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# AMT (3-(2-aminopropyl)indole) and 5-IT (5-(2-aminopropyl)indole): an analytical challenge and implications for forensic analysis

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**5-(2-Aminopropyl)indole (5-IT) and 3-(2-aminopropyl)indole (α-methyltryptamine, AMT) are isomeric substances and their differentiation can be a challenge under routine analytical conditions, especially when reference material is unavailable. 5-IT represents a very recent addition to the battery of new psychoactive substances that are commercially available from online retailers. This report illustrates how subtle differences observed under mass spectral and UV conditions can help to facilitate the differentiation between the two isomers. Analyses included $^{1}$H and $^{13}$C NMR, GC-EI/CI ion trap MS, applications of several U/HPLC-DAD and HPLC-MS methods. Investigations currently underway also highlight the confirmation that AMT was detected in a number of fatal intoxications. These findings also demonstrate that there is a potential risk of misidentification when dealing with both substances. Copyright © 2012 John Wiley & Sons, Ltd.**

**Keywords:** 5-IT; 3-IT; AMT; legal highs; tryptamines; forensic; clinical; isomers; internet

## Introduction

5-(2-Aminopropyl)indole (**1**, 5-IT, 5-API, Figure 1) was noted by Shulgin and Shulgin to show long-lasting stimulant properties in humans (~12 h at 20 mg p.o.).[1] Although the preparation of 5-IT, and some of its positional isomers, were reported in the early 1960s,[2,3] data on its psychoactive properties remain obscure. Very recently, 5-IT became available from suppliers operating via the Internet, consistent with the trend to offer commercially available psychoactive substances.[4]

α-Methyltryptamine (**2**, 3-(2-aminopropyl)indole, AMT, α-MT, 3-IT, IT-290, IT-403, U-14, 162-E, Ro 3-0926, NSC 97069, Indopan; Figure 1), on the other hand, is a positional isomer of 5-IT that also shows long-lasting psychoactive effects in humans[1] although further studies are needed to determine the differences or similarities between both psychopharmacological profiles. Following its first synthesis in 1947,[5] the interest in AMT, and other α-alkylated tryptamines, began to develop in the late 1950s when it was discovered that some of these analogues also displayed monoamine oxidase (MAO) inhibiting properties.[6,7] Interestingly, when all six possible isomeric 2-aminopropyl analogues were studied for their MAO inhibiting properties (pig liver homogenate with serotonin as the substrate) and anti-reserpine action in mice, pronounced levels of activity were observed in both assays for 5-IT, AMT and its 6-(2-aminopropyl)indole (6-IT) counterpart, respectively.[8] Orally administered dosage levels typically reported for AMT appear to range between 15–50 mg.[1,9] Long duration of effects (~10–24 h) have also been noted and one study described that two out of twelve subjects reported a duration of two days (20 mg, p.o.).[10] The nature of psychoactive effects induced by AMT points towards a wide range of dose-dependent interindividual differences between subjects which may range from psychedelic/hallucinogenic and antidepressant effects to severe psychological and physical discomfort and malaise,[1,10–16] which may explain why AMT appeared to play a comparatively modest role in the recreational context. Availability of AMT from online retailers was observed before[14] and there is also precedent of quantitative AMT detection in post-mortem samples in addition to a positive result for amphetamines in urine and gastric contents following immunoassay analysis.[17]

With the recent emergence of the 'legal highs' phenomenon, it became apparent that AMT has been increasingly added to the product catalogue of retailers operating online which adds to the need for research into prevalence of use and monitoring. 5-IT represents a very recent addition to the battery of new psychoactive substances and one of the key difficulties regularly encountered within the clinical and forensic work arises from the presence of positional isomers. Both 5-IT and AMT serve as such an example and their differentiation can be a challenging endeavour due to the obvious structural similarities. In particular, the isobaric nature of the compounds ($C_{11}H_{14}N_2 = M_W$ 174.1157) does not allow unambiguous identification even with the use of high resolution accurate mass spectrometry. This could be a concern given the increasing reliance and use of such a technique within clinical and forensic toxicology for rapid identification of drugs.

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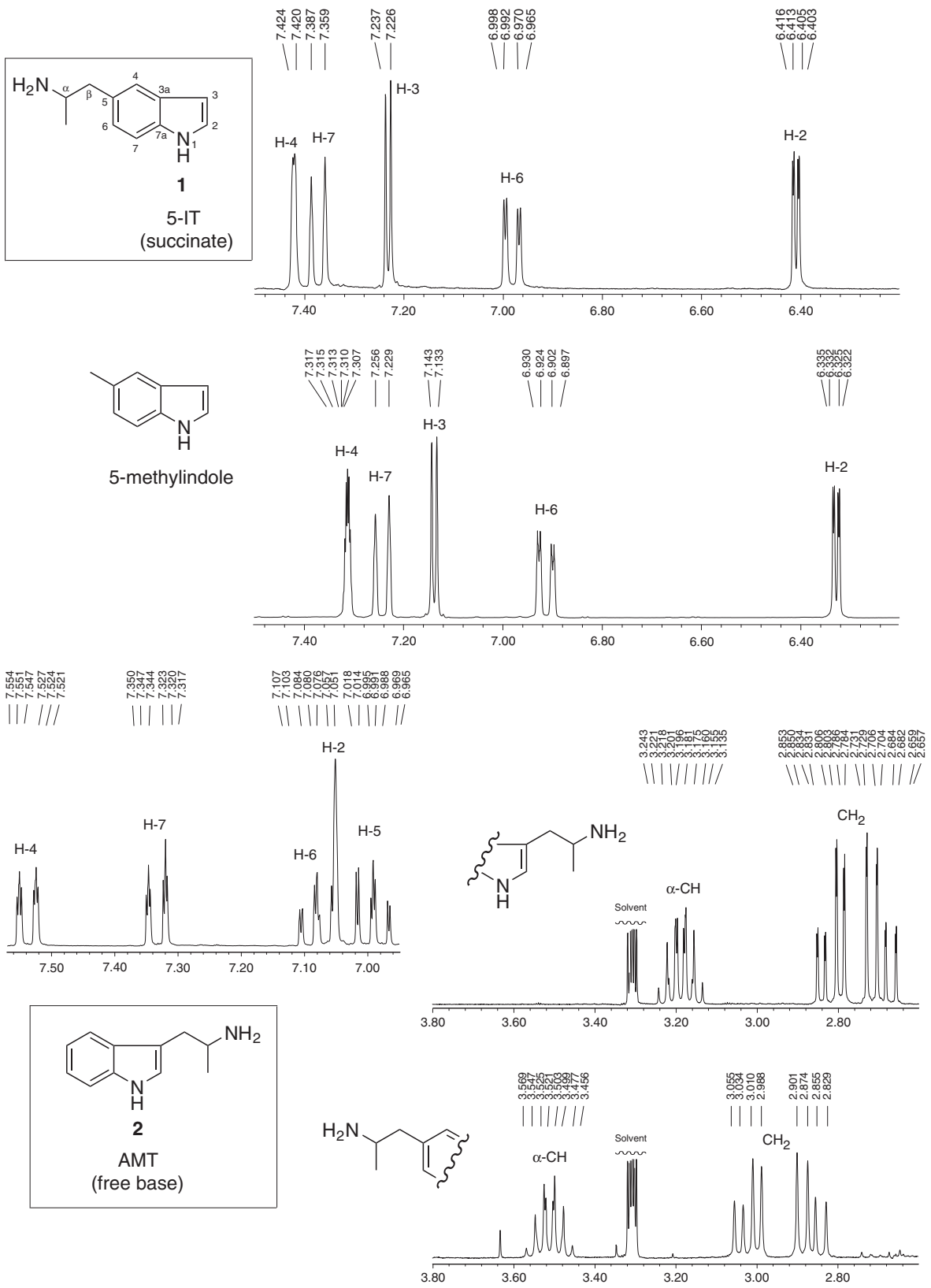


**Figure 1.** $^{1}$H NMR spectra of 5-IT succinate (1) and AMT free base (2) in $CD_3OD$. Spectra are divided into the aromatic region and side chain-related resonances to facilitate comparison. The aromatic region of 5- methylindole is also shown. See text for details.

The present paper provides analytical characteristics of the two compounds showing some subtle differences that may allow for the differentiation between the two isomers which seems crucial given that 5-IT does not yet appear to be commercially available as reference material. In addition, summarized fatal cases confirmed to involve AMT (rather than 5-IT) are highlighted.

## Experimental

5-(2-Aminopropyl)indole (**1**, 5-IT), advertised as the succinate salt, was obtained as a brown powder from an online retailer in April 2012. 5-Methylindole (99%) was from Sigma Aldrich (Dorset, UK), racemic α-methyltryptamine base (AMT, **2)** (99%) from Acros Organics (Geel, Belgium) and $CD_3OD$ (99.80%) was from VWR (Leicestershire, UK), respectively. All other solvents and chemicals, e.g. acetonitrile, methanol, formic acid, triethylammonium phosphate buffer, and ammonium formate, were of analytical grade or equivalent (Aldrich, Dorset, UK).

### Instrumentation

One- and two-dimensional nuclear magnetic resonance (NMR) spectra were recorded using a Bruker Avance 300 spectrometer. Samples were dissolved in $CD_3OD$ and chemical shifts are reported relative to TMS at $\delta = 0$ ppm.

*NMR data for 5-IT succinate (**1**)*

$^1$H NMR (300 MHz, $CD_3OD$): δ 7.42 (1 H, br d, $J = 1.1$ Hz, H-4), 7.37 (1 H, d, $J = 8.3$ Hz, H-7), 7.23 (1 H, d, $J = 3.2$ Hz, H-3), 6.98 (1 H, dd, $J = 8.3$ Hz, $J = 1.7$ Hz, H-6), 6.41 (1 H, dd, $J = 3.2$ Hz, $J = 0.8$ Hz, H-2), 3.57-3.45 (1 H, m (consistent with predicted dqd), α-CH), 3.02 (1 H, dd, $J_{gem} = 13.8$ Hz, $^3J = 6.5$ Hz, $CH_AH_B$), 2.86 (1 H, dd, $J_{gem} = 13.8$ Hz, $^3J = 8.0$ Hz, $CH_AH_B$), 2.51 (4 H, s, succinate), 1.26 (3 H, d, $^3J = 6.6$ Hz, $CH_3$). $^{13}$C NMR (75 MHz, $CD_3OD$): δ 179.4 (succinate), 137.0 (C-7a), 129.9 (C-3a), 127.5 (C-5), 126.3 (C-3), 123.5 (C-6), 121.8 (C-4), 112.6 (C-7), 102.2 (C-2), 50.8 (α-CH), 42.2 ($CH_2$), 32.9 ($CH_2$, succinate), 18.5 ($CH_3$).

*NMR data for α-methyltryptamine (**2**)*

$^1$H NMR (300 MHz, $CD_3OD$, assigned with the aid of a $^1$H/$^1$H-COSY): δ 7.54 (1 H, dt, $^3J = 8.1$ Hz, $J = 0.9$ Hz, H-4), 7.33 (1 H, dt, $^3J = 8.1$ Hz, $J = 0.9$ Hz, H-7), 7.11-7.05 (1 H, m, H-6), 7.05 (1 H, br s, H-2), 7.02-6.96 (1H, m, H-5), 3.25-3.13 (1 H, m (consistent with predicted dqd), α-CH), 2.82 (1 H, ddd, $J_{gem} = 14.1$ Hz, $^3J = 5.7$ Hz, $^4J_{H,H\text{-}2} = 0.9$ Hz, $CH_AH_B$), 2.69 (1 H, ddd, $J_{gem} = 14.1$ Hz, $^3J = 7.5$ Hz, $^4J_{H,H\text{-}2} = 0.6$ Hz, $CH_AH_B$), 1.12 (3 H, d, $^3J = 6.4$ Hz, $CH_3$). $^{13}$C NMR (75 MHz, $CD_3OD$): δ 138.3 (C-7a), 129.0 (C-3a), 124.0 (C-2), 122.3 (C-6), 119.6 (C-5), 119.5 (C-4), 113.4 (C-3), 112.3 (C-7), 49.9 (α-CH), 36.3 ($CH_2$), 23.0 ($CH_3$).

Gas chromatography-(electron ionization/chemical ionization)-mass spectrometry (GC-(EI/CI)-MS) analyses (scan range *m/z* 40–350) were carried out using a Varian 450-GC gas chromatograph coupled to a Varian 220-MS ion trap mass spectrometer. Samples were introduced (1 μl, ~0.5 mg/ml) with a Varian 8400 auto-sampler using a CP-1177 injector (280 °C) in split mode (1:50). Data manipulation was performed with the MS Data Review function of the Workstation software, version 6.91. Transfer line, manifold, and ion trap temperatures were set at 280, 80, and 220 °C, respectively. The liquid CI reagent was high performance liquid chromatography (HPLC) grade methanol. CI parameters (0.4 s/scan): CI storage level 19.0 *m/z*; ejection amplitude 15.0 *m/z*; background mass 55 *m/z*; maximum ionization time 2000 μs; maximum reaction time 40 ms; target TIC 5000 counts. A 30 m × 0.25 mm (0.25 μm film thickness) Supelco SLB-5 ms column (Bellefonte, PA, USA) was employed for separation. The temperature profile was as follows: The starting temperature was set at 80 °C and held for 1 min. The temperature then increased at 20 °C/min to 280 °C that was subsequently held constant for 9 min, leading to a total run time of 20 min.

Ultra high performance liquid chromatography-diode array detection (UHPLC-DAD) analyses were carried out using a Dionex 3000 Ultimate RLSC system (Thermo Dionex, St Albans, UK) with an UV spectral data acquisition range of 200–595 nm. A Dionex Acclaim Polar Advantage II (100 × 2.1 mm, 2.2 μm) column was used with a mobile phase of 25 mM triethylammonium phosphate buffer and acetonitrile. The column temperature was maintained at 40 °C.

For HPLC-DAD determinations, a Dionex 3000 Ultimate system (Thermo Dionex, St Albans, UK) was employed (UV full scan: 200–595 nm) using a Phenomenex Synergi Fusion column (150 × 2.0 mm, 4 μm) protected by a 4 mm × 3 mm Phenomenex Synergi Fusion guard column (Phenomenex, Cheshire, UK). The mobile phase consisted of 25 mM triethylammonium phosphate buffer and acetonitrile and the column temperature was set at 30 °C.

An ABSciex 3200 QTRAP LC/MS/MS instrument was used coupled to an Agilent 1200 HPLC-DAD system (ABSciex, Cheshire, UK). A Phenomenex Gemini column (150 × 2.0 mm, 5 μm) was protected by a 4 mm × 3 mm Phenomenex Gemini guard column (Phenomenex, Cheshire, UK) using a mobile phase of 1 mM ammonium formate with 1% formic acid and acetonitrile (column temperature 30 °C). Ionization was achieved with a Turbo V electrospray source. Liquid chromatography-mass spectrometry (LC-MS) data were obtained in positive enhanced mass spectrum (EMS) mode (scan range *m/z* 70–800) with information-dependent (above 10000 cps) enhanced product ion (EPI) scanning (between *m/z* 50–800). Product ions were formed using collision energies (CE) of 20, 30, and 50 eV in addition to collision energy spread (CES) of 35 V ± 15 eV. The following parameters were used: source temp: 500 °C, curtain gas: 40, gas 1: 40 units, gas 2: 55 units, ion spray voltage: 5000 V, collision gas: high, declustering potential: 40 V, entrance potential: 5 V, scan rate: 1000 amu/s (EMS), 4000 amu/s (EPI) and LIT fill-time: 20 ms.

### Analytical procedures

The HPLC-DAD and LC-MS procedures were based on the application of previously published methods.[18] HPLC used a 4–70% acetonitrile gradient ramp in 15 min with a 70% acetonitrile hold for 3 min and a flow rate of 0.6 ml/min producing a run time with equilibration of 18 min. UHPLC conditions employed a 6–70% acetonitrile gradient ramp in 3 min with a 70% acetonitrile hold for 1 min with a flow rate of 1.0 ml/min producing a run time with equilibration of 5 min. LC-MS used a 3–19% acetonitrile gradient ramp in 5 min then up to 25% acetonitrile in 5 min followed by an increase up to 65% acetonitrile in 9 min and held for 1 min with a flow rate of 0.8 ml/min producing a run time with equilibration of 21 min. A 'faster' LC-MS method was also included and involved a 3–65% acetonitrile gradient ramp in 3 min and a return to 3% acetonitrile in 3 min. The flow rate was 0.8 ml/min which led to a run time with equilibration of 6 min.

Biological fluid extraction for casework included a liquid-liquid 1-chlorobutane solvent extraction with 0.2 M sodium carbonate buffer followed by back extraction into 0.05 M sulfuric acid as previously published.[18]

## Results and discussion

*NMR spectroscopy data*

The $^1$H and proton-decoupled $^{13}$C NMR spectra were recorded for both 5-IT (**1**) and AMT (**2**) (see Experimental section for

chemical shift data). The $^{1}$H NMR data were compared with the spectra for 5-methyl and 3-methylindole in order to confirm the position of the side chain on the indole ring. For 5-IT succinate, the peak shapes and coupling constants for the aromatic region of the $^{1}$H NMR spectrum are comparable to those for 5-methylindole (Figure 1), confirming the side chain substitution on C-5. It should be noted that for 5-IT succinate, all aromatic peaks have been shifted downfield relative to 5-methylindole due to protonation of the nitrogen in the side chain. Each proton of the prochiral methylene group in the side chain of 5-IT is expectedly observed as a doublet of doublets due to geminal coupling and coupling to the α-CH group. As anticipated, the aromatic region of the $^{1}$H NMR spectrum of AMT bears little resemblance to 5-IT. Instead it is very similar to that recorded for 3-methylindole (skatole),[19] confirming side chain substitution on the 3-position for AMT. Of note, the prochiral methylene group of the side chain of AMT was unexpectedly observed as two ddd, with the fine 0.6 Hz and 0.9 Hz couplings attributed to $^{4}$J long range coupling to H-2 on the indole ring, confirmed in the $^{1}$H/$^{1}$H-COSY. In summary, there are sufficient differences in both the aromatic and side chain peaks in the $^{1}$H NMR spectra that this technique could be used to distinguish between relatively pure samples of AMT and 5-IT. The $^{13}$C NMR data are also reported for (**1**) and (**2**), however this technique is unlikely to be of use for the characterization of clinical samples due to its low sensitivity. Of note is the large difference in the chemical shift for C-2, recorded as 102.2 and 124.0 ppm for (**1**) and (**2**), respectively.

*GC-EI/CI-ion trap-MS data*

Both EI and CI mass spectra of 5-IT and AMT and their GC retention times are summarized in Figure 2. As expected, the similarity of both EI and CI spectra reflected the isomeric nature of both substances and followed a fragmentation pattern commonly observed with tryptamines.[20] Under CI conditions both protonated molecules exhibited a neutral loss of ammonia to yield the *m/z* 158 ion. A common issue that might be encountered when using a GC ion trap mass spectrometer is the occasional occurrence of an $[M+H]^{+}$ instead of the expected molecular ion at *m/z* 174 which might represent ion-molecule interactions within the trap when operating in the EI mode which might also include the formation of the *m/z* 158 species under these conditions. The EI spectrum observed for AMT appeared to be consistent with data of underivatized EI-MS previously published.[17,21]

*UHPLC-DAD and HPLC-DAD data*

Chromatographic analysis of 5-IT and AMT included two HPLC-DAD systems and were based on two different stationary phases. Both systems showed baseline separation of the compounds allowing for identification of retention parameters. Specifically, HPLC analysis resulted in a retention time (RT) of 3.9 min for AMT compared to a RT of 3.2 min for 5-IT (Figure 3A). UHPLC analysis resulted in a retention time (RT) of 1.3 min for AMT compared to a RT of 1.1 min for 5-IT when analysed concurrently.

Diode array UV spectroscopy has been previously shown to resolve structural isomers of some drugs, including 3- and 4-trifluoromethylphenylpiperazine (TFMPP).[18] In this case, the UV spectra of 5-IT and AMT showed similar features. However, there appeared to be slight differences in the secondary UV maximum, with 273 nm for 5-IT and 279 nm for AMT, respectively (Figures 3B and 3C). This was reproducible on different occasions and when using different diode array detectors, i.e. when using Dionex 3000 Ultimate and the Agilent 1200 Series DAD.

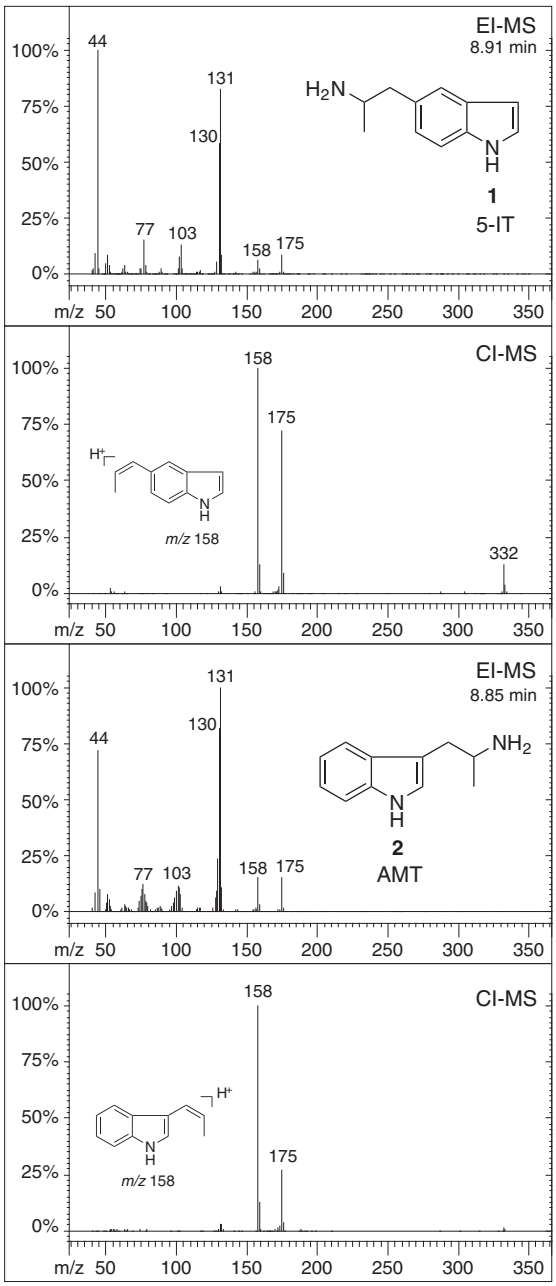


**Figure 2.** EI and CI ion trap mass spectra obtained for 5-IT succinate (**1**) and AMT free base (**2**). A neutral loss of ammonia led to the formation of the *m/z* 158 ion.

*HPLC-MS data*

Chromatographic analysis of 5-IT and AMT using two acetonitrile gradient conditions based on a different stationary phase to that used for both U/HPLC-DAD analyses, also showed baseline separation of the compounds allowing for retention parameter identification. Specifically, HPLC analysis with a longer gradient resulted in a RT of 2.6 min for AMT compared to a RT of 2.3 min for 5-IT when analysed concurrently. HPLC analysis with a much faster gradient resulted in a RT of 2.5 min for AMT compared to a RT of 2.3 min for 5-IT when analyzed concurrently. The relative similarity of retention times between the two gradient conditions is a reflection of the early elution characteristics of the compounds which was also apparent during U/HPLC-DAD analysis. This is

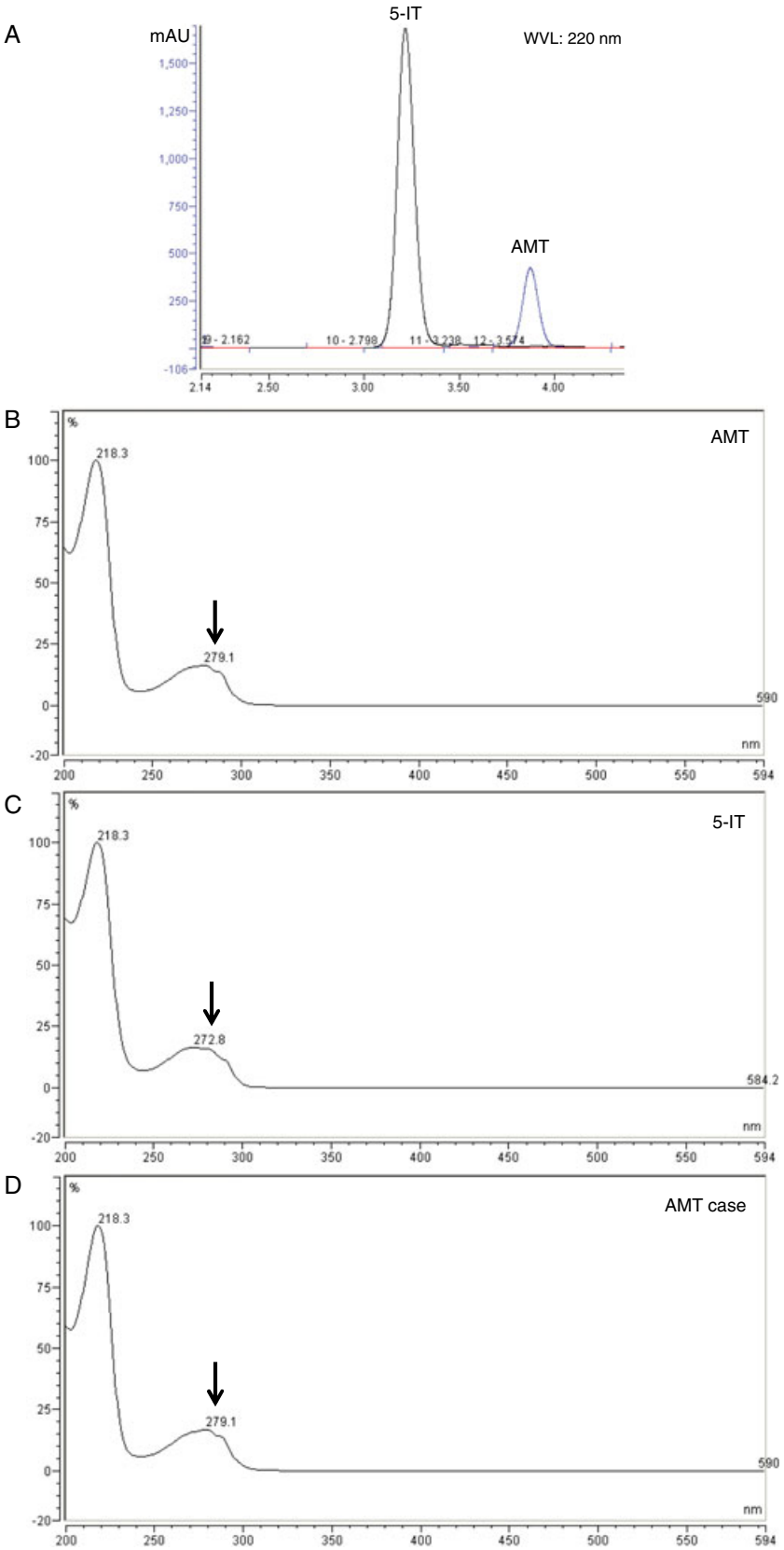


**Figure 3.** A: HPLC baseline chromatographic separation of 5-IT and AMT. B and C: UV full scan spectra of AMT and 5-IT showing discriminatory inflection and maximum. D: UV full scan trace applied to a casework sample confirming the identification of AMT.

compared to the observed void volume of 0.5 min for all HPLC conditions and 0.3 min for the UHPLC conditions applied.

Positive electrospray mass spectrometry at various collision energies (20, 35, and 50 eV) showed identical product ions for 5-IT and AMT (*m/z* 103, 117, 130, 143, 158) with some in-source fragmentation of the protonated molecule at *m/z* 175 (Figure 4). However, there were important and distinct differences in relative abundance (Figure 5), allowing for the potential use of ion ratios for multiple reaction monitoring (MRM) transitions. Specifically, for AMT: *m/z* 175/158 *m/z* (100% abundance), *m/z* 175/143 (78%), *m/z* 175/130 (30%) and for 5-IT: *m/z* 175/158 (100% abundance), *m/z* 175/143 (22%), *m/z* 175/130 (84%).

*Biological fluid case analysis currently underway*

Application of the presented U/HPLC-DAD and HPLC-MS methods allowed the detection and identification of AMT in 5 fatal cases investigated by ROAR Forensics laboratory. The analytical characteristics as outlined above were consistent with AMT and not 5-IT (Figures 3D and 6). Case 1 (used as the representative example in some of the figures) involved AMT at a measured post mortem (PM) blood concentration of 0.89 mg/l along with 3,4-methylenedioxypyrovalerone (MDPV) only. Case 2 involved AMT (0.48 mg/l PM blood) along with cocaine and amphetamine. Case 3 involved AMT (0.29 mg/l PM blood) along with 4-methyl-*N*-ethylcathinone (4-MEC) and amphetamine.

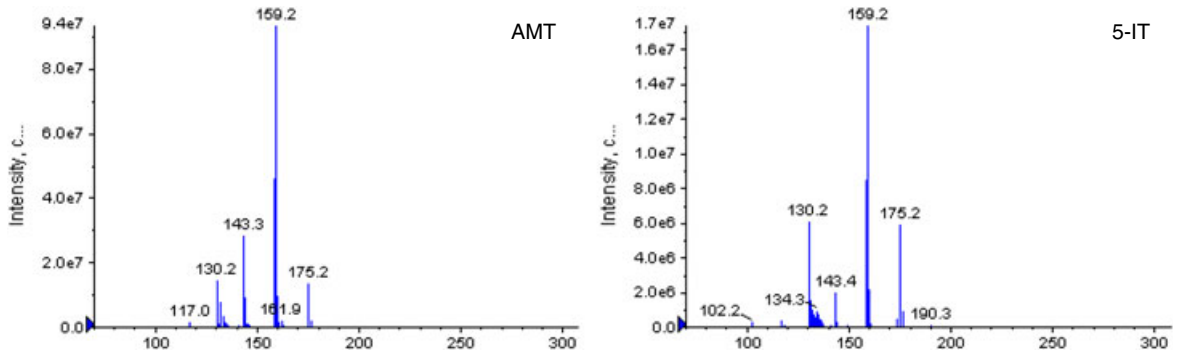


**Figure 4.** In-source fragmentation of AMT and 5-IT in positive mode electrospray LC-MS.

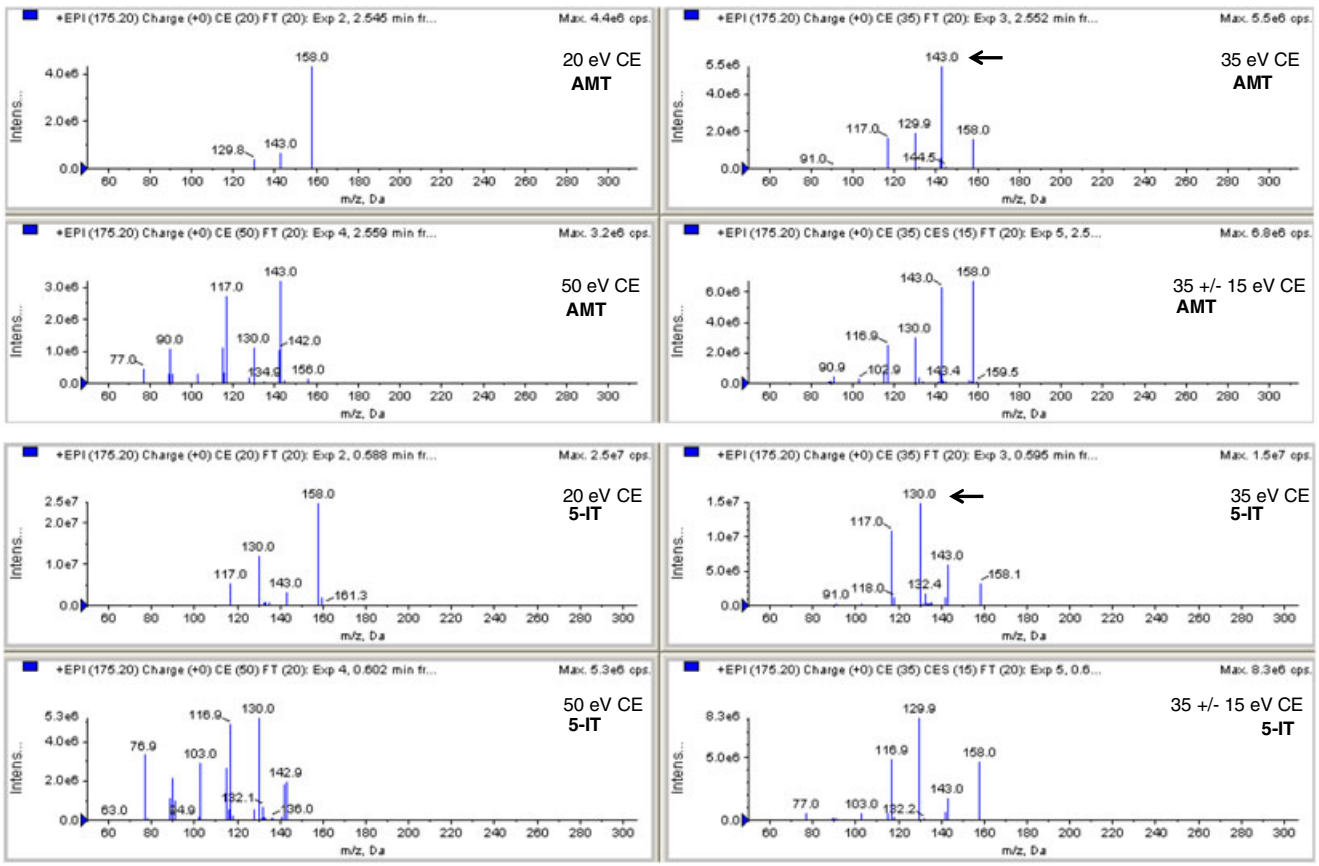


**Figure 5.** Enhanced Product Ion scans of AMT and 5-IT at varying collision energies (CEs) using electrospray LC-MS. The formation of distinct ion ratios allowed for differentiation between both isomers.

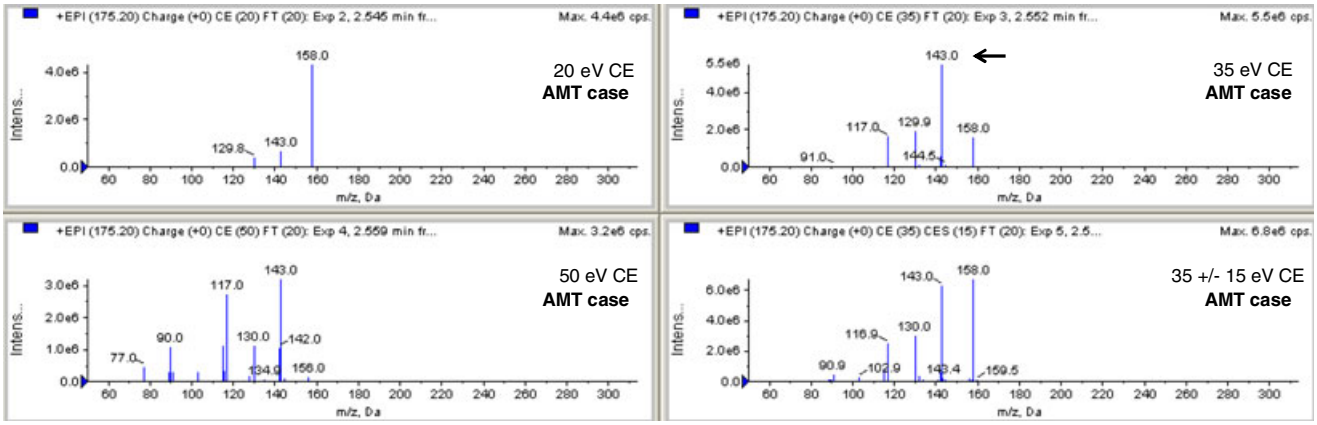


**Figure 6.** LC-MS Enhanced Product Ion scan results of AMT in a casework example.

Case 4 involved AMT (1.00 mg/l PM blood) along with MDMA and cannabinoids. The concentration of AMT and other drugs (5,6-methylenedioxy-2-aminoindane (MDAI), 5-iodo-2-aminoindane (5-IAI), 4-fluoro-*N*-methylcathinone (4-FMC), MDMA, MDPV, 3,4-methylenedioxy-*N*-methylcathinone (methylone) and methoxetamine) in Case 5 could not be determined due to the highly decomposed nature of the PM blood. The ability of the employed U/HPLC procedures allowed for the clear chromatographic separation of both isomeric analytes which helped to exclude co-elution in the cases highlighted showing 5-IT was not detected. If both substances were present in a case the retention time separation alone would be sufficient for dual identification.

## Conclusion

The fact that AMT (α-methyltryptamine) has been found in fatal intoxications raises concerns about this particular substance and more details about its pharmaco-toxicological profile require further studies. A review of the limited numbers of early clinical reports on AMT indicated a diverse range of less predictable dose-dependent psychoactive properties. Moreover, there are indications that at least in some cases the effects of AMT might take up to several hours to get noticed by the user which carries the additional risk of overdose and the possibility to consider drug combinations which may add to the potential to cause concern. In addition, it would also appear that AMT could be confused with 5-IT (5-(2-aminopropyl)indole), and vice versa, during routine laboratory analysis given its isomeric nature. It is hoped that the present report serves as an aid in the attempt to differentiate these two substances.

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