Enantiomeric resolution of methylamphetamine and ephedrine: does this affect the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ stable isotope ratios of the product?

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ABSTRACT:

The use of stable isotope ratio mass spectrometry as a profiling tool for methylamphetamine has evolved over the last decade. Stable isotope ratios of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and hydrogen ($\delta^{2}H$) of methylamphetamine are useful in determining the precursor used to manufacture methylamphetamine, and in many cases the synthetic origin of the methylamphetamine precursor. More recently samples of seized methylamphetamine show that a resolution step is being employed in the manufacturing process. We sought to determine whether the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values were affected by either a resolution performed on racemic methylamphetamine or a resolution on racemic ephedrine, a commonly used precursor to methylamphetamine. We found that for the types of resolution studied, IRMS is still able to provide useful information on the provenance of a methylamphetamine sample.

KEYWORDS:

Profiling; isotope ratios; ephedrine; methylamphetamine; enantiomeric resolution

Introduction:

Methylamphetamine is illicitly produced in large quantities throughout the world. In 2015, 132 tonnes of the drug was seized globally, with detections in East and South East Asia accounting for the greatest proportion of international seizures followed closely by North America.[1] The main methods and precursors used by clandestine operations to make methylamphetamine are shown in Figure 1.

Profiling of methylamphetamine to determine its method of manufacture and precursor employed, is carried out at the National Measurement Institute of Australia (NMIA). Until about a decade ago, the organic impurity profile of a seized methylamphetamine sample contained sufficient manufacturing by-products to determine the methylamphetamine precursor and route of manufacture. In recent years the majority of methylamphetamine profiled at NMIA is of high purity and has been described as almost pharmaceutical grade.[2] This 'pharmaceutical grade' methylamphetamine is so pure it contains insufficient manufacturing by-products which are valuable for determining the synthetic route and precursors used to make methylamphetamine. In these cases the use of isotope ratio mass spectrometry (IRMS) has proven to be most valuable. Using a combination of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values it is possible in most instances to distinguish methylamphetamine made from ephedrine/pseudoephedrine from methylamphetamine made from phenyl-2-propanone (P2P). Furthermore, using the stable isotope ratios of methylamphetamine it is possible to determine which of the three common industrial processes was used to make the ephedrine/pseudoephedrine: (i) natural - extracted from the *Ephedra* plant (ii) semi-synthetic or bio-synthetic – a procedure involving benzaldehyde and sugar or (iii) fully-synthetic – a method of synthesis starting with the precursor propiophenone.[3-12] The characteristic isotopic profiles of the methylamphetamine made from these different sources of ephedrine is graphically displayed in Figure 2. This figure is a scatter plot of $\delta^{15}N$ and $\delta^{13}C$ values for 4,350 methylamphetamine samples seized at the Australian Border between 2010 and 2017. These samples were determined to be synthesised from ephedrine/pseudoephedrine, by considering all three stable isotope ratios together with information from other chemical profiling signatures including organic impurity profiling, chiral analysis and elemental analysis. This capability has been the culmination of research work carried out over 10 years by NMIA, US Drug Enforcement Administration (USDEA) Special Testing and Research Laboratory (STRL) and research groups in Japan.[3-14] Research was also carried out to

investigate the impact that changes in synthesis conditions could have on the isotopic profile of a methylamphetamine sample. For each of the common synthetic routes, it was demonstrated that changes in synthesis conditions including stoichiometry, reaction temperature, reaction time and the pressure at which the reaction was conducted, had little effect on the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values of the final methylamphetamine product, within measurement uncertainty.[10]

In recent years, many of the seized methylamphetamine samples profiled at the NMIA indicate that a resolution step was employed in the manufacture of methylamphetamine. Most of this methylamphetamine is known to have originated from Mexico where large volumes of the drug are manufactured from P2P using a reductive amination procedure. The racemic methylamphetamine product is subjected to an efficient optical resolution process which often yields an enantiomerically pure product.[15, 16] Presumably this is to enrich the sample with the more physiologically active (+)-methylamphetamine. Enantiomerically pure (+)-methylamphetamine is formed from (-)-ephedrine or (+)-pseudoephedrine. This is typical if the ephedrine/pseudoephedrine is sourced from pharmaceutical preparations.[3] A racemic mixture of (±)-methylamphetamine is formed if P2P is used or if the ephedrine is of a fully-synthetic origin, i.e. made from propiophenone and therefore racemic.[11] In Mexico large quantities of (±)-methylamphetamine are being synthesised from P2P, which is then resolved to yield (+)-methylamphetamine.[2] There is also evidence to suggest that large quantities of (+)/(-)-methylamphetamine are produced from (+)/(-)-ephedrine and (+)/(-)-pseudoephedrine which are also being resolved. In light of this we sought to investigate what effect an enantiomeric resolution of the methylamphetamine product or precursor ephedrine would have on the final isotopic profile of the resolved product. Would any changes observed in the isotopic profile be significant enough to affect the classification of the synthetic origin of the precursor of methylamphetamine?

Experimental

Reagents and Chemicals

All reference materials, internal standards and surrogate standards used in the analysis of samples were obtained from the Chemical Reference Materials section of NMIA.

Phenylacetone (Product #159195000, Lot #A0240623, > 99%) and methylamine (40%) (Product #126230010, Lot #A0309285) were obtained from Acros Organics (Geel, Belgium). Hypophosphorous acid (50%) (Product #85454G, Lot #K23049606) was obtained from BDH Laboratory Supplies (Poole, England). Acetone, dichloromethane, diethyl ether, ethanol, glacial acetic acid (100%), methanol and propan-2-ol were obtained from Merck (Kilsyth, VIC, Australia). *N*-Methylhomoveratrylamine.HCl (98%) and hydrochloric acid (34-37%, Product code S010401-CHEQ09, Lot #4114121) were obtained from Seastar Chemicals Inc. (Sidney, Canada). *O,O'*-Dibenzoyl-L-tartaric acid (98%, Product #345849, Lot #STBC8391V), *O,O'*-dibenzoyl-D-tartaric acid (≥ 98%, Product #163449, Lot #STBC4216V), D-(-)-tartaric acid (99%, Product #483796, Lot #10804DDV), L-(+)-tartaric acid (99.5%, Product #251380, Lot #MKAA0180), sodium borohydride (≥ 98%, Product #452882, Lot #SHBB1090V), bromine (Product #207888, Lot #SHBF4242V) and 1-phenyl-1-propanone (99%, Product #P51605, Lot #1451881V) were obtained from Sigma-Aldrich (Castle Hill, NSW, Australia). Sodium hydroxide pellets, iodine and sodium sulfate (anhydrous, granular) were obtained from UNIVAR Ajax Finechem (Seven Hills, NSW, Australia). High purity oxygen (> 99.5%), ultra-high purity helium (> 99.99%), high purity carbon dioxide (> 99.5%), ultra-high purity hydrogen (> 99.99%) and ultra-high purity nitrogen (> 99.99%) were obtained from BOC gases (Sydney, Australia). Tin and silver capsules (3.3 mm x 5 mm) were obtained from IVA Analysentechnik (Meerbusch, Germany). DEA custom injection buffer was obtained from Microsolv (Eatontown, USA). Maleic acid (Product #QNMR010; Lot # 10_Q_02), (-)-ephedrine.HCl, (+)-ephedrine.HCl, (-)-pseudoephedrine.HCl, (+)-pseudoephedrine.HCl, (+)-methylamphetamine.HCl and (-)-methylamphetamine.HCl were obtained from the National Measurement Institute of Australia (North Ryde, NSW, Australia).

Synthetic Chemistry

Synthesis of Ephedrine hydrochloride

Minor modifications were made to the procedure outlined by Salouros *et al*. [11]

Synthesis of 2-bromo-1-phenyl-1-propanone

2-Bromo-1-phenyl-1-propanone was prepared by a variation of the method described by Schmidt [17] to yield a pale yellow oil (yield: 97%, purity: 93.5%). Product identity was confirmed by comparison to a certified reference material.

Synthesis of 2-methylamino-1-phenyl-1-propanone

2-Methylamino-1-phenyl-1-propanone was prepared from 2-bromo-1-phenyl-1-propanone using a modified method as described by DeRuiter *et al.* [18] to yield a dark yellow oil (yield: 85%, purity: 95%). Product identity was confirmed by comparison to a certified reference material.

Synthesis of ephedrine hydrochloride

(±)-Ephedrine hydrochloride was prepared by reducing 2-methylamino-1-phenyl-1-propanone with sodium borohydride, using a modification of the method described by Salouros *et al* [19]. This yielded a white crystalline solid (yield: 55%, purity 97%), whose identity was confirmed by comparison to a certified reference material. The ratios of ephedrine enantiomers were determined[1] based on peak areas calculated from chiral capillary electropherogram: (+)-ephedrine = 49%, (-)-ephedrine = 51%.

Synthesis of Methylamphetamine hydrochloride from Ephedrine hydrochloride

Methylamphetamine hydrochloride was synthesised from ephedrine hydrochloride using a HI / red phosphorus procedure as detailed in Collins *et al*.[7] to yield a white crystalline solid (yield: 75%, purity: 99.1%) whose identity was confirmed by comparison with a certified reference material.

Synthesis of Methylamphetamine hydrochloride from Phenyl-2-propanone

[1] Chiral analysis found minor amounts of (-) and (+)-pseudoephedrine in the combined bulk (±)-ephedrine.HCl. For simplicity, the (-)-pseudoephedrine has been included as part of the (+)-ephedrine pool, as these diastereomers will both generate (+)-methylamphetamine as the final product. Similarly, (+)-pseudoephedrine has been included in the (-)-ephedrine pool, as these diastereomers will both generate (-)-methylamphetamine. From the perspective of the investigation aims, it is the chirality of the final methylamphetamine product that is of importance. From this point, only (-)- and (+)-ephedrine will be referred to.

Methylamphetamine was synthesised from P2P via a reductive amination procedure as detailed by Salouros *et al.* [19] to yield a white crystalline solid (yield: 86%, purity 99%) whose identity was confirmed by comparison with a certified reference material.

Resolution of (±)-ephedrine with L- and D- *O,O'*-dibenzoyltartaric acid (DBTA)

The optical resolution of (±)-ephedrine was conducted using a method appropriated from the literature,[20,21] and a solvent system from Kozma and Fogassy[22] in which a two-phase, three component solvent system was employed for the resolution.

Resolution of (±)-ephedrine with D-DBTA

Step 1: Formation of the D-DBTA diastereomeric salt

(±)-Ephedrine.HCl (3.5 g) was dissolved in water, basified to pH 10-12 with 25% NaOH (aq) solution and extracted with dichloromethane. The dichloromethane was removed by rotary evaporation to yield (±)-ephedrine free base as a pale yellow waxy solid. The solid was dissolved in a mixture of dichloromethane and distilled water and stirred for 5 minutes. To this solution, dropwise, a solution of a 0.25 molar equivalent of D-DBTA dissolved in a dichloromethane and methanol mixture. The suspension was stirred for 10-15 minutes and left for 24 hours at 5 ^{o}C. The resulting precipitate was filtered to yield the diastereoisomeric salt of ephedrine and D-DBTA (5.3 g).

Step 2: Retrieval of enantiomerically enriched (-)-ephedrine.HCl

The (-)-ephedrine-D-DBTA salt formed in Step 1 was dissolved in dilute hydrochloric acid (2 M) and the aqueous layer washed with diethyl ether . The water from the aqueous layer was removed by rotary evaporation to yield enantiomerically enriched (-)-ephedrine.HCl, which was re-crystallised from hot absolute ethanol (average yield 85%).

Step 3: Retrieval of enantiomerically enriched (+)-ephedrine.HCl

The solvent mixture from the liquid filtrate generated in Step 1 was removed under reduced pressure and the resultant residue was dissolved in water, basified to pH 10-12 with 25% NaOH (aq) solution and extracted with dichloromethane. The organic solvent was removed under reduced pressure to yield a pale yellow oil. This was dissolved in cooled propan-2-ol, acidified with concentrated hydrochloric acid (37% v/v) and acetone added resulting in the precipitation of enantiomerically enriched (+)-ephedrine.HCl (average yield 85%).

Resolution of (±)-ephedrine with L-DBTA

A similar procedure for the resolution of (±)-ephedrine with L-DBTA was performed as was described above (Step 1 to Step 3). In this case, the L-DBTA resolving agent crystallised preferentially with (+)-ephedrine, leaving (-)-ephedrine in the filtrate.

Resolution of (±)-methylamphetamine using L- and D- tartaric acid (TA)

The optical resolution of (±)-methylamphetamine was conducted using the Pope-Peachey[23] method described by Kozma *et al*.[24]

Resolution of (±)-methylamphetamine with L-TA

Step 1: Formation of the L-TA diastereomeric salt

(±)-Methylamphetamine.HCl (1.3 g) was dissolved in water and basified to pH 10-12 with 25% NaOH (aq) solution. The aqueous solution was extracted with dichloromethane and the solvent was removed under reduced pressure yielding a pale yellow oil. The oil was combined with an equimolar amount of the same batch of (±)-methylamphetamine hydrochloride salt (1.3 g) and dissolved in absolute ethanol. To this was added, dropwise, a 0.5 molar equivalent solution of L-TA (1.05 g) dissolved in absolute ethanol and the mixture stirred at room temperature for 24 hours. This resulted in the formation of the diastereoisomeric salt of methylamphetamine and L-TA (2.1 g).

Step 2: Retrieval of enantiomerically enriched (-)-methylamphetamine.HCl

The (-)-methylamphetamine-L-TA diastereoisomeric salt was dissolved in water and basified to pH 10-12 with 25% NaOH (aq) solution. The aqueous layer was extracted with dichloromethane and the solvent was removed under reduced pressure to yield a pale yellow oil. The oil was dissolved in cooled propan-2-ol, acidified with concentrated hydrochloric acid (37% v/v) and diethyl ether added which resulted in the precipitation of enantiomerically enriched (-)-methylamphetamine.HCl as white crystals (average yield 70%).

Step 3: Retrieval of enantiomerically enriched (+)-methylamphetamine.HCl

Absolute ethanol from the filtrate in Step 1 was removed by rotary evaporation and the resultant solid re-crystallised from absolute ethanol to yield enantiomerically enriched (+)-methylamphetamine.HCl as white crystals (average yield 70%).

Resolution of (±)-methylamphetamine with D-TA

A similar procedure for the resolution of (±)-methylamphetamine with D-TA was performed with similar yields. In this case, the D-TA resolving agent complexed preferentially with (+)-methylamphetamine, leaving (-)-methylamphetamine in the filtrate.

Sample Identification

Enantiomeric composition by Capillary Electrophoresis (CE)

The enantiomeric composition of the ephedrine and methylamphetamine products (%, w/w) was determined using an Agilent Technologies 7100 capillary electrophoremeter (CE) with photodiode array detector (190-400nm) at a wavelength of 195 nm. Samples were separated using an Agilent HPCE standard capillary (i.d. 50 μL x 56 cm) using a DEA custom buffer (MicroSolv) at an applied voltage of 30 kV at 15 ºC. Data was acquired and reprocessed using 3D-CE Chemstation software version B.03.01. Methods were based on those published by Lurie *et al.*[25,26] Approximately 4 mg of ephedrine or methylamphetamine HCl was dissolved in 5 mL of DEA custom run buffer solution and further diluted with the buffer and *N*-methylhomoveratrylamine internal standard solution (~2 mg/mL in custom run buffer) to achieve a final concentration of 0.8 mg/mL. A mixed standard solution of (±)-ephedrine, (±)-pseudoephedrine, (±)-methylamphetamine and *N*-methylhomoveratrylamine, were used to identify and determine the isomeric composition.

Stable Isotope Ratio Mass Spectrometry

Measurements of the stable isotopes $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ of the precursor, intermediates, reagents and ephedrine products described here were determined using the isotope ratio mass spectrometry methods described in our previous work.[7, 9-12] Calibration and quality control of Elemental Analyser/Thermal Conversion – Isotope Ratio Mass Spectrometry (EA/TC-IRMS) measurements is outlined in our previous work.[7, 9-12]

The ability to determine isotopic fractionation patterns in the synthesis and resolution of ephedrine and methylamphetamine as being comparable or distinct is dependent on an estimation of measurement uncertainty. This was performed by combining bias and precision contributions in quadrature according to the "Guide to Uncertainty Measurement" (GUM) uncertainty framework.[27,28] 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 two high purity methylamphetamine.HCl and ephedrine.HCl quality control samples analysed every 3 samples, which were calibrated against international reference materials. The uncertainty estimates were used to assess whether the measured $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values were significantly different at a confidence level of 95%.

Results and Discussion:

Optical resolution of Methylamphetamine

The method used to resolve racemic methylamphetamine was selected on the basis of evidence of its use in illicit manufacture of methylamphetamine.[15] The "Pope-Peachey" method employs a half-molar equivalent of the resolving agent with the addition of an achiral agent of a similar ionisation state.[21,22] This functions by imparting an increased solubility of the salt of one enantiomer which then remains in solution, and permits the other enantiomer to be in equimolar proportions with the resolving agent in the solvent, thereby more readily precipitating out as a diastereoisomeric salt.[29] The resolving agent selected for this resolution, tartaric acid (TA), is diprotic but only forms a 1:1 tartrate salt with methylamphetamine.[29,30] The L- and D- enantiomers of TA preferentially crystallise with (-)- and (+)-methylamphetamine respectively, leaving behind the other enantiomer in the filtrate along with H^+ and Cl^- ions. Removal of the solvent from the filtrate yields the enantiomerically enriched methylamphetamine hydrochloride salt.

Impact on Stable Isotope Ratios

Methylamphetamine made from Racemic Ephedrine

Two batches of (±)-methylamphetamine hydrochloride were synthesised from two different sources of (±)-ephedrine hydrochloride. A total of twelve resolutions were carried out on the (±)-methylamphetamine hydrochloride (MA Synthesis Batch 1 and MA Synthesis Batch 2) producing twenty-four products: twelve samples of predominately (+)-methylamphetamine and twelve samples of predominately (-)-methylamphetamine. Half of the resolution experiments were conducted using L-TA (Resol._1 to Resol._4 and Resol._11 to Resol._12) and the other half using D-TA (Resol._5 to Resol._10). The stable isotope ratios of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and hydrogen ($\delta^{2}H$) of the precursors and resolved methylamphetamine products are shown in Table 1.

All twenty-four products of resolved methylamphetamine have $\delta^{13}C$ values that correspond within measurement uncertainty to the $\delta^{13}C$ value of the unresolved (±)-methylamphetamine (Table 1) demonstrating that enantiomeric resolution does not affect the $\delta^{13}C$ values of either enantiomeric resolved product, i.e. the (+)-methylamphetamine and the (-)-methylamphetamine products. This is not surprising given the carbons in the methylamphetamine molecule are non-reactive and non-exchangeable during the enantiomeric resolution process.

In most instances the $\delta^{2}H$ values of the resolved methylamphetamine were the same within measurement uncertainty to that of the unresolved methylamphetamine (Table 1). In the cases where isotopic mass balance was not conserved i.e. Resol._9 through to Resol._12, a possible explanation for this is equilibrium fractionation. A detailed discussion is given further on. Nonetheless the difference in $\delta^{2}H$ is insignificant in that it does not affect the ability to correctly determine the methylamphetamine precursor's synthetic origin. In other words the values are consistent with ephedrine made via a fully-synthetic procedure.[11]

The $\delta^{15}N$ values of the resolved methylamphetamine were more prone to isotopic fractionation than the other two elements. For example, in Table 1, Resolution #: Resol._9 (D-TA) shows that the (+)-methylamphetamine product (MA9_AS), which complexes preferentially with the D-TA, is more depleted in $\delta^{15}N$ than the (-)-methylamphetamine product (MA9_BL) which stays behind in the filtrate with H^{+} and Cl^{-} ions. One plausible explanation for this observed $\delta^{15}N$ depletion in the D-TA-(+)-methylamphetamine salt (MA9_AS) may be attributed mainly to equilibrium (or phase change) fractionation and in part to kinetic isotopic fractionation effects occurring during the process of diastereoisomeric salt formation. A similar scenario was presented by Casale *et al*.[31] and later, David *et al*.,[32] who noticed that the $\delta^{15}N$ values of free base cocaine and methylamphetamine, respectively, became more depleted in ^{15}N following successive attempts to convert the drugs to their hydrochloride salt forms; in other words a Rayleigh fractionation where the isotope ratio of the salt product is a function of the fraction precipitated from solution. In the process of salt formation, the nitrogen atom of the drug free base gains a proton from the hydrochloric acid source to become positively charged and associates with the negatively charged chloride, thereby precipitating as a hydrochloride salt from the solution. It was found that ^{15}N-containing cocaine and methylamphetamine molecules precipitated more readily from

solution relative to those containing ^{14}N. This is because the heavier isotope (^{15}N) is capable of forming a stronger bond to the proton which results in the ^{15}N-containing tertiary ammonium cation that is formed to be present in solution and precipitate with the chloride ion. The outcome of this isotopic fractionation was that the heavier isotope constitutes a larger proportion of the earlier precipitates than the latter ones. With respect to Experiment #: Resol._9 (D-TA), the (-)-methylamphetamine product (MA9_BL) which gets left behind in the filtrate is more enriched in ^{15}N owing to its stronger bond formation-interaction with the free protons in solutions, while the (+)-methylamphetamine product (MA9_AS) which complexes preferentially with the D-TA is more enriched in ^{14}N.

Regardless of the changes in the $\delta^{15}N$ values of the resolved methylamphetamine, these changes did not affect the ability to correctly assign the precursor synthetic origin, i.e. the $\delta^{15}N$ values are consistent with methylamphetamine made from fully-synthetic ephedrine.

One other contributing factor for the observed H and N isotopic fractionation in Resol._9 through Resol._12 may be attributed to the fact that these experiments had an average yield of 65% compared to Resol._1 through Resol._8 which on average had a yield of 90%. The experiments Resol._1 through Resol._8 demonstrate that isotopic mass balance can be achieved without isotopic fractionation of C, N and H when the majority of both isomers are recovered. This further illustrates that isotope ratios are not affected by resolution.

Methylamphetamine made from phenyl-2-propanone (P2P)

Four sets of optical resolutions, using the "Pope-Peachy" method, were carried out on the same batch of (±)-methylamphetamine (MA Synthesis Batch 3) made from P2P (Table 2). This resulted in eight products: four batches of predominately (+)-methylamphetamine (P1.1_AL, P2.1_AL, P3.1_BS, P4_BS) and four batches of predominately (-)-methylamphetamine (P1.1_BS, P2.1_BS, P3.1_AL, P4_AL). The $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values of the eight resolved products are the same within measurement uncertainty as those of the unresolved (±)-methylamphetamine. These findings are consistent with those obtained above for the resolution of (±)-methylamphetamine made from (±)-ephedrine. The ability to correctly classify the precursor used to make the methylamphetamine after having undergone resolution, based on the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values is not compromised.

Three seized methylamphetamine samples determined to have been synthesised from P2P were also resolved using the "Pope-Peachy" method (Table 2 – Sample A, B and C). Note the methylamphetamine precursor determination for these samples was based on a combination of the organic impurity profile, chirality, elemental composition and stable isotope ratios. Following optical resolution of Samples A, B and C, the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values remained the same within measurement uncertainty to those of the unresolved methylamphetamine (Table 2).

Table 2 also shows the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values of methylamphetamine which were resolved a second time. No changes, within measurement uncertainty, were noted in the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values of these samples (Resol._1.2, Resol._2.2, Resol._3.2, Resol. 5, Resol. 6 and Resol. 7). For example the methylamphetamine resolution product P1.1_BS from Resol_1.1 contained predominately (-)-methylamphetamine (69.9%) and (+)-methylamphetamine (30.1%). This product was resolved again with L-TA to yield a more enantiomerically enriched product P1.2_BS containing 88.4% (-)-methylamphetamine and 11.6% (+)-methylamphetamine. The resolved methylamphetamine products P1.1_BS (first resolution) and P1.2_BS (second resolution) have $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values the same within measurement uncertainty as those of the original batch of (±)-methylamphetamine; no apparent changes in the isotopic profile was observed in all the three replicate experiments.

Impact on Chirality

The level of enantiomeric enrichment achieved using the Pope-Peachey method of resolution was fairly modest, with the major enantiomer from individual resolutions ranging from 66.3% to 92.3%, and varying little with respect to the TA resolving agent enantiomer employed (Table 1 and Table 2). These results do not line up with what has been observed with the majority of seizures profiled at the NMIA. Many seized methylamphetamine samples known to have been synthesised from P2P are almost enantiomerically pure. It is feasible that clandestine facilities are conducting more than one resolution on the same batch of methylamphetamine product to yield a more enantiomerically enriched product. In our experiments we found that a second round of optical resolutions performed on already "once-enantiomerically enriched" methylamphetamine fractions, resulted in a higher level of enantiomeric enrichment (P1.2_BS, P2.2_BS, P3.2_BS, P5_S, P6_S and P7_S Table 2).

Optical resolution of Ephedrine

The resolution method employed for resolving mixtures of (±)-ephedrine, used a "two phase, three component" solvent system, which comprised a water/dichloromethane two-phase system and methanol as an intermediate solvent, miscible in both phases.[29] This method is known to be used by pharmaceutical companies for large-scale production [20, 33] and reported to have been used by clandestine laboratories.[15]

Impact on Stable Isotope Ratios

Two batches of (±)-ephedrine were synthesised and a number of resolutions conducted using a quarter-molar equivalent of DBTA resolving agent to yield products enantiomerically enriched in either (-)- or (+)-ephedrine. The resolved ephedrine products were subsequently converted to either (+)- or (-)-methylamphetamine respectively. The $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values are shown in Table 3.

The $\delta^{13}C$ values of the sixteen ephedrine products (E1_AL to E8_BL) range between -29.0‰ to -29.3‰ and are comparable to the $\delta^{13}C$ values of the unresolved (±)-ephedrine precursor ($\delta^{13}C$ = -29.1‰). Similarly the $\delta^{13}C$ values of the four ephedrine products (E9_AL to E10_BS) range between -27.9‰ to -28.0‰ and are the same within measurement uncertainty as the $\delta^{13}C$ values of the unresolved (±)-ephedrine precursor ($\delta^{13}C$ = -27.9‰). These results demonstrate that enantiomeric resolution of the precursor ephedrine does not affect the $\delta^{13}C$ isotope ratios. The twenty ephedrine products were then converted to methylamphetamine hydrochloride (E1_AL_MA to E10_BS_MA). No significant change was observed in the $\delta^{13}C$ values of the twenty methylamphetamine products compared to the $\delta^{13}C$ values of the ephedrine from which they were synthesised.

Table 3 shows that the resolution process did influence the $\delta^{2}H$ value of the resolved ephedrine product. For example for Resol._1 the two resolved products E1_AL (predominately (-)-ephedrine (97.5%)) and E1_BS (predominately (+)-ephedrine (97.5%)) had $\delta^{2}H$ values of -55‰ and -20‰ respectively. This difference can be explained if we consider the formation of the diastereomeric salt. The ephedrine nitrogen atom acquires a proton to gain a positive charge. Deuterium (^{2}H) forms a stronger bond to nitrogen than does hydrogen (^{1}H), and since this hydrogen gain is in rapid equilibrium, the deuterium-nitrogen species persists for longer in solution relative to hydrogen-nitrogen species.[34] What this

means is the deuterium-nitrogen species is more likely to associate with the negatively charged carboxyl oxygen of the resolving agent and therefore precipitate from solution as the diastereomeric salt.

Regardless of this isotopic effect observed in the resolved ephedrine products, when these resolved ephedrine products were converted to methylamphetamine the δ^2H values were the same within measurement uncertainty. The change in δ^2H value of the methylamphetamine products compared to the δ^2H values of the unresolved precursor ephedrine (Table 3) has been previously explained in our earlier work [7] however the difference in isotopic fractionation between one synthesis to the other is surprising. For example E1_AL_MA and E1_BS_MA had δ^2H values of -91‰ and -92‰ respectively, while their respective precursors E1_AL and E1_BS had δ^2H values of -55‰ and -20‰. A plausible explanation for the difference in isotopic fractionation following the conversion of ephedrine to the methylamphetamine could be due to the labile proton on the nitrogen. This labile proton which is responsible for the deuterium enrichment observed in the resolved ephedrine, is likely to undergo rapid exchange in aqueous solution to give an isotope ratio for N-H that reflects that of the bulk solution. To test this a sample of E1_AL (δ^2H -55‰) and E1_BS (δ^2H -20‰) were dissolved in an equivalent amount of hypophosphorous acid used in the methylampehtamine synthesis, and then heated for an hour. The δ^2H values of the ephedrine samples following this reaction were determined to be -53‰ and -51‰ respectively.

Even with these isotopic changes, they are insignificant in that they do not affect the precursor synthetic origin classification, i.e. the δ^2H values are consistent with methylamphetamine made from ephedrine derived from a fully-synthetic route.

As with the methylamphetamine resolution experiments discussed earlier, changes in δ^{15}N values were also observed in the resolved ephedrine products. A similar mechanism of (diastereoisomeric) salt formation occurs during the resolution of ephedrine (Figure 3). Here, a labile proton from the DBTA resolving agent, or from water in the resolution medium, may be acquired by the nitrogen atom of the ephedrine amine group, becoming a positively charged secondary ammonium ion. The ^{15}N forms a stronger bond with hydrogen, and given it is in rapid equilibrium, the ^{15}N-proton species stays in solution for longer compared to the ^{14}N-proton species. As a result the ephedrine containing ^{15}N is more likely to form an ionic

interaction with the negatively charged carboxylic oxygen atom of the resolving agent and precipitate from solution, resulting in more isotopically enriched ephedrine constituting a larger proportion of the diastereoisomeric salt precipitate.

It is noteworthy, regardless at which point the resolution was carried out the $\delta^{13}C$ and $\delta^{2}H$ values of the resolved final methylamphetamine products (i.e. both (+)-methylamphetamine and (-)-methylamphetamine products) are the same within the measurement uncertainty. Table 1 shows that $\delta^{13}C$ and $\delta^{2}H$ values for the resolved methylamphetamine products synthesised from racemic ephedrine averaged at -29.4‰ and -87‰ respectively. Table 3 shows that the $\delta^{13}C$ and $\delta^{2}H$ values for the resolved methylamphetamine products synthesised from resolved racemic ephedrine averaged at -29.3‰ and -91‰ respectively. These results indicate that if the same batch of racemic ephedrine is used to synthesise enantiomerically pure methylamphetamine then regardless if the resolution is carried out on the precursor (i.e. racemic ephedrine) or on the racemic methylamphetamine (made from the racemic ephedrine and then resolved), the $\delta^{13}C$ and $\delta^{2}H$ values are not measurably altered.

Impact on Chirality

This method of resolution proved to be quite effective, as supported by the findings of other studies.[22] Both the L-DBTA-(+)-ephedrine, and D-DBTA-(-)-ephedrine diastereoisomeric salt pairs exhibited decreased solubility and thus precipitated, while their more soluble counterparts (L-DBTA-(-)-ephedrine and D-DBTA-(+)-ephedrine, respectively) remained in the resolution filtrate. Six repetitions of this resolution were carried out using L-DBTA and four using D-DBTA. The results of each of these resolutions were comparable with the major enantiomer from individual resolutions ranging from 86.9 to 100%, and varying little with respect to the DBTA resolving agent enantiomer used (Table 3). While the enantioselectivity using DBTA proved better than the TA method, the isotope mass balance in this case was not great which resulted in fractionation.

Conclusion

Resolution of racemic methylamphetamine and racemic ephedrine resulted in products whose stable isotope ratios were not compromised in so far as the ability for these values to be used to classify the synthetic origin of the methylamphetamine precursor. The study also demonstrated that regardless at which point a resolution is conducted, i.e. at the precursor

level or on the final methylamphetamine product, the stable isotope values were consistent with those isotope values prior to resolution.

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Table 1: Chiral composition and IRMS results of optically resolved methylamphetamine fractions. [1] L and S refer to whether the resolution product came from the diastereomeric salt (the solid fraction, "S"), or from the filtrate (the liquid fraction, "L").

Precursor	Methylamphetamine resolution results						
	Resolution # (resolving agent)	Resolution Product[2]	% Enantiomer composition		$\delta^{13}C_{VPDB}$ (‰)	$\delta^{15}N_{Air}$ (‰)	$\delta^{2}H_{VSMOW}$ (‰)
			(+)-MA	(-)-MA			
(±)-Methylamphetamine.HCl (MA Synthesis Batch 1) *(-)-MA = 50.0 %* *(+)-MA = 50.0 %* $\delta^{13}C$: -29.3 ± 0.4 ‰ $\delta^{15}N$: -6.1 ± 0.5 ‰ $\delta^{2}H$: -84 ± 4 ‰ *Synthesised from:* **(±)-Ephedrine.HCl** (E Batch 1) *(-)-Ephedrine = 51.0 %* *(+)-Ephedrine = 49.0 %* $\delta^{13}C$: -29.1 ± 0.4 ‰ $\delta^{15}N$: -5.5 ± 0.5 ‰ $\delta^{2}H$: -52 ± 4 ‰	Resol._1 (L-TA)	MA1_AL	85.1	14.9	-29.4	-6.2	-87
		MA1_BS	19.3	80.7	-29.6	-6.1	-86
	Resol._2 (L-TA)	MA2_AL	81.1	18.9	-29.5	-6.1	-91
		MA2_BS	18.4	81.6	-29.6	-6.1	-87
	Resol._3 (L-TA)	MA3_AL	78.3	21.7	-29.4	-6.2	-87
		MA3_BS	20.5	79.5	-29.4	-6.2	-85
	Resol._4 (L-TA)	MA4_AL	75.7	24.3	-29.4	-6.1	-88
		MA4_BS	12.2	87.8	-29.5	-5.9	-87
	Resol._5 (D-TA)	MA5_AS	80.8	19.2	-29.4	-6.0	-84
		MA5_BL	23.5	76.5	-29.3	-6.1	-87
	Resol._6 (D-TA)	MA6_AS	79.6	20.4	-29.4	-6.0	-86
		MA6_BL	24.0	76.0	-29.4	-6.1	-92
	Resol._7 (D-TA)	MA7_AS	79.9	20.1	-29.5	-6.2	-85
		MA7_BL	24.4	75.6	-29.4	-6.1	-87
	Resol._8 (D-TA)	MA8_AS	81.6	18.4	-29.5	-6.0	-85
		MA8_BL	23.9	76.1	-29.4	-6.0	-91
(±)-Methylamphetamine.HCl (MA Synthesis Batch 2) *(+)-MA = 48.8 %* *(-)-MA = 51.2 %* $\delta^{13}C$: -28.3 ± 0.4‰ $\delta^{15}N$: -5.2 ± 0.5‰ $\delta^{2}H$: -104 ± 4‰ *Synthesised from:* **(±)-Ephedrine.HCl** (E Batch 2) *(-)-Ephedrine = 48.8 %* *(+)-Ephedrine = 51.2 %* $\delta^{13}C$: -27.9 ± 0.4‰ $\delta^{15}N$: -6.0 ± 0.5‰ $\delta^{2}H$: -69 ± 4‰	Resol._9 (D-TA)	MA9_AS	78.4	21.6	-27.9	-12	-120
		MA9_BL	33.7	66.3	-28.0	-7.5	-112
	Resol._10 (D-TA)	MA10_AS	76.8	23.2	-28.4	-16.3	-125
		MA10_BL	25.2	74.8	-27.9	-7.3	-112
	Resol._11 (L-TA)	MA11_AL	70.9	29.1	-28.2	-8.6	-112
		MA11_BS	25.2	74.8	-28.8	-14.1	-124
	Resol._12 (L-TA)	MA12_AL	68.3	31.7	-27.9	-5.4	-114
		MA12_BS	21.7	78.3	-28.5	-10.4	-117

Table 2: Chiral composition and IRMS results of optically resolved methylamphetamine fractions synthesised from P2P, following multiple resolutions using the Pope-Peachey method.
[1]L and S refer to whether the resolution product came from the diastereomeric salt (the solid fraction, "S"), or from the filtrate (the liquid fraction, "L").

Precursor	Methylamphetamine resolution results_1							Methylamphetamine resolution results_2						
	Resolution # (resolving agent)	Resolution Product[3]	% Enantiomer composition (+)-MA	% Enantiomer composition (-)-MA	$\delta^{13}C_{VPDB}$ (‰)	$\delta^{15}N_{Air}$ (‰)	$\delta^{2}H_{VSMOW}$ (‰)	Resolution # (resolving agent)	Resolution Product[1]	% Enantiomer composition (+)-MA	% Enantiomer composition (-)-MA	$\delta^{13}C_{VPDB}$ (‰)	$\delta^{15}N_{Air}$ (‰)	$\delta^{2}H_{VSMOW}$ (‰)
(±)-Methylamphetamine.HCl (MA Synthesis Batch 3) *(+)-MA = 49.9 %* *(-)-MA = 50.1 %* $\delta^{13}C$: -30.1 ± 0.4‰ $\delta^{15}N$: 19.4 ± 0.5‰ $\delta^{2}H$: -79 ± 4‰ *Synthesised from:* **P-2-P** $\delta^{13}C$: -28.3 ± 0.4‰ $\delta^{2}H$: -71 ± 4‰	Resol._1.1 (L-TA)	P1.1_AL	92.1	7.9	-30.1	19.5	-81	No resolution attempted						
		P1.1_BS	30.1	69.9	-30.1	19.2	-81	Resol._1.2 (L-TA)	Insufficient sample for analysis					
									P1.2_BS	11.6	88.4	-30.2	18.9	-81
	Resol._2.1 (L-TA)	P2.1_AL	91.3	8.7	-30.1	19.2	-82	No resolution attempted						
		P2.1_BS	29.9	70.1	-30.2	19.0	-80	Resol._2.2 (L-TA)	Insufficient sample for analysis					
									P2.2_BS	5.2	94.8	-30.2	18.9	-81
	Resol._3.1 (D-TA)	P3.1_AL	15.0	85.0	-30.1	19.3	-84	No resolution attempted						
		P3.1_BS	72.1	27.9	-30.2	18.9	-82	Resol._3.2 (D-TA)	Insufficient sample for analysis					
									P3.2_BS	96.9	3.1	-30.2	18.9	-81
	Resol._4 (D-TA)	P4_AL	7.7	92.3	-30.2	19.1	-82	No resolution attempted						
		P4_BS	78.2	21.8	-30.2	18.8	-85	No resolution attempted						

Seizure Samples	Seizure Samples Prior to Laboratory Resolution Experiment							Seizure Samples Following Laboratory Resolution Experiment						
Sample A	[redacted]		86.0	14.0	-32.2	5.3	-91	Resol._5 (D-TA)	P5_S	96.8	3.2	-32.2	5.2	-89
									Insufficient sample for analysis					
Sample B			30.0	70.0	-34.7	7.3	-98	Resol._6 (L-TA)	P6_S	8.3	91.7	-34.8	7.6	-94
									Insufficient sample for analysis					
Sample C			65.0	35.0	-30.9	9.5	-78	Resol._7 (D-TA)	P7_S	91.3	8.7	-30.5	9.1	-71
									Insufficient sample for analysis					

Table 3: Chiral composition and IRMS results of optically resolved ephedrine fractions and their methylamphetamine products. [1] L and S refer to whether the resolution product came from the diastereomeric salt (the solid fraction, "S"), or from the filtrate (the liquid fraction, "L")

Precursor	Ephedrine resolution results							Methylamphetamine synthesis results					
	Resolution # (resolving agent)	Resolution Product[4]	% Enantiomer composition (-)-Eph	% Enantiomer composition (+)-Eph	$\delta^{13}C_{V\ PDB}$ (‰)	$\delta^{15}N_{Air}$ (‰)	$\delta^{2}H_{VS\ MOW}$ (‰)	MA Synthesis Product[1]	% Enantiomer composition (+)-MA	% Enantiomer composition (-)-MA	$\delta^{13}C_{V\ PDB}$ (‰)	$\delta^{15}N_{Air}$ (‰)	$\delta^{2}H_{VS\ MOW}$ (‰)
(±)-Ephedrine.HCl (E Batch 1) *(-)-Ephedrine = 51.0 %* *(+)-Ephedrine = 49.0 %* $\delta^{13}C$: -29.1 ± 0.4‰ $\delta^{15}N$: -5.5 ± 0.5‰ $\delta^{2}H$: -52 ± 4‰	Resol._1 (L-DBTA)	E1_AL	97.5	2.5	-29.3	-8.7	-55	E1_AL_MA	97.9	2.1	-29.5	-9.2	-91
		E1_BS	2.5	97.5	-29.2	-5.1	-20	E1_BS_MA	2.1	97.9	-29.4	-5.7	-92
	Resol._2 (L-DBTA)	E2_AL	100.0	0.0	-29.2	-6.7	-58	E3_AL_MA	100.0	0.0	-29.4	-7.4	-93
		E2_BS	1.9	98.1	-29.2	-3.2	-21	E3_BS_MA	1.9	98.1	-29.4	-3.9	-90
	Resol._3 (L-DBTA)	E3_AL	95.9	4.1	-29.2	-6.1	-49	E4_AL_MA	97.3	2.7	-29.3	-6.6	-93
		E3_BS	0.0	100.0	-29.1	-3.4	-26	E4_BS_MA	0.0	100.0	-29.2	-3.7	-89
	Resol._4 (L-DBTA)	E4_AL	96.0	4.0	-29.2	-6.2	-51	E5_AL_MA	97.9	2.1	-29.3	-6.6	-91
		E4_BS	0.0	100.0	-29.1	-2.1	-19	E5_BS_MA	0.0	100.0	-29.3	-2.9	-89
	Resol._5 (D-DBTA)	E5_AS	98.4	1.6	-29.0	-2.6	-20	E6_AS_MA	100.0	0.0	-29.2	-3.1	-87
		E5_BL	2.7	97.3	-29.1	-5.7	-55	E6_BL_MA	0.00	100.0	-29.4	-6.4	-90
	Resol._6 (D-DBTA)	E6_AS	98.4	1.6	-29.1	-3.2	-19	E7_AS_MA	100.0	0.0	-29.3	-3.9	-88
		E6_BL	1.9	98.1	-29.1	-5.8	-54	E7_BL_MA	0.0	100.0	-29.4	-6.3	-93
	Resol._7 (D-DBTA)	E7_AS	97.7	2.3	-29.1	-2.5	-14	E8_AS_MA	100.0	0.00	-29.1	-2.6	-87
		E7_BL	13.1	86.9	-29.2	-8.4	-51	E8_BL_MA	5.5	94.5	-29.3	-6.9	-92
	Resol._8 (D-DBTA)	E8_AS	100.0	0.0	-29.2	-6.0	-17	E3_AS_MA	100.0	0.0	-29.3	-6.1	-89
		E8_BL	3.7	96.3	-29.3	-8.8	-52	E3_BL_MA	2.4	97.6	-29.3	-8.5	-96
(±)-Ephedrine.HCl (E Batch 2) *(-)-Ephedrine = 48.8 %* *(+)-Ephedrine = 51.2 %* $\delta^{13}C$: -27.9 ± 0.4‰ $\delta^{15}N$: -6.0 ± 0.5‰ $\delta^{2}H$: -69 ± 4‰	Resol._9 (L-DBTA)	E9_AL	100.0	0.0	-28.0	-6.6	-63	E9_AL_MA	0.0	100.0	-28.2	-6.4	-117
		E9_BS	0.0	100.0	-27.9	-2.3	-66	E9_BS_MA	100.0	0.0	-27.9	-2.6	-116
	Resol._10 (L-DBTA)	E10_AL	100.0	0.0	-28.0	-5.4	-64	E10_AL_MA	0.0	100.0	-28.1	-6.2	-119
		E10_BS	0.0	100.0	-28.0	-1.4	-49	E10_BS_MA	100.0	0.0	-28.0	-5.5	-116

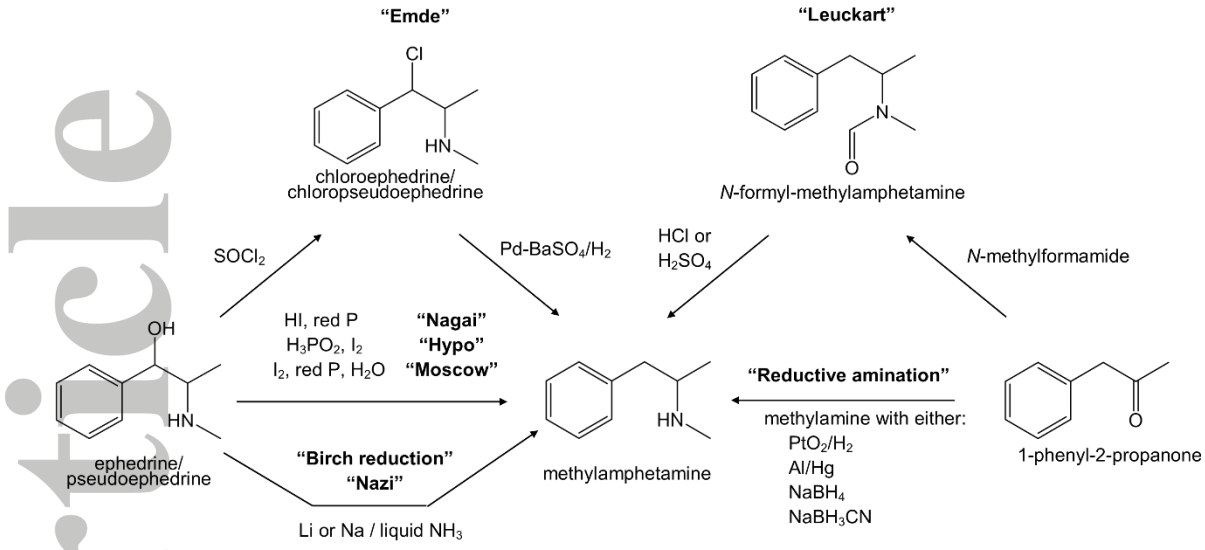

Figure 1: Common synthetic routes of clandestine methylamphetamine manufacture.

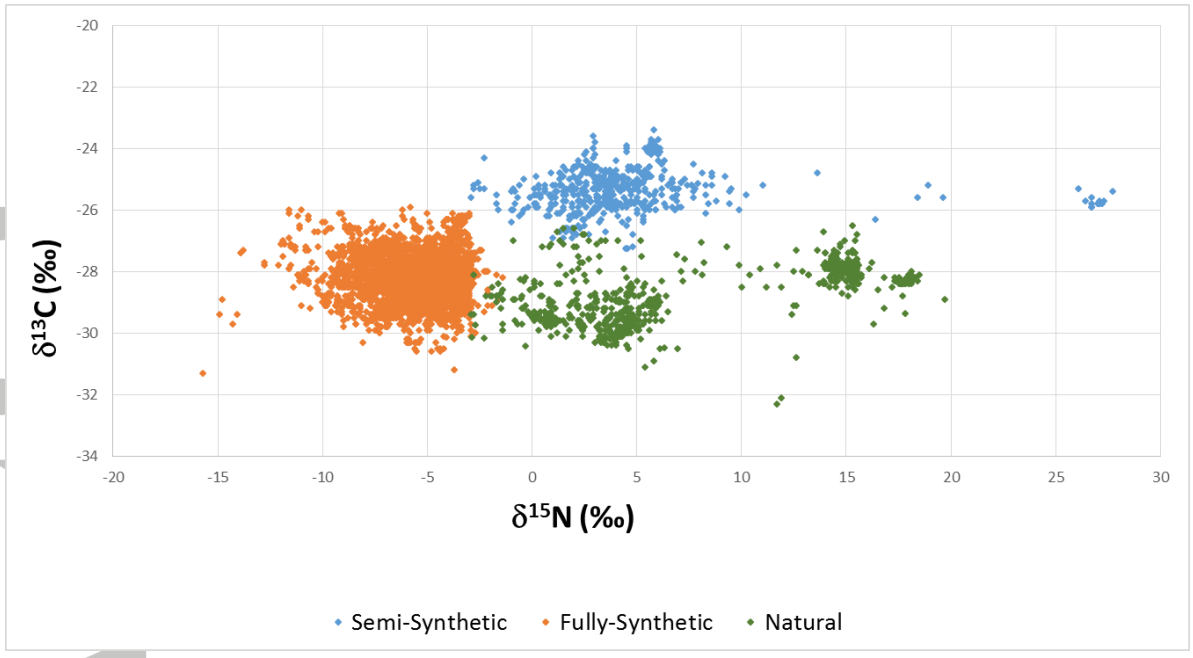

Figure 2: Nitrogen vs carbon stable isotope ratios of ephedrine/pseudoephedrine based methylamphetamine samples seized at the Australian Border between 2010 and 2017.

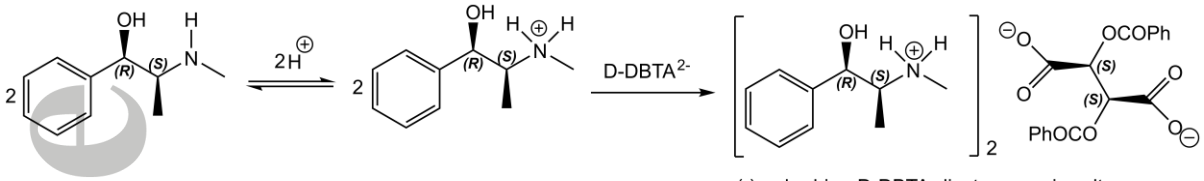

Figure 3: The (-)-ephedrine-D-DBTA salt is preferentially precipitated from solution during the resolution.