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Characterisation of a proposed internet synthesis of *N*,*N*-dimethyltryptamine using liquid chromatography/electrospray ionisation tandem mass spectrometry

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ABSTRACT

The psychoactive properties of *N*,*N*-dimethyltryptamine (DMT) are known to induce altered states of consciousness in humans. These properties attract great interest from clinical, neuroscientific, clandestine and forensic communities. The *Breath of Hope Synthesis* was reported on an internet website as a convenient two-step methodology for the preparation of DMT. The analytical characterisation of the first stage was the subject of previous publications by the authors and involved the thermal decarboxylation of tryptophan and the formation of tryptamine. The present study reports on the characterisation of the second step of this procedure which was based on the methylation of tryptamine. This employed methyl iodide and benzyltriethylammonium chloride/sodium hydroxide as a phase transfer catalyst. The reaction product was characterised by liquid chromatography/electrospray ionisation tandem mass spectrometry and orthogonal acceleration time-of-flight mass spectrometry. Quantitative evaluation was carried out in positive multiple reaction monitoring mode (MRM), which included synthesis of the identified reaction products. MRM screening of the product did not lead to the detection of DMT. Instead, 11.1% tryptamine starting material, 21.0% *N*,*N*,*N*-trimethyltryptammonium iodide (TMT) and 47.4% 1-*N*-methyl-TMT were detected. A 0.5% trace of the monomethylated *N*-methyltryptamine was also detected. This study demonstrated the impact on product purity of doubtful synthetic methodologies discussed on the internet.

1. Introduction

Many compounds with biological activity are derivatives of tryptamine **1** (Fig. 1). *N*,*N*-Dimethyltryptamine (DMT) **2** (Fig. 1) is a simple tryptamine derivative with powerful psychoactive properties in humans. It is a naturally occurring hallucinogen [1,2] that is also easily accessible through a variety of synthetic routes. DMT is not only structurally related to the neurotransmitter serotonin (5-hydroxytryptamine) but also forms the structural basis for a number of triptan-type anti-migraine drugs such as sumatriptan which is a *N*-methylmethanesulfonamide derivative of DMT. Due to a renewed interest in the role of such compounds in understanding mechanisms of mental functioning, DMT has become a popular target for several human clinical studies [3–10].

The implementation of quality control procedures plays a fundamental role in pharmaceutical and clinical analysis [11]. In addition, identification of potentially toxic contaminants and novel bioactive by-products present in illegally manufactured preparations of DMT and analogues is not well documented, but is important from the forensic, drug discovery and public health perspective [12,13].

The authors' interest in the analytical characterisation of a clandestine preparation of DMT arose from a two-stage procedure, known as *The Breath of Hope Synthesis* that was published on an internet website. This procedure was based on the thermolytic decarboxylation of commercially available tryptophan, followed by methylation to DMT using methyl iodide and benzyltriethylammonium chloride/sodium hydroxide employed as a phase transfer catalyst [14]. Previous work carried out by the authors involved the characterisation of the first stage. Thermolytic decarboxylation of tryptophan led to the formation of several solvent- and catalyst-dependent tetrahydro-β-carboline by-products in significant quantities. The desired decarboxylation product tryptamine **1** was detected but yields varied greatly depending on the choice of solvent and catalyst used [15,16].

The present paper reports on the analytical characterisation of the second step of *The Breath of Hope* procedure using liquid chromatography electrospray ionisation tandem mass spectrometry (LC–ESI-MS/MS). Identification and quantification of by-products were supported by organic synthesis of the target molecules.

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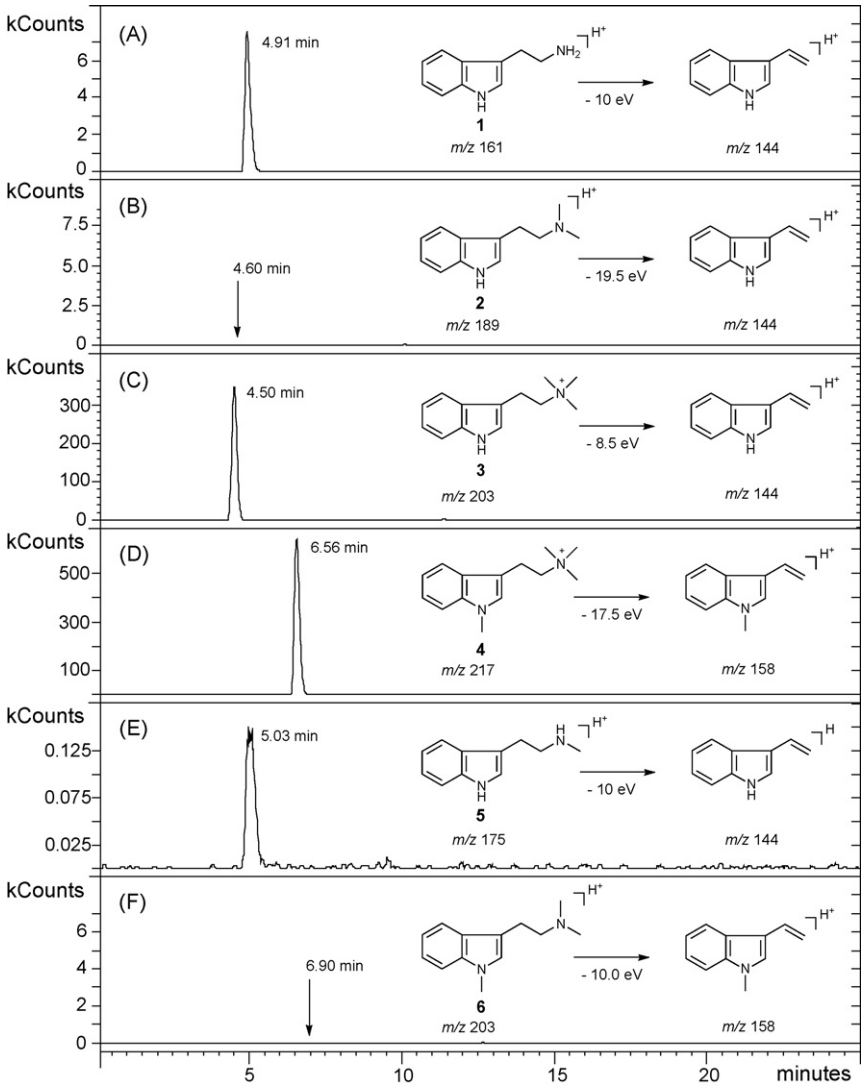

Fig. 1. Selected ion transitions, optimised collision energies and representative multiple reaction monitoring (MRM) traces of the crude product synthesised from tryptamine **1** following the *Breath of Hope* procedure. The desired product (DMT **2**) was not detected but instead, the major products were the quaternary ammonium salts **3** and **4**. For yields, see Table 1.

2. Experimental

2.1. Materials

Tryptamine **1** and 5-methoxytryptamine (internal standard, IS) were obtained from Aldrich (UK), while *N*,*N*-dimethyltryptamine (DMT) was available as a standard from previous work [17]. Silica gel for flash chromatography (particle size 40–63 μm) and silica gel aluminium thin-layer chromatography (TLC) plates were obtained from VWR (UK). All other solvents and reagents used for the synthesis of standards were purchased from Aldrich (UK) and were of analytical grade. Sodium hydride was employed as a 60% dispersion in mineral oil.

2.2. Instrumentation

A Varian 1200L LC–ESI-MS/MS system was used in positive ion mode. A Phenomenex Synergi MAX-RP column (250 mm × 4.60 mm, 4 μm) was employed for chromatographic separation. The solvent system consisted of 10 mM ammonium formate in 0.1% (v/v) aqueous formic acid solution (A) and 0.1% (v/v) formic acid in methanol (B). The LC gradient started at 30% B, ramped to 90% B within 15 min, held for 5 min. This was followed by returning to 30% B within 2 min and holding for 3 min for equilibration (total run time 25 min). The flow rate was set to 1 mL/min, followed by a post-column split, where 160 μL/min was directed towards the electrospray interface. Direct infusion of products was used to yield the optimized source parameters: nebulising and drying gas was nitrogen at 250 °C (5 psi). The collision gas was argon and collision-induced dissociation (CID) took place at 1.2 mTorr at 42 °C. The needle was held at 5000 V, capillary voltage was 40 V and shield at 600 V. Default voltages were used on Q_0, Q_1 and Q_3, respectively 6 V, 1 V and 1.9 V. The selected ion transitions used for quantitation are summarised in Fig. 1.

NMR spectra were recorded using a Bruker Avance 300 spectrometer at 300.1 MHz (^{1}H NMR) or 75.5 MHz (^{13}C NMR). NMR spectra were recorded in $CDCl_3$ and obtained by ^{1}H, proton decoupled ^{13}C, DEPT-135, HSQC and HMBC experiments. Chemical shifts are reported relative to TMS at $\delta = 0$ ppm. When d_6-DMSO was used, chemical shifts were determined relative to the residual solvent peak at $\delta = 2.51$ (^{1}H NMR) and $\delta = 39.6$ ppm (^{13}C NMR).

A Micromass LCT orthogonal acceleration time-of-flight mass spectrometer (Micromass, UK) equipped with an electrospray ionisation source was operated in positive mode. Samples were

introduced using a flow injection method *via* a Harvard Apparatus (Pump 11) (Massachusetts, USA) syringe pump at 20 μL/min. The instrument was tuned and calibrated in the mass range 100–1000 Da using a sodium formate solution (0.005 M in 50:50 acetonitrile–water). Exact mass measurements were based on protonated molecules $[M+H]^+$. Indole-3-yl-*N*-methylglyoxalylamide was detected as the $[M+Na]^+$ adduct. Leucine enkephalin (1 μg/mL) was used as lock mass standard after instrument calibration. Operation settings were—capillary voltage: 3000 V, sample cone voltage: 30 V, RF lens: 250 V, desolvation temperature: 150 °C, source temperature: 100 °C, acceleration: 200 V, cone gas flow: 22 L/h, desolvation gas flow: 602 L/h. Data acquisition was carried out using MassLynx version 4.0 SP2.

2.3. Synthesis of standards

N,*N*-Dimethyltryptamine (DMT) **2** was available from previous work [17].

2.3.1. Synthesis of N,N,N-trimethyltryptammonium iodide (TMT) *3*

Adapted from a published procedure [18]. Iodomethane (3.42 g, 24 mmol, 1.5 mL) was added to a solution of tryptamine (700 mg, 4.4 mmol), dissolved in isopropanol (30 mL), and stirred for 2 h at room temperature. The resulting solid product was filtered and washed four times with 30 mL isopropanol and dried overnight *in vacuo* over P_2O_5. Yield: 707 mg (2.1 mmol, 48%). ^{1}H NMR (d_6-DMSO): 10.99 (NH-1, br s), 7.65 (1H, d, H-4, *J* 7.9 Hz,), 7.39 (1H, d, H-7, *J* 7.9 Hz), 7.30 (1H, d, H-2, *J* 1.5 Hz), 7.12 (1H, t, H-6, *J* 7.3 Hz), 7.03 (1H, t, H-5, *J* 7.3 Hz), 3.63–3.58 (2H, m, CH_2-α), 3.21 (11H, m, overlapping CH_2-β, 3× N-CH_3). ^{13}C NMR: 136.5 (C-7a), 127.0 (C-3a), 123.8 (C-2), 121.7 (C-6), 118.9 (C-5), 118.7 (C-4), 111.9 (C-7), 110.8 (C-3), 65.5 (CH_2-α), 52.5 (N-CH_3), 19.0 (CH_2-β). HRESIMS theory $[M]^+$: 203.1548; observed: 203.1560.

2.3.2. Synthesis of 1-Me-N,N,N-trimethyltryptammonium iodide (1-Me-TMT) *4*

Adapted from a published procedure [18]. Iodomethane (383 mg, 2.7 mmol, 0.168 mL) was added to a solution of 1-methyl-*N*,*N*-dimethyltryptamine **6** (synthesis described below) (100 mg, 0.49 mmol), dissolved in isopropanol (20 mL), and stirred for 2 h at room temperature. Workup as described above for **3**. Yield: 75 mg (0.22 mmol, 45%). ^{1}H NMR (d_6-DMSO): 7.67 (1H, d, H-4, *J* 7.9 Hz,), 7.43 (1H, d, H-7, *J* 8.3 Hz), 7.27 (1H, s, H-2), 7.19 (1H, t, H-6, *J* 7.5 Hz), 7.08 (1H, t, H-5, *J* 7.3 Hz), 3.76 (3H, s, N_1-CH_3), 3.61–3.55 (2H, m, CH_2-α), 3.21 (11H, m, overlapping CH_2-β, N-CH_3). ^{13}C NMR: 137.0 (C-7a), 128.1 (C-2), 127.3 (C-3a), 121.8, 119.0 (overlapping C-5 and C-4), 110.2 (C-7), 108.0 (C-3), 65.5 (CH_2-α), 52.6 (N-CH_3), 32.8 (N_1-CH_3), 18.9 (CH_2-β). HRESIMS theory $[M]^+$: 217.1705; observed: 217.1693.

2.3.3. Synthesis of N-methyltryptamine (NMT) *5*

Indole (637 mg, 5.44 mmol) was dissolved in 150 mL anhydrous ether and stirred on ice for 30 min. Oxalyl chloride (2.07 g, 16.3 mmol) was added dropwise, stirred for 30 min on ice and kept at −20 °C for 4 h. The acid chloride was filtered and washed three times with cold anhydrous ether (50 mL) and dried *in vacuo* for 3 h at room temperature to give 1.02 g (4.90 mmol, 90%) indole-3-yl-glyoxalyl chloride. *N*-Methylamine (243 mg, 7.81 mmol, 0.170 mL) was added dropwise to an ice-cold solution of indole-3-yl-glyoxalyl chloride (540 mg, 2.60 mmol) dissolved in 100 mL anhydrous THF. The mixture was stirred on ice for 4 h. The solvent was evaporated under reduced pressure to give a crude solid that was purified by flash chromatography (DCM–MeOH, 9:1). The solvent was evaporated under reduced pressure, recrystallised from methanol and dried overnight under vacuum to give indole-3-yl-*N*-methylglyoxalylamide. Yield: 837 mg (4.14 mmol, 79%). Mp 220–222 °C, lit.: 222–223 °C [19]. ^{1}H NMR: 8.73 (1H, s, H-2), 8.24-8.16 (1H, m, H-4), 7.56–7.49 (1H, m, H-7), 7.27 (1H, td, H-6, J 6.9, 1.8 Hz), 7.24 (1H, td, H-5, *J* 7.2, 1.9 Hz), 2.73 (3H, s, N-CH_3). ^{13}C NMR: 182.2 (CO-β), 164.2 (CO-α), 138.6 (C-2), 136.3 (C-7a), 126.3 (C-3a), 123.5 (C-6), 122.6 (C-5), 121.4 (C-4), 112.6 (C-7), 112.2 (C-3), 25.6 (N-CH_3). HRESIMS theory $[M+Na]^+$: 202.0742; observed: 202.0731.

A solution of indole-3-yl-*N*-methylglyoxalylamide (303 mg, 1.5 mmol) in 40 mL anhydrous THF was added dropwise to a stirred, ice-cold slurry of lithium aluminium hydride (570 mg, 15 mmol) in 100 mL anhydrous THF under nitrogen. This reaction mixture was heated at reflux for 24 h and then cooled on ice. The excess hydride was destroyed by the dropwise addition of 5 mL water, followed by 5 mL 20% NaOH and 5 mL of water. The precipitated inorganic salts were filtered and washed three times with 30 mL THF. The filtrate was evaporated under reduced pressure and the resulting oily residue was dissolved in 75 mL $CHCl_3$, washed three times with water and once with saturated aqueous NaCl. The organic phase was dried over anhydrous $MgSO_4$, evaporated under reduced pressure. The resulting product was purified by flash chromatography ($CHCl_3$–MeOH–NH_4OH, 8.5:1.5:0.01). The free base product was dried overnight *in vacuo* over P_2O_5 to give **5**. Yield: 136 mg (0.78 mmol, 52%). ^{1}H NMR (d_6-DMSO): 10.75 (NH-1, br s), 7.51 (1H, d, H-4, *J* 7.9 Hz,), 7.34 (1H, d, H-7, *J* 7.9 Hz), 7.13 (1H, s, H-2), 7.06 (1H, t, H-6, *J* 7.5 Hz), 6.97 (1H, t, H-5, *J* 7.9 Hz), 2.86–2.81 (2H, m, CH_2-β), 2.78–2.73 (2H, m, CH_2-α), 2.32 (3H, s, N-CH_3). ^{13}C NMR: 136.5 (C-7a), 127.6 (C-3a), 122.9 (C-2), 121.2 (C-6), 118.6 (C-5), 118.5 (C-4), 112.7 (C-3), 111.6 (C-7), 52.3 (CH_2-α), 36.0 (N-CH_3), 25.2 (CH_2-β). HRESIMS theory $[M+H]^+$: 175.1235; observed: 175.1247.

2.3.4. Synthesis of 1-methyl-N,N-dimethyltryptamine (1-Me-DMT) *6*

Adapted from a published procedure [20]. *N*,*N*-Dimethyltryptamine free base **2** [17] (100 mg, 0.53 mmol) was dissolved in anhydrous DMF (8 mL) and stirred on ice for 5 min. To this solution were added 60% sodium hydride (25 mg, 0.62 mmol) and 2 mL DMF and stirred on ice for 5 min. This solution was then left to stir for 30 min at room temperature, followed by 5 min on ice. Iodomethane (91 mg, 0.64 mmol, 40 μL) was added and the mixture was left to stir overnight. Water (5 mL) was added, followed by three extractions with 50 mL $CHCl_3$. After evaporation under reduced pressure the crude reaction product was subjected to flash chromatography and workup as described for **5** to give **6**. Yield: 70 mg (0.35 mmol, 66%). ^{1}H NMR ($CDCl_3$): 7.59 (1H, d, H-4, *J* 7.5 Hz,), 7.27 (1H, d, H-7, *J* 7.9 Hz), 7.21 (1H, td, H-6, *J* 7.5, 1.5 Hz), 7.10 (1H, td, H-5, *J* 7.3, 1.5 Hz), 6.87 (1H, s, H-2), 3.71 (3H, s, N_1-CH_3), 2.96 (2H, t, CH_2-β, *J* 8.1 Hz), 2.66 (2H, t, CH_2-α, *J* 8.1 Hz), 2.36 (6H, s, N-CH_3). ^{13}C NMR: 137.4 (C-7a), 128.2 (C-3a), 126.8 (C-2), 121.9 (C-6), 119.3 (C-5), 119.1 (C-4), 112.9 (C-3), 109.6 (C-7), 60.8 (CH_2-α), 45.7 (N-CH_3), 33.0 (N_1-CH_3), 23.8 (CH_2-β). HRESIMS theory $[M+H]^+$: 203.1548; observed: 203.1538.

2.4. Breath of Hope procedure

Crushed NaOH (1.28 g, 32 mmol), benzyltriethylammonium chloride (0.06 g, 0.26 mmol) and DCM (30 mL) were combined and allowed to stir at room temperature for 15 min. Tryptamine (1 g, 6.24 mmol) was added and the mixture was allowed to stir for 1 h. Methyl iodide (2.2 g, 15.5 mmol) was then added to the solution and stirred overnight. The reaction mixture contained a solid material and a dark brown solution. The solid was separated by filtration under suction, washed three times with DCM (20 mL) and kept separately. The organic fractions were pooled and washed with distilled water (50 mL). The organic phase was evaporated under reduced pressure and the resulting product was dried overnight *in vacuo* over P_2O_5 to yield 1.24 g of the crude, brown product.

3. Results and discussion

The second step of the *Breath of Hope Synthesis* proposed on the internet was based on the synthesis of DMT **2** using tryptamine **1** as the starting material (Fig. 1). This procedure involved an S_N2 reaction using methyl iodide as the methylating agent, dichloromethane as the solvent and benzyltriethylammonium chloride/sodium hydroxide as a phase transfer catalyst (PTC) [14].

3.1. Identification of by-products

Preliminary studies, based on direct infusion of the *Breath of Hope* reaction product into the electrospray ionisation source, revealed that the expected protonated molecule of the desired product at *m/z* 189 (DMT **2**) was absent. Instead, two dominant species at *m/z* 203 and *m/z* 217 and one of minor intensity at *m/z* 161 were observed.

Two main indicators directed the identification process. The first was based on the chemistry involved in the reactivity of the tryptamine **1** starting material. The tryptamine molecule contains three potential abstractable protons, located at the indole nitrogen (N–H) and the ethylamine side chain ($-NH_2$) (Fig. 1(A)). Primary amines can be easily alkylated by alkyl halides and over alkylation is often observed, particularly when a short-chain representative such as methyl iodide is used. Correspondingly, tryptamine exposure to methyl iodide has been employed for the synthesis of the quaternary *N*,*N*,*N*-trimethyltryptammonium iodide **3** (TMT) (Fig. 1(C)) [18,21,22], hence resulting in the presence of *m/z* 203. It followed that the detection of *m/z* 217 could only be formed by the presence of an additional methyl group at the indole nitrogen, giving rise to 1-methyl-*N*,*N*,*N*-trimethyltryptammonium iodide **4** (1-Me-TMT) alkylated at the indole nitrogen (Fig. 1(D)).

The second indicative factor that facilitated product identification was based on collision-induced dissociation (CID) experiments. The use of an electrospray ionisation triple quadrupole tandem mass spectrometer (ESI-MS/MS) allowed for the detected species at *m/z* 161, *m/z* 203 and *m/z* 217 to be studied without chromatographic separation at a reduced flow rate of 30 μL/min. These individual species were selected in quadrupole Q_1 and product ions scanned in Q_3 after dissociation in collision cell q_2. Collision energies were automatically determined by the software using the breakdown curve function. Fig. 1 summarises the selected ion transitions and optimised collision energies. The determined ion transitions were consistent with a number of previously characterised psychoactive tryptamine derivatives where a key fragment was proposed to be represented by a protonated 3-vinylindole fragment. In other words, formation of a product ion at *m/z* 144 was indicative of a tryptamine nucleus without the presence of substituents on the indole ring [16,17,23]. Fig. 1 displays the proposed structural representation of those ion transitions where the 3-vinylindole fragment can be identified by either *m/z* 144 or *m/z* 158, depending on the absence or presence of the methyl group at the indole nitrogen.

In order to unambiguously confirm the identity of the detected side products and to enable their quantitative determinations, reference standards were obtained by organic synthesis. Considering that several stages of alkylation were conceivable when using methyl iodide, preparation of the monoalkylated *N*-methyltryptamine **5** (NMT) and 1-methyl-DMT **6** were also carried out. The preparation of differentially alkylated standards **2**–**6** allowed for the application of a suitable multiple reaction monitoring (MRM) procedure. Fig. 1 provides a representative LC–ESI-MS/MS chromatogram obtained after the analysis of a *Breath of Hope* product. Each trace represents one compound-specific ion transition used for screening purposes (Fig. 1(A)–(F)). As it can be seen, tryptamine **1** (Fig. 1(A)) was still detectable which indicated that the reaction had not reached completion. DMT **2**, trace (B) in Fig. 1, was not detectable at all, confirming the preliminary observation made during flow injection analysis. Both quaternary ammonium salts **3** and **4** (Fig. 1(C) and (D)) were clearly dominating, NMT **5** was detected at trace levels only (trace (E)) whereas 1-Me-DMT **6** appeared to be absent, as indicated in trace (F) of Fig. 1. The MS/MS experiment was particularly helpful for the differentiation of TMT **3** from 1-Me-DMT **6** since both species would appear at *m/z* 203. Indeed, direct infusion analysis of the *Breath of Hope* product using the LC-orthogonal acceleration time-of-flight (TOF) system indicated a potential molecular formula of $C_{13}H_{19}N_2$ for the *m/z* 203 species. This composition however would have been consistent with both compounds **3** and **6**. Application of the tandem MS procedure led to the formation of the *m/z* 144 product ion (Fig. 1(C)), representing the 3-vinylindole species, and confirmed the absence of the methylated indole nitrogen that would have caused dissociation of the protonated molecule into *m/z* 158 (Fig. 1(F)).

All yields are summarised in Table 1. The data supports the formation of 47.4% of 1-Me-TMT **4** and 21.0% TMT **3** from the *Breath of Hope* synthesis involving the reaction of tryptamine with methyl iodide.

3.2. Calibration and LC/ESI-MS/MS method validation

Calibration curves for compounds **1**–**6** were generated based on their injected on-column masses (OCM). The internal standard (IS, 5-methoxytryptamine) and reference materials were diluted in methanol. In the former case the IS concentration was kept constant in all samples. The preparation of the latter standards was carried out by serial dilution of the corresponding stock solutions in order to cover the calibration range described in Table 1. A linear regression approach was found to be appropriate for the correlation of peak areas with OCM. Table 1 provides a summary of the calibration data and correlation coefficients (r^2) which indicated good linearity.

Accuracy was determined as recovery percentage ($[M_o/M_s] \times 100\%$) between found (M_o) and known (M_s) amounts of the compounds of interest. Standards were run in triplicate at OCM levels (Table 1). System precision was determined from triplicate injections of standard solutions and expressed as relative standard deviation (RSD). The method precision was estimated by injecting standards **1**–**6** in triplicate. Recoveries were obtained by using the calibration equations in the lower part of the calibration ranges. Table 1 shows that recoveries ranged from 92.1% to 105.0% indicating a satisfactory level of accuracy. The system precision was determined from peak area ratios of analytes and IS based on triplicate injections and showed acceptable reproducibility. The method precision was assessed after triplicate measurements of **1**–**6** standards using calibration equations. Table 1 indicated that the obtained RSD values for compounds **1**–**6** were below 10%.

The use of crushed NaOH and quaternary benzyltriethylammonium chloride was claimed to produce DMT **2** in a 98% yield [14]. Discussion on the internet and separately, work in the authors' laboratory repeating the proposed method, indicated that it did not work well [24] but warranted a detailed investigation for clinical and forensic purposes due to its perceived appeal to the clandestine chemist.

Indole *N*-alkylation requires the presence of a strong base due to the weak nucleophilicity of the indole-N-H. Deprotonation causes the resulting anion to act as a strong nucleophile leading to its substitution reaction with methyl iodide [25]. Interestingly, Jaisankar and Srinivasan reported on the *N*-methylation of 2-methylindole-

Table 1
Precision, accuracy, yields and calibration data for analytes **1**–**6**.

Analyte[a]	Line of best fit (r^2)	M_s/μg[b]	M_o/μg[c] ± SD	Recovery/%[d]	RSD (%)	Range OCM/μg[e]	Yield (%)
1	$y = 0.0591x - 0.1736$ (0.9939)	7.5	7.1 ± 0.10	94.5	1.42	2.63–60	11.1
2	$y = 0.1077x - 0.0886$ (0.9951)	2.06	2.0 ± 0.01	97.3	0.70	0.59–25	nd[f]
3	$y = 0.7641x + 0.1756$ (0.9981)	6.3	6.25 ± 0.22	99.3	3.45	0.02–8.4	21.0
4	$y = 0.8042x + 0.2778$ (0.9989)	0.26	0.27 ± 0.02	92.1	5.67	0.03–43.3	47.4
5	$y = 0.3172x - 0.1661$ (0.9987)	5.9	5.6 ± 0.50	105.0	9.04	0.74–23.75	0.5
6	$y = 0.9124x + 0.5865$ (0.9964)	1.36	1.32 ± 0.07	103.0	4.97	0.016–26	nd[f]

[a] Structures of analytes are shown in Fig. 1.
[b] M_s: mass of analyte in standard.
[c] M_o: observed mean mass of analyte ($n = 3$).
[d] Percent recoveries were calculated by $(M_o/M_s) \times 100\%$.
[e] OCM: on-column mass before split.
[f] nd: not detected.

3-aldehyde under PTC conditions that were similar to the *Breath of Hope* PTC procedure [26] and several indole-*N*-alkylation reactions have been carried out under PTC conditions using aqueous NaOH [27,28].

The presence of starting material **1** and both quaternary ammonium salts **3** and **4**, combined with the claimed DMT **2** product being absent, raises interesting questions about their potential pharmacology and toxicology. Tryptamine **1**, and its monoalkylated counterpart NMT **5** do not produce psychoactive effects in humans [18]. It is conceivable that the polar derivatives **3** and **4** may not show significant hallucinogenic properties due to their inability to transfer across the blood-brain barrier. Takahashi et al. showed that ^{11}C-labelled TMT **3** was characterised by poor brain uptake when they were investigating the tissue distribution of this compound after parenteral administration in normal Wistar rats [22]. A structurally similar derivative is aeruginascin, a trimethylammonium analogue of psilocybin [4-(H_2O_3PO)-DMT] that has recently been isolated from the hallucinogenic mushroom *Inocybe aeruginascens*. The authors provided some indication that it may display peripheral effects, particularly at high dosages [29].

4. Conclusions

Application of the *Breath of Hope* procedure proposed on the internet for the preparation of DMT from tryptamine did not result in the formation of this product. The use of methyl iodide in combination with the particularly alkaline conditions led to the formation of tryptamine quaternary ammonium salt derivatives instead. This characteristic pattern of products is of interest in a forensic and clinical context. The application of a liquid chromatography/electrospray ionisation tandem mass spectrometry method showed how useful a multiple reaction monitoring procedure can be for quantitative screening of clandestine drug products.

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