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Detection of co-extracted impurities from methamphetamine seized in Tanzania: a prediction of the source of the precursors

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Abstract

Using UHPLC-HESI-MS/MS, the study investigated the co-extracted target compounds as chemically attributable signatures for the source of ephedrine. Through the investigation, we found EPH as the primary precursor used in the clandestine synthesis of methamphetamine used in this investigation. The samples also contained co-extracted impurities such as norephedrineedrine and methylephedrineedrine. N, N-dimethylamphetamine was also discovered not as a co-extracted impurity but as a degraded product of methylephedrineedrine. The impurities profiles indicate the source of EPH from an Ephedra plant. The findings can help shed light on the origins of the ephedrine used in the clandestine synthesis of methamphetamine and the practices that existed prior to the synthesis of methamphetamine.

Keywords: *Co-extraction; Intelligence; Iphedrine; Methamphetamine; Pseudoephedrine*

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Introduction

Methamphetamine is an illicit drug that has been increasingly emerging in the Tanzania drug market. Recently, large volumes of seizures whose sources have not been established have been capsized along the Tanzanian coast. Although the drug is majorly produced in East and South Asia and North America, East Africa has become a significant conduit for illicit drugs from South Asia to other parts of the world (Makangara and Mulima, 2021; I. Onoka, Banyika, Banerjee, Makangara, and Dujourdy, 2020).

Profiling methamphetamine (MA) based on impurity profiles is one of the significant intelligence approaches used to establish strategic, operational and tactical intelligence of illicit drugs c. The impurities help to establish synthetic routes, elemental signatures, and tracking enriched through different precursor chemicals, reagents, equipment, and various synthetic stages (Andersson, Lock, *et al.* 2007; Baechler *et al.* 2015; L. Dujourdy *et al.*, 2008; Kunalan, Daéid, Kerr, Buchanan, and McPherson, 2009; Li, Brown, and Toske, 2018; Medder, Nash, and Kirkbride, 2021; Isaac Onoka, Toyi, Nath, Makangara, and Dujourdy, 2020). Forensic intelligence of methamphetamine based on impurity profiles is a monitoring process that involves establishing chemical signatures from a methamphetamine sample. From a forensic perspective, chemical signatures are defined based on the amount of MA in the sample, type and amount of adulterants, type and amount of diluents and recipients, trace compounds such as organic impurities, residual solvents, and inorganic impurities, as well as isotope ratios (L.

Dujourdy *et al*. 2003; Laurence Dujourdy and Besacier, 2008; Lee *et al.* 2008; Toske *et al.,* 2019; Toske, Morello, Berger, and Vazquez, 2014; Van Deursen, Lock, and Poortman-Van Der Meer, 2006)

Phenyl-2-propanone (P2P), ephedrine (EPH) ($C_{10}H_{15}NO$), and pseudoephedrine (PSE) ($C_{10}H_{15}NO$) are the commonly used precursors for the clandestine synthesis of *d, l*-methamphetamine(Kunalan, Daéid, Kerr, Buchanan, and McPherson, 2009; Kunalan, Kerr, and NicDaéid, 2012; Kurashima, Makino, Sekita, Urano, and Nagano, 2004). The precursor chemicals are influenced by synthetic chemicals, reagents, synthetic stages and reaction conditions, each resulting in organic impurities profiles used to establish strategic, operational and tactical intelligence of illicit drugs (Andersson, Lock, et al. 2007; Baechler *et al.* 2015; L. Dujourdy *et al.,* 2008)

Based on the single convention of the narcotic drugs and the revised schedules of 1991, P2P, EPH and PSE are controlled substances of Schedule 3. Their precursors are under strict control in Tanzania under the Drugs and Prevention of Illicit Traffic in Drugs, Government Act, No. 9 1995; Drugs Control and Enforcement Act, No. 5 of 2015; Drug Control and Enforcement Act, of 2016. EPH alkaloids in a matrix of organic material, such as natural products, dietary supplements, drug products, and herbs containing PSE or EPH that the chemicals may offer a chance for the illicit manufacture of methamphetamine.

Due to the stringent measures taken against commercially available precursor chemicals, clandestine laboratories illegally acquire the precursors by extracting precursors from biosynthetic plants (biotransformation) and cold medicines containing EPH or PSE or through chemical synthesis from readily available starting materials or the fermentation of sugar followed by amination, bromination of propiophenone followed by amination (Kurashima, Makino, Sekita, Urano, and Nagano, 2004). The latter case is less favourable in the clandestine synthesis of EPH/PSE. It results in a racemic mixture of PSE/EPH, which requires a subsequent enantiomeric purification before conversion to their illicit end-product (Stojanovska *et al.,* 2013), (Kaja, Surendranath, Radhakrishnanand, Satish, and Satyanarayana, 2010). Nonetheless, a large number of impurity profiling programs track the chemical profiles back to the precursor level (Lee *et al.* 2008; Morelato *et al.*, 2015; Sanggil Choe, Jaesin Lee, Hyeyoung Choi, Yujin Park, Heesang Lee, Jiyeong Jo, Yonghoon Park, Eunmi Kim, Jaesung Pyo, Hun Joo Lee, 2016; Tsujikawa *et al*., 2013). Other known studies have focused on tracing the origin of the precursors based on stable isotope analysis, which focuses on determining the geo-source of the precursors (Kurashima *et al.,* 2009; Liu, Liu, Jia, and Fan, 2018). In the present study, we used Ultra-High Performance-Liquid Chromatography-Mass Spectrometry (UHPLC-MS/MS) with water 1% formic acid: acetonitrile 1% formic acid as the mobile phase. We applied heated electrospray ionization (HESI) to introduce the samples. The present study was performed to elucidate the chemically attributable signatures based on the co-extracted impurities from methamphetamine samples seized from the Tanzania illicit drug market.

Materials and Methods

Materials

The Government Chemist Laboratory Authority (Tanzania) (GCLA) provided methamphetamine seizures used in this study. The sample was seized by the Tanzania Police Force and was presumed to contain methamphetamine and its associated precursors and other impurities. Disodium hydrogen phosphate heptahydrate, sodium dihydrogen phosphate monohydrate, sodium hydroxide, and hydrochloric acid were procured from Sigma Aldrich. In addition, ethyl acetate, acetonitrile: water (1:1, v: v), acetonitrile and phosphate buffer were also obtained from Sigma Aldrich. Unless otherwise specified, all chemicals were of analytical grade. A Hypersense® SPE 500 mg/2.8 extraction ml C18 cartridge in a junior format was used.

Solid-phase extraction

A reversed-phase hydrophobic phase Thermo Scientific™ HyperSep™ C18 Cartridge obtained fractions rich in the target compounds. The surface area of the adsorbent was 530 m^2/g, the particle size was 60 µm, and the pore size was 60 Å. The target compounds were separated into

four categories: In step one, 10 mg of methamphetamine sample was dissolved in 1000 µL of methanol, followed by 1000 µL) of phosphate buffer. The mixture was vortexed for 3 minutes before being shaken for 2 minutes in an 11,000 rpm centrifuge. The SPE column was conditioned with 1000 µL of methanol and 1000 µL of phosphate buffer in step 2. In the third step, 1000 µL of methamphetamine sample solution was loaded into the conditioned SPE column at a 1 ml/min flow rate. In step 4, the buffer fraction was collected by passing 1000 µL of phosphate buffer through the SPE column and then washing it twice with 1000 µL of distilled water followed by elution using ethyl acetate (300 ml). The fractions were separated and labelled as water 1 and 2. The phosphate buffer, water , and ethyl acetate were dried at 40°C and 20 psi before being reconstituted with 1000 µL of 1:1 water/acetonitrile, vortexed for 3 minutes, and transferred to LC-MS vials for analysis (Alsante, Hatajik, Lohr, and Sharp, n.d.; Andersson, Jalava, *et al*., 2007; Huidobro, Rupérez, and Barbas, 2007; Marín, Espada, Vidal, and Barbas, 2005; Tomá Pluskal, Castillo, Villar-briones, and Ore, 2010).

UHPLC-HESI-MS/MS performance conditions

The samples were analysed using a UHP-LC-MSMS (Q-orbitrap) Thermofisher. The stereometric co-extracted impurities were separated by an accurecore PFP -column with dimensions of 4.6 x 100 mm, 2.6 (m at 40 °C and an isocratic elution at a flow rate of 0.40 mL/min. Further, temperature adjustments, different eluents ratios, and flow rates were optimized for efficient separation using acetonitrile and 20 mM ammonium acetate solution containing 0.1% formic acid (80: 20, v/v).

Data processing

The raw LC-MS/MS data files were processed with the open-source software mzMine(Tomáš Pluskal, Castillo, Villar-Briones, and Orešič, 2010). First, using a centroid mass detector, a list of masses of the individual ionisable compound was detected and converted to a list of masses with pairs of m/z and intensity values under a noise level of 15,000 in MS level 1. The detected masses were then used to construct an Extracted Ion Chromatogram (EIC) using the Automated Data Analysis Pipeline (ADAP) workflow chromatogram builder; new algorithms for constructing extracted ion chromatograms and detecting chromatographic peaks proposed by Du lab (Du-Lab Team, 2019) and Myers *et al.*,(Myers, Sumner, Li, Barnes, and Du, 2017).

A PubChem-based mzMine identification interface search was used to identify the detected target impurities, and the results were compared with the NIST and Metacyc databases (NIH, 2021)(KenehisLab, 2021). The platform computes the neutral molecular mass for each molecular ion subjected to the identification. The ion charge was set to 1, and positive ionization mode and K^+ ionization adduct were chosen as operational parameters. The equation below was used to calculate the neutral mass.

$$m_{neutral} = \frac{m}{z} x\ z$$

Where: $m_{neutral}$ is the primary term for database search within the user-specified m/z range. The variation of the targeted impurities was evaluated using the log scale scatter plot platform in mzMine.

Results

Variation of EPH (precursor) in the SPE fractions

The traces of the impurities in the precursor compounds used to synthesize MA were investigated in the buffer, water and ethylacetate SPE fractions. The three SPE fractions were analysed using UHPLC-MS/MS. The results were processed using a modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data, mzMine, as proposed by Pluskal *et al*. (2010) (Tomáš Pluskal *et al.*, 2010). Using a RANSAC method visualizer, the m/z values were aligned against retention times (rt) of each impurity using the expression:

$$RW_i = [(m, r) \mid m_i - rm_0 \le m \le m_i + rm_0 \text{ and } r_i - rr_0 \le r \le r_i + rr_0],$$

Where r_i = the average retention time of all individual peaks in the row and m_i = average m/z of all individual peaks in the row.

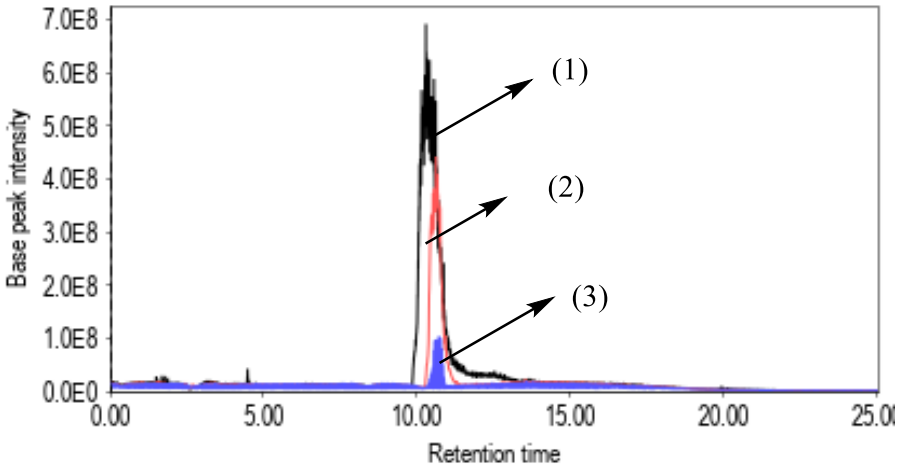
To investigate the variability of the precursor in the buffer, water ethyl acetate SPE fractions, base peak chromatogram shows avariation pf the

target compounds in the phosphate buffer water and ethylacetate fractions (Figure 1). Both fractions were extracted from the same methamphetamine sample. The polarity index of the buffer and water solvents used to separate the targeted impurities from the methamphetamine sample introduces systematic variability in this comparison. The impurities varied significantly across the three SPE fractions. At this point, our primary goal was to identify and trace the precursor (EPH) in the three fractions and connect it to the synthesis of methamphetamine.

EPH (m/z 165.1465) (PubChem CID: 9294) was detected at a retention time (RT) = 10.93 in the buffer SPE fraction with a peak height of 2 $x10^5$ and peak area of 2.6 x 10^6. The results show that water 1 and ethylacetate had no precursor used to synthesise MA in the analysed samples. The chromatograms is demonstrated in Figure 1.

Figure 1

mzMine Plots of (a) Base peak chromatogram of the SPE fractions (1) Ethylacetate fraction, (2) buffer fraction and 3 water fraction

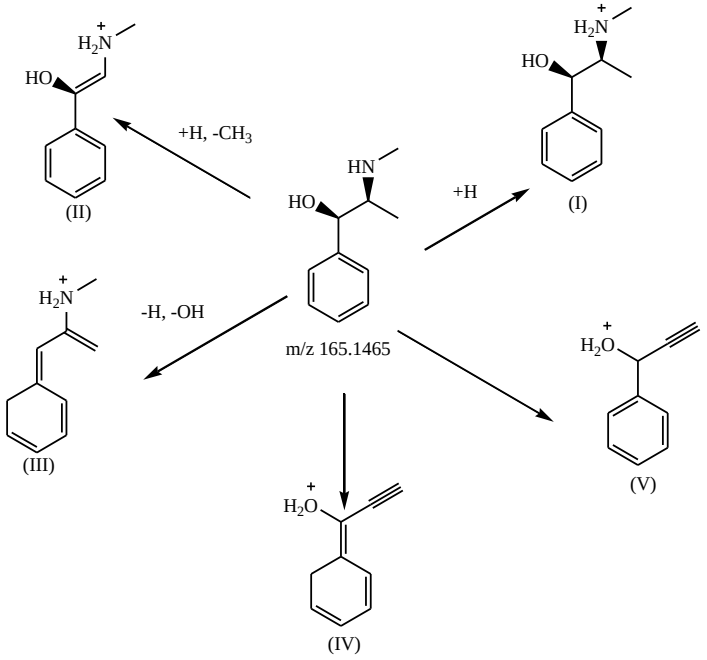


The detection of EPH indicates its use as a precursor for synthesising MA samples used in this analysis. To provide further insight into the fragmentation pattern, spectrum revealed prominent fragments with m/z values of (165.1225), 150.1276, 148.1120, 135.0440, and 139.0501. As shown in Figure 2, the precursor converts to MA via Emde, Nagai, Moscow, Hypo, and Nazi methods. It was detected at RT: 10.93, EI with the molecular ion at m/z 165.1465 $(M+H)^+$. The extracted ion was primarily seen in the buffer fraction and was identified as EPH.

Synthetic route determination is profiling information needed to establish intelligence information. One of the crucial steps in route determination entails determining the synthetic route precursors, i.e. the starting materials used to manufacture the drugs. MA can be achieved through various routes, leaving behind chemical profiles that discriminate the routes (I. Onoka *et al.*, 2020). This stage can directly detect the precursors or determine the by-products or impurities derived from the precursors. For example, in methamphetamine, this step may involve directly detecting EPH/PSE or phenyl-2-propanone or tracking down impurities such as *N, N*-dimethylamphetamine.

Figure 2

Characteristic fragments of the detected EPH: 5(I) Molecular ion peak at m/z 165.1225,5 (II) m/z 150.1275, 5(III) m/z 148.1120, 5(IV) m/z 135.0440 and 5(IV) m/z 139.0501



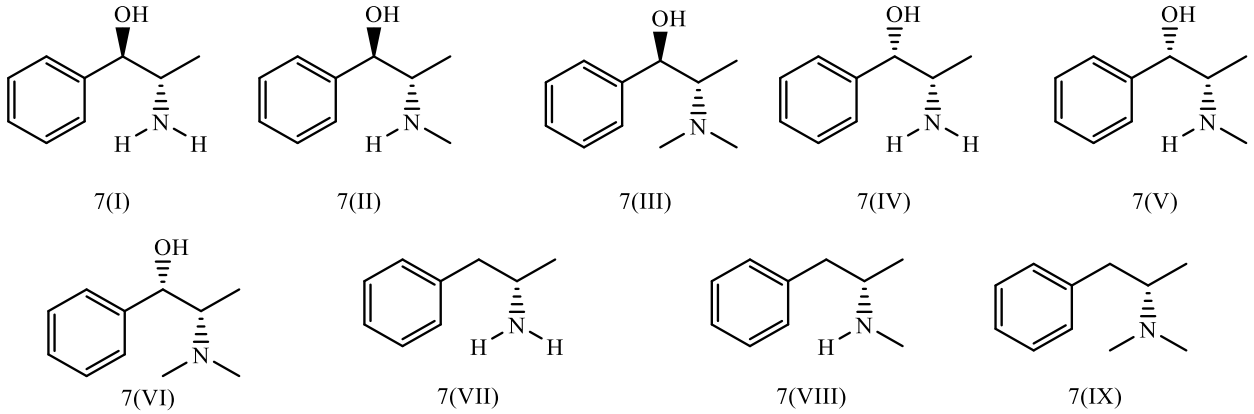
Origin of the impurities

So far, the study has identified EPH/PSE as the most likely precursors used in the clandestine synthesis of methamphetamine. In general, the origin of EPH and PSE have recently been investigated (Maxwell and Brecht, 2011; Windahl *et al*., 1995). However, our previous paper, Onoka *et al.,* [9], demonstrated that these precursors could be derived from *Ephedra* plants, over-the-counter medications, brominated propiophenone, and sugar fermentation. *Ephedra* plant is a gymnosperm shrub species in the family Ephedraceae, with over 30 species containing alkaloids, and EPH/PSE. Examples of the species include the *Ephedra sinica*, *E. equisetina*, *E. intermedia* and *E. distachya*. The shrub is primarily found in dry areas, such as the southwest United States, Mexico, South America and Asia. Africa is no exception, as previous studies have indicated the presence of *E. foliate*, *E. alte, E. aphylla and E. foemineai*n Djibouti, Ethiopia, Somalia, Sudan and Kenya (Freitag andFreitag, 2003). Therefore, it has been identified as a natural source of EPH/PSE used in the illicit production of MA. Impurities in EPH and PSE extracted from ephedra plants can be used to pinpoint the source of the precursors. Barker and Antia, as shown in Figure 3,(Barker and Antia, 2007) identified alkaloids (6(I)-VI)) associated with EPH/PSE extracted from the ephedra plant.

We identified co-extracted organic impurities 7(I), 7(II), 7(III), and 7(V) as co-extracted chemically attributable signatures of MA and 7(IX) detected as a product of the co-extracted impurities in this study. The impurities are detected at RT intervals ranging from 10 to 17.43 minutes. In addition, they have varying intensities and peak areas, indicating a variable concentration of impurities. Table 1 shows the general characteristics of the impurities.

Figure 3

The six physiologically active EPH-related alkaloids found in Ephedra: 7(I) (-)- norephedrineedrine, 7(II) (-)-EPH, 7(III) (-)-Methylephedrineedrine, 7(IV) norpsedoephedrine, 7(V) (+)-PSE, 7(VI) (+)-methylpseudoephedrine and their three reduction products: 7(VII) (+)-amphetamine, 7(VIII) (+)-methylamphetamine, and 7(IX) (+)-N, N-dimethylamphetamine (N, N-dimethyl-1-phenylpropan-2-amine)



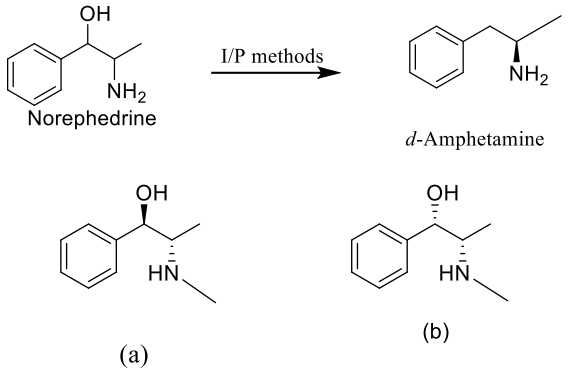
Norephedrine is a well-known sympathomimetic that primarily acts by causing norepinephrine release and has direct agonist activity at some adrenergic receptors. Its most common applications are as a nasal vasoconstrictor and an appetite suppressant. The impurity is a well-known alkaloid *Ephedrae Herba* plant alongside EPH, PSE, and methylephedrine (EPH alkaloids) (Okamura *et al.*, 1999). It is widely used as a traditional medicinal drug to treat various ailments. Traditional medicinal decoctions containing norephedrine include Mao-to, Makyo-youkai-to, Makyo-kaiseki-to, Yokuinin-to, Sho-seiryu-to, Keima-kakuhan-to, Kakkon-to and Kakkon-to-ka-senkyu-sin' well known in Japan as Mao-drugs (Okamura *et al.*, 1999). The impurity with an m/z value of 151.0815 and an RT = 10.55 has been detected in the buffer fraction and identified as norephedrine (PubChem). It has a peak height of 1.6 x 10^6 and an area of 3.2 x 10^5. The impurity extracted ion chromatogram (XIC) is shown in Figure 8. The impurity is a well-known alkaloid *Ephedrae herba* plant alongside EPH, PSE, and methylephedrine (EPH alkaloids) (Okamura *et al.*, 1999). It is widely used as a traditional medicinal drug to treat various ailments. Conventional therapeutic regimens containing norephedrine include Mao-to, Makyo-yokukan-to,Makyo-kaiseki-to, Yokuinin-to, Sho-seiryu-to, Keima-kakuhan-to, Kakkon-to and Kakkon-to-ka-senkyu-sin'i.

PSE (**V**), on the other hand, has been identified as an impurity with m/z 165.1273 detected RT = 15.06 and has a peak shape of a height of 6.00 x 10^4 and a peak area of 1.20 x 10^6.

According to the structure shown in Figure 4, the pro-S hydrogen at position 2 is replaced by a methyl group, which reverses the impurity enantiomers with the –OH group with EPH. They have not mirrored images of one another, which is detected at RT = 15.06. Figure 10 shows the base peak chromatogram of the impurity. PSE has sympathomimetic properties, according to reports. The simultaneous identification of EPH and PSE is one of the significant challenges of GC-MS-based methods. The two impurities are diastereoisomer and co-elute at the same retention time (Andrighetto, Stevenson, Pearson, Henderson, and Conlan, 2014). However, based on the electrospray ionization (ESI) method, ESI has separated and identified the co-extracted diastereoisomer. The technique was initiated by a simple SPE method followed by optimized conditions for LC-MS analysis.

Figure 4

Chemical structures of the detected co-extracted impurities: (a) PSE compared with (b) EPH



PSE displaces norepinephrine from presynaptic neurons' storage vesicles, releasing norepinephrine into the neuronal synapses, primarily stimulating alpha-adrenergic receptors. It also has weak direct agonistactivity at alpha- and beta-adrenergic receptors. Receptor stimulation results in vasoconstriction and decreases nasal and sinus congestion.

Table 1

Detected target impurities of methamphetamine

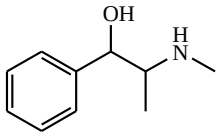
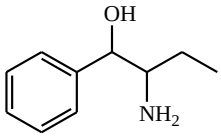
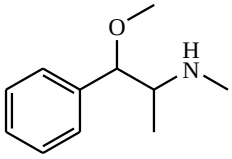
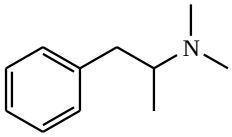
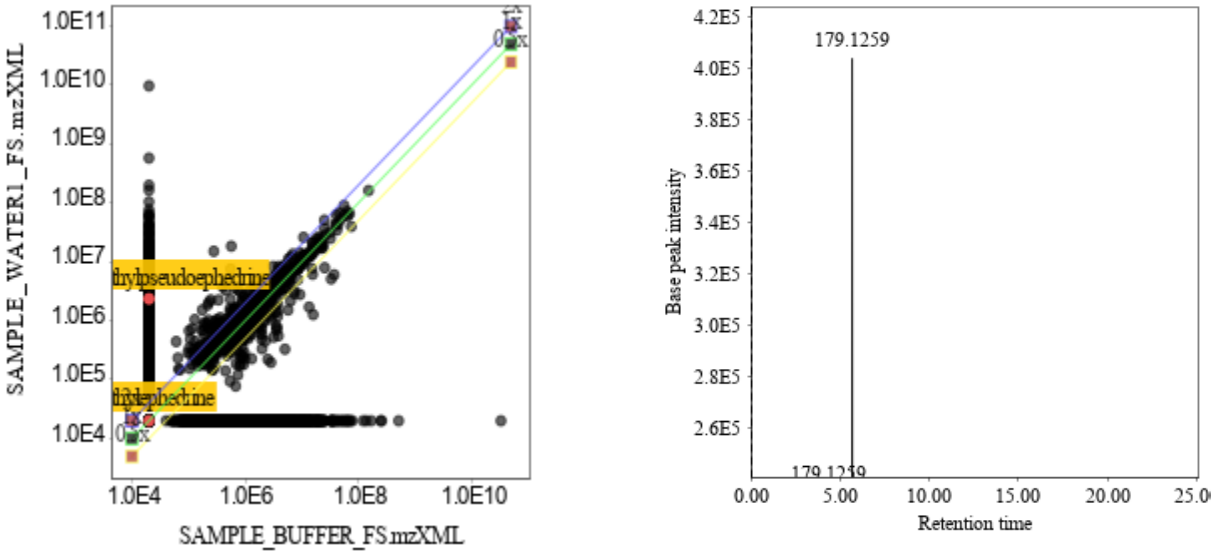
S/N	Co-extracted impurity	SPE fraction	Peak shape	m/z	RT	Peak height	Peak Area
1	EPH	Buffer		165.12	10.93	6.0E4	1.26
2	Norephedrine	Buffer		151.0515	10.55	1.60E5	3.2E5
3	Methylephedrine	Water		179.1258	14.76	1.5E5	5.3E6
4	*N,N*-dimethylamphetamine (N,N-dimethyl-1-phenylpropan-2-amine)	Ethylacetate		163.1479	17.43	5.1E4	1.1E6

Figure 5

Variation of Methylpseudoephedrine/Methylephedrine in the SPE fractions: (a) a scatter plot of the detected Methylpseudoephedrine/Methylephedrine and (b) Base peak chromatogram of methylpseudoephedrine

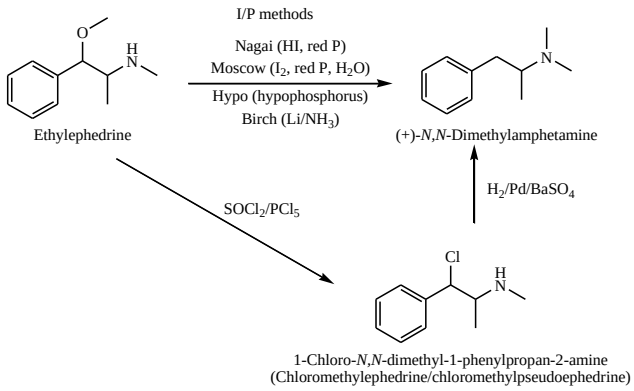


As with EPH, PSE has an equal chance of forming MA through one of the synthetic routes presented in presented by one of our previous research (I. Onoka *et al.*, 2020). However, the current study considers the impurity as a co-extracted impurity along the EPH used as a precursor for the synthesis of MA.

The other co-extracted impurities detected in this study's water 1 sample were (+)-*N*-methylpesudoephedrine/ethylethylephedrine. A 2-fold scatter plot of the buffer against the water 1 fraction is presented in Figure 5 (a), while Figure 5 (b) shows the XIC of methylephedrine. The two target compounds provide yet another important clue as to the precursor source used in synthesising the samples used in this study.

Figure 6

Reaction for the formation of N, N-dimethylamphetamine as a signature for the use of EPH as a precursor



The detection of *N, N*-dimethylamphetamine is linked to the reduction of *l*-methyl ephedrine (III)/methylpseudoephedrine (VI) via iodine/phosphorus methods: (Nagai route using HI, red P), Moscow route via (I2, red P and H_2O, Hypo route using hypophosphorous, or Birch route via (Li/NH_3). In addition, the Birch route can also be achieved via intermediates such as chlromethylephedrine/chlromethylephedrine, which converts to *N, N*-dimethylamphetamine H2/Pd/BaSO4. The intermediates are formed by SOCl2/PCl5 reduction of *l*-methylephedrine (III)/methylpseudoephedrine (VI). Figure 6 depicts the proposed mechanism's scheme. The detection of *N, N*-dimethylamphetamine confirms the presence of co-extracted impurity methylephedrine in the sample analysed. As a derivative of methylephedrine/methylpseudoephedrine present in the *Ephedra* plant, the impurity might have been generated from methylephedrine/methylpseudoephedrine, the co-extracted impurities of EPH (Stojanovska *et al.*, 2013). The reduction of methylephedrine/methylpseudoephedrine results in *N N*-dimethylamphetamine, one of the key signatures for EPH derived from the Ephedra plant (Barker and Antia, 2007). Since this study has not detected intermediates, claiming route 1 as the most likely synthetic route remains valid.

Conclusion

It has been shown the origin of methamphetamine synthesised from EPH, or PSE, can be traced by using the co-extracted impurities using Ultra-High Performance Liquid Chromatography. The identification of (+) -(*1S*, 2*S*)-PSE (V), norephedrine (I), (+)-*N*-Methylpseudoephedrine (VI), Methylephedrine (III), (+)-*N*, *N*-dimethylamphetamine (*N*, *N*-dimethyl-3-phenylpropan-1-amine) (IX) is essential in discriminating the MA synthesised from EPH/PSE derived from ephedra plant against the bromination of propiophenone, fermented sugar and from pharmaceutical drugs. This study shows that it is possible to get the impurities using UHP-LC-MS/MS and consequently use them in the chemical profiling of MA. Establishing the relative concentration of the co-extracted impurities may also be necessary for further studies.

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