Analytical methods for psychoactive *N,N*-dialkylated tryptamines

Simon D. Brandt, Cláudia P.B. Martins

Many *N,N*-dialkylated tryptamine derivatives can induce altered states of consciousness in humans. The term ''hallucinogen'' is often used when describing the effect on the human mind, and the extent of psychoactivity depends on the nature of substituents attached to the ethylamine side chain and the indole ring. Naturally-occurring tryptamines (e.g., *N,N*-dimethyltryptamine and psilocybin) have increasingly been investigated in human clinical studies, which increased interest within a number of scientific communities and the public. Many of these derivatives are controlled substances and an increasing number of previously unreported and structurally modified *N,N*-dialkylated ''designer'' tryptamines with unknown bioactive profiles have become available.

This review provides an overview of analytical methodologies published in recent years on detection and characterization of 40 *N,N*-dialkylated derivatives. The majority of literature available utilized reversed-phase high-performance liquid chromatography, gas chromatography and/or capillary electrophoresis. Derivatization was not normally required for sufficient separation and detection.

Bioanalytical applications and characterization of natural products have not been included due to space limitations. The majority of analytical data described in the literature indicated the dominance of *N,N*-dialkylated derivatives carrying a methoxy group or hydrogen at the 5-position of the indole ring. A consistent body of data has been produced and should set the scene for the detection of derivatives in order to inform healthcare providers, clinicians, forensic scientists and potential consumers.

Keywords: Capillary electrophoresis; Clinical; Derivative; Forensic; Gas chromatography; Hallucinogen; High-performance liquid chromatography; *N,N*-dialkylated tryptamine; Pharmaceutical; Psychoactivity

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1. Introduction

N,N-Dimethyltryptamine (DMT) **1** (Table 1) can be considered as one of the classic tryptamine hallucinogens with short-acting effects when smoked as the free base or injected as a suitable salt [1,2]. Psychoactive properties appear to be affected mostly by structural modification of the indole ring at the 4- and 5-positions, the α-carbon and side-chain nitrogen, respectively (Table 1). So far, it appears that *N,N*-dialkylation of the ethylamino group is an important feature for the retention of human psychoactivity. Most of the currently-known derivatives may show oral activity, although homologation of the *N,N*-dialkyl chain appears to reduce potency [1,3].

The impact on neurotransmitter systems is incompletely understood but current knowledge indicates an involvement of 5-HT_{2A} and various 5-HT_1 receptor subtypes [4–7]. Interestingly, it was recently observed that DMT **1** served as an agonist at the sigma-1 receptor [8]. In addition, DMT and a number of *N,N*-dialkylated tryptamines (**6**, **11** and **12**) were also found to be substrates at both the plasma membrane serotonin transporter and the vesicle monoamine transporter, respectively [9].

The need for development and application of detection methods arises from the renewed interest in human clinical studies and also from increased availability of *N,N*-dialkylated tryptamines on the market sold as research chemicals. These products may derive from extracted plant materials and mushrooms where a variety of *N,N*-dimethylated derivatives are abundantly present [10,11] or from purchase *via* the Internet. A number of clinical studies have shown that DMT **1** and psilocybin **16** appear to be well tolerated when studied under controlled conditions [12–15]. Little is known about their structurally-modified counterparts and their neuroactive properties. Many of these derivatives are controlled substances which adds a level of analytical complexity when dealing with illegally prepared and contaminated products. Complex extraction procedures are normally not required for simple products and the most common approaches involved a straightforward acid-base extraction or dissolution into a suitable solvent.

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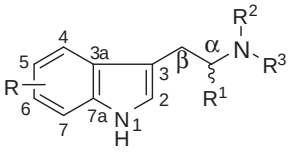
Table 1. Structural representations of *N,N*-dialkylated tryptamine derivatives reported in recent years. Commonly used abbreviations are shown in bold. The presence of H, HO or MeO at positions 4 and 5 play a key role. The hydroxyl group, especially at the 4-position, may be derivatized to give a suitable pro-drug as is the case, for example, with psilocybin **16**



No.	R	R^1	R^2	R^3	Name (Abbreviation)
1	H	H	Me	Me	*N,N*-Dimethyltryptamine (**DMT**)
2	H	H	Me	Me	(with N^1-Me) 1-Methyl-*N,N*-dimethyltryptamine (**1-Me-DMT**)
3	H	H	Me	CH_2CN	*N*-Methyl-*N*-cyanomethyltryptamine (**MCMT)**
4	H	H	Me	Et	*N*-Methyl-*N*-ethyltryptamine (**MET**)
5	H	H	Me	Pr	*N*-Methyl-*N*-propyltryptamine (**MPT**)
6	H	H	Me	iPr	*N*-Methyl-*N*-isopropyltryptamine (**MIPT**)
7	H	H	Me	iBu	*N*-Methyl-*N*-isobutyltryptamine (**MIBT**)
8	H	H	Et	Et	*N,N*-Diethyltryptamine (**DET**)
9	H	H	Et	Pr	*N*-Ethyl-*N*-propyltryptamine (**EPT**)
10	H	H	Et	iPr	*N*-Ethyl-*N*-isopropyltryptamine (**EIPT**)
11	H	H	Pr	Pr	*N,N*-Dipropyltryptamine (**DPT**)
12	H	H	iPr	iPr	*N,N*-Diisopropyltryptamine (**DIPT**)
13	H	H	iBu	iBu	*N,N*-Diisobutyltryptamine (**DIBT**)
14	H	H	Bu	Bu	*N,N*-Dibutyltryptamine (**DBT**)
15	H	Me	H	H	α-Methyltryptamine (**AMT**)
16	4-H_2O_3PO	H	Me	Me	4-Phosphoryloxy-*N,N*-dimethyltryptamine (**Psilocybin**)
17	4-HO	H	Me	Me	4-Hydroxy-*N,N*-dimethyltryptamine, 4-HO-DMT (**Psilocin**)
18	4-HO	H	Me	iPr	4-Hydroxy-*N*-methyl-*N*-isopropyltryptamine (**4-HO-MIPT**)
19	4-HO	H	Pr	Pr	4-Hydroxy-*N,N*-dipropyltryptamine (**4-HO-DPT**)
20	4-HO	H	iPr	iPr	4-Hydroxy-*N,N*-diisopropyltryptamine (**4-HO-DIPT**)
21	4-AcO	H	Me	iPr	4-Acetoxy-*N*-methyl-*N*-isopropyltryptamine (**4-AcO-MIPT**)
22	4-AcO	H	iPr	iPr	4-Acetoxy-N,N-diisopropyltryptamine, **4-AcO-DIPT**)
23	5-HO	H	Me	Me	5-Hydroxy-*N,N*-dimethyltryptamine, 5-HO-DMT (**Bufotenin**)
24	5-HO	H	iPr	iPr	5-Hydroxy-*N,N*-diisopropyltryptamine (**5-HO-DIPT**)
25	5-MeO	H	Me	Me	5-Methoxy-*N,N*-dimethyltryptamine (**5-MeO-DMT**)
26	5-MeO	H	Me	Et	5-Methoxy-*N*-methyl-*N*-ethyltryptamine (**5-MeO-MET**)
27	5-MeO	H	Me	Pr	5-Methoxy-*N*-methyl-*N*-propyltryptamine (**5-MeO-MPT**)
28	5-MeO	H	Me	iPr	5-Methoxy-*N*-methyl-*N*-isopropyltryptamine (**5-MeO-MIPT**)
29	5-MeO	H	Me	iBu	5-Methoxy-*N*-methyl-*N*-isobutyltryptamine (**5-MeO-MIBT**)
30	5-MeO	H	Et	Et	5-Methoxy-*N,N*-diethyltryptamine (**5-MeO-DET**)
31	5-MeO	H	Et	Pr	5-Methoxy-*N*-ethyl-*N*-propyltryptamine (**5-MeO-EPT**)
32	5-MeO	H	Et	iPr	5-Methoxy-*N*-ethyl-*N*-isopropyltryptamine (**5-MeO-EIPT**)
33	5-MeO	H	Pr	Pr	5-Methoxy-*N,N*-dipropyltryptamine (**5-MeO-DPT**)
34	5-MeO	H	iPr	iPr	5-Methoxy-*N,N*-diisopropyltryptamine (**5-MeO-DIPT**)
35	5-MeO	H	iPr	iPr	(with β-keto) 5-Methoxy-β-keto-*N,N*-diisopropyltryptamine (**5-MeO-bk-DIPT**)
36	5-MeO	H	iPr	iPr	(with β-HO) 5-Methoxy-β-hydroxy-*N,N*-diisopropyltryptamine (**5-MeO-β-HO-DIPT**)
37	5-MeO	H	iBu	iBu	5-Methoxy-*N,N*-diisobutyltryptamine (**5-MeO-DIBT**)
38	5-MeO	H	Bu	Bu	5-Methoxy-*N,N*-dibutyltryptamine (**5-MeO-DBT**)
39	5-MeO	Me	H	H	5-Methoxy-α-methyltryptamine (**5-MeO-AMT**)
40	5-MeO	H	Allyl	Allyl	5-Methoxy-*N,N*-diallyltryptamine (**5-MeO-DALT**)

2. Analysis of tryptamine products and mixtures

A previous review detailed the analytical profiling of synthetic routes to *N,N*-dialkylated tryptamines, focusing on mass spectrometry (MS) [16].

The present review provides an account of analytical methods published in recent years on the analysis of synthetic products or mixtures. Bioanalysis and characterization of natural products have been excluded, due to space limitations.

In recent years, a total of 40 *N,N*-dialkylated derivatives have been detected and analytically characterized (see Table 1 for structural representations) and individual compounds described in each publication are summarized

in Table 2. However, only *N,N*-dialkylated compounds have been included, with a comment to indicate whether non-tryptamine drugs, primary, secondary or quaternary ammonium derivatives were also part of the publication.

Comments on analytical details are summarized in the following sections and in Table 3.

2.1. Gas chromatography

Tryptamine derivatives can be conveniently subjected to analysis and characterization by gas chromatography (GC) due to their volatile properties. One of the first reports on the identification of so-called ''designer'' drug 5-methoxy-*N,N*-diisopropyltryptamine (5-MeO-DIPT) **34** was presented after GC-MS analysis of powdered samples and the corresponding electron ionization (EI) mass spectra, and Fourier-transform infrared (FT-IR) spectra of four tryptamines (Table 2) were also displayed [17]. Qualitative GC-MS analysis of submitted sugar cubes, following an acid-base extraction with 1% citric acid, sodium carbonate and butyl chloride, revealed the presence of 5-methoxy-α-methyltryptamine (5-MeO-AMT **39**).

This seemed unusual at first, since products based on this matrix would traditionally lead the analyst to expect the presence of *d*-lysergic acid diethylamide (LSD). However, application of the sodium nitroprusside reagent test

Table 2. Overview of individual derivatives reported in recently published literature

N,N-Dialkylated tryptamine derivative number	Comment[a]	Ref.
15, 25, 34, 39	A	[17]
39	B	[18]
17	A	[19]
1, 4-14, 25-34, 37, 38	B	[20,21]
1, 6, 8, 11, 12, 15, 20, 21, 22, 28, 34, 39	–	[22]
11, 25, 28, 34, 39	–	[23]
1, 15–17, 20, 23, 25, 34, 39	A	[24]
25, 34	A	[25]
1, 8, 11, 12, 14, 15, 25, 34, 39	–	[26]
1, 8, 11, 12, 14, 15, 25, 34, 39	–	[27]
15, 25, 28, 34	A	[28]
1, 8, 11, 20, 21–23, 25, 28, 30, 32–34, 39, 40	A + B	[29]
1	A	[30]
1	A + B	[31,32]
1–3	A + B	[33]
16	A	[34]
16, 17	A	[35]
16, 17	A	[36]
16, 17	–	[37]
11, 19 or 20, 25, 34, 39[b]	A	[38]
28	A	[39]
1, 8, 15, 23, 25, 34	B	[40]
24, 34–36	–	[41]
1, 2	B	[42]
16, 17	–	[43]
25	A + B	[44]
1, 8, 11, 12, 14, 15, 25, 34, 39	A	[45]
17	A	[46]
17	A	[47]
1, 8, 11, 12, 14, 15, 25, 34, 39	–	[48]
1, 8, 11, 16, 16-BSTFA, 17, 17-BSTFA, 34	A	[49]
17	–	[50]
17	–	[51]
11, 12, 15, 22, 25, 28, 33, 34	A + B	[52]
16, 17	–	[53]
25	A + B	[54]
35	B	[55]
25, 34	A + B	[56]

[a]A: Plus additional non-tryptamine analyte(s). B: Plus additional tryptamine(s) such as primary and secondary amines and quaternary ammonium salts.
[b]Both isomeric derivatives **19** and **20** could not be differentiated on exact masses alone. The detection of 5,6-MeO-MIPT was indicated but considered a false positive.

Table 3. Analytical details

Comment	Separation	Column	Analysis/Detection[a]	Ref.
Analysis of tan or white powdered samples	GC	30 m × 0.32 mm, 0.25 μm, DB-1	Q (EI), FT-IR, NMR	[17]
Analysis of spiked sugar cubes	GC	12 m × 0.20 mm, 0.33 μm, HP-1	Q (EI), various color tests	[18]
Analysis of mushroom/chocolate mixture	GC	Not specified	Q (EI)	[19]
Synthesis and analytical characterization of a synthetic route including precursors	GC	30 m × 0.25 mm, 0.25 μm, CP-Sil 8 CB Low Bleed/MS	QqQ-MS/MS and in-source CID, TOF (all ESI+), IT-MS (EI), 1-D/2-D NMR, IT-MS (CI-MS/MS)	[20,21]
Mass spectral characterization of twelve standards	GC –	Not specified Direct infusion	IT-MS (EI) IT-MS^n (ESI+)	[22]
Analytical characterization of five standards	GC HPLC	30 m × 0.25 mm, 0.25 μm, DB-1 125 × 3.2 mm, 5 μm, Partisil ODS(3)	Q (EI), FID, various color tests, FTIR/ATR DAD (220–340 nm), ^{1}H-NMR	[23]
Method development and application to 123 drug products, mainly consisting of tablets, capsules, powder and liquids	GC HPLC	30 m × 0.25 mm, 0.25 μm, DB-5MS 50 × 2.0 mm, 5 μm, Atlantis dC18 and 10 × 2.0 mm, 5 μm guard column	Q (EI) Q-SIM (ESI+), DAD (210 nm)	[24]
Analysis of powdered extracts sold as *Amanita* mushroom products	GC	30 m × 0.25 mm, 0.25 μm, DB-5MS	Q (EI)	[25]
Comparison of HPLC, GC and CE-based separations using standards	CE GC HPLC	90.0/77.0 cm × 50 μm i.d. fused silica 30 m × 0.25 mm, 0.25 μm, HP-5MS 250 × 4.6 mm, 5 μm, Cosmosil 5 C-18 MS	UV (270 nm), CZE-UV (280 nm), Q (EI)	[26]
MS characterization of twelve standards	GC	30 m × 0.25 mm, 0.25 μm, HP-5MS	Q (EI), IT-MS/MS (ESI+) infusion, MALDI-TOF	[27]
Analytical characterization and detection in purchased drugs products	GC	30 m × 0.25 mm, 0.25 μm, DB-5MS	Q (EI), ^{1}H/^{13}C-NMR	[28]
Analytical characterization and detection in purchased drug products	GC HPLC	30 m × 0.25 mm, 0.25 μm, DB-5MS 150 × 4.6 mm, 5 μm, L-column ODS, SymmetryShield RP-18, Atlantis dC18	Q (EI), DAD (199–360 nm)	[29]
Hadamard transform technique applied to GC-MS and LC-MS	GC HPLC	30 m × 0.25 mm, 0.25 μm, HP-5MS 150 × 4.6 mm, 5 μm, C_{18} T-5	Q (EI) SIM-MS IT-SIM-MS (ESI+)	[30]
DMT **1** sensitivity towards dichloromethane	GC HPLC	30 m × 0.25 mm, 0.25 μm, CP-Sil 8 CB Low Bleed/MS 250 × 4.6 mm, 4 μm, Synergi Max-RP	TOF (ESI+), IT-MS (EI), 1-D/2-D NMR, IT-MS (CI-MS/MS) UV (280 nm)	[31]
DMT **1** sensitivity towards dichloroethane, dibromomethane and MS studies using deuterated derivatives	GC	30 m × 0.25 mm, 0.25 μm, CP-Sil 8 CB Low Bleed/MS	TOF (ESI+), IT-MS (EI), 1-D/2-D NMR, IT-MS (CI-MS/MS)	[32]
Characterization of DMT **1** synthesis *via* reductive amination	GC	30 m × 0.25 mm, 0.25 μm, DB-1ms	IT-MS (EI/CI), 1-D/2-D NMR	[33]
Development of a fast LC-ESI-MS/MS method using standards	HPLC	50 × 4.6 mm, C-18 Chromolith SpeedRod	IT-MS/MS (ESI+)	[34]
Separation of diverse compound classes	UPLC	100 & 30 × 2.1 mm, 1.7 μm Acquity BEH C-18;	UV (210 and 240 nm)	[35]
Separation of diverse compound classes under high temperature conditions	HPLC	50–100 × 2.1 mm, 1.8 μm Zorbax Rapid Resolution HT Stable Bond C18	DAD (210 plus additional wavelengths)	[36]
MS characterization of two standards	HPLC	150 × 4.6 mm, 5 μm, Prodigy	IT-MS (full scan, MS/MS and in-source CID MS/MS)(ESI+)	[37]
Seized drugs	HPLC	100 × 2 mm, 3 μm, Luna C-18(2) and 4 × 2 mm precolumn	TOF, DAD (230/210–400 nm), CLND	[38]
Analysis of powdered sample	HPLC GC	100 × 4.0 mm, 3 μm, Inertsil ODS-3 30 m × 0.25 mm, 0.32 μm, HP-5	DAD (210–400 nm) Q (EI)	[39]
Seized drugs	CE HPLC	66.0/46.5 cm × 75 μm i.d. fused silica 250 × 4 mm, 5 μm, LiChrospher LiChroCart RP select B	(UV)LIF (λex 266 nm) DAD (222 nm)	[40]
Characterization of 5-MeO-DIPT **34** synthesis	–	Direct infusion	QqQ-MS/MS and in-source CID, TOF (all ESI+), 1-D/2-D NMR	[41]
Characterization of DMT **1** synthesis *via* alkylation	HPLC	250 × 4.6 mm, 4 μm, Synergi Max-RP	QqQ-MS/MS and in-source CID, TOF (all ESI+), 1-D/2-D NMR	[42]
Stability of aqueous standard solutions	HPLC	150 × 4.6 mm, 4 μm, Synergi Max-RP	UV (269 nm)	[43]
Optimization of normal phase separation of various amines	HPLC	150 × 2.0 mm, 5 μm, Kromasil SIL, CN and Diol	DAD (254 and 280 nm)	[44]

(*continued on next page*)

Table 3 (*continued*)

Comment	Separation	Column	Analysis/Detection[a]	Ref.
Separation of standards	HPLC	250 × 4.6 mm, 5 μm, Cosmosil 5 C-18 MS	UV (280 nm), IT-MS/MS (ESI+); underivatized and derivatization with acetic anhydride	[45]
Impact of gas flow parameters on CE-MS performance	CE	80.0 cm × 50 μm fused silica 90.0 cm × 50 μm fused silica	IT, QqQ (both ion spray (+)) in SIM mode	[46]
Coupling of poly(dimethylsiloxane) chip with ESI-QqQ	–	Direct infusion	QqQ nanospray source (+)	[47]
Separation and online sample concentration using MEKC	CE	80.0/67.0 cm × 50 μm i.d. fused silica	UV (280 nm)	[48]
Analytical characterization of drugs	GC	15 m × 0.25 mm, 1.0 μm, DB-1	IR, Q (EI), 2/6 derivatized with BSTFA[a]	[49]
Synthesis of psilocin hapten and coupling with bovine serum albumin	–	–	MALDI-TOF, ^{1}H-NMR, Q (EI)	[50]
Synthesis of psilocin derivative and coupling to carrier proteins; followed by preparation of monoclonal antibodies	–	Purification of conjugates by dialysis	Enzyme immunoassay	[51]
Optimization of TLC-based detection method	TLC GC	Silica gel and RP-18 12 m × 0.2 mm, 0.33 μm, DB-1ms	UV (365 nm), FL, Q (EI)	[52]
Method development using standards	FIA	–	Photomultiplier; acidic potassium permanganate and tris(2,2′-bipyridyl)ruthenium(II) chemiluminescence	[53]
Impact of solvent polarities on absorption and fluorescence spectra	–	–	UV-Vis, FL	[54]
NMR and computational studies incl. product and precursor	–	–	1-D/2-D NMR	[55]
Optimization of Scott test applied to variety of drug standards	–	–	Color test, UV (622–626 nm)	[56]

[a]Abbreviations: ATR, Attenuated total reflectance; BSTFA, N,O-bis(trimethylsilyl)trifluoroacetamide; CE, Capillary electrophoresis; CI, Chemical ionization; CID, Collision-induced dissociation; CLND, Chemiluminescence nitrogen detection; DAD, Diode-array detection; EI, Electron ionization; ESI, Electrospray ionization; FIA, Flow-injection analysis; FID, Flame-ionization detection; FL, Fluorescence; FT-IR, Fourier-transform infrared; GC, Gas chromatography; HPLC, High-performance liquid chromatography; IT, Ion trap; MALDI, Matrix-assisted laser desorption/ionization; MEKC, Micellar electrokinetic chromatography; MS, Mass spectrometry; NMR, Nuclear magnetic resonance; Q, Single quadrupole; QqQ, Triple quadrupole; SIM, Selected-ion monitoring; TLC, Thin-layer chromatography; TOF, Time-of-flight; (UV)LIF, (Ultraviolet) laser-induced fluorescence; UPLC, Ultra-performance liquid chromatography; UV-Vis, Ultraviolet-visible.

pointed towards the presence of a tryptamine derivative, which was supported by qualitative GC-MS analysis by comparison with four standards, including **39** [18].

A rapid extraction of a submitted mushroom/chocolate concoction using acetic acid facilitated another qualitative GC-MS determination of psilocin **17**, and, as expected, caffeine and theobromine have also been detected, reflecting the presence of chocolate [19]. A total of 12 symmetrically and 13 asymmetrically substituted tryptamines and their precursors were prepared by the Speeter & Anthony procedure and characterized by 1D/2D nuclear magnetic resonance (NMR). MS characterization was carried out in EI-MS and chemical ionization tandem MS (CI-MS/MS) modes, followed by comparison with softer ionization techniques {e.g., electrospray ionization time-of-flight MS (ESI-TOF-MS) and ESI-triple-quadrupole-tandem mass spectrometry, (ESI-QqQ-MS/MS), respectively} [20,21]. EI-induced MS patterns were in agreement with a report on a total of 12 derivatives also characterized by GC-EI-MS [22].

Key MS patterns are shown in Fig. 1A–D using 5-MeO-DIPT **34** as a representative example. The extent of fragmentation depends on the ionization mode used and a summary of key dissociations is provided in Fig. 1E. Generally, when using atmospheric pressure ionization (API) sources commonly employed with liquid chromatography (LC), implementation of MS/MS gives rise to the formation of [3-vinylindole]$^{+}$-type species that are reflecting substitution at the indole ring (*m*/*z* 174 for 5-MeO-DIPT **34**). Iminiumion formation (CH_2=N^{+}(iPr)$_2$,

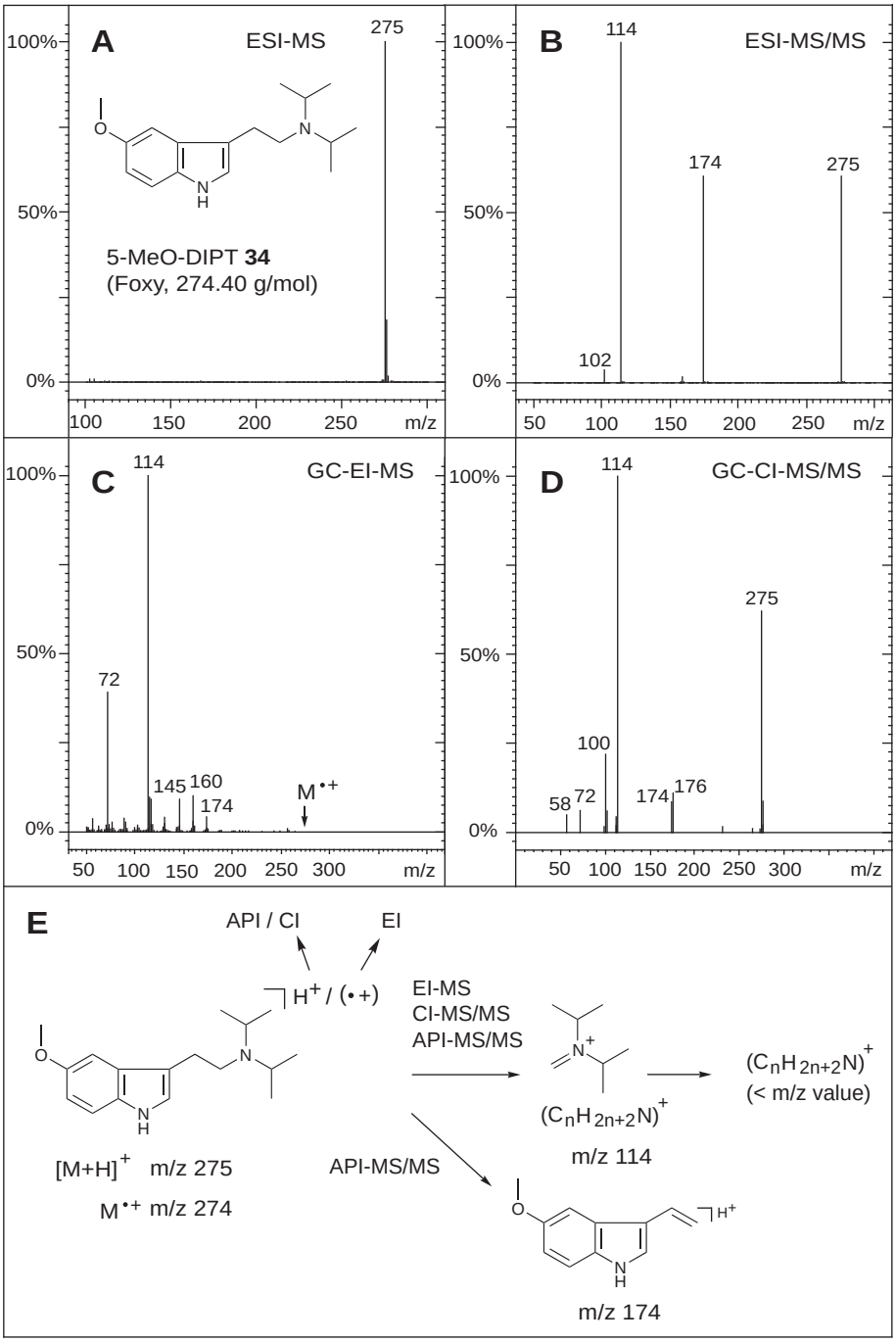


Figure 1. Representative mass spectra of 5-MeO-DIPT **34** obtained from various ionization sources. A) Electrospray ionization (ESI) and formation of the expected protonated molecule $[M + H]^+$ at *m/z* 275. B) Collision-induced dissociation (CID) under triple quadrupole and ESI conditions. C) Electron ionization (EI) mass spectrum and absence of the molecular ion. D) Chemical ionization ion trap tandem MS (CI-IT-MS/MS) spectrum using methanol as the liquid CI reagent. E) The detection of key fragments is characterized by formation of the side-chain related iminium ion at *m/z* 114 ($C_nH_{2n+2}N^+$). Secondary fragmentations of this species occur most frequently under EI and CI-MS/MS conditions, thus leading to iminium ions of lower mass. The $[3\text{-vinylindole}]^+$-type species at *m/z* 174 is particularly abundant when using ESI and other atmospheric pressure ionization (API) techniques.

m/z 114 for **34**) provides side-chain specific information. Consequently, these two abundant dissociations are often selected as a starting point during method development when designing a target-screening procedure using these selected ion transitions under LC-MS^2 conditions. On the other hand, secondary fragmentations of iminium ion are normally more pronounced under EI and chemical-ionization MS/MS (CI-MS/MS) conditions {e.g., leading to the detection of *m/z* 72 ($CH_2{=}N^+(iPr)_2$) for **34**}. EI and CI are particularly useful where differentiation

between isomeric derivatives is required, since secondary fragmentation of iminium ions increases information content (for more details, see [16,20,21]).

Analytical profiles of five tryptamine derivatives were described employing color tests (Marquis and Ehrlich), GC with flame-ionization detection (GC-FID), FT-IR/ATR, ^{1}H-NMR, HPLC and GC-MS [23]. A GC-MS method was developed for qualitative and quantitative analysis of a diverse class of psychoactive drugs.

The methods were then applied to the analysis of 123 drug products obtained from shops and Internet sources and compared with HPLC (see below). Ultrasonication and extraction into methanol appeared to be suitable. Nine tryptamines were included in the method development and characterization of these product revealed that eight of 99 kinds of product contained 5-MeO-DIPT **34**. Some products also contained α-methyltryptamine (AMT) **15** and DMT **1** [24]. A number of products, sold as dried *A. muscaria* extracts (fly agaric), were found to contain 5-MeO-DIPT **34**, 5-MeO-DMT **25**, tropane and β-carboline derivatives, instead of the expected constituents (i.e. ibotenic acid and muscimol) (without quantitation). Extractions were carried out by a modified Stas-Otto's isolation method [25]. A GC-MS method was applied to the separation of nine tryptamine derivatives and full-scan MS. Limit of detection (LOD) values were 0.5–15 μg/mL, where two primary amines (i.e. AMT and 5-MeO-AMT **39**) appeared to provide reduced signal responses compared to their tertiary counterparts (Fig. 2A).

The LOD values compared less favorably with capillary electrophoresis MS (CE-MS) and HPLC-UV analysis (see below) [26]. The same nine derivatives were also subjected to a GC-MS procedure with a slightly modified

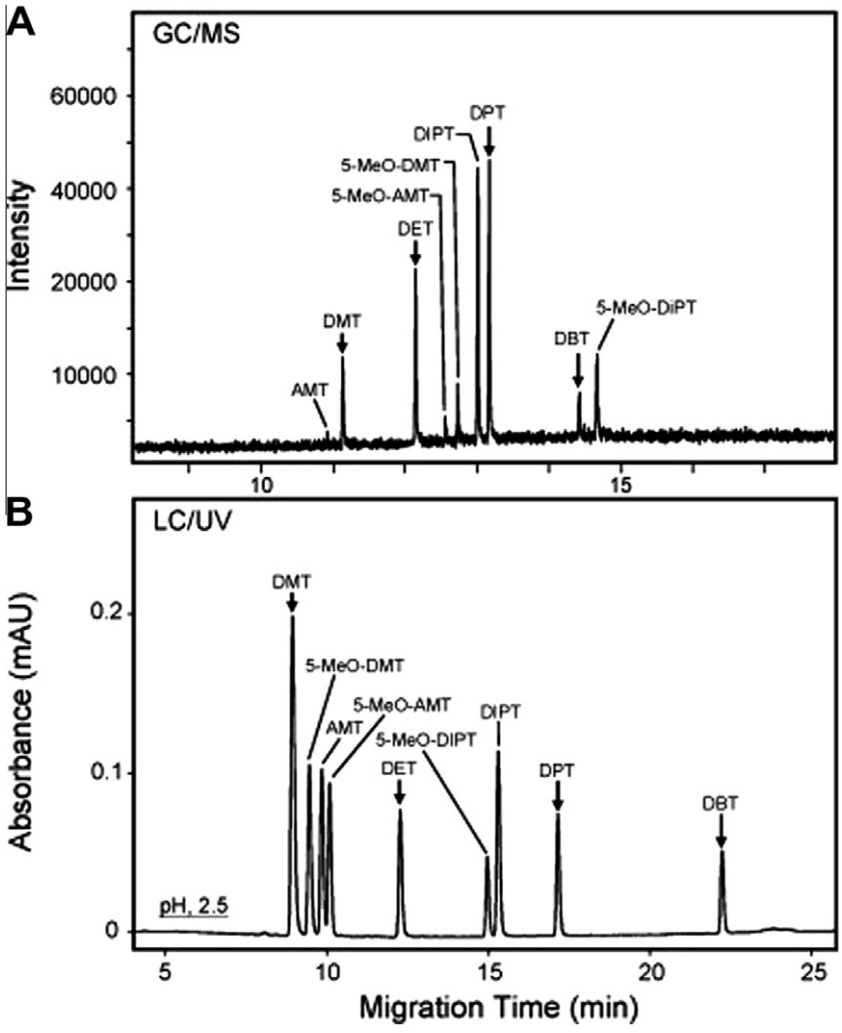


Figure 2. Representative chromatograms for nine tryptamines. A) GC total ion current chromatogram at 10 μg/mL following a 1 μL injection. B) HPLC-UV trace (280 nm) at 10 μg/mL following a 10 μL injection. See Tables 2 and 3 for details. The same mixture was separated under electrophoretic conditions and is shown in Fig. 3 below. Modified from [26].

temperature profile followed by a comparison between ionization techniques {e.g., EI, matrix-assisted laser desorption/ionization (MALDI) and ESI [27]}.

Four tryptamines have been characterized by GC-MS and $^{1}H/^{13}C$-NMR, and analysis of 178 drug products obtained from Internet sources showed that 73 of 178 were represented by tryptamine derivatives [28].

Further studies included implementation of a designer drugs data library based on GC-MS and HPLC-photodiode array (HPLC-PDA) analysis (see below) and consisted of 104 psychoactive drug derivatives based on a variety of compound classes. Some 26 tryptamines were included in characterization, and analysis of 205 commercially-available products confirmed detection of tryptamine derivatives in 72 cases [29]. A novel injection device and the application of the Hadamard transform technique have been applied to single-ion monitoring analysis (GC-SIM-MS) using DMT **1** as one of the model compounds. Increased signal-to-noise ratios were obtained using this approach [30].

DMT was also found to be sensitive towards a number of halogenated solvents either during storage or work-up, which resulted in the formation of various quaternary ammonium salts. When exposed to GC-MS analysis, a number of heat-induced re-arrangement products were detected and fully characterized by organic synthesis, GC-EI/CI ion-trap (IT)-MS, MS/MS and NMR [31,32].

One simple route for the preparation of DMT is based on reductive amination between formaldehyde and the tryptamine starting material in acidic media, often carried out in methanol. A commonly used reducing agent is $NaBH_3CN$, which enables the reduction of an intermediate imine or iminium salt. An analytical characterization of this synthetic route was carried out using GC-EI/CI-IT-MS, where the impact of stoichiometric modifications on side-product formation was also investigated. Tryptamine, *N*-methyltryptamine, DMT **1**, tetrahydro-β-carboline (THBC), 2-methyl-THBC, *N*-methyl-*N*-cyanomethyltryptamine **3** and 2-cyanomethyl-THBC were detected under a variety of conditions [33].

2.2. High-performance liquid chromatography

An LC-ESI-IT-MS/MS method using a C18 monolithic column was developed for the detection of a structurally diverse set of compounds of forensic interest, among which was psilocybin **16**. The LOD obtained for **16** was 0.05 μg/mL. Individual detection of all 14 compounds was facilitated by extracted ion chromatograms within 2.5 min [34]. This work showed that use of monolithic silica technology can lead to dramatic acceleration of the analytical process, reflecting the usefulness of porous monolithic material instead of particle-packed stationary phases.

A structurally diverse selection of 24 different drugs, including both psilocybin **16** and psilocin **17**, was separated under elevated pressure HPLC-UV conditions (UPLC) using a 1.7 μm C_{18} hybrid column. When using a column dimension of 10.0 cm × 2.1 mm, full separation was achieved in less than 13.5 min. When using a shorter 3 cm column and a faster gradient, all solutes were at least partially resolved within 2.4 min [35].

A number of forensically important compound classes (including **16** and **17**) were successfully separated using two 1.8 μm C_{18} columns, exploring a temperature range of 15–95°C. Both psilocybin **16** and psilocin **17** were successfully separated within 0.5 min [36].

The characterization of **16** and **17** was also described using LC-ESI-IT-MS under MS/MS and in-source CID conditions in order to increase information content [37].

Some 21 seized street drugs were analyzed by a combination of LC-TOF-MS and LC-CLND (chemiluminescence nitrogen detection) without using standards, based on accurate-mass determination using a target library of 735 exact monoisotopic masses [38].

Several tryptamines were identified using the TOF-MS method and were partially confirmed by a reference method that indicated that isomeric derivatives could not be differentiated based on MS alone [38].

Quantitative HPLC-UV analysis of three seized street drugs revealed the presence of 5-MeO-DIPT **34** in two cases, whereas DMT **1** was identified in the remaining sample. The HPLC chromatogram of the DMT sample also led to detection of additional compounds that were identified as *N*-methyltryptamine and α-ethyltryptamine. One derivative remained unknown. A powdered sample was analyzed in response to intoxication from a product obtained from the internet. It was reported that the sample consisted of about 60% methylone (3,4-methylenedioxymethcathinone) and 38% 5-MeO-MIPT **28**. The characterization was reported to be based on GC-MS and HPLC-diode array detection (DAD) and a HPLC-UV trace was depicted. Further details on quantitative aspects were not provided [39].

Eight tryptamine standards were also separated with LOD values of 15.9–47.0 μg/L. The HPLC approach was then compared with a CE method (see below) [40].

A fingerprint analysis was reported for the synthesis of 5-MeO-DIPT **34**, obtained from the Speeter and Anthony procedure, where several key impurities were identified. These included two incompletely reduced derivatives, 5-MeO-bk-DIPT **35** and 5-MeO-β-OH-DIPT **36**, 5-HO-DIPT **24** and 5-MeO-tryptophol. Identification was carried out by 1D/2D-NMR experiments, ESI-TOF-MS and direct-infusion ESI-QqQ-MS/MS studies. Further confirmation was obtained by organic synthesis [41]. Tryptamine was suggested on the Internet to give DMT under a specified set of conditions using methyl iodide and a phase-transfer catalyst. The reaction product was characterized by LC-ESI-QqQ-MS/MS and ESI-TOF-MS and it was found that DMT **1** was not detected under these conditions. Instead, 21.0% *N*,*N*,*N*-trimethyltryptammonium iodide

(TMT) and 47.4% 1-*N*-methyl-TMT (1-Me-TMT) were observed to be present. In addition, 11.1% tryptamine starting material and 0.5% trace of *N*-methyltryptamine were also detected – which indicated that the reaction did not go to completion [42].

Quaternary ammonium-salt formation can often render the use of GC-based analysis useless due to dealkylation and thermal degradation. HPLC-based characterization circumvents this issue due to the ability to dissolve in the mobile phase. One HPLC-UV study investigated the stability of aqueous psilocybin **16** and psilocin **17** standard solutions and it was found that exposure to light had significant detrimental effects on stability. Careful protection from light during storage and analysis increased solution stability to seven days [43].

The analysis of 123 drug products mentioned in the GC section (2.1 above) was also carried out by LC-ESI-MS in SIM mode and nine tryptamine standards were also analyzed by LC-UV, and that indicated the feasibility of both separation methods [24].

A normal-phase HPLC-UV method was described for a number of primary, secondary and tertiary amines, including three tryptamine representatives. The retention behavior was investigated using silica, diol, and cyano stationary phases. Both selectivity and resolution were deemed suitable on a cyano column using a mobile phase consisting of ethoxynonafluorobutane, methanol and *n*-propylamine [44]. Separation of nine derivatives under LC-UV conditions yielded LOD values of 0.3–1 μg/mL, which were compared with both GC-MS and CE approaches [26] (Fig. 2B). The LC-UV separation of this mixture was published again but with the addition of LC-ESI-SIM-MS analysis. Both separations were compared following derivatization of analytes with acetic anhydride and the impact on LOD values was evaluated. Mixed results were obtained and they appeared to be compound-dependent [45].

Implementation of the designer drugs data library mentioned above included HPLC-PDA analysis in addition to GC-MS. Three HPLC columns were involved for the determination of capacity factors and retention times. The full scan UV spectrum was displayed for nine tryptamines [29].

The Hadamard transformation method mentioned in the GC section (2.1 above) was also successfully applied to LC-SIM-MS analysis [30].

2.3. Capillary electrophoresis

Utilization of CE methods was comparatively underrepresented regarding the characterization of psychoactive tryptamine products, with the exception of several promising examples.

One optimization study reported on the use of CE-MS. Two sheath-liquid CE-MS interfaces were employed and an emphasis was placed on the impact of gas-flow parameters on CE separation. A major factor of influence on mobility and sensitivity was found to be the nebulizing gas velocity. The only tryptamine derivative involved in this study was psilocin **17** [46].

The detection of **17** was then extended to fabricated poly(dimethylsiloxane) (PDMS) electrospray devices where the ESI-MS spectrum was obtained by direct infusion using the PDMS chip [47].

Nine tryptamine derivatives were separated by CE followed by ultraviolet laser-induced fluorescence detection (LIF) [40]. It was found that addition of α-cyclodextrin to the buffer increased the relative fluorescence intensity and cross-validation was carried out *via* analysis of seized real samples in comparison with HPLC-DAD and good agreement between results was achieved. With the help of a stacking procedure, LOD values obtained under CE-LIF conditions were in the range 0.1–6 μg/L [40].

A CE-UV method was reported for optimization and separation of nine tryptamines using micellar electrokinetic chromatography (MEKC) and sweeping-MEKC modes [26].

The associated LOD values were found to depend on the separation mode and, with CZE-UV, were in the range 0.5–1 μg/mL. Significant improvements were determined under sweeping-MEKC conditions resulting in LOD values of 2–10 ng/mL [26] (Fig. 3). In a subsequent study using the same mixture, LOD values were obtained in the range 1.0–1.8 μg/mL.

Application of the sweeping-MEKC method resulted in an LOD in the range 2.4–8.0 ng/mL, whereas further optimization of the sample-concentration approach under cation-selective exhaustive injection-sweep-MEKC conditions (CSEI-sweep-MEKC) provided improved LODs of 1.3–2.7 ng/mL. All compounds eluted within ~25 min when using this particular mode [48].

2.4. Miscellaneous methods

Six derivatives were described in the form of IR and EI-MS spectra within a book chapter on the analysis of hallucinogens [49]. A psilocin derivative was prepared by a reaction with 4-bromobutyronitrile to give a 1-alkyl derivative of psilocin. The cyano group was subsequently converted to a carboxylic acid group that allowed coupling of this psilocin moiety with bovine serum albumin (BSA) to yield a BSA-hapten conjugate. Purification was carried out using a Sephadex column and dialysis.

MALDI-TOF analysis indicated detection of 4–5 molecules of psilocin hapten per molecule of BSA – which was aimed at developing a radioimmunoassay for psilocin **17** in human plasma [50]. A succinamic acid derivative of psilocin, connected *via* the hydroxyl 4-position, was synthesized and conjugated with BSA, keyhole limpet hemocyanin and horseradish peroxidase, respectively. This was followed by preparation of monoclonal antibodies.

Analysis using an enzyme immunoassay revealed cross-reactivity with DMT **1** but not with psilocybin

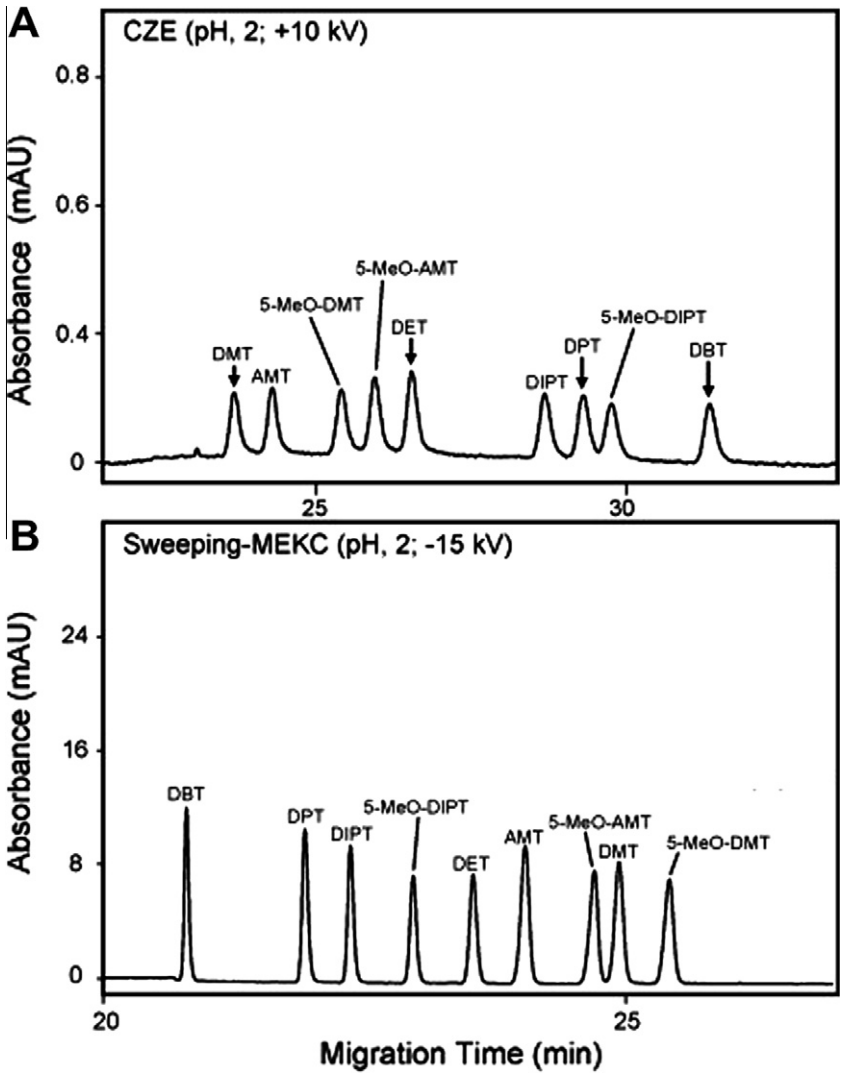


Figure 3. A) CZE-UV electropherogram (280 nm) of nine tryptamines. B) Sweeping-MEKC-UV conditions leading to improved LOD values. See Tables 2 and 3 for details. Modified from [26].

16 – which indicated potential suitability for the detection of **16** in mushrooms [51].

Thin-layer chromatography (TLC) has been used extensively for the analysis of indole and tryptamine derivatives. Although TLC-based applications have disappeared in recent years, its value must not be underestimated for routine analysis. A recent study investigated the implementation of silica gel and C_{18} reversed-phase TLC. Ten tryptamine derivatives were mostly separated and detected by heating after spraying with sodium hypochlorite, hydrogen peroxide or potassium hexacyanoferrate(III)-sodium hydroxide reagent. Visualization of the fluorescent products were obtained at a wavelength at 365 nm and LOD values were found in the range 0.01–0.06 μg on the plate [52].

A flow-injection method was reported for the detection of psilocybin **16** and psilocin **17**, based on acidic potassium permanganate and tris(2,2′-bipyridyl)ruthenium(II) chemiluminescence. LODs were 0.3 nM and 0.9 nM for **16** and **17**, respectively. The authors found that the emission intensity was highly pH dependent with an optimum at pH 1.5.

A synthesis of **17** was also reported by the group *via* *N*-tert-butoxycarbonyl-2-iodo-3-methoxyaniline and 4-methoxy-DMT intermediates. High-resolution ESI-MS, ^{1}H and ^{13}C NMR data were also provided [53].

The effects of solvents on absorption and fluorescence spectra of two indole and two tryptamines were investigated and a prominent red shift in fluorescence band on increased polarity was observed [54].

1D/2D NMR data of three tryptamine derivatives, including 5-MeO-DIPT **34**, were presented and supplemented by optimized geometry calculations [55].

During an investigation on the specificity of the Scott test, which is commonly used for the analysis of cocaine, it was discovered that 5-MeO-DIPT **34** was one of the

compounds that produced a false-positive response. The sample amount was found to be crucial, and, after some minor modification of the three-step procedure, it was possible to differentiate between free-base cocaine and **34** [56].

3. Conclusion

Analytical characterization of synthetic tryptamine drugs and products is a comparatively recent field of research, and the scene has now been set for the ability to identify these derivatives unambiguously. Use of single-stage and multi-stage MS will continue to play a key role when dealing with novel derivatives that have not yet been described. If possible, verification should also be obtained from organic synthesis of the target molecules. This overview should also be of interest within the pharmaceutical context, as a number of *N,N*-dialkylated tryptamines might also be prepared for human clinical studies.

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