

Test purchase of new synthetic tryptamines via the Internet: Identity check by GC-MS and separation by HPLC

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ABSTRACT

Over the past few years, a continuous alteration of the recreational drug market took place. Among other novel psychoactive drugs, new synthetic tryptamine derivatives appeared on the market. These compounds are mainly traded via the Internet, which has become an important marketplace for the sale of recreational drugs. The goal of our research was to check, if 13 new synthetic tryptamines obtained by test purchase via different online vendors meet the promised identity. Analysis was performed by GC-MS, using a common 30 m HP-5MS capillary column as stationary phase. Subsequently, a simple HPLC method for the separation of these tryptamines was developed. Therefore, the aim was to establish a method to separate a broad spectrum of tryptamines simultaneously within short time. Measurements were performed by a LiChrospher® RP-18e column and a mobile phase consisting of 0.1% triethylammonium acetate buffer, methanol and acetonitrile. Both presented methods were found to be suitable for the identification as well as separation of tryptamines as the analysis times were short and the selectivity sufficient. Moreover, enantioseparation of 3 chiral tryptamine derivatives purchased via the Internet by HPLC-UV and an Astec® Cyclobond I™ 2000 as CSP was performed. All of them were sold as racemic mixtures.

INTRODUCTION

Over the past decade the availability and diversity of novel psychoactive drugs on the European drug market has progressively increased. According to the European Drug Report 2015, 101 new and not previously reported psychoactive compounds were reported in 2014. Compared with the drug report of 2013 this represents an increase of 25%. Besides synthetic cathinones, cannabinoids, opioids, phenethylamines and arylamines, synthetic tryptamines represent a further group of misused psychoactive substances (EMCDDA, 2015). Tryptamine, a monoamine alkaloid, and its derivatives can be found in plants, fungi and animals. Lots of organisms, especially plants, produce tryptamines as metabolites. Substances with a tryptamine-similar structure are *e.g.* the neurotransmitter serotonin, melatonin and the amino acid tryptophan. Well-known natural representatives are the hallucinogens of magic mushrooms, namely psilocybin and its hydrolysis product psilocin. A further metabolite derived from tryptamines is the alkaloid bufotenin, which exists in the skin of some species of toads of the genus *Bufo* (Gibbons, 2012). Besides the naturally

occurring tryptamines, there is a huge spectrum of synthetic derivatives. Via substitution at the aromatic ring or at the nitrogen atom a diversified range of legal psychoactive compounds is given. Hallucinogenic tryptamines bear a substituted tryptamine structure. The indole ring, which is a particular structure of the tryptamines, is known from other substances such as lysergic acid diethylamide (LSD). Due to the fact that tryptamines show a similar but a bit weaker psychoactive effect than LSD, this compound class is used as legal alternative for LSD (Araujo *et al.*, 2015). The effects of tryptamines include a marked release and inhibited reuptake of serotonin. In addition to the hallucinations, the psychotropic effects of the tryptamines also cover euphoria, empathy and a feeling of love. Furthermore, visual and auditory disturbances and distortions may occur. Because of the mind-expanding effect, these substances have become popular in the drug scene, where they are called “happy pills”, “love pills” or “hi-trips”.

Due to the psychotropic effects, tryptamines have been used for medical purposes and religious ceremonies since ancient times. There are different traditional ways how shamans and natives have applied them for rituals, healing ceremonies or recreational use. They can either be used as a powder, which is insufflated or snuffed, as hallucinogenic drink or as magic mushrooms (also known as psychedelic mushrooms). In South

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America and on the West-Indian islands the seeds, which contain hallucinogenic tryptamine derivatives, (*e.g.* dimethyltryptamine (DMT), bufotenin and 5-methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT)) of *Anadenanthera species* are grinded to powder. Identified as “yopo” or “cohoba”, this powder is snuffed or blown through a bamboo tube into the nostrils. “Epena”, a hallucinogenic tryptamine rich powder from the *Virola species*, is still used as insufflation from the medicine men from the Amazon. “Ayahuasca”, which has its origin at the Amazon too, is a sacred brew of the indigenous population. It is mainly prepared out of stems of *Banisteriopsis caapi* and leaves of *Psychotria viridis* and *Diplopterys cabrerana*. Dependent from the part of the plant and the origin, the main components are DMT, 5-MeO-DMT and the MAO-inhibitors harmine and harmaline (Arunotayanun and Gibbons, 2012). There are worldwide over 100 sorts of mushrooms. But in Europe, the most common one is the *Psilocybe semilanceata*, better known as liberty cap. The ingredients of the hallucinogenic mushrooms are mainly psilocin, psilocybin, baeocystin and norbaeocystin.

Dipropyltryptamine (DPT), diethyltryptamine (DET), DMT as well as other methoxytryptamines were the first fully synthetic prepared tryptamines. Since DMT was used as additive to prepare the ayahuasca decoctions in the Amazon for centuries, it was synthesized the first time by the Canadian chemist Richard Helmuth Fredrick Manske in 1931 (Manske, 1931).

Some tryptamine derivatives synthesized for pharmaceutical purposes *e.g.* sumatriptan or zolmitriptan (for migraine treatment) are misused as recreational drugs by addicts (UNODC, 2013).

The American chemist Alexander Shulgin dedicated his research also to tryptamines. After his former book PiHKAL he synthesized a large number of derivatives of this compound class and published the methods of the syntheses as well as the dosage conditions in his book TiHKAL (“Tryptamines I Have Known And Loved”) (Shulgin and Shulgin, 1991; Shulgin and Shulgin, 1997).

In literature, several articles dealing with analysis, identification and separation by various separation techniques of novel psychoactive compounds, including tryptamines as new compound class, are available. Some articles report on a comparison of separation methods including HPLC, CE and GC for tryptamines (Wang *et al.*, 2008; Brandt and Martins, 2010).

Recently, Armenta and his group detected and characterized amphetamines, cathinones, tryptamines and synthetic cannabinoids by ion mobility spectrometry (Armenta *et al.*, 2015).

Arunotayanun *et al.* reported on the structure elucidation as well as receptor binding profiles of the synthetic tryptamines α -methyltryptamine and 5-methoxy-*N,N*-diallyltryptamine (Arunotayanun *et al.*, 2013).

In 2013 Kanu *et al.* successfully analysed and identified psychoactive cathinones and tryptamines using APIM (TOF)-MS (atmospheric pressure ion mobility time-of-flight mass spectro-

meter) (Kanu *et al.*, 2013).

Moreover several papers deal with the analysis of traditional dosage forms of tryptamines, especially ayahuasca (Gabelunghe *et al.*, 2008; McIlhenny *et al.*, 2009; Pires *et al.*, 2009; Gaujac *et al.*, 2013).

Additionally, literature about the synthesis and cytotoxic properties (Salikov *et al.*, 2015) as well as characterization of psychotropic tryptamines is available (Brandt *et al.*, 2010; Tearavarich *et al.*, 2011; Brandt *et al.*, 2012; Elliott *et al.*, 2013). Hsiao and co-workers reported on the simultaneous separation and detection of phenethylamine and tryptamine derivatives using HPLC-UV and LC/ESI MS (Hsiao *et al.*, 2009).

In 2015, LC-MS/MS was reported to be successful for screening procedures, detection as well as quantification of novel psychoactive drugs including tryptamine derivatives in blood (Adamovicz and Tokarczyk, 2015; Ambach *et al.*, 2015) and urine (Ambach *et al.*, 2015) samples.

In Austria, tryptamines are controlled by the Novel Psychoactive Substances Law (NPSG, BGBl.I Nr. 48/2013). According to this new law only trade of new psychoactive compounds such as novel tryptamines is prohibited.

Since different new tryptamines were seized by Austrian police during the last years they represent an important category of novel psychoactive drugs (NPDs). Due to an increased availability of these compounds on the illicit drug market and the fact that they are often used as an alternative to illegal drugs such as LSD, this compound class has developed to a social problem.

A remarkable spectrum of synthetic tryptamines is available via the Internet. In order to prove purity of the offered compounds online vendors disclose product analyses by nuclear magnetic resonance (NMR) and mass spectroscopy (MS) on their Internet pages. However, data of the published information can not be verified. Thus, the aim of our work was to check identity of 13 synthetic tryptamines purchased by mystery shopping between the years 2012 and 2014 via the Internet by GC-MS. To be precise, 5-API (5-(2-aminopropyl)indole) is a positional isomer of AMT (α -methyltryptamine) and belongs chemically rather to phenethylamines than to the compound class of tryptamines.

Four tryptamine derivatives were additionally bought as research chemicals from an official supplier in order to separate them simultaneously with the test purchased compounds. Since these commercially available chemicals were analytical standards there was no need to prove their identity. Afterwards a separation method by HPLC was developed.

Generally, chiral recreational drugs are traded as racemates and thus their enantiomers may show different pharmacological effects, as it is known from several chiral pharmaceutical ingredients, cathinones and amphetamine.

As 3 of the 17 tryptamine derivatives investigated possess a stereogenic centre, a further emphasis of this contribution was enantioseparation by HPLC-UV to check, if the purchased samples were sold as racemic mixtures or pure enantiomers.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals were of analytical grade. Acetonitrile (ACN), methylene chloride, methanol, phosphoric acid (85%), sodium hydroxide and anhydrous sodium sulfate were obtained from VWR (Darmstadt, Germany). Triethylamine (TEA) and acetic acid (100%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultra-pure water was prepared in our laboratory (Millipore, Darmstadt, Germany). Because of their novelty, the majority of the analytes were not available at official suppliers and therefore they were purchased from different Internet shops. 5-MeO-DALT (5-methoxy-*N,N*-diallyltryptamine) was from vip-legals.com. 5-API (5-(2-aminopropyl)indole) was bought from sensearomatic.com. MiPT (*N*-methyl-*N*-isopropyltryptamine), 5-MeO-DMT, 5-MeO-DiPT (5-methoxy-*N,N*-diisopropyl tryptamine), 4-MeO-MiPT (4-methoxy-*N*-methyl-*N*-isopropyl tryptamine), 4-OH-MiPT (4-hydroxy-*N*-methyl-*N*-isopropyl tryptamine), 4-OH-MET (4-hydroxy-*N*-methyl-*N*-ethyl tryptamine), 4-AcO-DPT (4-acetoxy-*N*-dipropyltryptamine), 4-AcO-DiPT (4-acetoxy-*N,N*-diisopropyltryptamine), 4-AcO-DET (4-acetoxy-*N,N*-diethyltryptamine), 5-MeO-MiPT (5-methoxy-*N*-methyl-*N*-isopropyltryptamine) were obtained from buybestre.com. AMT (α -methyltryptamine) was from futurelabs.eu. DiBT (*N,N*-diisobutyltryptamine), DPT (*N,N*-dipropyltryptamine), DALT (*N,N*-diallyltryptamine) and *N*-OH-AMT (*N*-hydroxy- α -methyltryptamine) were obtained from THC Pharm GmbH (Frankfurt/Main, Germany). Prior to separation experiments, compounds were characterized by gas chromatography-electron impact mass spectrometry and if necessary by NMR. Table 1 shows the chemical structure of the investigated tryptamines.

Instrumentation

Gas chromatography–mass spectrometry

GC-MS was carried out with an Agilent (Waldbronn, Germany) HP 6890 GC coupled to an Agilent (Waldbronn, Germany) HP 5972 mass selective detector. As stationary phase an HP-5MS capillary column with a length of 30 m, an inner diameter of 250 μ m and film thickness of 0.25 μ m was used. The column temperature program was as follows: the initial oven temperature was held at 50°C for 1 min, before continuous increase to 310°C (10°C/min). Afterwards, the column temperature was held at the final temperature (310°C) for 3 min. Helium served as mobile phase at a maintaining flow rate of 0.8 ml/min. Injection (1 μ l) was performed automatically with a split ratio of 2:1 at an inlet temperature of 280°C. To elucidate the mass fragments of each tryptamine, data were received in scan mode with a scan range of *m/z* 35–400 with 4 min solvent delay. Data collection was performed with MSD Chemstation Rev. E. 02.00.493 (Agilent Technologies, CA, USA) software.

High-performance liquid chromatography

HPLC analyses were performed using an HP Hewlett Packard Series II, 1090, Liquid Chromatograph, provided with an

autosampler and a diodearray detector. Measurements were done at 35°C under isocratic conditions with a flowrate of 1 ml/min and an injection volume of 5 μ l. Ultraviolet detection was carried out at 280 nm. Data evaluation was performed with a Chemstation Rev. A. 0903 (Agilent Technologies, Waldbronn, Germany) software. A LiChrospher® 100 RP-18e, 250 mm x 4 mm, 5 μ m from Merck KGaA (Darmstadt, Germany) served as stationary phase.

Enantioseparation measurements were carried out using an Agilent 1100 Series Liquid Chromatograph, equipped with an autosampler and an UV-detector. The column compartment was maintained at 25 °C. Chiral separation was performed under isocratic conditions at a flow rate of 0.6 ml/min. Experiments were conducted with an injection volume of 1 μ l and a wavelength of 240 nm. Data processing was done with a ChemStation for LC 3D Systems Rev. B. 04.03 (Agilent Technologies, Waldbronn, Germany) software. As chiral stationary phase an Astec® Cyclobond I™ 2000, 250 mm x 4.6 mm, 5 μ m from Sigma-Aldrich (St. Louis, MO, USA) was used.

Mobile phases were prepared by mixing the components in the required ratios. Afterwards the solutions were degassed for 2 minutes with helium and filtered through a 0.45 μ m pore size cellulose filter (Carl Roth, Karlsruhe, Germany).

Sample preparation

For GC-MS, the basic aqueous solution of the analytes was extracted with methylene chloride to obtain the free base of the compounds in the organic layer. Therefore, 0.5 mg of each analyte was dissolved in 1.5 ml nanopure water, 20 μ l of 0.2 N NaOH were added and extraction was performed by 1.5 ml methylene chloride. The organic solvent, which contained the free base, was dried over anhydrous sodium sulfate.

As sample preparation for HPLC experiments, 0.5 mg of each analyte was dissolved in 1 ml nanopure water and ultrasonicated for 1 min. In case of low solubility, 10 μ l methanol and 10 μ l 2 N hydrochloric acid were added.

RESULTS AND DISCUSSION

Tryptamines with various substitution patterns show psychedelic activity. Many of the tryptamine derivatives show an unspecific binding profile and exhibit a neuronal interaction with 5-HT serotonin receptor subtypes. It is presumed that the agonistic activity at 5-HT_{2A} receptor is primarily responsible for the psychedelic effects.

Generally, all 17 analytes are basic compounds and can be structurally divided in 5 groups: a) tryptamines without any substituent on the indol ring, b) tryptamines with an acetoxy group on position 4 of the indol ring, c) tryptamines with a methoxy group on position 4 or 5 of the indol ring, d) tryptamines with a hydroxy group on position 4 of the indol ring and e) indol derivatives (Table 1).

Table 1: Chemical structure of the tryptamines investigated in this study.

Group a) Tryptamines without any substituent on the indol ring					
α -Methyltryptamine (AMT) **	<i>N,N</i> -Diallyltryptamine (DALT) *	<i>N,N</i> -Diisobutyltryptamine (DiBT) *	<i>N,N</i> -Dipropyltryptamine (DPT) *	<i>N</i> -Methyl- <i>N</i> -isopropyltryptamine (MiPT) **	<i>N</i> -Hydroxy- α -methyltryptamine (<i>N</i> -OH-AMT) *
Group b) Tryptamines with an acetoxy group on position 4 of the indol ring					
4-Acetoxy- <i>N,N</i> -diethyltryptamine (4-AcO-DET) **	4-Acetoxy- <i>N,N</i> -diisopropyltryptamine (4-AcO-DiPT) **	4-Acetoxy- <i>N</i> -dipropyltryptamine (4-AcO-DPT) **			
Group c) Tryptamines with a methoxy group on position 4 or 5 of the indol ring					
5-Methoxy- <i>N,N</i> -diallyltryptamine (5-MeO-DALT) **	5-Methoxy- <i>N,N</i> -diisopropyltryptamine (5-MeO-DiPT) **	5-Methoxy- <i>N,N</i> -dimethyltryptamine (5-MeO-DMT) **	4-Methoxy- <i>N</i> -methyl- <i>N</i> -isopropyltryptamine (4-MeO-MiPT) **	5-Methoxy- <i>N</i> -methyl- <i>N</i> -isopropyltryptamine (5-MeO-MiPT) **	
Group d) Tryptamines with a hydroxy group on position 4 of the indol ring			Group e) Indol derivative		
4-Hydroxy- <i>N</i> -methyl- <i>N</i> -ethyltryptamine (4-OH-MET) **	4-Hydroxy- <i>N</i> -methyl- <i>N</i> -isopropyltryptamine (4-OH-MiPT) **	5-(2-Aminopropyl) Indole (API) **			

* obtained from an official supplier, ** obtained via mystery shopping.

Analysis and identification of tryptamines by gas chromatography

Gas chromatography was mainly performed to verify identity with the coupled MS-detector. In Table 2, analysis data including retention time, molecular formula, molecular weight and characteristic main fragment are presented. Retention times ranged between 15 and 23 minutes, which correlated well with increasing molecular weight. Comparing retention times of tryptamines bearing no substituents on the indol ring and those of tryptamines, which contain a methoxy or acetoxy group, retention times increased with functional groups. Regarding 4-MeO-MiPT

and 4-OH-MiPT, which differ only in the methoxy or hydroxy group on the indol ring, it is obvious that the functional groups cause only slight differences of effects on the selectivity and on the interaction of the compounds and the stationary phase.

By means of retention time, molpeak and main fragment of each compound, listed in Table 2, identification of all 17 analytes was possible. With this GC method, up to 8 tryptamines were separated simultaneously. In Fig. 1 a chromatogram of 8 resolved tryptamines is presented.

Additionally, a further method for separation of novel psychoactive drugs by HPLC was developed.

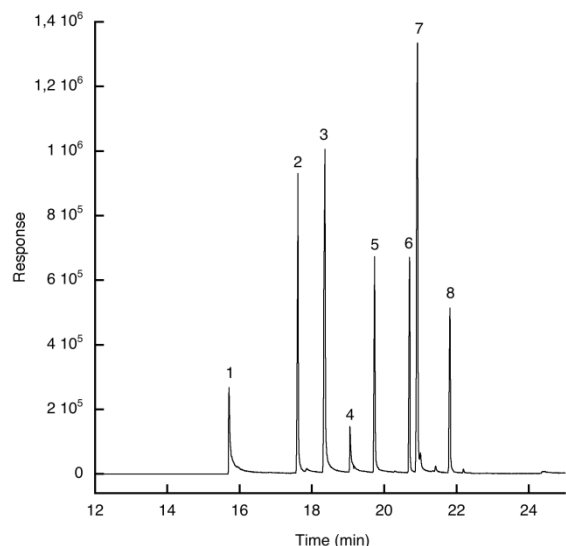


Fig. 1: Simultaneous identification of (1) AMT, (2) MiPT, (3) 5-MeO-DMT, (4) 4-OH-MET, (5) 5-MeO-MiPT, (6) 5-MeO-DiPT, (7) 5-MeO-DALT, (8) 4-AcO-DiPT by GC-MS. Conditions: Capillary column: 30 m x 250 μ m x 0.25 μ m nominal, HP-5MS 5% phenyl methyl siloxane; mobile phase: helium; temperature profile: 1 min 50°C, 10°C/min to 310°C, 3 min postrun; flow: 0.8 ml/min; injection: 1 μ l.

Table 2: Retention time, molecular formula, molecular weight and main fragment of the 17 analysed tryptamines by GC-MS.

Compound	Rt (min)	molecular formular	Mr (g/mol)	Main fragment (m/z)
4-AcO-DET	20.95	C ₁₆ H ₂₂ N ₂ O ₂	274.36	86
4-AcO-DiPT	22.05	C ₁₈ H ₂₆ N ₂ O ₂	302.42	114
4-AcO-DPT	22.24	C ₁₈ H ₂₆ N ₂ O ₂	302.42	114
AMT	15.93	C ₁₁ H ₁₄ N ₂	174.24	44
DALT	18.93	C ₁₆ H ₂₀ N ₂	240.34	110
DiBT	19.69	C ₁₈ H ₂₈ N ₂	272.43	142
DPT	18.97	C ₁₆ H ₂₄ N ₂	244.38	114
5-API	15.97	C ₁₁ H ₁₄ N ₂	174.24	44
5-MeO-DALT	21.14	C ₁₇ H ₂₂ N ₂ O	270.37	110
5-MeO-DiPT	20.93	C ₁₇ H ₂₆ N ₂ O	274.40	114
5-MeO-DMT	18.53	C ₁₃ H ₁₈ N ₂ O	218.29	58
4-MeO-MiPT	19.64	C ₁₅ H ₂₂ N ₂ O	246.35	86
5-MeO-MiPT	19.99	C ₁₅ H ₂₂ N ₂ O	246.35	86
MiPT	17.81	C ₁₄ H ₂₀ N ₂	216.33	86
N-OH-AMT	16.11	C ₁₁ H ₁₄ N ₂ O	190.24	130
4-OH-MET	19.29	C ₁₃ H ₁₈ N ₂ O	218.29	72
4-OH-MiPT	20.09	C ₁₄ H ₂₀ N ₂ O	232.32	86

Conditions: Capillary column: 30 m x 250 μ m x 0.25 μ m nominal, HP-5MS 5% phenyl methyl siloxane; mobile phase: helium; temperature profile: 1 min 50°C, 10°C/min to 310°C, 3 min postrun; flow: 0.8 ml/min; injection: 1 μ l.

Analysis of tryptamines by high-performance liquid chromatography

Based on previous research, different mobile phases consisting of phosphate-buffers (e.g. 13 mM, 26 mM, 39 mM) or TEAA-buffers with organic solvents such as methanol or acetonitrile in various ratios were used. We started with a mobile phase consisting of phosphate buffer (13 mM, pH 2.5) and acetonitrile (80:20). However, this composition led to a marked peak tailing. To improve peak shape, 10% methanol were added. Retention times increased and tailing got worse. After optimization experiments of the mobile phase still no optimal results were obtained. In a next step, in order to check the

influence of different buffers, the phosphate buffer was replaced by a 0.1% TEAA buffer. With the final composition of 0.1% TEAA buffer (pH 2.5): MeOH: ACN (70:10:20), suitable retention times as well as good peak shapes were obtained. 10% Methanol were added to the mobile phase to increase selectivity. Retention times ranged between 2 and 19 minutes. In Table 3 optimized results of the analysed tryptamines are shown. Besides single determination, simultaneous separation up to 8 tryptamines was feasible (Fig. 2).

Table 3: Retention time of 17 tryptamines analysed by HPLC.

Analytes	t _{Ret} (min)
4-AcO-DET	4.39
4-AcO-DiPT	6.33
4-AcO-DPT	8.69
AMT	3.53
DALT	7.32
DiBT	18.73
DPT	9.61
5-API	2.98
5-MeO-DALT	6.36
5-MeO-DiPT	6.05
5-MeO-DMT	3.24
4-MeO-MiPT	6.54
5-MeO-MiPT	4.26
MiPT	4.56
N-OH-AMT	4.26
4-OH-MET	2.76
4-OH-MiPT	3.03

Conditions: Column: LiChrospher® 100 RP-18e, 250 mm x 4 mm; mobile phase: 0.1% TEAA (pH 2.5) : methanol : acetonitrile (70:10:20); flow: 1 ml/min; temperature: 35°C; UV: 280 nm; injection: 5 μ l.

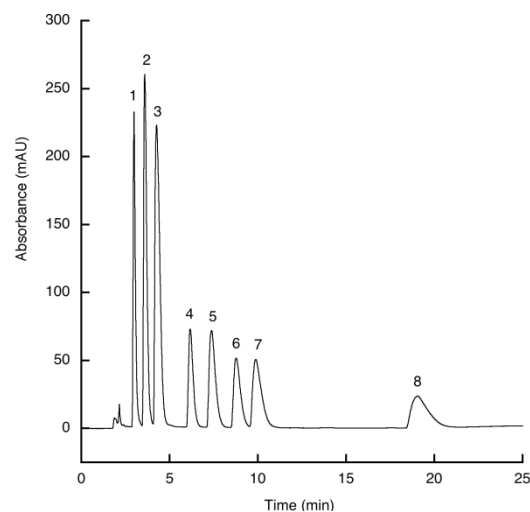


Fig. 2: Determination of 8 tryptamines simultaneously by HPLC: (1) 5-API, (2) AMT, (3) N-OH-AMT, (4) 5-MeO-DiPT, (5) DALT, (6) 4-AcO-DPT, (7) DPT, (8) DiBT. Conditions: Column: LiChrospher® 100 RP-18e, 250 mm x 4 mm; mobile phase: 0.1% TEAA (pH 2.5) : methanol : acetonitrile (70:10:20); flow: 1 ml/min; temperature: 35°C; UV: 280 nm; injection: 5 μ l.

Additionally, a validation of the method by means of 4-AcO-DiPT was performed (Table 4). The relative standard deviation (RSD) of the intra- and interday retention time was between 0.13% and 0.70%. To check reproducibility, the analyte was injected 20 times a day, where 0.27% RSD of the retention time was obtained.

The proposed HPLC method is advantageous, since due to the isocratic conditions it is very economical compared to other approaches described in literature. There are articles dealing with similar separation techniques of tryptamine derivatives but they report on gradient elution which is more complex (Hsiao *et al.*, 2009; Wang *et al.*, 2008). A further benefit of the present method is the opportunity to distinguish between the two isomeric compounds AMT and 5-API. Also, Elliott *et al.* (2013) described the analytical challenge for forensic analysis to differentiate these two analytes.

Comparing HPLC and GC separation results, retention times of both methods are quite short and provide clear information about identity. Simultaneous separation up to a maximum of 8 tryptamines was achieved.

Table 4: Repeatability and reproducibility data including the retention time by means of 4-AcO-DiPT.

Repeatability	t_{Ret} (min)
Intraday n=5	6.37±0.01, RSD=0.13%
Interday n=10	6.42±0.05, RSD=0.70%
Reproducibility	t_{Ret} (min)
Intraday n=20	6.40±0.02, RSD=0.27%

Conditions: Column: LiChrospher® 100 RP-18e, 250 mm x 4 mm; mobile phase: 0.1% TEAA (pH 2.5) : methanol : acetonitrile (70:10:20); flow: 1 ml/min; temperature: 35°C; UV: 280 nm; injection: 5 µl.

Enantioseparation of 5-API, AMT and N-OH-AMT by HPLC-UV

Enantioseparation of 5-API, AMT and N-OH-AMT was performed using two different direct chiral separation methods. As first approach a chiral Astec® Cyclobond I™ 2000, modified with native β-cyclodextrin was applied for enantioseparation experiments. As second approach, based on previous chiral separation studies, sulfated β-cyclodextrin was used as chiral mobile phase additive using a common RP-18e, 250 x 4 mm column as stationary phase (Taschwer *et al.*, 2014).

A mobile phase consisting of 1% TEAA buffer (pH 4.1) and methanol was used for optimization process of the Astec® Cyclobond I™ 2000 column. Therefore the mobile phase ratio was adjusted as well as the flow and the injection volume were optimized. With the final composition of the mobile phase 1% TEAA buffer (pH 4.1) and methanol (99:1) baseline separation of 5-API was achieved. The retention times of the two peaks were 9.77 min and 10.53 min ($R_s=1.72$). N-OH-AMT was partially separated within 9 minutes (8.00 min and 8.32 min; $R_s=1.10$). In Fig. 3, a chromatogram of the enantioseparation of 5-API is presented. Obviously AMT showed too little interaction with the stationary phase. Hence no chiral separation was obtained (6.66 min). Finally, the method was validated by means of 5-API. In Table 5 repeatability as well as reproducibility data of the retention times and the resolution factor are shown.

With the second approach using sulfated β-cyclodextrin as mobile phase additive 5-API was only partially separated, AMT and N-OH-AMT showed no chiral separation.

Chiral separation results obtained with the Astec® Cyclobond I™ 2000 column might be a possible evidence that the

psychoactive compounds had their origin in a clandestine laboratory as non-enantioselective synthesis is easier and cheaper. Although AMT was not chirally separated by the presented methods, there is evidence for a racemic mixture as it was already baseline separated into its enantiomers by former CE experiments in our group using sulfated β-cyclodextrin as chiral additive in the background electrolyte (unpublished results).

With the introduced methods it was feasible to elucidate real life samples (AMT, 5-MeO-MiPT, DMT) seized by Austrian police and to give further background concerning the syntheses and the origin of the psychoactive compounds.

Table 5: Repeatability and reproducibility data including the retention time and resolution by means of 5-API using the Astec® Cyclobond I™ 2000 column.

Repeatability	t_1 (min)	t_2 (min)	R_s
Intraday n=5	9.77±0.01,	10.53±0.01,	1.72±0.02,
	RSD=0.07%	RSD=0.08%	RSD=1.08%
Interday n=10	9.66±0.13,	10.39±0.15,	1.72±0.02,
	RSD=1.30%	RSD=1.40%	RSD=1.00%
Reproducibility	t_1 (min)	t_2 (min)	R_s
Intraday n=20	9.49±0.03,	10.21±0.03,	1.71±0.02,
	RSD=0.32%	RSD=0.34%	RSD=1.43%

Conditions: Column: Astec® Cyclobond I™ 2000, 250 mm x 4.6 mm; mobile phase: 1% TEAA (pH 4.1); methanol (99:1); flow: 0.6 ml/min; temperature: 25°C; UV: 240 nm; injection: 1 µl.

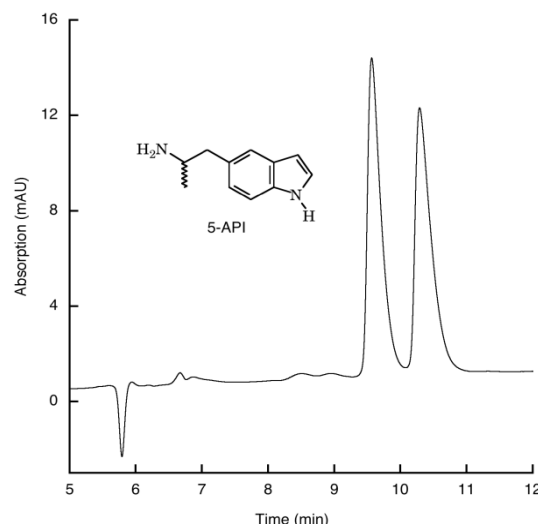


Fig. 3: Chiral separation of 5-API by HPLC-UV. Conditions: Column: Astec®Cyclobond I™ 2000, 250 mm x 4.6 mm; mobile phase: 1% TEAA (pH 4.1); methanol (99:1); flow: 0.6 ml/min; temperature: 25°C; UV: 240 nm; injection: 1 µl.

CONCLUSION

Based on natural occurring tryptamines *e.g.* in toads, fungi and cacti, new chemically designed synthetic tryptamine derivatives have become a competitive compound class of abused drugs in recent years. Many of these substances are legal and as they induce similar psychotropic effects like illegal recreational drugs, they have become very popular in the scene. For many of the NPDs only few analytical methods for identification are available. Consequently, there is a demand to develop new chromatographic separation methods. In the course of this research

tryptamine derivatives, obtained by mystery shopping, were analysed by GC-MS. It was verified that all 13 compounds were in accordance with the specified identity given by the online vendors. Separation was carried out with HPLC by developing quite an inexpensive and simple method. The introduced approaches provide a good opportunity for identification and separation of new tryptamines. Run times are rather short and the tryptamines are clearly distinguishable.

Additionally, a chiral separation method was developed to provide further information concerning the quality and origin of 5-API, AMT and *N*-OH-AMT. The presented enantioseparation results verify the fact that recreational drugs are sold as racemic mixtures which is in conformity with results of NPDs reported in other publications (Taschwer *et al.*, 2014; Weiss *et al.*, 2015; Taschwer *et al.*, 2014; Aturki *et al.*, 2014).

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Declaration/Conflict of Interest

The authors have declared no conflict of interest.

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