Analytical characterization of bioactive N-benzyl substituted phenethylamines and 5-methoxytryptamines

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Abstract:	RATIONALE: Substances based on the N-(2-methoxybenzyl)phenethylamine template ('NBOMe' derivatives) play an important role in medicinal research but some of these derivatives have also appeared as 'research chemicals' for recreational use which attracted attention worldwide. A major challenge associated with newly emerging substances includes the lack of analytical data and the ability to correctly identify positional isomers. METHODS: Six N-benzylphenethylamines based on the 2,5-dimethoxy-4-iodophenethylamine structure ('25I') and twelve substituted N-benzyl-5-methoxytryptamines ('5MT') have been prepared and extensively characterized. Techniques used for characterization were gas chromatography ion trap mass spectrometry in electron and chemical ionization mode, liquid chromatography diode array detection (DAD), infrared spectroscopy, electrospray high mass accuracy quadrupole time-of-flight tandem mass spectrometry, and triple quadrupole tandem mass spectrometry. RESULTS: The characterization of eighteen 'NBOMe' analogs provided a comprehensive collection of chromatographic and spectral data. Four

	groups of three positional isomers, i.e. 25I-NB2OMe, 25I-NB3OMe, 25I-NB4OMe, 25I-NB2B, 25I-NB3B, 25I-NB4B and their 5-methoxytryptamine counterparts, were included and assessed for ability to differentiate between them. Six meta-substituted N-benzyl derivatives of 5-methoxytryptamine (CF3, F, CH3, Cl, I, SCH3) were also studied. CONCLUSIONS: The implementation of mass spectral techniques was helpful for the differentiation between isomers, for example, when considering the difference in a number of ion ratios. This was considered beneficial in cases where chromatographic separation was only partially achieved under liquid chromatography (LC) conditions. The use of LC/DAD analysis was also found to be valuable for this particular purpose, which confirmed the integrative value of complementary techniques for areas related to forensic toxicology.

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Running title: *N*-benzyl substituted phenethylamines and 5-methoxytryptamines

Abstract

RATIONALE: Substances based on the *N*-(2-methoxybenzyl)phenethylamine template ('NBOMe' derivatives) play an important role in medicinal research but some of these derivatives have also appeared as 'research chemicals' for recreational use which attracted attention worldwide. A major challenge associated with newly emerging substances includes the lack of analytical data and the ability to correctly identify positional isomers.

METHODS: Six *N*-benzylphenethylamines based on the 2,5-dimethoxy-4-iodophenethylamine structure ('25I') and twelve substituted *N*-benzyl-5-methoxytryptamines ('5MT') have been prepared and extensively characterized. Techniques used for characterization were gas chromatography ion trap mass spectrometry in electron and chemical ionization mode, liquid chromatography diode array detection (DAD), infrared spectroscopy, electrospray high mass accuracy quadrupole time-of-flight tandem mass spectrometry, and triple quadrupole tandem mass spectrometry.

RESULTS: The characterization of eighteen 'NBOMe' analogs provided a comprehensive collection of chromatographic and spectral data. Four groups of three positional isomers, i.e. 25I-NB2OMe, 25I-NB3OMe, 25I-NB4OMe, 25I-NB2B, 25I-NB3B, 25I-NB4B and their 5-methoxytryptamine counterparts, were included and assessed for ability to differentiate between them. Six *meta*-substituted *N*-benzyl derivatives of 5-methoxytryptamine (CF_3, F, CH_3, Cl, I, SCH_3) were also studied.

CONCLUSIONS: The implementation of mass spectral techniques was helpful for the differentiation between isomers, for example, when considering the difference in a number of ion ratios. This was considered beneficial in cases where chromatographic separation was only partially achieved under liquid chromatography (LC) conditions. The use of LC/DAD analysis was also found to be valuable for this particular purpose, which confirmed the integrative value of complementary techniques for areas related to forensic toxicology.

Introduction

The diverse nature of serotonin (5-HT) receptor activation forms a fundamental basis of the functioning of biological systems including human physiology and regulation and modulation of psychological operation.[1] In humans, profound alterations in mood, thought, and cognition can be induced by a range of substances that activate the 5-HT_{2A} receptor, which is believed to play a central role in the mediation of psychoactive effects associated with so-called 'classic' hallucinogens or psychedelics, such as lysergic acid diethylamide (*d*-LSD), psilocybin and a number of ring-substituted phenethylamines and amphetamines.[2-5] Although many of the highly selective 5-HT_{2A} receptor agonists remain in the pre-clinical space, a diverse range of these derivatives has been encountered as commercially available 'research chemicals' used for recreational purposes.[6,7] 5-HT_{2A} receptor agonist activity appears to be a necessary requirement for a compound to possess psychedelic effects although this might not always be sufficient.[8] It is this particular feature, however, that has led to the recreational and commercial exploration, including manufacturing and sale via various outlets, such as online retailers or 'head shops'.

The translation of laboratory-based research into a wider commodified open market has attracted concern worldwide. A number of *N*-(2-methoxybenzyl)phenethylamines became known as 'NBOMe' derivatives and common examples include 25I-NBOMe (**1a**) 25B-NBOMe and 25C-NBOMe (Figure 1A), although other substituted *N*-benzyl groups also have been encountered. Between 2011–2013, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) received notifications from partners of the EU Early Warning System on the detection of 12 'NBOMe' derivatives.[9-11] The United Nations Office on Drugs and Crime (UNODC) also confirmed the detection of 'NBOMe' compounds in various UN Member States.[12] The World Health Organization, supported by the Expert Committee on Drug Dependence (ECDD), has recently held the 36th ECDD meeting to consider the available data on 25I-NBOMe (**1a**), 25B-NBOMe, and 25C-NBOMe for options regarding potential international control[13] and recommendations made are expected to be announced in due course.

In Europe, the Scientific Committee of the EMCDDA convened a risk assessment on 25I-NBOMe,[14] which was later followed by a European Council decision to subject this substance to EU-wide control.[15] In the United States, 25I-NBOMe, 25B-NBOMe, and 25C-NBOMe have been temporarily scheduled into the Controlled Substances Act as Schedule 1 substances.[16]

A common challenge associated with the ability to identify newly emerging psychoactive substances includes the lack of analytical data, which can reflect the fact that many of them may have only been described in the patent literature or medicinal and pharmacological literature, where comprehensive analytical characterization is often not carried out.[6] Once a particular analog of interest has been identified, further questions commonly arise when considering the presence of positional isomers, because reference standards for these isomers are often not available.

The present study presents the analytical characterization of six *N*-benzylphenethylamines (**1a**) – (**1f**) and twelve *N*-benzyl-5-methoxytryptamines (**2a**) – (**3f**) (Figure 1B). Four groups of three positional isomers that differed in the position of substituents on the *N*-benzyl group, e.g. 25I-NBOMe (**1a**) and its 3- and 4-methoxybenzyl analogues (**1b**) and (**1c**), were included to determine the potential for differentiation using techniques commonly employed in forensic toxicology. The analytical characterizations were carried out by gas chromatography ion trap mass spectrometry in electron and chemical ionization mode, liquid chromatography diode array detection, infrared spectroscopy, electrospray high mass accuracy quadrupole time-of-flight tandem mass spectrometry and triple quadrupole tandem mass spectrometry.

Experimental

HPLC-grade acetonitrile was supplied by Rathburn Chemicals Ltd (Walkerburn, Scotland, UK) and triethylammonium phosphate (TEAP, 1.0 M) (pH 3.0) was obtained from Fluka (Gillingham, Dorset, UK). All other solvents and reagents were from Aldrich (Gillingham, Dorset, UK). All eighteen substituted *N*-benzyl derivatives (**1a**) – (**3f**) were synthesized following established procedures.[5,17,18] Syntheses and pharmacological data will be reported elsewhere.

Gas chromatography ion trap mass spectrometry

GC/MS data for all eighteen compounds (0.5 mg/mL in methanol) were obtained in electron (EI) and chemical ionization (CI) mode (scan range *m/z* 41 – *m/z* 500) using a Varian 450-GC gas chromatograph (Walnut Creek, CA, USA) coupled to a Varian 220-MS ion trap mass spectrometer. A Varian 8400 autosampler was employed with a Varian CP-1177 injector (275 ºC) in split mode (1:50). Data acquisition was performed with the Varian MS Data Review function of the Workstation software, version 6.91. Transfer line, manifold and ion trap temperatures were set at 310, 80 and 220 ºC, respectively. The carrier gas was helium at a flow rate of 1 mL/min using the EFC constant flow mode. The liquid CI reagent was HPLC grade methanol. The

default settings for CI ionization parameters (0.4 s/scan) were used: CI storage level *m/z* 19.0; ejection amplitude *m/z* 15.0; background mass *m/z* 55; maximum ionization time 2000 µs; maximum reaction time 40 ms; target TIC 5000 counts. A 30 m × 0.25 mm (0.25 µm film thickness) Agilent J&W VF-5ms GC column (Agilent, Cheadle, UK) was employed for separation. The starting temperature was set at 130 ºC and held for 1 min. The temperature then increased at 20 ºC/min to 280 ºC and held constant for 11.50 min to give a total run time of 20.00 min.

Electrospray triple quadrupole mass spectrometry

Electrospray triple quadrupole tandem mass spectrometry experiments were carried out by direct infusion (10 µL/min at 0.01 mg/mL) of compounds using a Waters Micromass Quattro Premier triple quadrupole MS/MS system (Waters Micromass, Wythenshawe, Manchester, UK) operated under Masslynx v.4.1 software. MS optimizations were performed in MS scan and in product ion scan. Product ion scans were obtained in positive ion mode and optimized source condition settings were as follows: capillary 3.12 kV, cone 28 V, rf lens 0.1 V, source temperature 100 °C, desolvation temperature 200 °C and the multiplier voltage was 650 V. Nitrogen was used as the cone gas (50 L/h) and desolvation gas (200 L/h) whilst the collision gas was argon (0.3 mL/min flow). The $[M + H]^+$ ion corresponding to all 18 substances were selected for MS/MS experiments. Product ions were collected over the range *m/z* 45 and *m/z* 500. Scan time for each channel was 0.5 s and interscan delay was 0.1 s. The cone voltage was set at 28 V in all cases. The collision energy used for (**1d**) – (**1f**) was 10 eV while a value of 20 eV was chosen for the tryptamine based compounds (**2a**) – (**3f**), respectively.

High-resolution electrospray mass spectrometry

Analyses were carried out by characterization using UHPLC-QTOF-MS/MS as described previously.[19,20] Briefly, mobile phases used for UHPLC separation consisted of 100% acetonitrile (1% formic acid) and an aqueous solution of 1% formic acid. The column temperature was set at 40 °C (0.6 mL/min) and data were acquired for 5.5 min. The elution was a 5–70% acetonitrile gradient ramp over 3.5 min, then increased to 95% acetonitrile in 1 min and held for 0.5 min before returning to 5% acetonitrile in 0.5 min. QTOF-MS data were acquired in positive mode scanning from *m/z* 100 – *m/z* 1000 with and without auto MS/MS fragmentation. Ionization was achieved with an Agilent JetStream electrospray source and infused internal reference masses. Agilent 6540 QTOF-MS parameters: gas temperature 325 °C, drying gas 10 L/min and sheath gas temperature 400 °C. Internal reference ions at *m/z* 121.05087 and *m/z* 922.00979 were used.

High performance liquid chromatography diode array detection

HPLC-DAD analyses[19,20] were carried out on a Dionex 3000 Ultimate system coupled to a UV diode array detector (Thermo Fisher, St Albans, UK), using a Phenomenex Synergi Fusion column (150 mm x 2 mm, 4 µm) that was protected by a 4 mm x 3 mm Phenomenex Synergi Fusion guard column (Phenomenex, Macclesfield, UK). The mobile phases were made from 70% acetonitrile with 25 mM

TEAP buffer and an aqueous solution of 25 mM TEAP buffer. Elution was achieved with a gradient that started with 4% acetonitrile and ramped to 70% acetonitrile in 15 min and held for 3 min. The total acquisition time was 18 min at a flow rate of 0.6 mL/min. The diode array detection window was set at 200 nm to 595 nm (collection rate 2 Hz).

Results and discussion

The high potency associated with substances such as 25I-NBOMe (**1a**)[21,22] and the possibility that some of these 5-HT_{2A} receptor agonists may not be orally active,[23] might reflect the fact that these substances are often encountered in the form of absorbent paper (blotters), which are ingested via sublingual and buccal routes. However, the presence of powdered forms, capsules and liquids has also been reported.[14] Although the availability of bulk drugs may be amenable to a large range of analytical investigations, detection and identification in biofluids often requires the implementation of mass spectrometry based techniques due to the high potency associated with some of these derivatives.[24] Whilst knowledge about the formation of fragment and product ions form an important basis for the development of suitable screening techniques, further complexities might arise from the need to differentiate between isomers.[19]

In addition to the study of bioactive *N*-benzylphenethylamines, a number of their 5-methoxytryptamine counterparts were investigated. For example, in the rat tail artery assay, *N*-(2-methoxybenzyl)-5-methoxytryptamine (**2a**) has also been shown to have appreciable partial 5-HT_{2A} receptor agonist activity (pEC_{50} = 7.08; E_{max} = 54% relative to serotonin),[25] which indicated potential for further exploration. Indeed, all of the characterized substituted *N*-benzylphenethylamines and 5-methoxytryptamines (**1a**) – (**3f**) (Figure 1B) characterized in the present study have recently undergone a comprehensive investigation into serotonin receptor affinity, functional activity and mouse head-twitch response in order to acquire insights into their structure-activity relationships and the results will be reported in due course. One of the observations made, and which will be reported in due course, was that *meta*-substitution in the *N*-benzyl derivatives of 5-methoxytryptamine were important for their affinity to the 5-HT_2 receptor family and functional activity *in vitro* and *in vivo*.

In the interest of clarity and brevity, a more detailed discussion of analytical data obtained for *N*-(methoxybenzyl)phenethylamine isomers (**1a**) – (**1c**) and *N*-(methoxybenzyl)-5-methoxytryptamine isomers (**2a**) – (**2c**) is included in this manuscript to serve as representative examples. The data for all remaining derivatives, including infrared spectroscopy of all eighteen substances, are shown as supplementary information.

Gas chromatography electron and chemical ionization ion trap mass spectrometry

Gas chromatography (GC) retention times and ion trap (IT) electron- and chemical ionization (EI/CI) mass spectra for *N*-(methoxybenzyl)phenethylamine (**1a**) – (**1c**) are shown in Figure 2A–C as representative examples while the remaining mass spectra including all GC chromatograms are available as supplementary information. All

three sets of positional isomers, i.e. (**1a**) – (**1f**) and (**2a**) – (**2f**), could be separated under GC conditions and the elution order followed a similar pattern with increasing retention times in the order of *ortho*-, *meta*- and *para*-substitution which also was observed with a range of other isomeric *N*-(methoxybenzyl)phenethylamine analogs.[26] The EI-IT mass spectra obtained for the three 25I-NBOMe isomers (**1a**) – (**1f**) were largely consistent with the EI mass spectra reported previously using a quadrupole mass analyzer and included key fragments at *m/z* 121 (methoxylated tropylium ion) and *m/z* 150 (iminium ion formation following α-cleavage).[26] Correspondingly, the mass spectra associated with the three *N*-(bromobenzyl) substituted phenethylamine isomers (**1d**) – (**1f**) show the tropylium and iminium counterparts at *m/z* 169/171 and *m/z* 198/200, respectively (supplementary data). In contrast to *N*-(methoxybenzyl) isomers (**1a**) – (**1c**) that gave the same base peak at *m/z* 121, the brominated counterparts (**1d**) – (**1f**) revealed increasing relative abundance values for the equivalents at *m/z* 169/171 following the order of *ortho*- (**1d**), *meta*- (**1e**), and *para*-substitution (**1f**), respectively (supplemental data). Suggested EI-MS key fragments are shown in Figure 3A, which included suggestions incorporated from Zuba and colleagues on structurally related substances.[27-29] Representative GC-CI-IT-MS for *N*-(methoxybenzyl)phenethylamine analogs (**1a**) – (**1c**) are depicted in Figure 2D–F and revealed facile detection of the $[M + H]^+$ ion at *m/z* 428 to confirm the mass of the molecule, which was more challenging under EI-IT-MS conditions due to extensive fragmentation of the molecular ion. Some variations in the relative abundance of key fragments, e.g. *m/z* 91, *m/z* 121 and *m/z* 150, have been reported previously for (**1a**) – (**1c**) when using a single quadrupole mass analyzer.[26] Similar variations have been observed in the present study under three-dimensional ion trap conditions (Figure 2A–C), which might point towards the ability to differentiate between isomers. A more pronounced difference that might allow for differentiation between the *N*-(methoxybenzyl)phenethylamine isomers on mass spectral grounds was observed under CI-IT-MS conditions (Figure 2D–F) when inspecting the relative abundance values for the *m/z* 121 ion. In the case of 25I-NB4OMe (**1c**), this species formed the base peak whereas it was absent in the mass spectrum of the *N*-(3-methoxybenzyl) analog (**1b**). By contrast, 25I-NB2OMe (**1a**) yielded a relative abundance value of 20% for *m/z* 121. Interestingly, in the case of the three *N*-(bromobenzyl) isomers (**1d**) – (**1f**) the stability of the $[M + H]^+$ ion at *m/z* 476/478 dominated throughout the three CI mass spectra with negligible abundance values for the brominated tropylium counterpart at *m/z* 169/171 (supplemental data).

GC retention times and EI/CI IT-MS data for *N*-(methoxybenzyl)-5-methoxytryptamine isomers (**2a**) – (**2c**) are provided in Figure 2G–L. Similar to the corresponding phenethylamine 25I-NB4OMe (**1c**) (Figure 2C), the relative abundance of the *m/z* 150 iminium ion appeared to be significantly lower in the tryptamine counterpart (**2c**) (Figure 2I) when compared with the increased abundance observed in the EI-IT-MS of the remaining isomers. The tropylium ion at *m/z* 121 also formed the base peak in all three isomeric cases, similar to the phenethylamines (**1a**) – (**1c**). The main difference found in the EI-IT mass spectra, however, which facilitated the identification of the 5-methoxytryptamine moieties, was seen in key ions at *m/z* 160, *m/z* 145 and *m/z* 117. In addition, the key indicator that differentiated between a potentially existing *N*,*N*-dialkyltryptamine from a *N*-mono substituted compound, as is the case with the *N*-benzyl species, was the occurrence

of a strong *m/z* 161 ion which, for example, would not be observed with *N*,*N*-dialkylated compounds (Figure 2G–I and 3B).[30] Interestingly, apart from 5MT-NB3Me (**3c**), which yielded the methyltropylium base peak at *m/z* 105, all remaining *N*-benzyltryptamines (**2d**) – (**2f**), (**3a**), (**3b**) and (**3d**) – (**3f**), formed the base peak at *m/z* 161 (supplementary information). The CI-IT-MS data for *N*-benzyltryptamines (**2a**) – (**2c**) (Figure 2J–L) provided confirmation of the $[M + H]^+$ ion as expected although, as observed with the phenethylamines, distinct differences where observed due to collision-induced dissociation, which might be useful for the ability to obtain differential mass spectra from these three isomers.

Some moderate differences were noted in the abundance values associated with the *N*-(methoxybenzyl) group, i.e. *m/z* 121 and *m/z* 150. One feature that did not appear to be detected in the CI mass spectra of *N*-(methoxybenzyl)phenethylamine counterparts (**1a**) – (**1c**) (Figure 2D–F) was the appearance of an ion at *m/z* 294 that indicated a potential loss of ammonia from $[M + H]^+$ (Figure 2J–L). As shown below, a similar loss of ammonia was observed under electrospray (ESI) tandem mass spectrometry conditions. It also appeared that the *m/z* 174 ion (also described below under ESI conditions), which has only been detected to a minor extent in *N*,*N*-dialkylated tryptamines,[31] showed a comparatively higher abundance that differed between isomers (**2a**) – (**2c**) under CI conditions. Another ion of interest was at *m/z* 162 (Figure 2 and supplementary data), which may be characteristic for *N*-monosubstituted tryptamines when using GC-CI-IT-MS, i.e. absent in *N*,*N*-dialkyltryptamines.[30,31]

Electrospray ionization mass spectrometry

All *N*-benzyl substituted phenethylamines (**1a**) – (**1f**), and 5-methoxytryptamines (**2a**) – (**2f**) and (**3a**) – (**3f**) were characterized by electrospray (ESI) high mass accuracy quadrupole time-of-flight tandem mass spectrometry (QTOF-MS/MS) and ESI triple quadrupole (QqQ) tandem mass spectrometry. The mass spectral data for *N*-(methoxybenzyl) isomers (**1a**) – (**1c**) are presented in Figure 4. A comparison between QTOF-MS/MS (Figure 4A–C) and QqQ-MS/MS (Figure 4D–F) data showed an essentially similar trend with regards to the extent of product ion formation. The most extensive dissociation of the $[M + H]^+$ ion to yield the largest number of product ions was observed in the spectra of 25-NB3OMe (**1b**), whereas 25I-NB4OMe (**1c**) appeared to give the lowest level of product ion diversity. Figure 4D–F also shows a summary of the ion ratios determined under QqQ-MS/MS conditions, which displayed the extent of differential dissociation. All three isomers gave the expected *m/z* 121 ion as a base peak. A selection of proposed structural representations of product ions that might have formed during MS/MS analysis is shown in Figure 5A, which were in part adapted from previously published work on related substances.[27-29] Figure 5A also lists the calculated exact masses, whereas Table 1 lists the masses and suggested elemental formulae that could be detected by QTOF-MS/MS analysis with acceptable mass accuracy. In some cases, however, the product ion counts observed under QTOF-MS/MS conditions did not yield sufficient signal intensity to confirm the suggested elemental composition with acceptable accuracy although some species were more abundant when employing QqQ-MS/MS detection.

The ESI MS/MS data for *N*-(methoxybenzyl)tryptamines (**2a**) – (**2c**) can be seen in Figure 6 and the mass spectral information of the remaining twelve substances have been added as supplemental data. As observed with the phenethylamine counterparts, the relative abundance values observed with QTOF-MS/MS and QqQ-MS/MS showed marked differences, which translated into distinct ion ratios (e.g. Figure 6D–F). Interestingly, the *m/z* 121 product ion formed the base peak in two rather than all three isomers. 5MT-NB3OMe (**2b**) produced a base peak at *m/z* 174. Visual inspection of both QTOF and QqQ spectra indicated comparable relative abundance values with regards to some of the ion ratios encountered. For example, the distinct ratios observed for the *m/z* 121 and *m/z* 174 species (e.g. Figure 6D–F), might serve as a helpful feature when dealing with these isomers in the absence of reference material. A summary of suggested product ions, in part adapted from previously published work on *N*,*N*-dialkylated tryptamines[30] is shown in Figure 5B and Table 2.

The UHPLC-QTOF/MS method did not result in sufficient separation of the *N*-(methoxybenzyl) isomers (**1a**) – (**1c**) and (**2a**) – (**2c**), which led to an implementation of an HPLC-DAD method that has been successfully applied previously as a screening method in forensic casework.[19,20,32,33] Figure 7 shows the retention times and overlaid DAD spectra following separation by HPLC. Both 25I-NB2OMe (**1a**) and 5MT-NB2OMe (**2a**) were separated from the remaining two NB3OMe and NB4OMe isomers. Although NB3OMe and NB4OMe isomers (**1b**)/(**1c**) and (**2b**)/(**2c**) could not be separated, it was found that they could be distinguished by their full scan UV spectra (Figure 7A). *N*-(methoxybenzyl)phenethylamines (**1a**) – (**1c**) showed a slight shift of increasing wavelengths with regards to the secondary UV maximum. The difference was especially pronounced in the case of the two co-eluting isomers (**1b**) (283.5 nm) and (**1c**) (298.3 nm). In addition, 25I-NB4OMe (**1c**) displayed a distinct shoulder at 226.1 nm that was absent in the *ortho*- and *meta*- NBOMe isomers (**1a**) and (**1b**). Whilst some of the spectral differences were seemingly small, experiences in casework has previously shown how valuable these can be when dealing with isomers and other closely related compounds.[19,20] Similarly, although the 5MT-based *N*-(methoxybenzyl) isomer (**2a**) could be separated from the *meta*- and *para*-substituted isomers (**2b**) and (**2c**), inspection of their UV full scan spectra confirmed the presence of distinctive differences to facilitate their differentiation (Figure 7B). By contrast, all three 5MT-based *N*-(bromobenzyl) isomers (**2d**) – (**2f**) were fully separated under identical conditions (supplemental information).

Conclusion

As is the case with most new psychoactive substances, the appearance of potent 5-HT_2 receptor agonists on the 'research chemicals' market highlighted the need to provide analytical data. The present study undertook a comprehensive analytical characterization of six *N*-benzylphenethylamines and twelve *N*-benzyl-5-methoxytryptamines and the majority of these substances have not yet been described in the analytical literature. Differentiation between positional isomers may be obtained by mass spectral investigations to some extent but the implementation of

chromatographic techniques with additional methods such as diode array detection were found to offer additional value. The collection of spectral data provided in the present study was aimed to support those researchers in the field who have to deal with exposure to these particular substances, such as clinicians, forensic providers and law enforcement.

Figure captions

Figure 1. A: Three representative 5-HT_{2A} receptor agonists and *N*-benzylphenethylamine derivatives 25I- (**1a**), 25B-, and 25C-NBOMe that are available as recreational psychoactive substances. B: chemical structures of 'NBOMe' derivatives (**1a**) – (**3f**) subjected to analytical characterization.

Figure 2. Gas chromatography retention times, electron (EI)- and chemical (CI) ionization ion trap (IT) mass spectra of *N*-(methoxybenzyl) substituted isomers (**1a**) – (**1c**) and (**2a**) – (**2c**).

Figure 3. Structural representations of suggested key ions formed during analysis by electron ionization ion trap mass spectrometry. A: 25I-NBOMes (**1a**) – (**1f**). B: 5-MT-NBOMes (**2a**) – (**3f**).

Figure 4. Electrospray ionization mass spectra obtained from positional 25I-NBOMe isomers (**1a**) – (**1c**). A – C: UHPLC-QTOF-MS/MS. The retention time of (**1c**) in trace C, i.e. associated with the peak top was 2.66 min. The given retention time of 2.82 min represented the attempt to obtain a less saturated signal response for the base peak ion. D – F: direct infusion triple quadrupole MS/MS and corresponding ion ratios.

Figure 5. Structural representations of suggested key ions formed during analysis by QTOF-MS/MS and their calculated theoretical *m/z* values. A: 25I-NBOMes (**1a**) – (**1c**). B: 5-MT-NBOMes (**2a**) – (**2c**).

Figure 6. Electrospray ionization mass spectra obtained from positional 5-MT-NBOMe isomers (**2a**) – (**2c**). A – C: UHPLC-QTOF-MS/MS. D – F: direct infusion triple quadrupole MS/MS and corresponding ion ratios.

Figure 7. HPLC retention times and full scan UV spectra (DAD) for the *N*-(methoxybenzyl) substituted isomers. A: (**1a**) – (**1c**) and B: (**2a**) – (**2c**). Differentiation was possible by the combination of UV full scan and retention time data.

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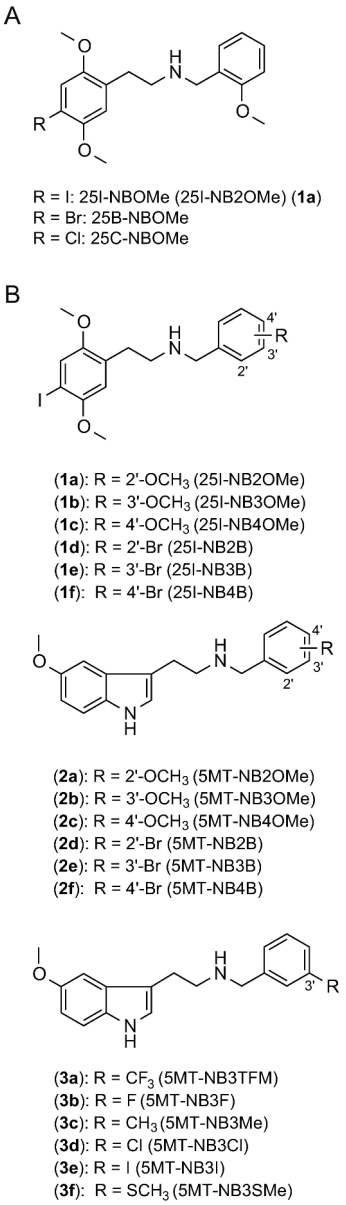

Figure 1. A: Three representative 5-HT2A receptor agonists and N-benzylphenethylamine derivatives 25I-(1a), 25B-, and 25C-NBOMe that are available as recreational psychoactive substances. B: chemical structures of 'NBOMe' derivatives (1a) – (3f) subjected to analytical characterization.
243x874mm (300 x 300 DPI)

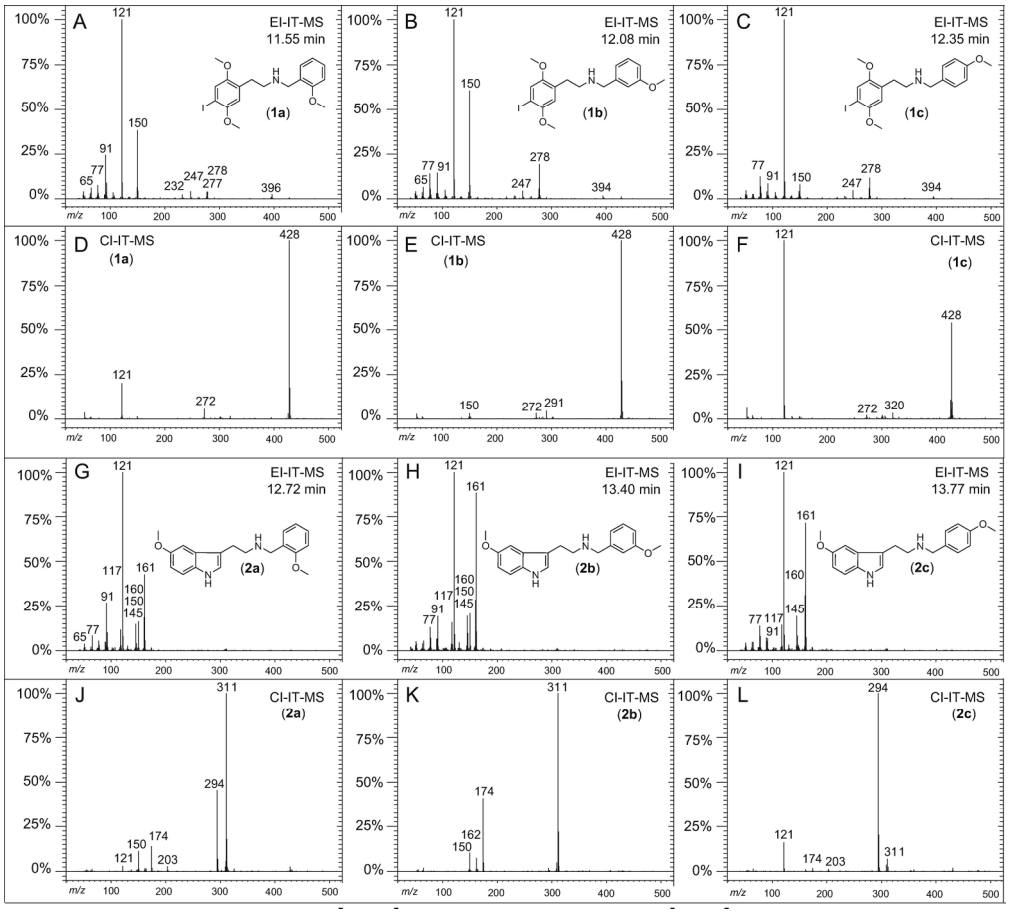

Figure 2. Gas chromatography retention times, electron (EI)- and chemical (CI) ionization ion trap (IT) mass spectra of N-(methoxybenzyl) substituted isomers (1a) – (1c) and (2a) – (2c).
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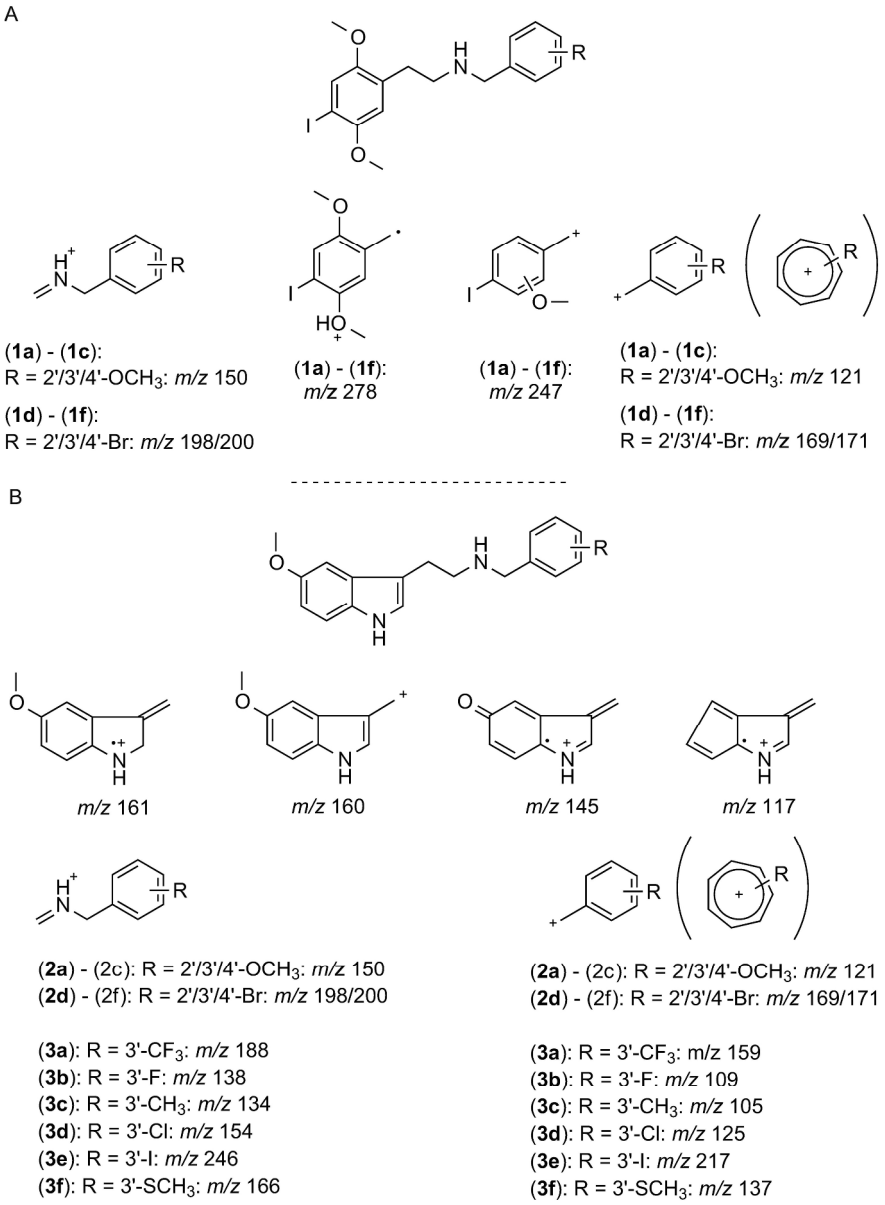


Figure 3. Structural representations of suggested key ions formed during analysis by electron ionization ion trap mass spectrometry. A: 25I-NBOMes (1a) – (1f). B: 5-MT-NBOMes (2a) – (3f).
245x336mm (300 x 300 DPI)

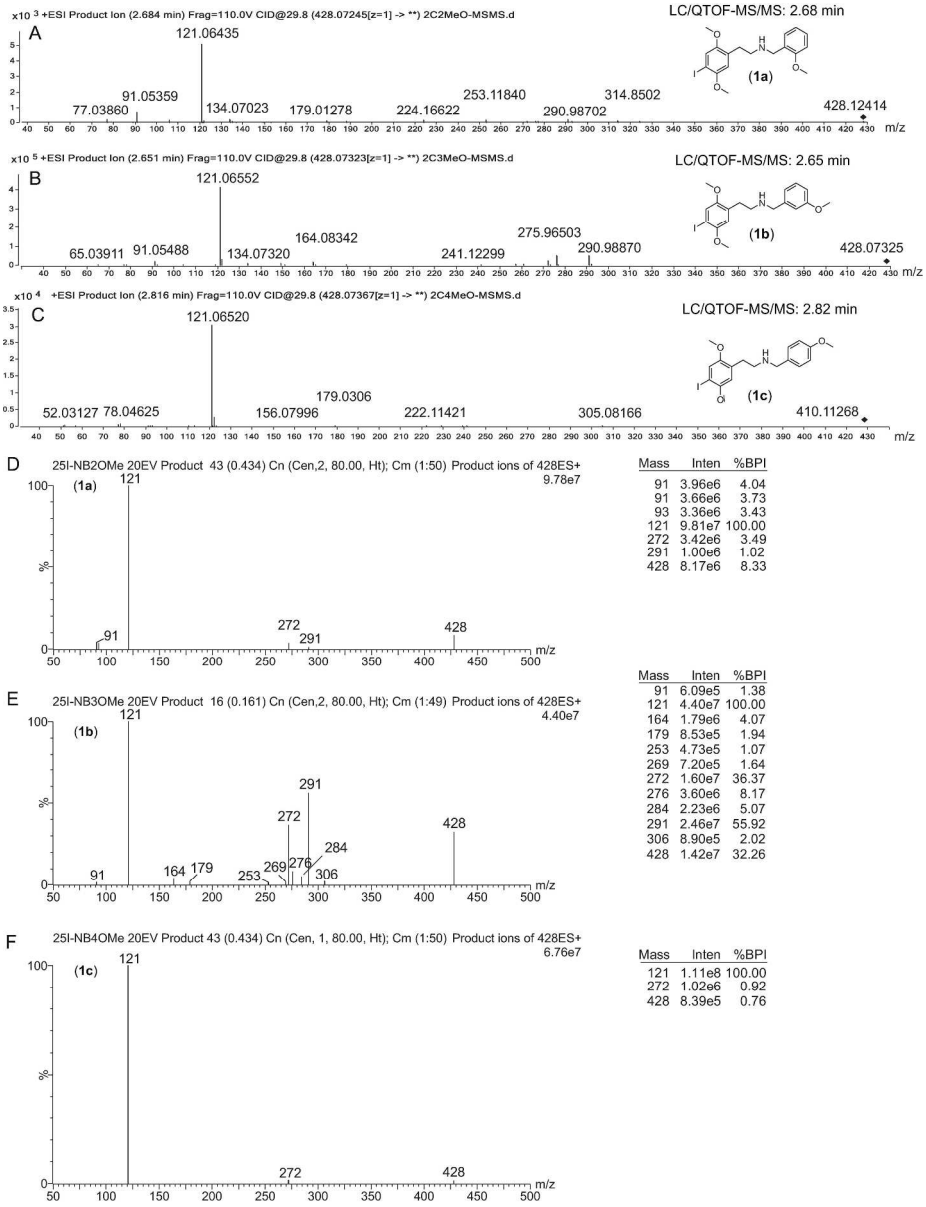


Figure 4. Electrospray ionization mass spectra obtained from positional 25I-NBOMe isomers (1a) – (1c). A – C: UHPLC-QTOF-MS/MS. The retention time of (1c) in trace C, i.e. associated with the peak top was 2.66 min. The given retention time of 2.82 min represented the attempt to obtain a less saturated signal response for the base peak ion. D – F: direct infusion triple quadrupole MS/MS and corresponding ion ratios. 268x350mm (300 x 300 DPI)

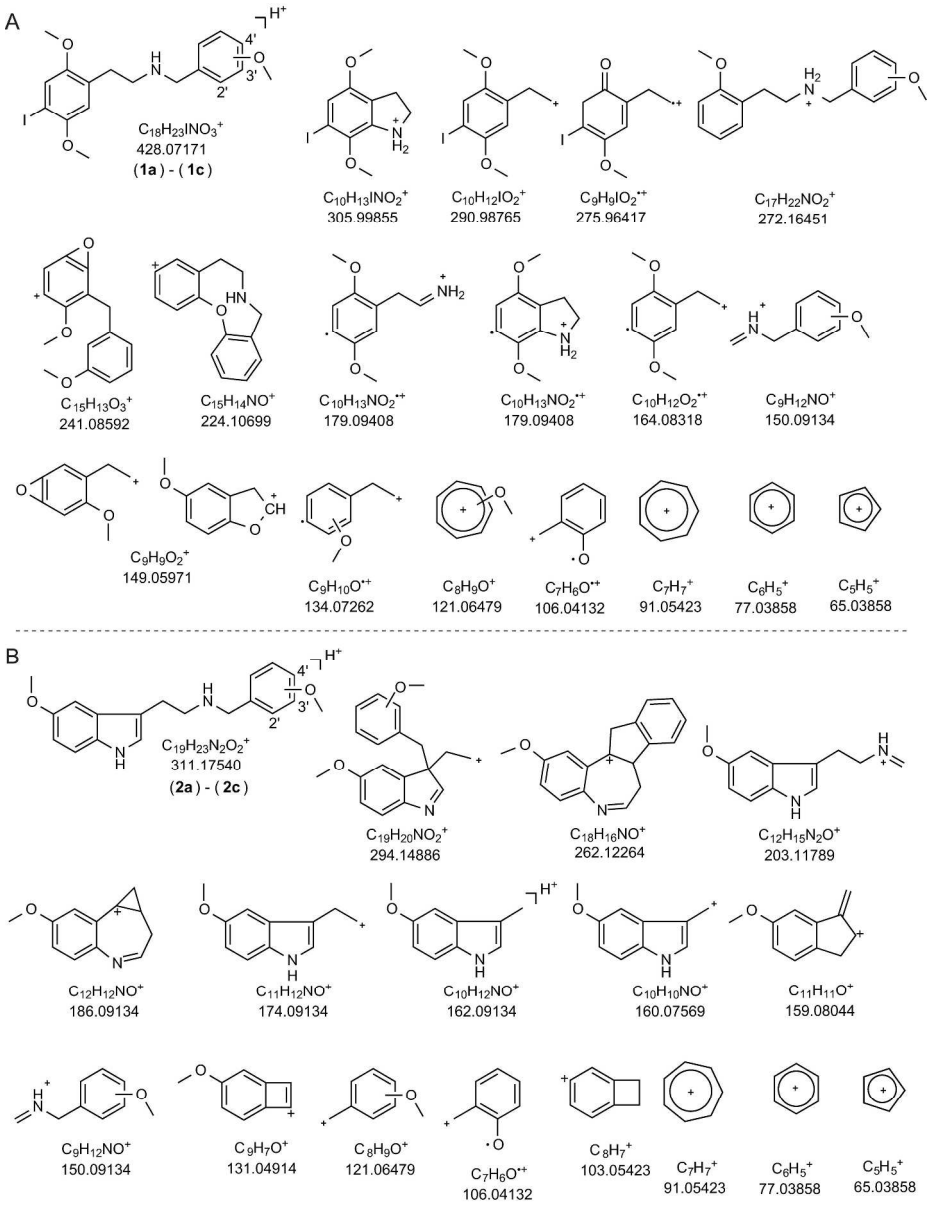


Figure 5. Structural representations of suggested key ions formed during analysis by QTOF-MS/MS and their calculated theoretical mass values. A: 25I-NBOMes (1a) – (1c). B: 5-MT-NBOMes (2a) – (2c).
270x351mm (300 x 300 DPI)

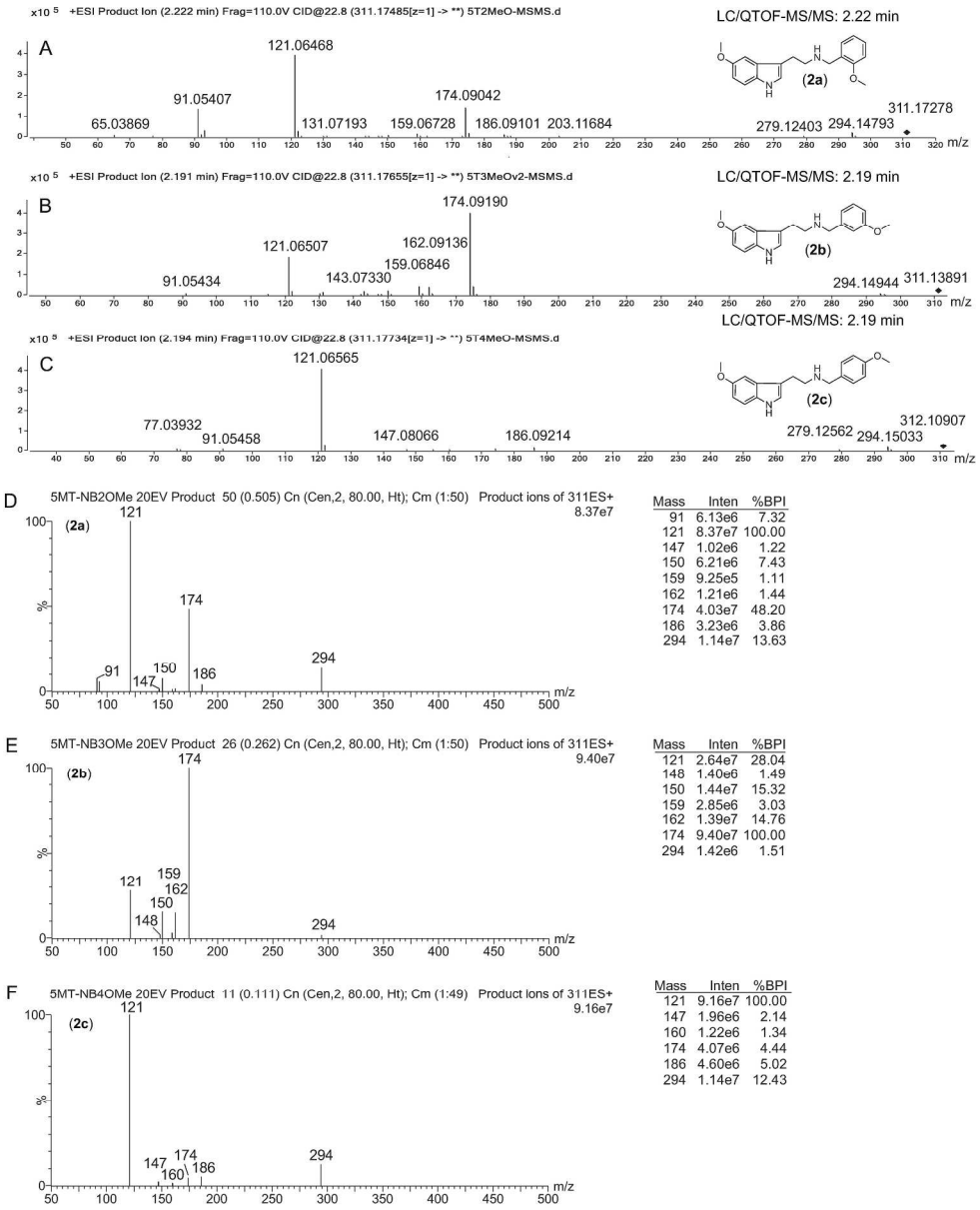
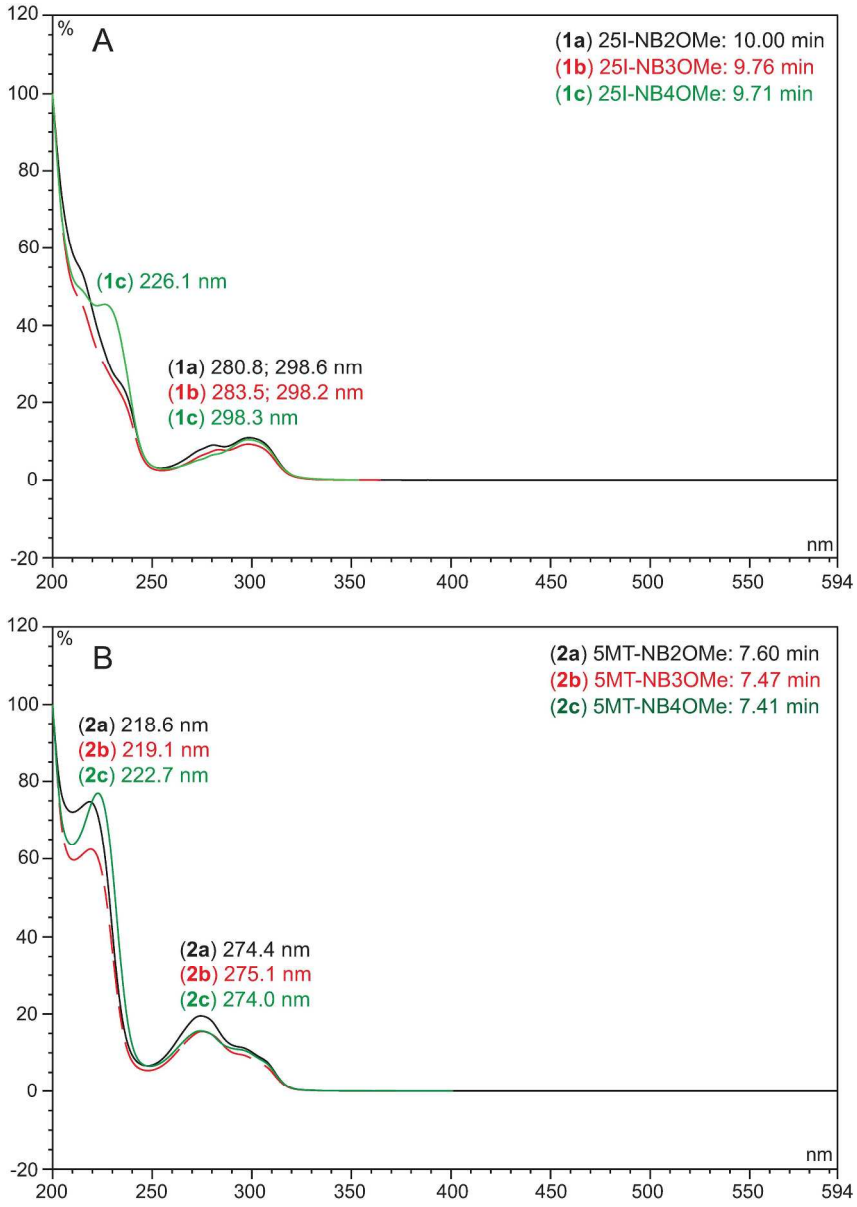


Figure 6. Electrospray ionization mass spectra obtained from positional 5-MT-NBOMe isomers (2a) – (2c). A – C: UHPLC-QTOF-MS/MS. D – F: direct infusion triple quadrupole MS/MS and corresponding ion ratios. 257x316mm (300 x 300 DPI)



241x342mm (300 x 300 DPI)

Table 1. Product ions obtained from *N*-(methoxybenzyl) substituted phenethylamines (**1a**) – (**1c**). Suggested elemental formulae of proposed product ions (Figure 5A) that could be detected by QTOF-MS/MS analysis with acceptable mass accuracy.

Compound	Measured *m/z* value	Suggested formula	Calculated *m/z* value	Δm [ppm]
(**1a**) 25I-NB2OMe	428.07239 (PM)	$C_{18}H_{23}INO_3^+$	428.07171	- 1.59
	314.08502	*No formula*	314.08	
	290.98702	$C_{10}H_{12}IO_2^+$	290.98765	+ 2.17
	179.01278	$C_{12}H_{13}O_2^{\bullet+}$	179.01276	- 0.11
	134.07023	$C_9H_{10}O^{\bullet+}$	134.07262	- 17.8
	121.06435 (BP)	$C_8H_9O^+$	121.06479	+ 3.68
	106.04068	$C_7H_6O^{\bullet+}$	106.04068	- 6.04
	91.05359	$C_7H_7^+$	91.05423	+ 7.07
(**1b**) 25I-NB3OMe	428.07319 (PM)	$C_{18}H_{23}INO_3^+$	428.07171	- 3.46
	306.99932	$C_{10}H_{13}INO_2^+$	305.99855	- 2.53
	290.98870	$C_{10}H_{12}IO_2^+$	290.98765	- 3.62
	275.96503	$C_9H_9IO_2^{\bullet+}$	275.96417	+ 3.12
	179.09422	$C_{10}H_{13}NO_2^{\bullet+}$	179.09408	+ 0.78
	164.08342	$C_{10}H_{12}O_2^{\bullet+}$	164.08318	+ 1.46
	149.05990	$C_9H_9O_2^+$	149.05971	- 1.31
	134.07320	$C_9H_{10}O^{\bullet+}$	134.07262	+ 4.33
	121.06552 (BP)	$C_8H_9O^+$	121.06479	- 6.07
	91.05488	$C_7H_7^+$	91.05423	- 6.26
	65.03911	$C_5H_5^+$	65.03858	- 8.33
(**1c**) 25I-NB4OMe	428.07302 (PM)	$C_{18}H_{23}INO_3^+$	428.07171	
	121.06520 (BP)	$C_8H_9O^+$	121.06479	- 3.40
	106.04158	$C_7H_6O^{\bullet+}$	106.04132	+ 2.45
	91.05437	$C_7H_7^+$	91.05423	- 1.59
	77.03923	$C_6H_5^+$	77.03858	- 8.59

PM: $[M + H]^+$ ion; BP: base peak.

Table 2. Product ions obtained from *N*-(methoxybenzyl) substituted 5-methoxytryptamines (**2a**) – (**2c**). Suggested elemental formulae of proposed product ions (Figure 5B) that could be detected by QTOF-MS/MS analysis with acceptable mass accuracy.

Compound	Measured *m/z* value	Suggested formula	Calculated *m/z* value	Δm [ppm]
(**2a**) 5MT-NB2OMe	311.17548 (PM)	$C_{19}H_{23}N_2O_2^+$	311.17540	- 0.24
	294.14793	$C_{19}H_{20}NO_2^+$	294.14886	+ 3.16
	262.11985	$C_{18}H_{16}NO^+$	261.11536	+ 10.69
	203.11684	$C_{12}H_{15}N_2O^+$	203.11789	+ 5.19
	186.09101	$C_{12}H_{12}NO^+$	186.09134	+ 1.79
	174.09042 (BP)	$C_{11}H_{12}NO^+$	174.09134	+ 5.32
	162.09067	$C_{10}H_{12}NO^+$	162.09134	+ 4.16
	121.06468	$C_8H_9O^+$	121.06479	+ 0.93
	103.05381	$C_8H_7^+$	103.05423	+ 4.08
	91.05407	$C_7H_7^+$	91.05423	+ 1.74
	65.03869	$C_5H_5^+$	65.03858	- 1.77
(**2b**) 5MT-NB3OMe	311.17650 (PM)	$C_{19}H_{23}N_2O_2^+$	311.17540	- 3.53
	294.14838	$C_{19}H_{20}NO_2^+$	294.14886	+ 1.62
	174.09190 (BP)	$C_{11}H_{12}NO^+$	174.09134	- 3.23
	162.09136	$C_{10}H_{12}NO^+$;	162.09134	- 1.8
	150.09104	$C_9H_{12}NO^+$	150.09134	+ 2.02
	131.07282	$C_9H_9N^{\bullet+}$	131.0735	- 5.19
	121.06507 (BP)	$C_8H_9O^+$	121.06479	- 2.32
	91.05434	$C_7H_7^+$	91.05423	- 1.26
	65.03869	$C_5H_5^+$	65.03858	- 1.77
	77.03878	$C_6H_5^+$	77.03858	- 2.68
(**2c**) 5MT-NB4OMe	311.17632 (PM)	$C_{19}H_{23}N_2O_2^+$	311.17540	- 2.95
	294.15033	$C_{19}H_{20}NO_2^+$;	294.14886	- 5.03
	174.09207	$C_{11}H_{12}NO^+$	174.09134	- 4.22
	160.07655	$C_{10}H_{10}NO^+$	160.07569	- 5.40
	147.08066	$C_{10}H_{11}O^+$	147.08044	- 1.50
	121.06565 (BP)	$C_8H_9O^+$	121.06479	- 7.15
	106.04105	$C_7H_6O^{\bullet+}$	106.04186	- 7.64
	91.05458	$C_7H_7^+$	91.05423	- 3.92
	77.03932	$C_6H_5^+$	77.03858	- 9.78

PM: $[M + H]^+$ ion; BP: base peak.