was estimated to be ±0.4 ‰, ±0.5 ‰ and ±4 ‰, respectively. These uncertainty estimates were considered to be fit-for-purpose based on the range of values recorded for a high purity methylamphetamine HCl quality control analyzed every 3 samples.

$\delta^{13}C$ and $\delta^{15}N$ analysis by EA-IRMS

Daily jump calibrations enabled a change in continuous monitoring of *m/z* 28 and 29 to *m/z* 44, 45 and 46 for the determination of $\delta^{15}N$ and $\delta^{13}C$ in the same analysis. A folded tin foil capsule containing no sample material was the first and final analysis performed in a sequence to demonstrate that the system was void of contamination. A typical sequence comprised 10 samples run in triplicate, preceded and followed by a triplicate analysis of acetanilide standard and triplicate analysis of a high purity methylamphetamine check sample after every third sample. Crimped tin capsules containing sample material were introduced into a Thermo Scientific chamber and pressurized with high purity oxygen gas (BOC gases, Sydney, Australia) to exclude the contribution of nitrogen from ambient air. The sample entered the combustion furnace operated at 980 °C where it burns exothermically in a stream (250 mL/min, 3 seconds) of high purity (> 99.5 %) oxygen. The oxidized sample was reduced *in situ* in the presence of copper before water was removed from the resultant gas stream using a magnesium perchlorate filled trap. A post-reactor GC column, operated at 65°C separated evolved N_2 and CO_2. Ultra high purity (> 99.99 %) helium (BOC gases, Sydney, Australia) pressure was set to 100 kPa to enable a run time of 650 seconds. Two N_2 and CO_2 reference gas pulses were applied prior to the sample N_2 signal and following the sample CO_2 signal, respectively.

δ²H analysis by TC-IRMS

Silver-foil capsules containing sample material were introduced into the TC reactor consisting of an alumina ceramic outer containing a glassy carbon tube with glassy carbon granulate and silver wool packing. The thermal conversion furnace was operated at 1450 °C and the post-reactor GC column at 85 °C. Helium pressure was set to 330 kPa to enable a run time of 300 s. A typical sequence comprised of 10 samples run in triplicate, preceded and followed by a triplicate analysis of C36 *n*-alkane hexatriacontane, icosanoic acid methyl ester standards and triplicate analysis of a high purity methylamphetamine check sample after every third sample.

Mathematics of the clustering algorithms

k-means clustering

k-means clustering[11] (implemented as 'kmeans' in Matlab (7.12.0 R2011a), The Mathworks, USA) assigns each sample to the cluster that minimizes the sum of the Euclidean distances of each point from the centroid of its group. The algorithm is a two phase process, both phases iterative, and is seeded by a random assignment of samples. In the first phase, each iteration consists of reassigning points to their nearest cluster centroid, all at once, followed by recalculation of cluster centroids. In the second phase, points are individually reassigned if doing so will reduce the sum of distances, and cluster centroids are recomputed after each reassignment. Each iteration during the second phase consists of one pass through all the points. The second phase will converge to a local minimum, although there may be other local minima with lower total sum of distances. After sufficient random restarts the global minimum may usually be found.

The ranges of the delta values differ (~10 ‰ for $\delta^{13}C$, ~35 ‰ for $\delta^{15}N$, and ~350 ‰ for $\delta^{2}H$), so the data was scaled to produce a range of 1 for each variable before

classifying, and then returned to the original scale for display and reporting of classifying equations. The output of the algorithm is the classification of each point, which then allows determination of the equation of the boundary plane between any two groups. The equations for the boundaries then allow new samples to be classified.

In particular, the boundary planes are midway between, and orthogonal to, the pairs of cluster centroids in the transformed space, though this no longer holds after rescaling back to the original space. Hence, a point $x = [\delta^2\text{H}, \delta^{13}\text{C}, \delta^{15}\text{N}]^{\text{T}}$ lying on the boundary plane between two k-means clusters satisfies the equation:

$$(m_i - m_j)^{\text{T}} S^2 (x - (m_i + m_j)/2) = 0, \quad (1)$$

where m_i is the centroid of group i in the original scale, and S is a diagonal matrix containing the scale factors used for each variable. That is,

$$S = \begin{bmatrix} 1/\text{range}(\delta^2\text{H data}) & 0 & 0 \\ 0 & 1/\text{range}(\delta^{13}\text{C data}) & 0 \\ 0 & 0 & 1/\text{range}(\delta^{15}\text{N data}) \end{bmatrix}. \quad (2)$$

EM algorithm - Gaussian mixture model

In the second classification method, a Gaussian mixture model, with three components, is used to describe the probability density function of the data. For the three components of the mixture model, let μ_i, Σ_i and τ_i be the mean, covariance matrix and weight for component i, i = 1, .., 3; $\theta_i = \{\mu_i, \Sigma_i, \tau_i\}$; and $\theta = \{\theta_i\}$. Denote the set of data $X = \{x_k\}$, $k = 1,..,K$, where $x_k = [\delta^2\text{H}_k, \delta^{13}\text{C}_k, \delta^{15}\text{N}_k]^{\text{T}}$. Then the likelihood, $L(\theta; X)$, is the product of the sum of weighted probability densities for each Gaussian component:

$$L(\theta; X) = P(X \mid \theta) = \prod_{k=1}^{K} \sum_{i=1}^{3} \tau_i f_i(x_k; \theta), \quad (3)$$

where

$$f_i(x;\theta) = (2\pi)^{-3/2} \mid \Sigma_i \mid^{-1/2} \exp((x-\mu_i)^{\mathrm{T}} \Sigma_i^{-1} (x-\mu_i)) \quad (4)$$

and

$$\sum_{i=1}^{3} \tau_i = 1. \quad (5)$$

The EM algorithm finds the best fit parameters θ. For the original paper on the EM algorithm, see Dempster et al.[12]; for a more readable account of applying the EM algorithm to a Gaussian mixture model, see McLachlan and Peel.[18]

To classify sample *x* once θ is estimated, the probability density of *x* given *x* belongs to cluster *i*, weighted by the probability of *x* belonging to cluster *i*, $T_i = \tau_i f_i(x;\theta)$, is calculated for each cluster i. Then the probability that *x* belongs to cluster *i* is:

$$P(x \in i) = T_i / (T_1 + T_2 + T_3). \quad (6)$$

In use, once the parameter θ is determined, T_i and therefore probabilities of new samples can be calculated in a spreadsheet. A rule such as "the probability of membership of a class must exceed 99 % to signify a match" could then be applied to new samples.

Results and Discussion

Analysis of seized samples

The raw data (δ^{15}N, δ^{2}H and δ^{13}C values) from 782 samples of seized methylamphetamine are given in the Supplementary Information. Initial inspection shows a group with increased δ^{2}H and δ^{13}C values which fit the profile of methylamphetamine synthesized from ephedrine (pseudoephedrine) produced via the 'semi-synthetic' industrial process as demonstrated in earlier work[5 12]. Samples with decreased δ^{2}H values (–30 ‰ to –240 ‰) and decreased δ^{13}C values (< –26 ‰) are likely to represent methylamphetamine produced from ephedrine (pseudoephedrine) made by the remaining two industrial processes, i.e. of 'natural' origin (extracted

from one of the *ephedra* species) or 'fully-synthetic' origin (produced from propiophenone).

The latter group can be separated into two groups when their $\delta^{15}N$ values were considered. One group had decreased $\delta^{15}N$ values with the majority more negative than –2 ‰. Based on Makino's[8] earlier work with several samples of known provenance it seemed possible that many of the samples with decreased $\delta^{15}N$ values represent methylamphetamine produced using the 'fully-synthetic' form of ephedrine (pseudoephedrine). Evidence to support this hypothesis was furnished by analysis of the samples synthesized in our laboratory.

Analysis of synthesized samples

Thirty syntheses of ephedrine employing the 'fully-synthetic' method and using different sources of propiophenone and methylamine were performed. The methylamine sources used had $\delta^{15}N$ values ranging from –7.7 ‰ to +7.8 ‰. From these 30 prepared ephedrine samples ten were selected to convert to methylamphetamine. Table 1 includes $\delta^{15}N$ values for each methylamine precursor and the final ephedrine product. Makino et al.[8] observed that ephedrine with an established 'fully-synthetic' provenance had decreased $\delta^{15}N$ values. Each of the 30 ephedrine syntheses in this work gave a product with decreased $\delta^{15}N$ values compared to its methylamine precursor in agreement with Makino's[8] observations.

Table 1. $\delta^{15}N$, $\delta^{2}H$ and $\delta^{13}C$ values in ephedrine synthesized from propiophenone and methylamine. The final column is the difference in $\delta^{15}N_{Air}$ between starting material methylamine and product ephedrine. The measurement uncertainty is expressed as an expanded uncertainty with 95 % probability.

Source of methylamine; $\delta^{15}N_{Air}$	$\delta^{15}N_{Air}$ (±0.5 ‰)	$\delta^{2}H_{VSMOW}$ (±4 ‰)	$\delta^{13}C_{VPDB}$ (±0.4 ‰)	Depletion of $\delta^{15}N_{Air}$ (±0.7 ‰)
Aldrich (HCl); N = –4.6 ‰	–6	–81	–28.1	–1.4
Alfa Aesar (HCl); N = –5.9 ‰	–7.9	–65	–26.5	–2.0
Fluka (HCl); N = –7.7 ‰	–9.8	–67	–28.4	–2.1
Sigma (aqueous); N = +4.4 ‰	–4.5	–82	–27.6	–8.9
Alfa Aesar (aqueous); N = +0.8 ‰	–8.1	–58	–25.9	–8.9
Aldrich (aqueous); N = +7.8 ‰	–8.1	–68	–26.6	–15.9
TCI (aqueous); N = –1.3 ‰	–8.5	–65	–27.0	–7.2
TCI (aqueous); N = –3.9 ‰	–6.7	–74	–28.0	–2.8
Acros (aqueous); N = +2.7 ‰	–5.1	–69	–29.2	–7.8
Acros (HCl); N = –2.2 ‰	–3.7	–78	–28.1	–1.5
Aldrich (aqueous); N = +7.8 ‰	–5.5	–59	–29.4	–13.3
Sigma (aqueous); N = +4.4 ‰	–6.7	–65	–29.2	–11.1
Alfa Aesar (aqueous); N = +0.8 ‰	–8.4	–50	–28.3	–9.2
Alfa Aesar (HCl); N = –5.9 ‰	–8.7	–45	–28.3	–2.8
Acros (aqueous); N = +2.7 ‰	–5.1	–48	–29.2	–7.8
Aldrich (HCl); N = –4.6 ‰	–6.8	–60	–29.7	–2.2
Fluka (HCl); N = –7.7 ‰	–9.4	–53	–30.2	–1.7
Acros (HCl); N = –2.2 ‰	–4.6	–64	–29.6	–2.4
TCI (aqueous); N = –1.3 ‰	–9.8	–49	–29.2	–8.5
TCI (aqueous); N = –3.9 ‰	–7.4	–54	–30.4	–3.5
Aldrich (HCl); N = –4.6 ‰	–7.3	–77	–29.4	–2.7
Alfa Aesar (HCl); N = –5.9 ‰	–16.6	–70	–28	–10.7
Fluka (HCl); N = –7.7 ‰	–10.8	–71	–29.1	–3.1
Sigma (aqueous); N = +4.4 ‰	–6.8	–70	–28.3	–11.2
Alfa Aesar (aqueous); N = +0.8 ‰	–7.1	–78	–28.8	–7.9
Aldrich (aqueous); N = +7.8 ‰	–4.8	–84	–29.3	–12.6

TCI (aqueous); N = –1.3 ‰	–9.5	–65	–28.6	–8.2
TCI (aqueous); N = –3.9 ‰	–8.9	–76	–29.5	–5.0
Acros (aqueous); N = +2.7 ‰	–4.7	–82	–29.3	–7.4
Acros (HCl); N = –2.2 ‰	–5.3	–71	–28.6	–3.1
Average Depletion				–7.1

Table 2: Values of $\delta^{15}N_{Air}$ in ephedrine and methylamphetamine synthesised from propiophenone and methylamine.

Source of methylamine; $\delta^{15}N_{Air}$	Product	$\delta^{15}N_{Air}$ (±0.5 ‰)	$\delta^{2}H_{VSMOW}$ (±4 ‰)	$\delta^{13}C_{VPDB}$ (±0.4 ‰)
Aldrich (HCl); N = –4.6 ‰	ephedrine	–7.3	–77	–29.4
	methamphetamine	–8.1	–95	–29.7
Alfa Aesar (HCl); N = –5.9 ‰	ephedrine	–16.6	–70	–28
	methamphetamine	–17.4	–82	–28
Fluka (HCl); N = –7.7 ‰	ephedrine	–10.8	–71	–29.1
	methamphetamine	–11.2	–82	–29.2
Sigma (aqueous); N = +4.4 ‰	ephedrine	–6.8	–70	–28.3
	methamphetamine	–7.3	–90	–28.3
Alfa Aesar (aqueous); N = +0.8 ‰	ephedrine	–7.1	–78	–28.8
	methamphetamine	–6.8	–88	–28.6
Aldrich (aqueous); N = +7.8 ‰	ephedrine	–4.8	–84	–29.3
	methamphetamine	–5.7	–104	–29.2
TCI (aqueous); N = –1.3 ‰	ephedrine	–9.5	–65	–28.6
	methamphetamine	–9.5	–84	–28.3
TCI (aqueous); N = –3.9 ‰	ephedrine	–8.9	–76	–29.5
	methamphetamine	–9	–90	–29.2
Acros (aqueous); N = +2.7 ‰	ephedrine	–4.7	–82	–29.3
	methamphetamine	–5.5	–95	–29.2
Acros (HCl); N = –2.2 ‰	ephedrine	–5.3	–71	–28.6
	methamphetamine	–5.7	–88	–28.4

The smallest depletion observed was 1.4 ‰ and the largest was 15.9 ‰, with an average depletion of 7.1 ‰. All 30 ephedrine samples prepared had $\delta^{15}N$ values less than or equal to –3.7 ‰ including those samples of ephedrine produced using methylamine having $\delta^{15}N$ values up to +7.8 ‰. Ten samples of the ephedrine prepared by the 'fully-synthetic' method were then selected to convert into methylamphetamine. The $\delta^{15}N$, $\delta^{2}H$ and $\delta^{13}C$ values for the resulting methylamphetamine samples are presented in Table 2. As might be expected, within measurement uncertainty, little or no change was observed in the $\delta^{15}N$ value in going from ephedrine to methylamphetamine where only the benzylic hydroxyl group is being removed. But, importantly, in each case ^{15}N remains depleted compared to ^{15}N of the methylamine used in the preparation of the ephedrine precursor.

Classification into groups

k-means clustering

Figure 2 shows the result of k-means clustering on the $\delta^{15}N$, $\delta^{2}H$ and $\delta^{13}C$ values of 782 seized methylamphetamine samples plus the ten samples synthesized in our laboratory. The three groups are separated by planes calculated as described above.

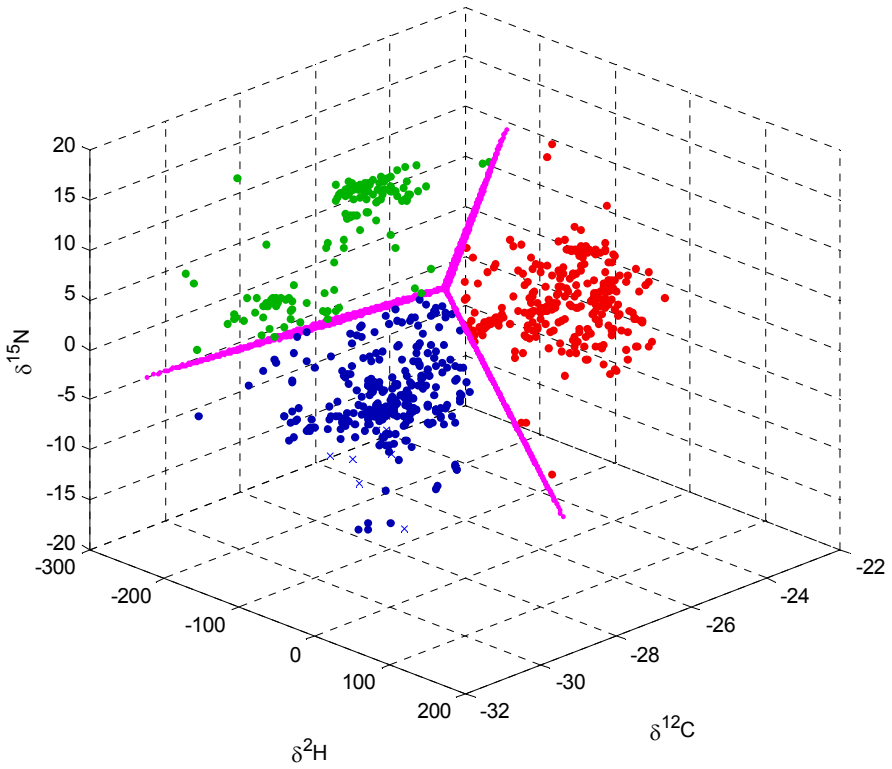

Figure 2. Scatter plot of $\delta^{15}N$, $\delta^{2}H$ and $\delta^{13}C$ values from seized methylamphetamine (points) and synthesised methylamphetamine (crosses) assigned to groups by the k-means clustering algorithm. Boundary planes between pairs of clusters are shown. Assignment: red = 'semi-synthetic' route to ephedrine (pseudoephedrine), blue = 'fully-synthetic' route, green = 'natural' route.

We assign the groups to the routes to ephedrine (pseudoephedrine) as follows. Samples known to have been produced via the 'fully-synthetic' industrial procedure, and samples synthesized by us, have decreased (i.e. negative) $\delta^{15}N$ values[4e, 5d, 8]. Therefore we assign the blue-colored group of Figure 2 to the 'fully-synthetic' route. In all samples isotopically profiled in this laboratory having increased $\delta^{2}H$ and $\delta^{13}C$ values consistent with a 'semi-synthetic' source of ephedrine (pseudoephedrine) the $\delta^{15}N$ values were also increased and usually positive. We have taken the delta values

of samples of known 'semi-synthetic' provenance from Table 2 of reference[5d] and determined that they clearly fall in the 'red group' of Figure 2, which is thus assigned to 'semi-synthetic'. The remaining group (green in Figure 2) is assigned to the 'natural' route. A single sample with data in Table 2 of [5d] was known to be synthesized by the natural route and fell in the green group. This assignment is consistent with results from our laboratory which associate the 'natural' route with increased $\delta^{15}N$ values. It is also noted that the 'natural' group is less tightly clustered about its centroid. With the potential for different geographical origin of the *Ephedra* plants and species variation, it is expected that multiple locations and different harvest times would lead to a greater spread of delta values.

From the k-means clustering algorithm, we obtain tests that specify which side of each of the three dividing planes a point, $[\delta^2H, \delta^{13}C, \delta^{15}N]^T$, lies on, as follows:

$$test\_12 = 0.001184*(\delta^2H + 70.34) + 0.04466*(\delta^{13}C + 26.92) - 0.005468*(\delta^{15}N - 7.970);$$

$$test\_13 = 0.000636*(\delta^2H + 36.26) + 0.04753*(\delta^{13}C + 27.01) + 0.005465*(\delta^{15}N - 0.5665);$$

$$test\_23 = -0.000547*(\delta^2H + 109.9) + 0.002871*(\delta^{13}C + 28.50) + 0.01093*(\delta^{15}N - 4.269).$$

Then the k-means classification is given by:

$$Class = \begin{cases} \text{Semi-synthetic}, & \text{if } (test\_12 > 0) \text{ AND } (test\_13 > 0), \\ \text{Natural}, & \text{if } (test\_12 < 0) \text{ AND } (test\_23 > 0), \\ \text{Fully Synthetic}, & \text{if } (test\_13 < 0) \text{ AND } (test\_23 < 0). \end{cases}$$

The numbers of samples assigned to each class are given in Table 3.

Table 3. Numbers of methylamphetamine samples (782 seized + 10 synthesized) classified in each group by k-means clustering and the EM algorithm.

	k-means clustering	EM algorithm	In agreement
Semi-synthetic	296	312	293
Natural	171	179	166
Fully-Synthetic	325	301	297
Total	792	792	756

EM algorithm - Gaussian mixture model

The results of EM classification were very similar to that of k-means (see Table 3 and Figure 3). Although there is some overlap, particularly between 'natural' and 'fully-synthetic', the clusters allow clear assignment of most of the samples.

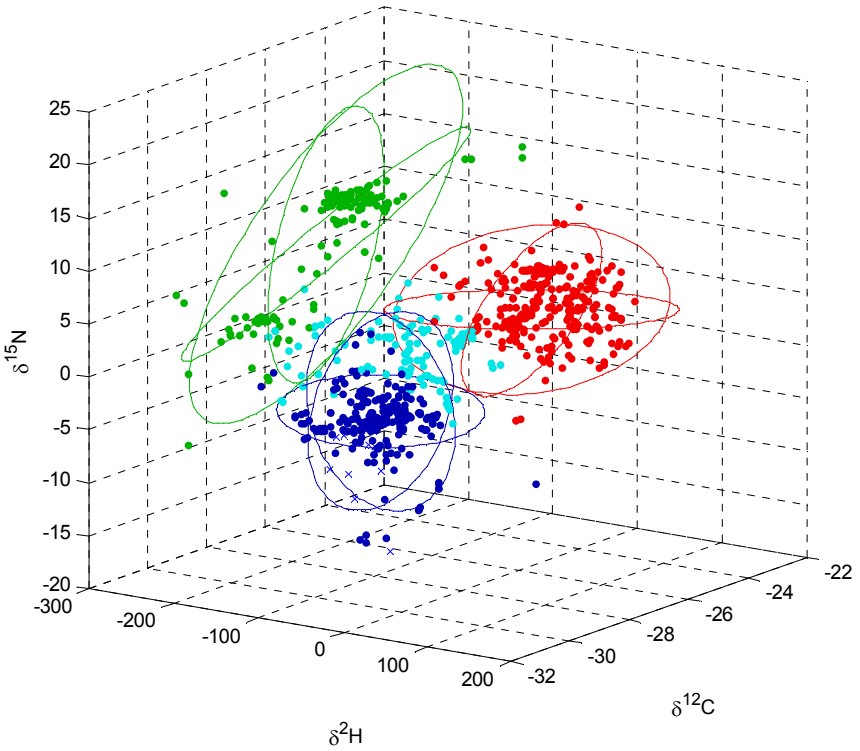

Figure 3. Scatter plot of δ^2H, δ^{13}C and δ^{15}N values from seized methylamphetamine (points) and synthesized methylamphetamine (crosses) assigned to groups by the EM algorithm. 95% probability contours for each cluster are shown. Assignment: red = 'semi-synthetic' route to ephedrine (pseudoephedrine), blue = 'fully-synthetic' route,

green = 'natural' route. Cyan points have less than 99 % probability of membership to an assigned class.

The fitted parameters are given in Table 4, and allow any new sample with δ^2H, $\delta^{13}C$ and $\delta^{15}N$ values to be assigned probabilities of belonging to each of the three classes, by equations (4)~~(4)~~ and (6)~~(6)~~.

Table 4. Gaussian mixture model parameters (prior probability, mean and covariance matrix) of the three classes fitted by the EM algorithm. The order of delta values is δ^2H, $\delta^{13}C$, $\delta^{15}N$.

	Mean (μ)	Prior (τ)	Covariance matrix (Σ) /1000
Semi-synthetic	$\begin{bmatrix} -2.086 \\ -25.52 \\ 4.095 \end{bmatrix}$	0.3901	$\begin{bmatrix} 2.6696 & 0.0075 & 0.0151 \\ 0.0075 & 0.0007 & 0.0010 \\ 0.0151 & 0.0010 & 0.0061 \end{bmatrix}$
Natural	$\begin{bmatrix} -144.4 \\ -28.41 \\ 11.15 \end{bmatrix}$	0.2266	$\begin{bmatrix} 1.2339 & 0.0218 & 0.1312 \\ 0.0218 & 0.0009 & 0.0036 \\ 0.1312 & 0.0036 & 0.0331 \end{bmatrix}$
Synthetic	$\begin{bmatrix} -71.59 \\ -28.64 \\ -3.376 \end{bmatrix}$	0.3834	$\begin{bmatrix} 0.5245 & 0.0056 & -0.0184 \\ 0.0056 & 0.0008 & -0.0001 \\ -0.0184 & -0.0001 & 0.0110 \end{bmatrix}$

Rule of Thumb

The samples isotopically profiled in the authors' laboratory that had increased δ^2H and $\delta^{13}C$ values consistent with a 'semi-synthetic' source of ephedrine (pseudoephedrine) usually had positive $\delta^{15}N$ values. Similarly, those samples classed as having a 'natural' provenance had increased $\delta^{15}N$ values. To date only samples known to have been produced via the 'fully-synthetic' industrial procedure, i.e. this work and the earlier observations of Makino[8], have been observed to have decreased

$\delta^{15}N$ values. Therefore a general 'rule of thumb' can be proposed. 'semi-synthetic' has $\delta^{13}C > -26.5$ ‰; 'natural' has $\delta^{15}N > 2.5$ ‰, and is not 'semi-synthetic'; and 'fully-synthetic' is not 'semi-synthetic' and not 'natural'. These rules are shown as a flow chart in Figure 4. Note that this method of assignment does not employ a knowledge of $\delta^{2}H$. In our previous work we demonstrated that in cases where ^{2}H is profoundly enriched, i.e. $\delta^{2}H > 0$ ‰, the sample was classified as having been synthesized from a semi-synthetic source of ephedrine (pseudoephedrine)[5(d)]. This assignment holds for all but two samples here having delta values: $\delta^{15}N = -3.7$ ‰, $\delta^{2}H = +5.0$ ‰ and $\delta^{13}C = -28.4$ ‰, and $\delta^{15}N = -11.0$ ‰, $\delta^{2}H = +30.9$ ‰ and $\delta^{13}C = -26.4$ ‰. The former sample is 'unknown' by EM, and 'fully-synthetic' by rule of thumb and k-means. The latter sample is 'semi-synthetic' by the rule of thumb and k-means, but the negative $\delta^{15}N$ classes it on the borders of 'fully synthetic' by the EM algorithm. The boundaries of the 'rule of thumb' classification are compared to the EM algorithm classification in Figure 5.

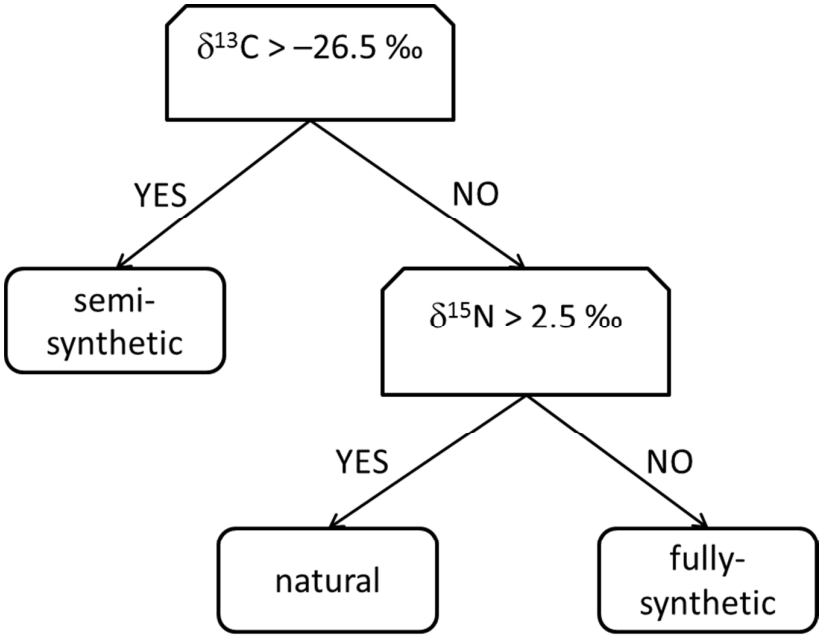

Figure 4. Rule of thumb assignments shown as a decision-tree chart.

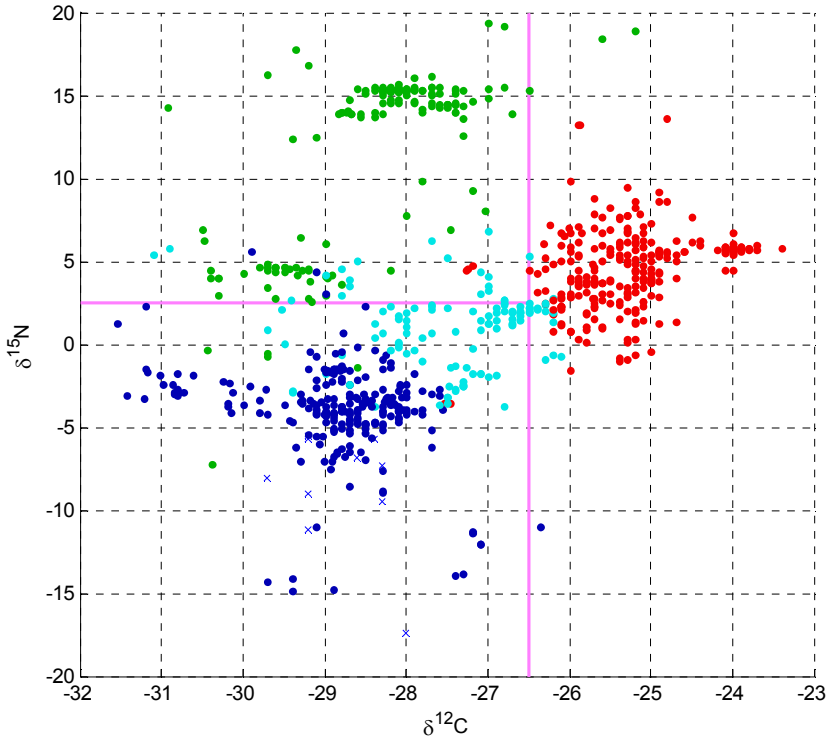

Figure 5. Scatter plot of $\delta^{13}C$ and $\delta^{15}N$ values from seized methylamphetamine (points) and synthesized methylamphetamine (crosses), comparing the EM algorithm and 'rule of thumb' classifications. Colors specify the EM algorithm assignment: red = 'semi-synthetic' route to ephedrine (pseudoephedrine), blue = 'fully-synthetic' route, green = 'natural' route, cyan = < 99 % probability of membership to the assigned class. Magenta lines indicate the 'rule of thumb' boundaries.

Comparison of classification methods

In assessing the usefulness of the classification methods we note that although each sample can have only one provenance, there is no reason why the different groups should not overlap, i.e. two routes can have the same set of delta values. However as more samples are obtained the groups can be refined and the assignment models improved, and we invite other research groups to build on the database of values given in the Supplementary Information. Apart from the laboratory synthesized

samples and some samples of 'semi-synthetic' and 'natural' ephedrine (pseudoephedrine) of known provenance[5d], we do not definitively know the origins of our samples, so can only offer percentages of agreement/disagreement between the classification methods (Table 5). Having only 4.5 % disagreement between the two statistical approaches encourages the view that the methods are giving genuine insight into the true groupings.

Table 5. Comparison of classifications obtained from the three methods i) k-means, ii) EM algorithm (EM), iii) Rule of thumb (RoT).

Methods compared	Agree (count)	Disagree (count)	Disagreement (%)
k-means vs EM	756	36	4.5 %
k-means vs RoT	755	37	4.7 %
EM vs RoT	724	68	8.6 %

Conclusion

The delta values $\delta^{15}N$, $\delta^{2}H$ and $\delta^{13}C$ have been measured for 782 samples of seized methylamphetamine and for 30 ephedrine and ten methylamphetamine samples synthesized in our laboratory. Based on present observations of $\delta^{15}N$ values and the previous work by Kurashima et al.[3, 7], Makino et al.[8] and Collins et al.[5d] on $\delta^{2}H$ and $\delta^{13}C$ values it seems possible that careful measurement of $\delta^{15}N$, $\delta^{2}H$ and $\delta^{13}C$ values in a methylamphetamine sample may indicate the industrial source of the ephedrine (pseudoephedrine) used. Different classification methods are described including a simple 'rule of thumb' on $\delta^{15}N$ and $\delta^{13}C$ values only, and an expectation maximization algorithm that allows expression of probabilities, or likelihood ratios, for the different routes. This is a desirable outcome for law enforcement attempting to disrupt the diversion of legitimate industrial chemicals.

Acknowledgements

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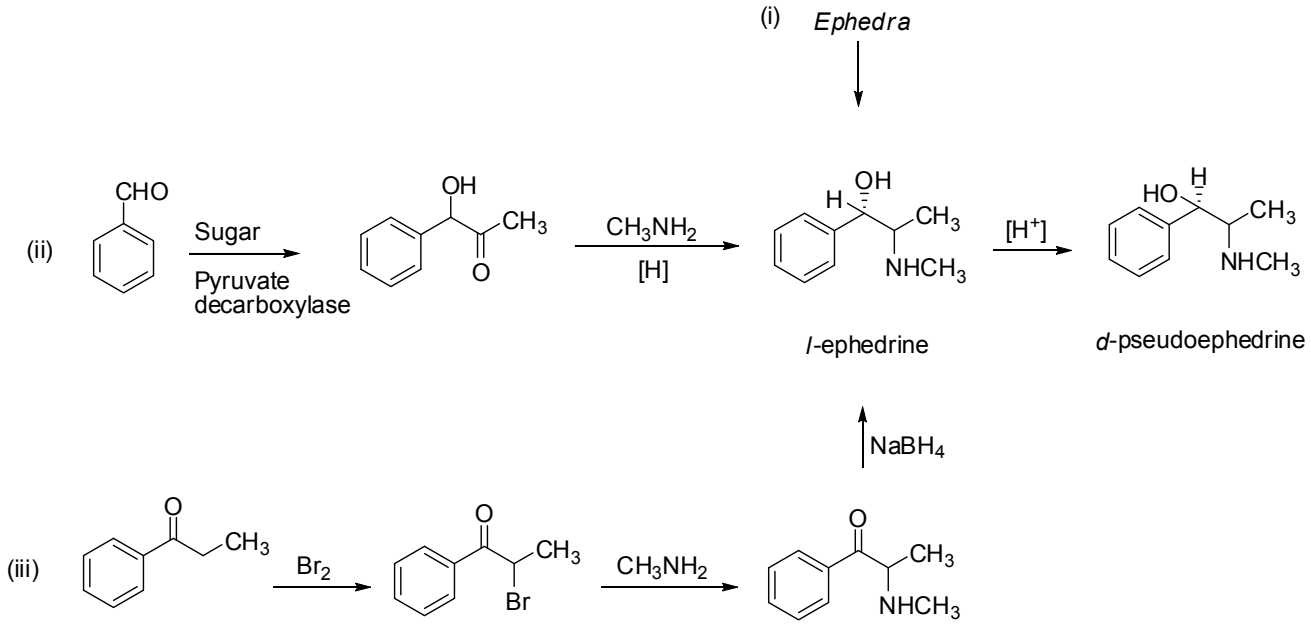
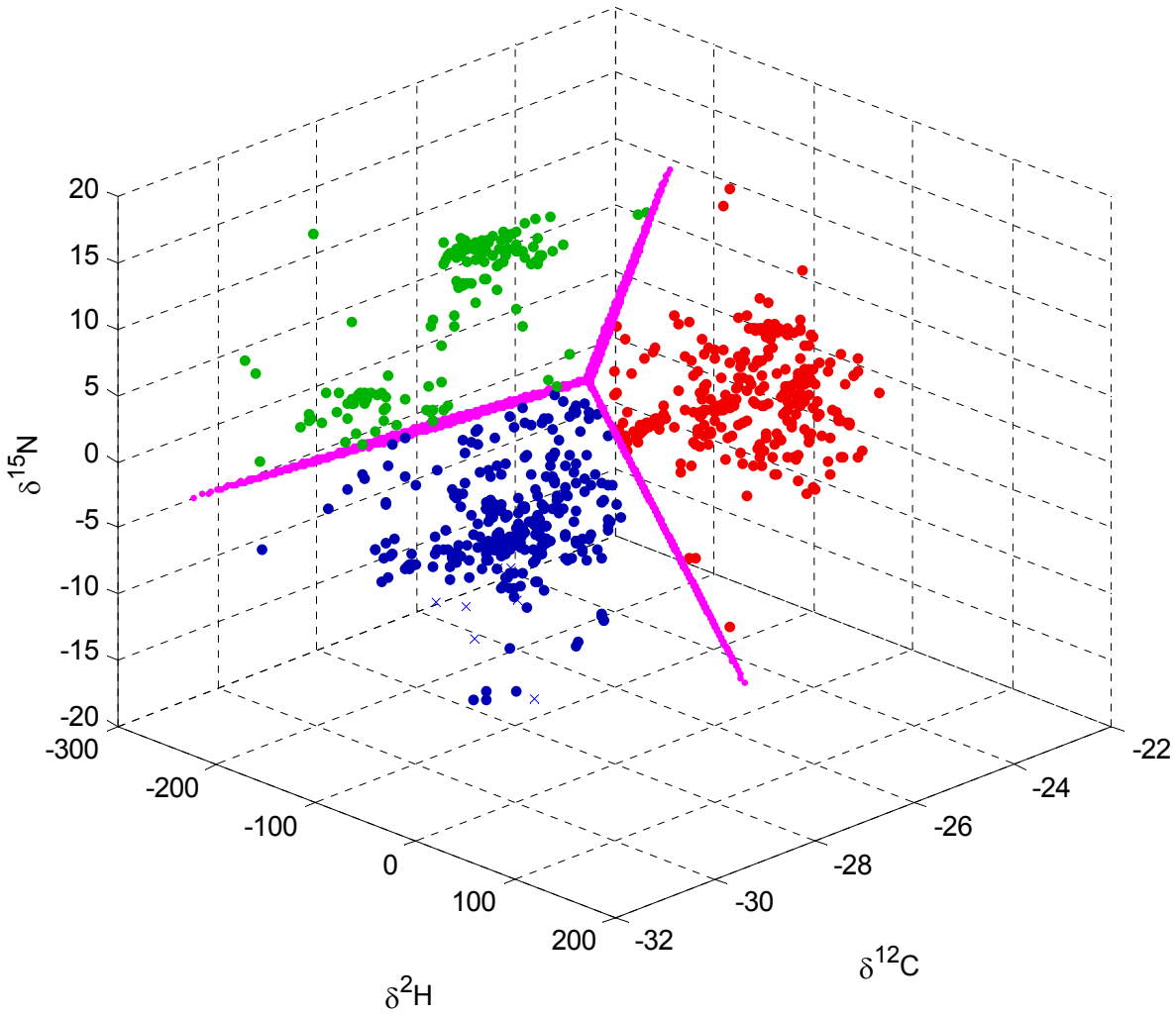

(i) Ephedra
(ii)
CHO
Sugar
Pyruvate decarboxylase
OH
CH3
O
CH3NH2
[H]
OH
H
CH3
NHCH3
l-ephedrine
[H+]
HO
H
CH3
NHCH3
d-pseudoephedrine
NaBH4
(iii)
O
CH3
Br2
O
CH3
Br
CH3NH2
O
CH3
NHCH3

Figure 1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15
10
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δ15N
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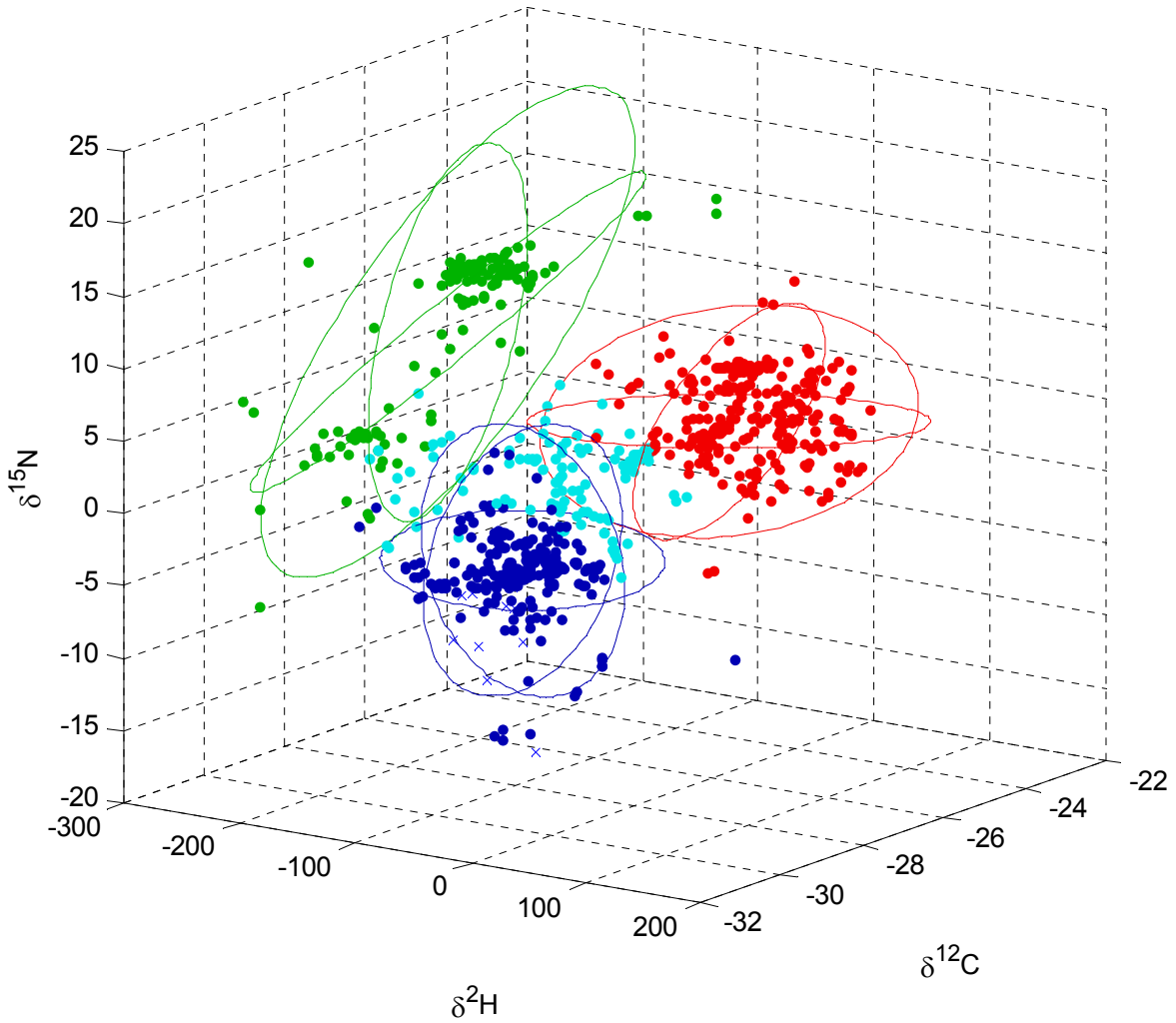
δ12C

Figure 2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δ15N
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-30
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δ12C

Figure 3

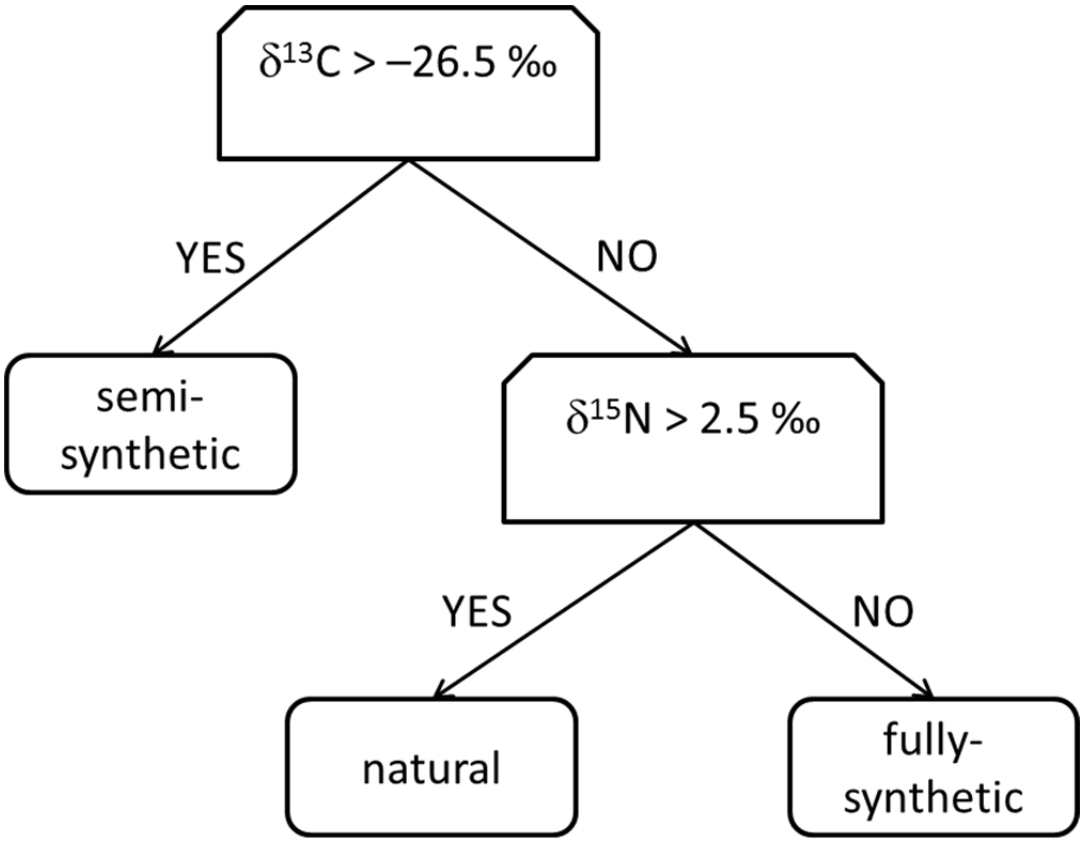

δ13C > –26.5 ‰
YES
NO
semi-synthetic
δ15N > 2.5 ‰
YES
NO
natural
fully-synthetic

Figure 4

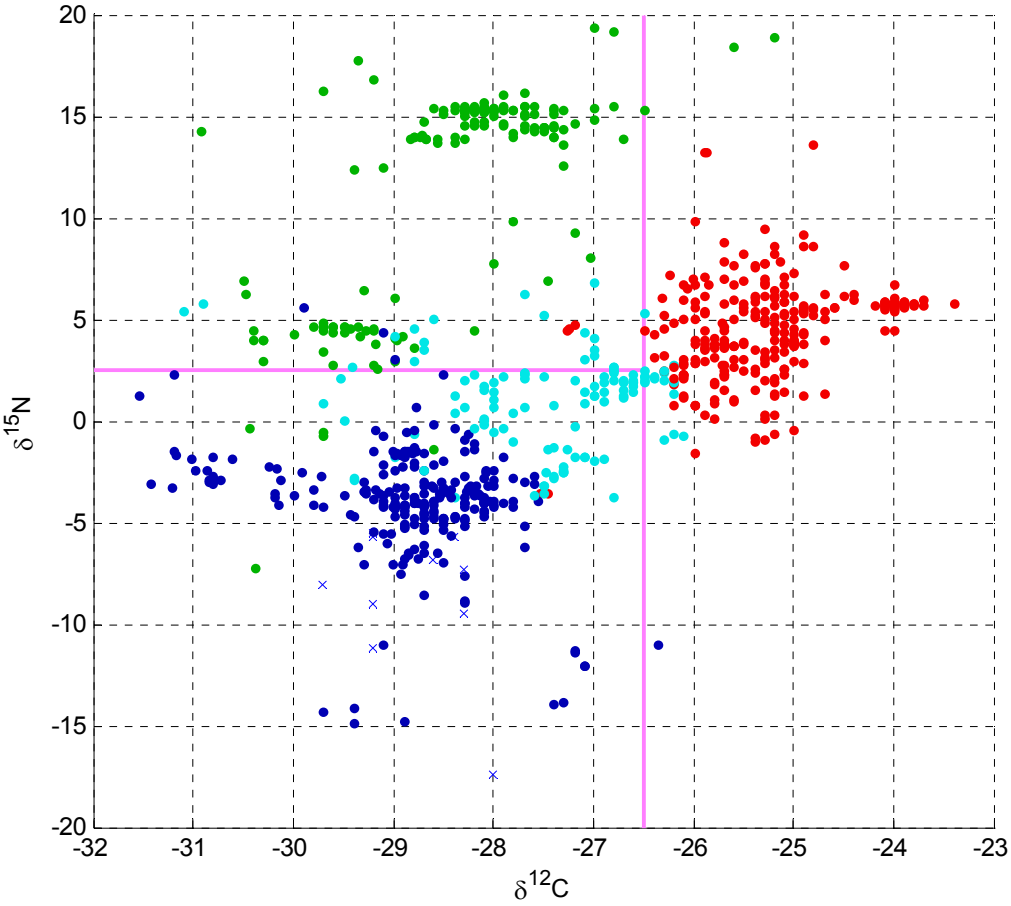
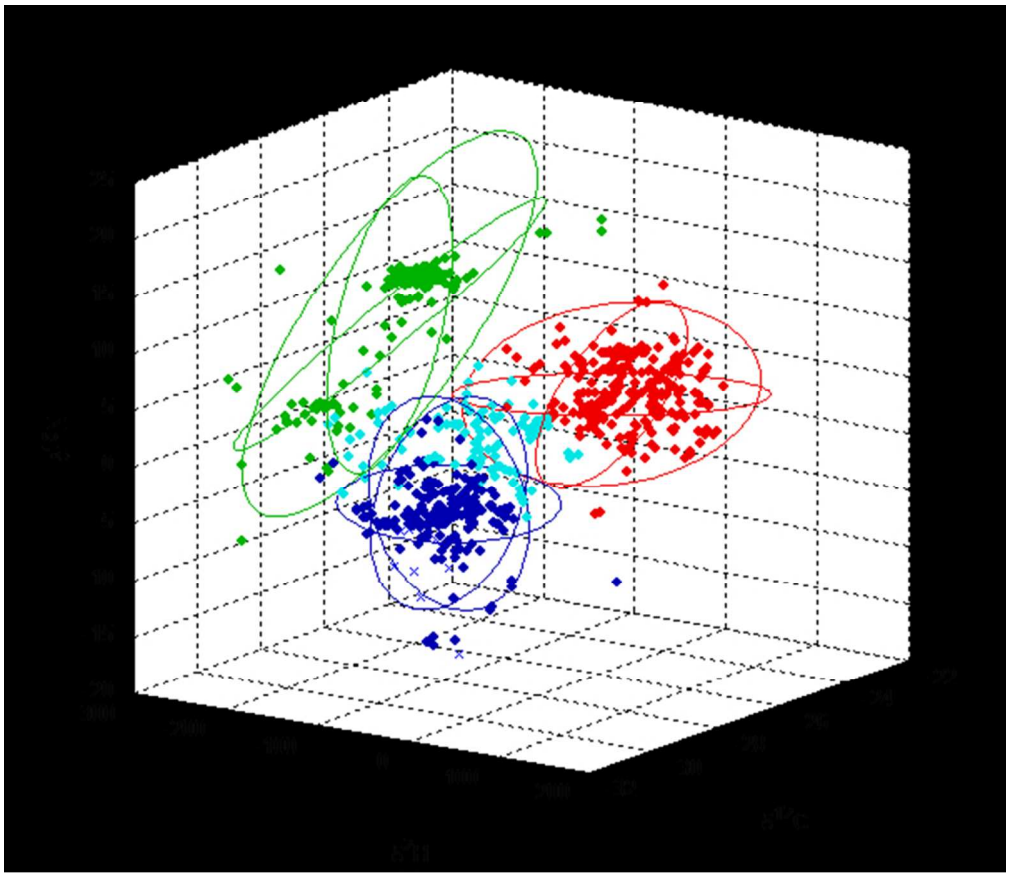


Figure 5



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