Drug Testing
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# Identification and characterization of

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## Abstract

4-Methylmethcathinone　　　　　　(2-methylamino-1-(4-methylphenyl)propan-1-one, mephedrone) is a psychoactive substance that has been associated with recreational use worldwide. Analytical data related to mephedrone are abundantly available but the characterization of by-products obtained during organic synthesis remains to be explored. This study presents the identification of a 1,2,3,5-tetramethyl-4-(4-methylphenyl)-1*H*-imidazol-3-ium salt (TMMPI), which was formed during the synthesis of mephedrone. When diethyl ether was added to the crude reaction product, solid material precipitated from the solution. Analytical characterization of TMMPI employed a range of analytical techniques including chromatographic analysis in combination with various mass spectrometric detection methods, nuclear magnetic resonance spectroscopy and crystal structure analysis. Additional confirmation was obtained from organic synthesis of the imidazolium by-product. When TMMPI was subjected to analysis by gas chromatography-mass spectrometry (GC-MS), isomerization and degradation into two distinct compounds were observed, which pointed toward thermal instability under GC conditions. An LC-MS based investigation into a micro scale synthesis of mephedrone and three additional analogues revealed that the corresponding TMMPI analogue was formed. Interestingly, storage of mephedrone freebase in a number of organic solvents also gave rise to TMMPI and it appeared that its formation during storage was significantly reduced in the absence of air. The present study was aimed to support clandestine forensic investigations by employing analytical strategies that are applicable to manufacturing sites. The imidazolium salts will most likely be found amongst the waste products of any clandestine lab site under investigation rather than with the desired product.

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**Keywords:** forensic; mephedrone; cathinones; synthesis; imidazolium, by-product; TMMPI.

**Introduction**

The cathinone molecular scaffold gives rise to a large range of biologically active compounds and a considerable number of substituted cathinones has gained popularity amongst drug users. These substances were often accessible to the general public, for example, via specialized street shops (e.g. head shops/smart shops), and the Internet that included retailers operating in the 'surface' net or hidden 'dark' net environment. The rapid emergence of these largely un-tested new psychoactive substances (NPS) has raised concerns worldwide from emergency personnel, clinicians, policy makers, law enforcement and the general public.[1,2, 3] Amongst the more frequently encountered substituted cathinones, which showed some form of diffusion into specific subpopulations was 4-methylmethcathinone (mephedrone). When mephedrone was controlled under the Irish misuse of drugs legislation, it was encountered on the Irish illicit market where it was typically sold either as a powder purporting to be cocaine or in a tablet form purporting to represent 3,4-methylenedioxymethamphetamine (MDMA). [4,5]

The synthesis of mephedrone was first published in 1929 by Saem de Burnaga Sanchez.[6] The route involved the bromination of 4-methylpropiophenone to yield 4-methyl-2-bromopropiophenone which, when reacted with methylamine hydrochloride and triethylamine as an acidic scavenger, gave mephedrone. The reaction was quenched with hydrogen chloride (aqueous or gaseous) that provided the racemic hydrochloride mephedrone salt. A Friedel-Crafts acylation route, starting with toluene

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and propanoyl chloride in the presence of a suitable Lewis acid as a catalyst, may also be used to produce the 4-methylpropiophenone intermediate.[7] Another potential route to mephedrone synthesis involves the oxidation of 4-methylephedrine in a simple one step reaction process or a Grignard reaction using 4-methylbenzaldehyde as the starting material.[8]

Detailed published information on possible synthetic mephedrone impurities and route-specific by-products are currently unavailable although it has been recognized that the formation of iso-cathinones may be relevant for the chemistry of synthetic cathinones.[9-11] Mephedrone pyrolysis has also been reported to yield a range of degradation products.[12] In addition, GC-based heat-induced formation of artifacts or decomposition products may be observed during the analysis of substituted cathinones, which deserves further study.[13-18]

As part of the authors' ongoing collective research efforts regarding the systematic preparation and forensically relevant characterization of targeted NPS, the present study reports on the formation of a by-product during mephedrone synthesis. During a series of experiments involving the use of a 33% methylamine solution and a number of different solvents containing the α-bromo-4-methylpropiophenone, one experiment included the addition of diethyl ether to the crude mephedrone product (in preparation for forming a mephedrone hydrochloride salt). A solid product, however, was observed to precipitate from the solution, initally, this solid was presumed to be mephedrone freebase and accordingly filtered, dried and stored in a freezer for further work.

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This product was subsequently identified as a 1,2,3,5-tetramethyl-4-(4-methylphenyl)-1*H*-imidazol-3-ium salt (TMMPI). Analytical characterization included chromatographic techniques coupled with various forms of mass spectrometry, nuclear magnetic resonance spectroscopy, crystal structure analysis and additional confirmation by synthesis of this imidazolium by-product. The presence of TMMPI was also observed in the crude reaction mixtures of subsequent syntheses along with mephedrone and a mephedrone dimer. Given the polar salt nature of TMMPI, the data presented is also aimed to inform forensic investigators and researchers involved in the examination of waste products derived from clandestine manufacturing facilities suspected of synthesizing substituted cathinones.

## Experimental

### *Reagents and standards*

All reagents and dry solvents used in the syntheses were obtained from Sigma Aldrich Ltd. (Arklow, Ireland). LC-MS grade solvents were obtained from Fisher Scientific (Dublin, Ireland).

### *Isolation of the imidazolium by-product from a mephedrone reaction mixture.*

A solution of α-bromo-4-methylpropiophenone (5.68 g, 25 mmol) and ethanolic methylamine (6.25 mL, 8 M, 50 mmol) in absolute ethanol (50 mL) was stirred overnight at room temperature. The mixture was evaporated to a small volume under vacuum at room temperature and diethyl ether (150 mL) was added. After vigorous mixing, the mixture was filtered. The residue was extracted with acetonitrile (30 mL)

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and filtered. The filtrate was evaporated to a small volume to afford a precipitate. The

remaining solvent was removed with a pipette and the solid was washed with

acetonitrile (3 x 1 mL). The residue was dissolved in dichloromethane (15 mL),

filtered and the filtrate was evaporated to dryness under vacuum to give a colorless

solid (172 mg). A sample was recrystallized (slow evaporation) from dichloromethane

to afford colorless crystals (m. pt. 224 - 226 $^{o}$C uncorrected ): $^{1}$H NMR (DMSO) δ

7.42 (d, *J*=7.5 Hz, 2H, CH Ar), 7.42 (d, *J*=7.5 Hz, 2H, CH Ar), 3.74 (s, 3H, CH$_3$),

3.74 (s, 3H, CH$_3$), 2.68 (s, 3H, CH$_3$), 2.42 (s, 3H, CH$_3$), 2.22 (s, 3H, CH$_3$) ;

$^{13}$C NMR (d$_6$ DMSO) δ 144.2 (C imidazolium), 140.1 (C phenyl), 130.7 (C phenyl),

129.5 (C imidazolium), 129.3 (C phenyl), 123.4 (C phenyl), 33.2 (C imidazolium),

32.6 (C imidazolium), 21.4 (C phenyl methyl), 10.5 (C imidazolium), 9.2 (C

imidazolium. HR-ESIMS found 215.1548 (theory M$^{+}$: C$_{14}$H$_{19}$N$_2$, 215.1548)


### *1,2,3,5-Tetramethyl-4-(4-methylphenyl)-1H-imidazol-3-ium iodide synthesis*


A mixture of α-bromo-4-methylpropiophenone (1.22 g, 5.4 mmol), acetamidine

hydrochloride (1.04 g, 10.9 mmol) and anhydrous potassium carbonate (2.20 g) in

acetonitrile (120 mL) was refluxed for 15 hr. The mixture was cooled, filtered and

evaporated to dryness to afford a light yellow powder (729 mg), which was dissolved

in DMF (3 mL). A suspension of sodium hydride (115 mg, 4.8 mmol) in DMF (3 mL)

was added and the mixture was stirred at room temperature for 2 h. Iodomethane (2

mL) was added and the mixture was stirred for 3 h at room temperature. More

iodomethane (2 mL) was added and the mixture was stirred overnight at room

temperature. The mixture was diluted with acetonitrile (100 mL), filtered and the

volatiles were removed under vacuum. The residue was dissolved in diethyl ether (100 mL) and the precipitated solid was collected by filtration. The solid was mixed with dichloromethane (20 mL), filtered and the filtrate was concentrated to give a light yellow solid (234 mg). This was recrystallized from dichloromethane/*tert*-butyl methyl ether to afford a colorless solid (92 mg, m.p. 220 - 222 $^{\circ}$C uncorrected ): $^1$H NMR (DMSO) δ 7.40 (d, *J*=7.5 Hz, 2H, CH Ar),  7.34 (d, *J*=7.5 Hz, 2H, CH Ar), 3.73 (s, 3H, CH$_3$),  3.56 (s, 3H, CH$_3$),  2.68 (s, 3H, CH$_3$),  2.40 (s, 3H, CH$_3$), 2.21 (s, 3H, CH$_3$) ; $^{13}$C NMR (d$_6$ DMSO) δ 143.6 (C imidazolium), 139.5 (C phenyl), 130.2 (C phenyl), 129.7 (C imidazolium), 129.0 (C phenyl), 126.6 (C phenyl), 33.7 (C imidazolium), 31.9 (C imidazolium), 20.9 (C phenyl methyl), 10.1 (C imidazolium), 9.7 (C imidazolium. HR-ESIMS found 215.1548 (theory M$^+$: C$_{14}$H$_{19}$N$_2$, 215.1543)

### *Micro scale reactions for LC-MS analysis*

A solution of the α-bromo ketone (0.25 mmol) and the amine (0.5 mmol) in the specified solvent (500 μL) was allowed to stand at room temperature overnight. This solution was diluted with acetonitrile/water (1/1, containing 0.1 % formic acid, 1.5 mL), an aliquot (8 μL) was further diluted with acetonitrile/water (1/1, containing 0.1 % formic acid, 1 mL) and analyzed by LC-MS.

### *Micro scale formation of TMMPI from freebase mephedrone*

A solution of freebase mephedrone in acetonitrile (0.5 mmol/500 μL acetonitrile, 100 μL) and acetonitrile (100 μL) was allowed to stand overnight (in the presence or absence of air) at room temperature. This solution was diluted with acetonitrile/water (1/1, containing 0.1 % formic acid, 600 μL), an aliquot (8 μL) was further diluted

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with acetonitrile/water (1/1, containing 0.1 % formic acid, 1 mL) and analyzed by LC-MS. The by-product formation was observed in the presence of air, but very significantly, this was observed to drop by over 90 % in the absence of air.

### *Preparative formation of TMPPI from mephedrone freebase.*

A solution of mephedrone free base (prepared from 2.15 g of the HCl salt, 10 mmol) was dissolved in acetonitrile and stirred at room temperature for 48 h. The solution was evaporated to dryness and the residual brown oil was washed with diethyl ether (4 x 10 mL). The residue was dissolved in dichloromethane and hydrogen chloride in 2-propanol (5-6 M) was added. The mixture was concentrated to a small volume under vacuum, the supernatant was removed and drying was continued under vacuum to afford a light brown solid (219 mg). The supernatant was concentrated to give a brown oil (178 mg) which was analyzed by NMR and also used for further chromatographic analysis.

### *Preparation of crude mephedrone hydrochloride.*

A solution of α-bromo-4-methylpropiophenone (1.13 g, 5 mmol) and ethanolic methylamine (1.25 mL, 8 M, 10 mmol) in absolute ethanol (10 mL) was stirred overnight at room temperature. The mixture was evaporated to dryness and, taken up in dichloromethane and a solution of hydrogen chloride in diethyl ether (2 M) was added. Removal of the volatiles afforded a brown powder (1.41 g)

## *Instrumental analysis*

### *Gas chromatography - mass spectrometry*

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Samples were analyzed on an Agilent 6890N gas chromatograph coupled to a 5973 MSD. A HP-5MS column (30 m × 0.25 mm × 0.25 μm) was used in splitless mode with helium carrier gas at a constant flow of 1 mL/min. The injection port and transfer line temperatures were set at 250 °C and 280 °C, respectively. The initial oven temperature was 90 °C, held for 1 min, then ramped at 15 °C/min to 280 °C and held for 5 min, followed by a ramp of 20 °C/min to 300 °C with a final hold time of 1 min. The mass spectra data was collected after a 5 minute solvent delay. The ionization energy was set at 70 eV and the mass range was *m/z* 40-550.

### *Liquid chromatography - mass spectrometry*

LC-MS analyses, equipped with an electrospray ionization source, were performed on an Agilent 1100 LC system. The column (Allure PFP Propyl, 5 μm, 50 × 2.1 mm) was from Restek (Bellefonte, PA, USA) and the aqueous mobile phase A consisted of 0.05% formic acid in water whereas mobile phase B was prepared from 0.05% formic acid in acetonitrile, respectively. The Agilent LC-MSD settings were as follows: positive electrospray mode, capillary voltage 3000 V, drying gas ($N_2$) 12 L/min at 350 °C, nebulizer gas ($N_2$) pressure 60 psi, *m/z* 50-500, fragmentor voltage either 50 V or 120 V. Samples for LC-MS analysis (2 μL injection volume) were dissolved in acetonitrile/water (1:1, containing 0.1% formic acid) at a concentration of 5 μg/mL. The following gradient elution program was used: 0-5 min 12% A and then increased to 35% over 30 min using a linear gradient. The flow rate was 1 mL/min and the column temperature was 30 °C.

### *Nuclear magnetic resonance spectroscopy*

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Samples were prepared in DMSO-$d_6$. $^1$H (600 MHz) and $^{13}$C (150 MHz) NMR spectra were recorded on a Bruker AV600 NMR spectrometer using a 5 mm TCI cryoprobe. $^1$H NMR spectra were referenced to an external TMS reference at $\delta = 0$ ppm.

### *High-resolution electrospray mass spectrometry*

HR-ESI mass spectra were recorded by direct injection into a LTQ Orbitrap Discovery (Thermo Fisher, UK). Samples were dissolved in acetonitrile/water (1:1, containing 0.1% formic acid) and infused at a rate of 5 μL/min. Full accurate high-resolution (30000) mass scans were performed in positive electrospray mode. Measured accurate masses were within ± 5ppm of the theoretical masses. The following conditions were used: drying gas ($N_2$) 10000 mL/min, capillary temperature 310 °C, spray voltage 4 V, capillary voltage 22 V and tube lens 77 V.

### *X-ray crystallography*

Data for the by-product $C_{14}H_{19}BrN_2$ (colourless needle, 0.060 mm x 0.090 mm x 0.220 mm) were collected on a Bruker APEX DUO using Mo Kα radiation (λ = 0.71073 Å) using a MiTeGen micromount and at 100(2) K (Oxford Cobra Cryosystem). Bruker APEX2 software was used to collect and reduce data, determine the space group, solve and refine the structure.[19] A total of 2942 frames were collected. The total exposure time was 12.26 hours. Absorption corrections were applied using SADABS 2012.[20] All final refinements were performed with SHELXL 2014/3.[21] All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were assigned to calculated positions using a riding model.

***Crystal Data***: $C_{14}H_{19}BrN_2$, $M$ = 295.22, Monoclinic, $a$ = 11.2256(3), $b$ = 7.4874(2), $c$ = 16.0597(5) Å, $\beta$ = 99.3230(10)°, $U$ = 1332.00(7) Å$^3$, $T$ = 100 K, space group $P2_1/c$, $Z$ = 4, $\mu$ (Mo K$\alpha$) = 3.067 mm$^{-1}$, $\rho$ = 1.472 Mg/cm$^3$, 86862 reflections collected, 4895 independent (R$_{int}$ = 0.0263), $^aR_1$ = 0.0205, wR2 = 0.0517 ($I > 2\sigma(I)$), $S$ = 1.045.

$^aR_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, w$R_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$.

(CCDC 1033669 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.)

## Results and discussion

The analytical characterization of synthetic cathinones is a relatively recent research field. Typically, the unique determination of substances in most forensic and law enforcement laboratories rely on various separation technologies linked to mass spectrometric detection. In the analysis of submitted samples where cathinone structures are suspected, particular attention is paid to the base peak that is generally formed from whatever side chain substitution pattern is present in the cathinone. The molecular ion for substituted cathinones in EI mass spectrometry (70 eV) may not be easily identifiable; hence molecular weights may not easily be ascertained. Typical cathinone fragmentation patterns are dominated by alpha-cleavage and formation of immonium ions. In cases where charge retention is observed following alternative alpha cleavage, substituted benzoyl ions are then detected.[22] Although analytical data on mephedrone are abundantly available, published information on synthetic routes, possible by-products or possible route specific compounds are not available.

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The present investigation reports on the identification and characterization of 1,2,3,5-tetramethyl-4-(4-methylphenyl)-1*H*-imidazol-3-ium salt (TMMPI) obtained as a by-product during the synthesis of mephedrone. As previously stated, when diethyl ether was added to synthesised crude mephedrone product (in preparation of forming a mephedrone salt), a solid product precipitated out of solution that was filtered, dried and stored at -20 °C for further work. Initially, GC-MS analysis was performed on this product and the chromatogram obtained which surprisingly showed two distinct peaks at 15.58 min and 17.54 min, respectively (see supplemental 1a). The GC retention time and mass spectra associated with these two peaks were not consistent with the retention time or mass spectra of the expected synthesis product mephedrone. The GC-MS spectra of the synthesis by-product were similar to each other each having a base peak at *m/z* 200 but some differences were also noted. The fragmentation obtained for the compound at 17.54 min showed the second largest peak at *m/z* 185 following the loss of a methyl group from the base peak at *m/z* 200. This *m/z* 185 was much less abundant in the compound eluting at 15.58 min. Interestingly, a relatively large *m/z* 132 present in the species at 15.58 min was observed (suggesting loss of a neutral $C_4H_6N$ moiety from the base peak) but this was not detected in the chromatographic peak at 17.54 min. A potential explanation is that the formation of the *m/z* 132 ion would require the breakage of two double bonds in the imidazolium structure proposed for the by-product, which is energetically less favoured compared to only one double bond breakage that could account for the *m/z* 132 ion formation associated with the compound eluting first. Two interconverting structures could give rise to the mass spectra obtained via loss of either $C_4H_6N$ or $C_5H_9N$ and subsequent rearrangement of the imidazole ring (see supplemental 1b).

An LC-MS analysis was performed on the mephedrone synthesis product and the

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chromatogram consisted of a single peak, which supported the suggestion that the presence of two GC chromatographic peaks were formed artificially under GC-MS conditions. The electrospray ionization mass spectrum (180 V) showed a base peak at $m/z$ 215 $(C_{14}H_{19}N_2^+)$ and the second most abundant product ions was observed at $m/z$ 132, presumably due to the loss of $C_5H_9N$ from the base peak and even-electron molecular mass (Figure 1a). Ions observed at $m/z$ 200, $m/z$ 199 and $m/z$ 185 might have reflected the loss of $CH_3$, $CH_4$ and $C_2H_6$, respectively. The imidazolium ring may also undergo opening with the loss of $C_3H_5N$ or $C_3H_6N$ to yield $m/z$ 160 and $m/z$ 159, and further losses from $m/z$ 159 (H or $CH_3$) gave $m/z$ 158 and $m/z$ 144 respectively. A suggested mechanism is provided in Figure 1b. The difference between the GC-MS base peak and the LC-MS base peak also indicated possible thermal degradation during GC-MS analysis (loss of a methyl moiety) since ions above the $m/z$ 200 base peak in the GC-MS spectrum were not detected.

The solid state crystal structure of the mephedrone by-product was also obtained. The structure of the mephedrone by-product was found to be a bromide salt and shown to consist of a *para*-methyl substituted phenyl ring attached to a tetra-methyl substituted five-membered heterocyclic imidazole ring (Figure 2). The bromine atom was only weakly coordinated to the organic moiety via CH…Br interactions (3.75 - 3.98Å) and each Br⁻ linked four organic moieties together. The twist between the rings (torsion angle N7-C9-C10-C16, 133.6(1) Å) allowed the alternating packing of the imidazolium/phenyl system into a stack parallel to the b-axis (supplemental 2). This arrangement allowed the rings to overlap and align with an intermolecular centroid (C13-C15-C16-C10-C11-C12) to centroid (N3-C5-N7-C9-C2) distance of ca. 3.6Å. The characterization of the mephedrone by-product added to the few structurally

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known tetramethylimidazolium-$R^+X^-$ compounds (R = Me, X = I; R = $CH_2I$, X = I; R = Me, X = $(SCN)_4Crphenanthroline$; R = Me, X = $(SCN)_4Cr(NH_3)_2$; R = Me, X = $N(SO_2CF_3)_2$). [22-27]

A mechanism of formation is proposed to account for the formation of TMMPI during mephedrone synthesis (Figure 3). The carbonyl group on mephedrone was thought to undergo a reduction to the alcohol followed by dimerization and subsequent loss of water. This intermediate in which the carbonyl functional group is directly attached to the 4-methylphenyl moiety was proposed to undergo losses of 4-methylbenzaldehyde and a hydride ion to yield the imidazolium by-product.

Confirmation of the mechanisms proposed by-products was obtained when a series of micro scale experiments were performed using cathinone substituents in which the alpha-alkyl group was either H, $CH_3$, $CH_2CH_3$ and $CH_2CH_2CH_3$. The corresponding substituted by-products were observed when the reaction solvent was methanol, ethanol, acetonitrile and tetrahydrofuran. It is significant and noteworthy that in the absence of air during these micro experiments, a considerable reduction > 90% was observed in the formation of TMMPI by-product. This reduction may be due to the absence of an efficient mechanism to regenerate the hydride ion in the proposed mechanism in the absence of air and hence a corresponding reduction in by-product formation. The regeneration of the hydride ion may have some analogous mechanism to those found in biological processes. For example, dihydrobenzo-imidazoles have been the subject of extensive studies for hydride transfer mechanisms which might make the phenyl-1*H*-imidazol-3-ium by-products observed in this study useful as potential organic hydride donors.[28]

The results of the micro scale experiments are presented in Figure 4A-D where the TMMPI product was shifted in mass based on the nature of the alpha-alkyl group (R) during LC-MS analysis, i.e. at $m/z$ values of 187, 215, 243 and 271, respectively. When R was an ethyl-substituted cathinone, a peak was observed in the expected LC window of the R as a methyl substituted cathinone at $m/z$ 215. Co-injection into the LC-MS of the $CH_3$ and $CH_2CH_3$ micro experiments, clearly show that the second peak observed in the m/z 215 target window is an artifact and not the mephedrone by-product (see figure 4 B, C. co-injected).

Additional confirmation for the unambiguous identification of the mephedrone by-product as TMMPI was obtained from its organic synthesis via the reaction of 4-methyl-1-phenyl-2-bromo-propan-1-one with acetamidine and potassium carbonate (Figure 5). The synthesized compound and the mephedrone by-product was then compared using GC-MS, LC-MS and NMR and found to be identical (supplemental data 3, NMR data).

Analysis of the reaction products derived from in-house synthesis of mephedrone showed that the imidazolium by-product was observed in the crude reaction mixture along with mephedrone and a mephedrone dimer. GC-MS data clearly showed the presence of two peaks at the expected retention times (supplemental data 4a). LC-MS data also showed the presence of a single peak at the corresponding retention time expected for TMMPI in addition to the expected ESI mass spectrum (supplemental data 4b).

Mephedrone freebase was placed in acetonitrile and allowed stand for 48 hours, which resulted in the formation of both an oil and a solid. Mephedrone was not

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detected in both forms but analysis of these reaction products indicated that along

with the formation of TMMPI an additional compound was detected. Proton NMR of

the solid indicated the presence of extra peaks in addition to those expected for

TMMPI, thus suggesting that a para substituted aromatic compound with a methyl

substituent was also present in the solid (supplemental data 5a).

 LC-MS (negative electrospray ionization) analysis of both the oil and solid indicated

that this additional compound had a deprotonated [M-H]⁻ *m/z* 135  (supplemental data

5b). GC-MS analysis of both forms provided indication that it was consistent with 4-

methyl benzoic acid (supplemental data 5c). The scheme outlining the formation of

the TMMPI (Figure 3) showed the loss of 4-methylbenzaldehyde from an

intermediate and its subsequent oxidation could have been consistent with the

formation of 4-methyl benzoic acid or it could have been formed by another oxidative

pathway.


In the investigation of sites where the manufacture of mephedrone (or other

substituted cathinones) is suspected, it is important that identification of substances

that can be related to a synthesis route are fully explored. The analytical data

presented here will be beneficial to such investigations, as any imidazolium by-

product found will give an indication as to what substituted cathinone was

synthesized. The imidazolium salts will most likely be found amongst the waste

products at a clandestine lab site. It is often the case, that waste products are either

dumped in remote locations or are the only items remaining at such illicit synthesis

sites when enforcement personnel actually get to examine a premises suspected of

having been used in illicit clandestine manufacture. This may be the case were

significant by-products are identifiable and associated with a particular class of

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cathinones. The typical forensic analytical approach taken when examining items seized at a suspected clandestine laboratory facility or of suspected waste material generated at such a manufacturing site and dumped at a different location is to initially screen such samples by GC-MS. If this is the sole screening methodology, then challenges may be encountered when attempting to identify the imidazolium salts due to GC-induced isomerization and thermal degradation. It is vital that analytical strategies utilized for clandestine laboratory investigations are fit for purpose and investigators should be aware that some traditional screening technologies may have unforeseen limitations.

## Conclusion

The majority of forensic examinations of drug submissions involve samples with questionable origin. Verifiable data for samples derived from clandestine manufacturing conditions remain illusive unless such operations are found while in production and with products being available. The ability to determine a potential link to clandestine production with the identification of a by-product linkable to a known synthesis route would be a very valuable forensic option. This study explored the relationship between a synthetic route commonly used for mephedrone production and the formation of a specific imidazolium by-product identified as a 1,2,3,5-tetramethyl-4-(4-methylphenyl)-1*H*-imidazol-3-ium salt. Furthermore, it was also established that mephedrone freebase was unstable in several organic solvents. Given the polar nature of the imidazolium salt formed during a mephedrone synthesis, it is conceivable that it might be found amongst the waste products generated at such

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clandestine manufacturing sites or it may be formed post synthesis and as in our initial belief presumed to be mephedrone. The present study was aimed to support clandestine forensic investigations by targeting specific compounds and the analytical strategies which are applicable to the analysis of waste products that are generally either dumped or remain abandoned at sites after production has ceased.

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**Supplemental data**

**1A**        **GC-MS of imidazolium by-product.**

**1B**        **Two interconverting structures for TMMPI in GC inj port**

**2**         **X-Ray crystallography supplemental 2.**

2. Packing diagram of the mephedrone by-product viewed down the b-axis. (A) hydrogen atoms shown with dashed lines indicating weak CH…Br interactions.(B) Hydrogen atoms removed for clarity to show the alternating head-to-tail packing assembly of the organic moiety.

**3**         **TMPPI synthesized and the mephedrone by-product NMR**
**4**         **Crude mephedrone mixture analysis by GC-MS (4a), LC-**

              **MS (4b)**

**5**         **Mephedrone freebase mixture analysis by NMR (5a), LC-**

              **MS (5b) and GC-MS (5c)**

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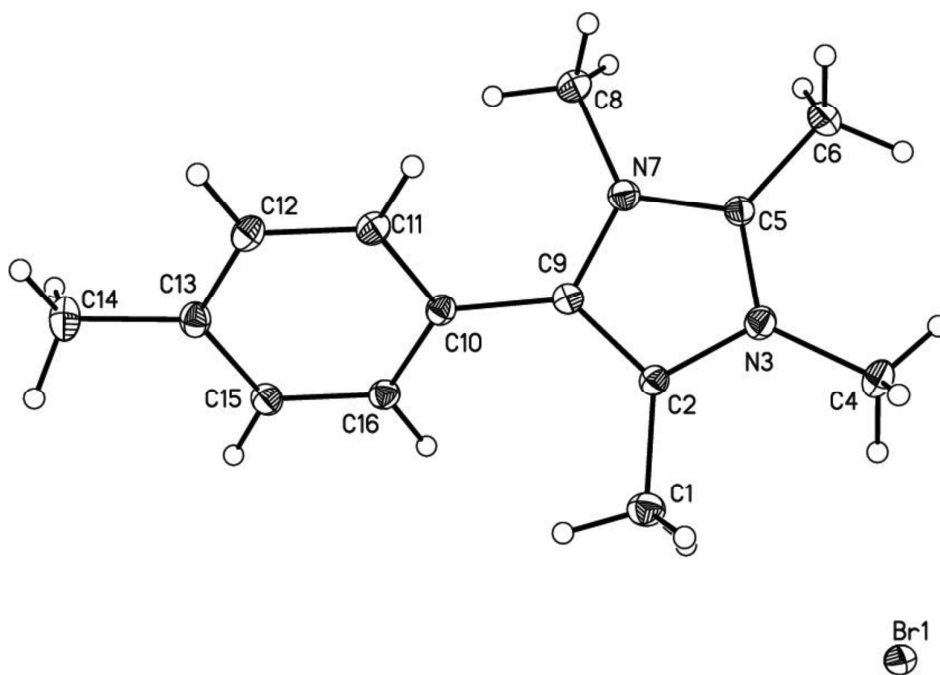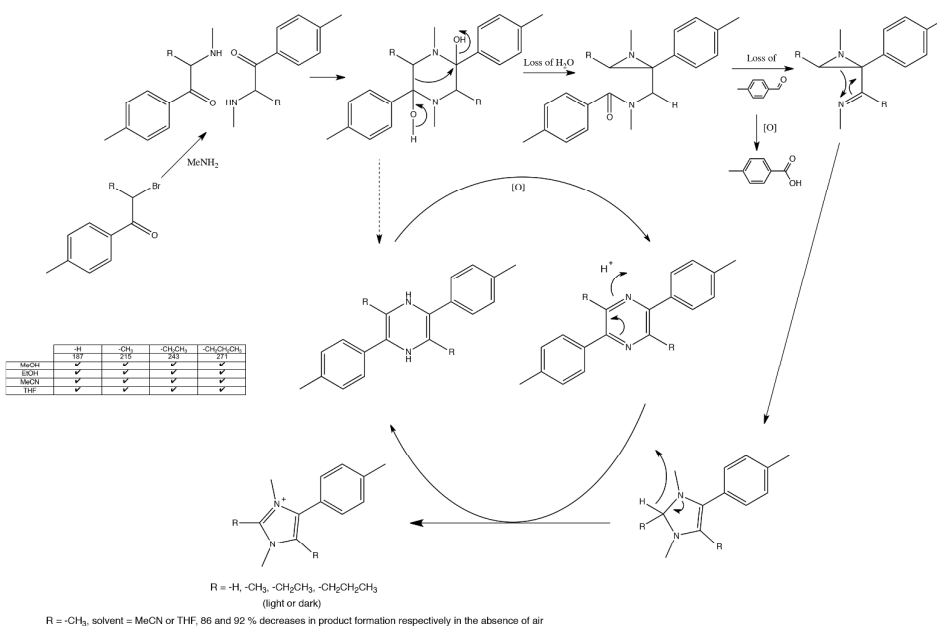58
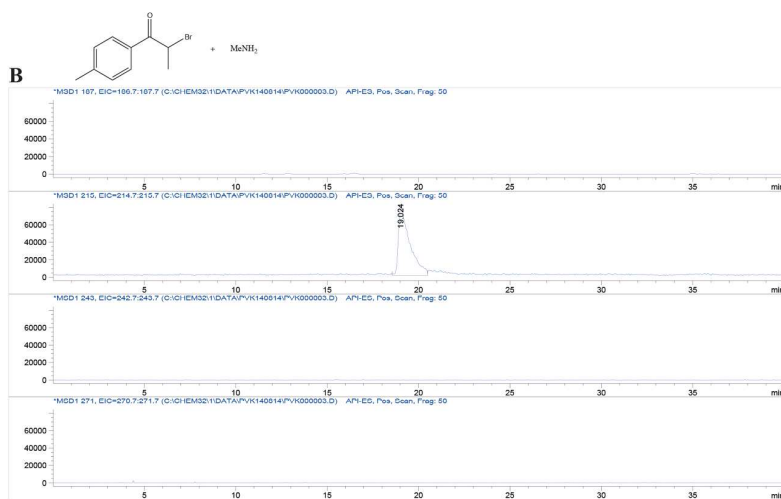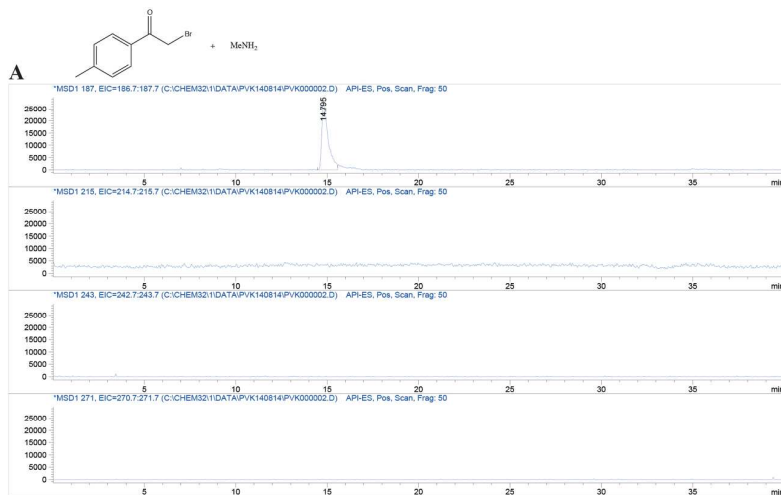59
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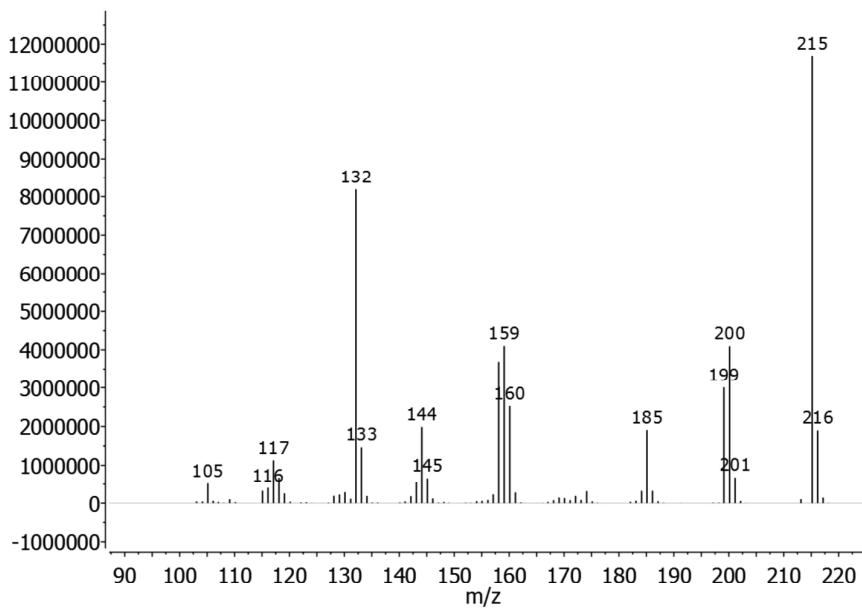


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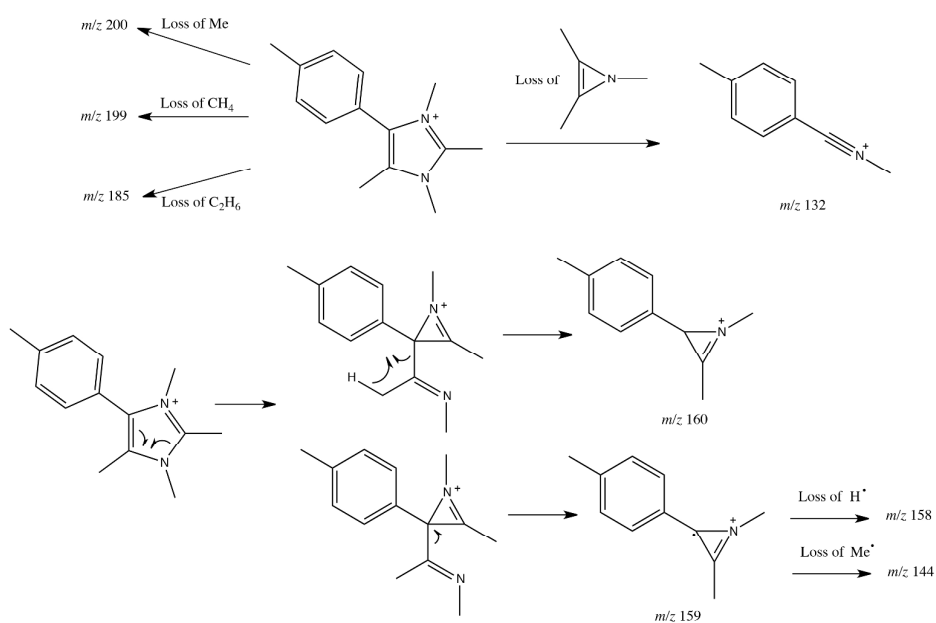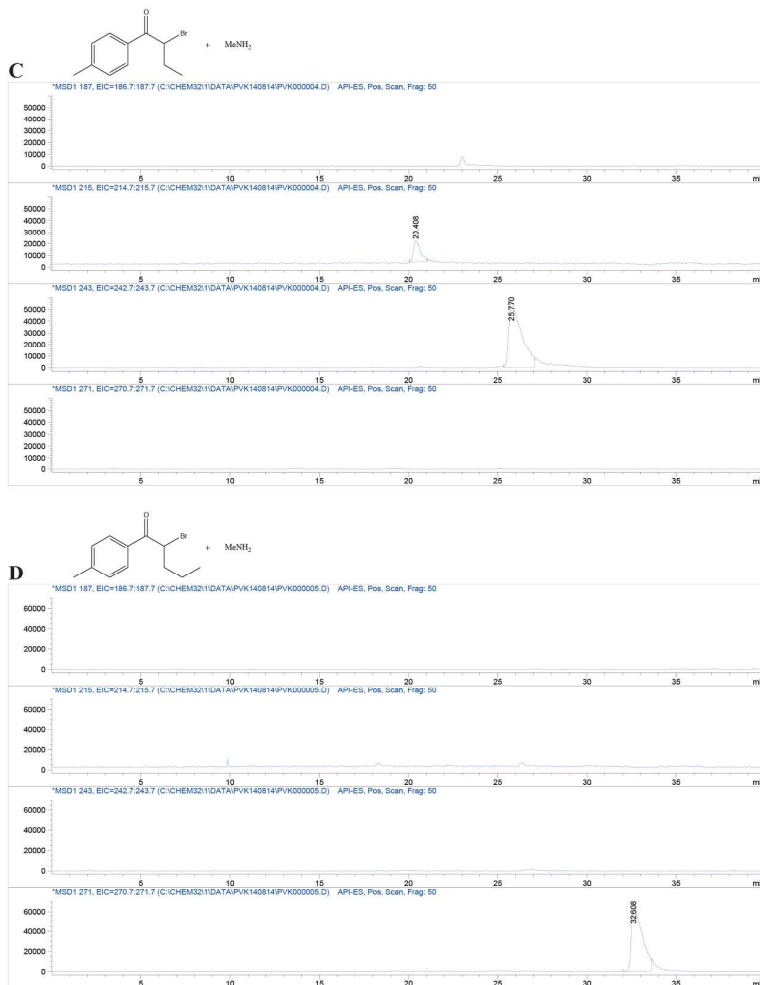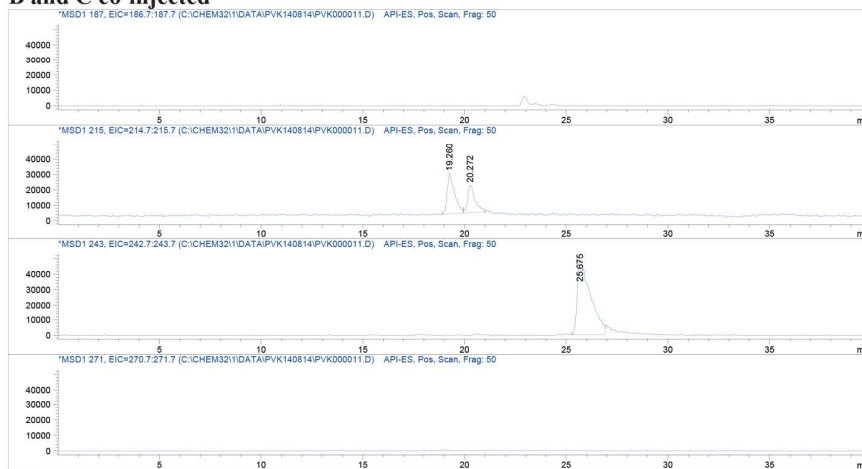
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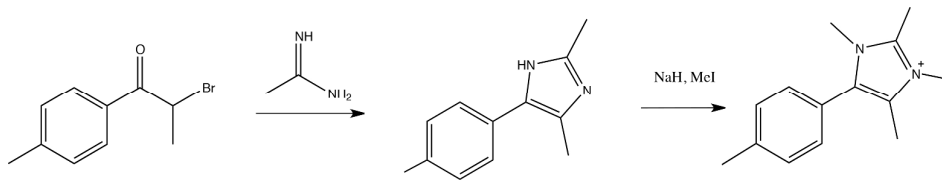55
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**B and C co-injected**



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