Identification and assessment of new psychoactive substances: a European network **(NPS-EURONET)**

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Synthesis α-methyltryptamine

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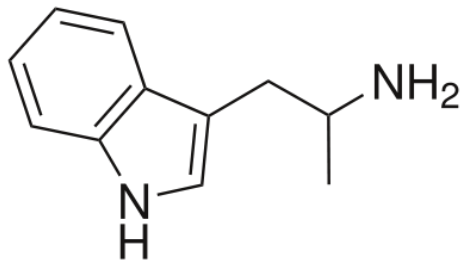
Reference standards of New Psychoactive Substance (NPS) tentatively identified in urine and/or wastewater are to be purchased in order to confirm the identity. However, reference standards are not available for all substances and therefore the synthesis of some substances is required. In this document we describe the synthesis of α-methyltryptamine. The analyte is then thoroughly identified and characterized by NMR and High Resolution Mass Spectrometry (HRMS).

Alpha-methyltryptamine is a psychedelic, stimulant, and entactogen drug of the tryptamine class. This NPS was tentatively identified in urine samples provided by Instituto Nacional de Medicina Legal e Ciencias Forenses (INMLCF, partner P4)(Lisbon, Portugal) and Norwegian Institute for Water Research (NIVA, associate partner P1) (Oslo, Norway). The urine and pooled urine samples were taken from a hospital in Lisbon and a music festival in Norway, respectively.

	**α-methyltryptamine**
	IUPAC name: 1-(1H-Indol-3-yl) propan-2-amine
	ChemSpider ID: 8930
	SMILES: CC(Cc1c[nH]c2c1cccc2)N
	Formula: $C_{11}H_{14}N_2$
	$[M+H]^+$: 175.1235

***Synthesis of* α-methyltryptamine[1].** A prepared solution of 25.75 g indole in 100 mL dimethylformamide (DMF). A second solution was also prepared by cooling 80 mL DMF in an external ice bath (internal temperature about 12°C), stirring well, and adding 20 mL phosphoryl chloride (POCl3) dropwise over the course of 30 min. This was then warmed to 25°C and the first solution of indole in DMF was added, dropwise (with continued stirring), over an additional 30 min. Stirring was continued for yet another 45 min, during which time the temperature was raised to 40°C. Yellow solids formed during this period. The reaction mixture was poured onto chipped ice, which produced a clear red solution. This was made basic with the addition of 200 mL 5N sodium hydroxide (NaOH), which allowed the separation of a yellow solid. This was diluted by the addition of 200 mL hot water (H_2O) and, after cooling again, the product was removed by filtration and washed with cold H_2O. This can be recrystallized from aqueous DMF to yield, after air-drying, 25.5 g (84%) of indole-3-carboxaldehyde as faint orange needles.

A solution of 4.35 g indole-3-carboxaldehyde in 17.2 mL nitroethane was treated with 0.77 g ammonium acetate and heated, with occasional swirling, on the steam bath of 2.5 h. The excess reagent was removed under vacuum and the resulting yellow solids washed with H_2O and air-dried. Trituration under 25 mL dry methanol (MeOH), filtration and air-drying gave 5.22 g (86%) 1-(3-indolyl)-2-nitroprop-1-ene as a yellow powder with mp 190-192°C.

A suspension of 10.7 g lithium aluminium hydride (LAH) in 100 mL anhydrous tetrahydrofuran (THF) was placed under an inert atmosphere, stirred, and treated, dropwise, with a solution of 10 g 1-(3-indolyl)-2-nitroprop-1-ene in anhydrous THF over the course of 2.5 h. The reaction mixture was brought to reflux temperature, held there for 2 h, and then returned to room temperature. The excess hydride was destroyed with an aqueous THF solution (80 mL of 25% solution) and there was then added 10 mL of 50% NaOH. There was added 150 mL diethyl ether (Et_2O), and stirring was continued until no more solids formed. The reaction mixture was filtered and the filter cake washed with 150 mL Et_2O. The filtrates and washings were combined, dried over potassium carbonate (K_2CO_3), and the solvent removed under vacuum. The residue weighed 9.2 g and was distilled at 130-140°C at 1 mm/Hg to give a white oil the crystallized and had a mp of 96-98°C. This was recrystallized from an ethyl acetate/petroleum ether mixture, and had a mp of 97-100°C. The yield was 6.3 g (73%). IR (in cm^{-1}): 750, 818, 911, 1093, 1111. MS

[1] Alexander Shulgin and Ann Shulgin (1997) TIHKAL #48 α-methyltryptamine. ISBN: 0-9630096-9-9

(in *m/z*): $C_2H_6N^+$ 44 (100%); indolemethylene$^+$ 130, 131 (44%, 43%); parent ion 174 (2%). A sample dissolved in 10 volumes of MeOH, treated with one equivalent of glacial acetic acid, and taken to dryness under vacuum gave the acetate salt which, on recrystallization form ethyl acetate and air-drying to constant weight, yielded the product α-methyltryptamine (α-MT) as fine white crystals with a mp of 143-144°C. The fumarate salt, formed by the addition of ethyl acetate to a hot solution of free base α-MT in methanol which had been neutralized with fumaric acid, was isolated as fine white needles with a mp of 200-203°C.

Confirmation of the identity.

Nuclear Magnetic Resonance (NMR)

High-field 1H and 13C{1H} NMR analyses were recorded with Varian NMR System 500 MHz spectrometer at 303 K using CDCl3 (Varian, Palo Alto, CA, USA). The residual solvent signals [CHCl3 (1H: δ = 7.26) and CDCl3 (13C: δ = 77.16)] were used as the internal references.

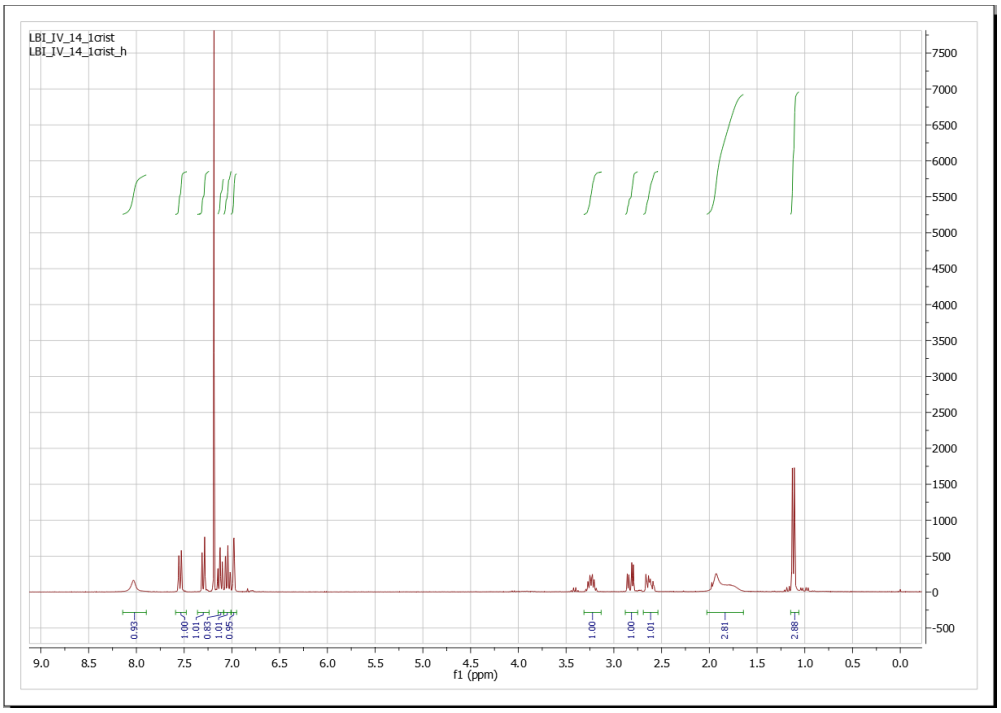


Figure 1: ^{1}H NMR spectrum of α-methyltryptamine

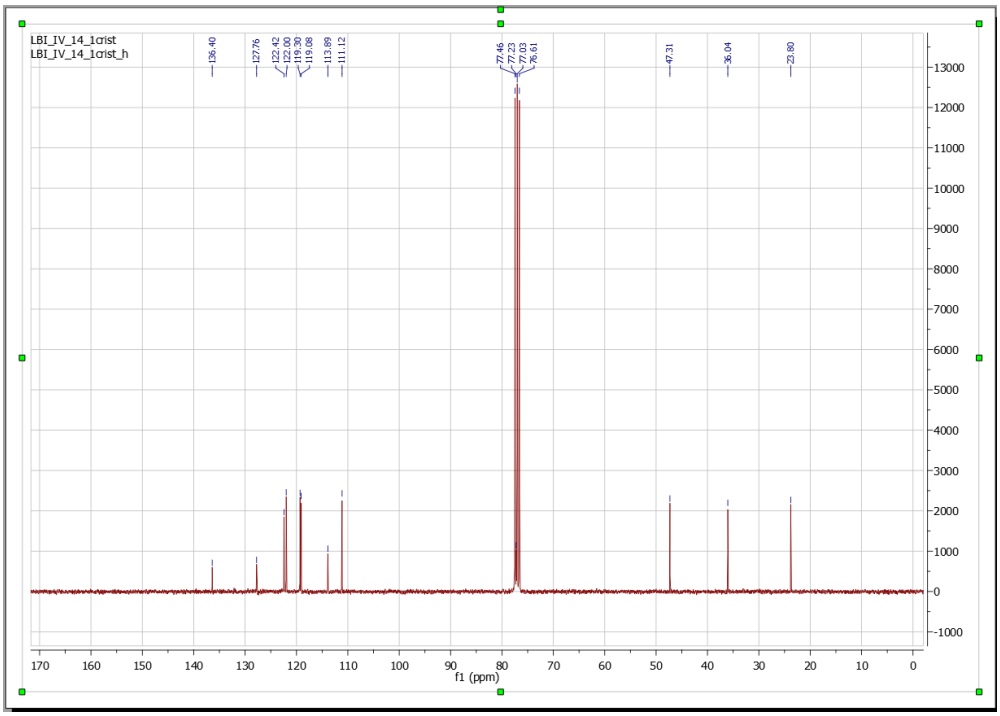


Figure 2: ^{13}C NMR spectrum of α-methyltryptamine

The ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of α-methyltryptamine displayed expected five aromatic signals for a 3-substituted indole ring (two doublets and two triplets corresponding to *ortho*-substituted phenyl ring and one singlet corresponding to the proton at the 2 position of the indole ring) and the signals of the 2-propanamine moiety (ABX pattern and doublet corresponding to the methyl at 1.11 ppm), NH signals appear as broad singlets at 8.00 ppm (indole ring) and 1.65-1.90 ppm (NH_2). The ^{13}C NMR (125 MHz, $CDCl_3$) spectrum was consistent with the structure and allowed the assignments of carbon resonances. Eight aromatic signals were observed: two of them upfield shifted at 136.40 and 127.76 ppm corresponding to quaternary carbons of positions 8 and 9 of the indole ring respectively, the other four signals of the phenyl ring were observed at 122.40, 122.00, 119.30 and 119.08 ppm, and positions 2 and 3 of the indole ring at 113.89 and 111.12 ppm. The signals from 2-propanamine were at 47.31 ppm for the nitrogenated carbon, and for the aliphatic carbons: 36.04 ppm and 26.80 ppm (methyl).

High Resolution Mass Spectrometry

A Waters Acquity UPLC system (Waters, Milford, MA, USA) was coupled to a quadrupole-orthogonal acceleration-TOF mass spectrometer (XEVO G2 QTOF, Waters Micromass, Manchester, UK), with an orthogonal Z- spray- ESI interface operating in positive ion mode. A Cortecs C_{18} 2.1x100 mm with 2.7 µm particle size was employed for chromatographic separation. Mobile phase, at a flow rate of 0.3 mL min^{-1}, consisted of water/methanol gradient both with 0.01% of formic acid. The percentage of organic modifier (B) was changed linearly as follows: 0 min, 10% B; 14.00 min. 90% B; 16.00 min. 90% B; 16.01 min, 10% B; 18.00 min, 10% B. The column temperature was set at 40 ºC. MS data were acquired on the *m/z* range of 50 - 1200. A capillary voltage of 0.7 kV was used with a cone voltage of 20 V. Collision gas was argon 99.995% (Praxair, Valencia, Spain). The interface temperature was set to 650 ºC and the source temperature at 120 ºC. For automated accurate mass measurement, the lock-spray probe was used, using a lock mass solution of leucine enkephalin (10 mg L^{-1}) in acetonitrile:water (1:1) at 0.1 % HCOOH pumped at 30 µL min^{-1} through the lock-spray needle. The protonated molecule of leucine enkephalin at *m/z* 556.2771 was used for recalibrating the mass axis and ensuring a robust accurate mass measurement along time. For MS^E, two acquisition functions with different collision energies were generated. The low collision energy function (LE) with a collision energy of 4 eV, and the high collision energy function (HE) with a collision energy ramping from 15 to 40 eV. MS data in centroid mode were processed with ChromaLynx XS application manager (within MassLynx v4.1; Waters Corporation).

It should be noted that all the exact masses shown have a deviation of 0.55mDa from the “true” value, as the calculation performed by the MassLynx software uses the mass of hydrogen instead of a proton when calculating $[M+H]^+$ exact mass. However, because this deviation is also applied during mass axis calibration, there is no negative impact on the mass errors presented.

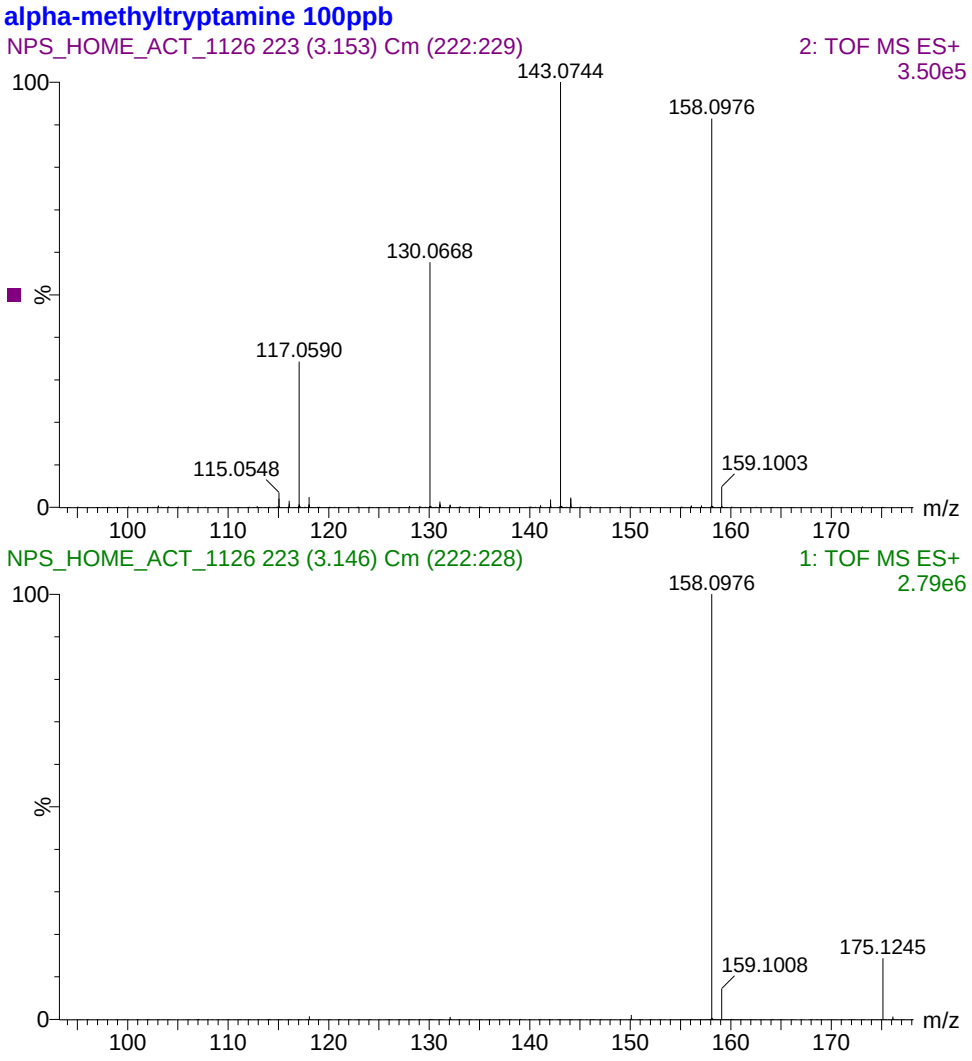
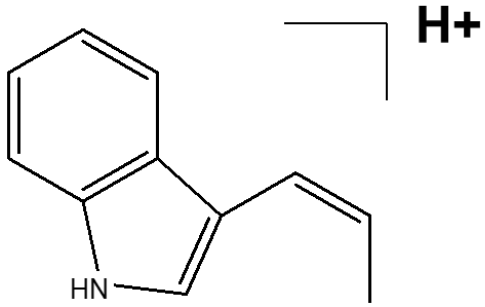
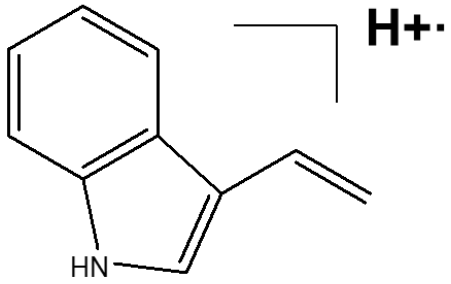
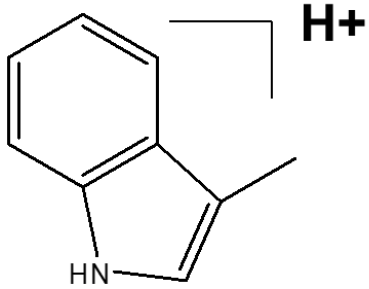
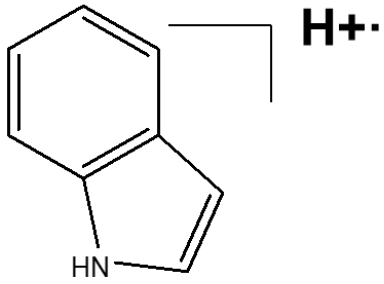


Figure 3: High resolution mass spectra of α-methyltryptamine (Bottom, LE spectrum; Top, HE spectrum)

The retention time of α-methyltryptamine using the conditions as described above was 3.15 min. In the low energy spectrum (**Figure 3, Bottom**) the protonated molecule can be observed with *m/z* 175.1245 with relative mass error of 5.7 ppm (absolute mass error, 1 mDa). The following fragment ions can be observed in the high energy spectrum (**Figure 3, Top**) and coincide with what is reported in the literature[2]

[2] Elliott SP, Brandt SD, Freeman S, Archer RP *AMT (3-(2-aminopropyl)indole) and 5-IT (5-(2-aminopropyl)indole): an analytical challenge and implications for forensic analysis*. Drug Test. Analysis 2013, 5, 196 - 202

Fragment ion	Exact mass *m/z*	Accurate mass *m/z*	Relative mass error (ppm)
	**158.0970**	**158.0976**	**3.7**
	**143.0735**	**143.0744**	**6.2**
	**130.0655**	**130.0668**	**9.9**
	**117.0578**	**117.0590**	**10.2**