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

Cláudia P.B. Martins[a], Sally Freeman[b], John F. Alder[a], Simon D. Brandt[c,*]

[a] School of Chemical Engineering and Analytical Science, The University of Manchester, Sackville Street, P.O. Box 88, Manchester M60 1QD, UK
[b] School of Pharmacy and Pharmaceutical Sciences, The University of Manchester, Oxford Road, Manchester M13 9PL, UK
[c] School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK

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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.

* Corresponding author. Tel.: +44 151 231 2184; fax: +44 151 231 2170.
*E-mail address:* s.brandt@ljmu.ac.uk (S.D. Brandt).

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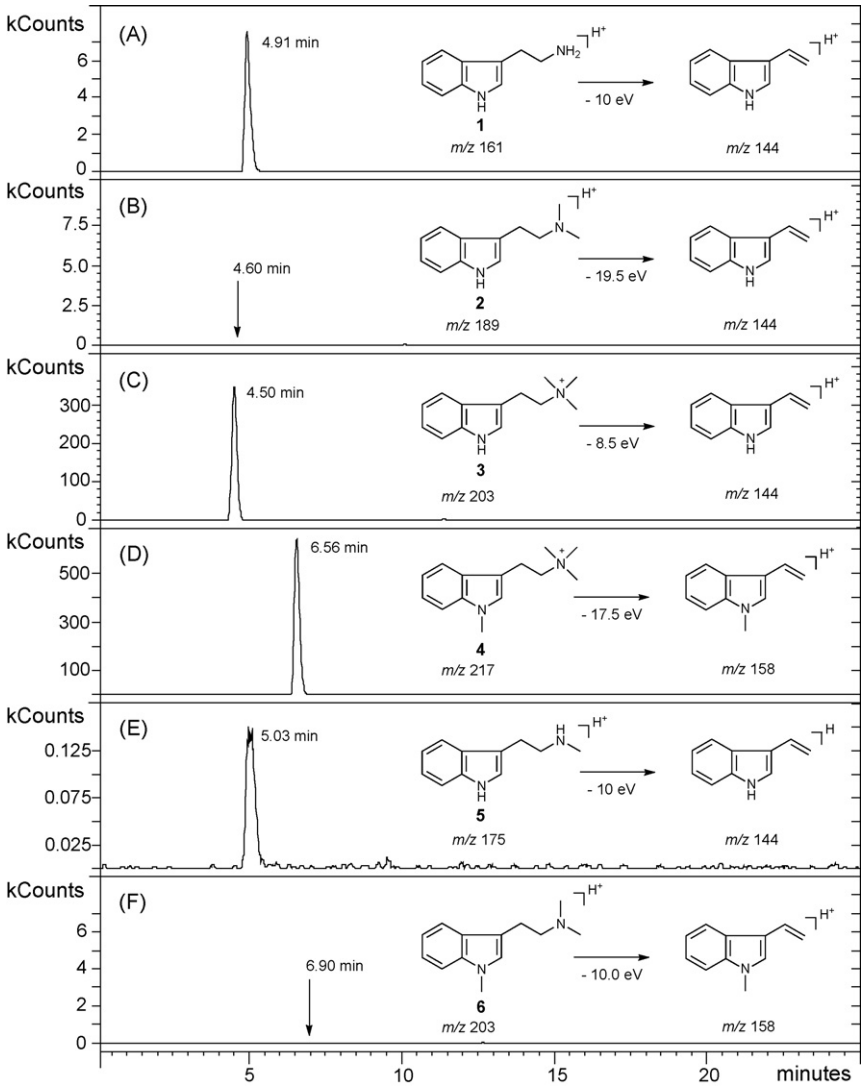


**Fig. 1.** Selected ion transitions, optimised collision energies and representative multiple reaction monitoring (MRM) traces of the crude product synthesised from tryptamine **1** following the *Breath of Hope* procedure. The desired product (DMT **2**) was not detected but instead, the major products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