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Accepted but not corrected

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Journal:	*Drug Testing and Analysis*
Manuscript ID:	DTA-15-0028.R1
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	n/a
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Keywords:	1,2-Diphenylethylamines, 'Research chemicals', New psychoactive substances, Methoxyphenidine , Diphenidine
Abstract:	The structurally diverse nature of the 1,2-diphenylethylamine template provides access to a range of substances for drug discovery work but some have attracted attention as 'research chemicals'. The most recent examples include diphenidine, i.e. 1-(1,2-diphenylethyl)piperidine and 2-methoxydiphenidine, i.e. 1-[1-(2-methoxyphenyl)-2-phenylethyl]piperidine (MXP, methoxyphenidine, 2-MXP) that have been associated with uncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist activity. Challenges encountered during chemical analysis include the presence of positional isomers. Three powdered samples suspected to contain 2-MXP were obtained from three Internet retailers in the United Kingdom and subjected to analytical characterization by gas-, and high performance liquid chromatography (GC-, HPLC) coupled to various forms of mass spectrometry (MS). Nuclear magnetic resonance

	spectroscopy, infrared spectroscopy and thin layer chromatography were also employed. This was supported by the synthesis of all three isomers (2-, 3- and 4-MXP) that were obtained from two different synthetic routes. The analytical data obtained for the three purchased samples were consistent with the synthesized 2-MXP standard. The differentiation between the isomers was possible. Distinct stability differences were observed for all three isomers during in-source collision-induced dissociation of the protonated molecule when employing detection under HPLC selected-ion monitoring detection, which added to the ability to differentiate between them. Furthermore, the analysis of a 2-MXP tablet by matrix assisted inlet ionization Orbitrap mass spectrometry confirmed that it was possible to detect the protonated molecule of 2-MXP directly from the tablet surface following addition of 3-nitrobenzonitrile as the matrix.

Test purchase, synthesis and characterization of 2-methoxydiphenidine (MXP) and differentiation from its *meta*- and *para*-substituted isomers

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Running title: Characterization of 2-, 3- and 4-methoxydiphenidine isomers

Abstract

The structurally diverse nature of the 1,2-diphenylethylamine template provides access to a range of substances for drug discovery work but some have attracted attention as 'research chemicals'. The most recent examples include diphenidine, i.e. 1-(1,2-diphenylethyl)piperidine and 2-methoxydiphenidine, i.e. 1-[1-(2-methoxyphenyl)-2-phenylethyl]piperidine (MXP, methoxyphenidine, 2-MXP) that have been associated with uncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist activity. Challenges encountered during chemical analysis include the presence of positional isomers. Three powdered samples suspected to contain 2-MXP were obtained from three Internet retailers in the United Kingdom and subjected to analytical characterization by gas-, and high performance liquid chromatography (GC-, HPLC) coupled to various forms of mass spectrometry (MS). Nuclear magnetic resonance spectroscopy, infrared spectroscopy and thin layer chromatography were also employed. This was supported by the synthesis of all three isomers (2-, 3- and 4-MXP) that were obtained from two different synthetic routes. The analytical data obtained for the three purchased samples were consistent with the synthesized 2-MXP standard. The differentiation between the isomers was possible. Distinct stability differences were observed for all three isomers during in-source collision-induced dissociation of the protonated molecule when employing detection under HPLC selected-ion monitoring detection, which added to the ability to differentiate between them. Furthermore, the analysis of a 2-MXP tablet by matrix assisted inlet ionization Orbitrap mass spectrometry confirmed that it was possible to detect the protonated molecule of 2-MXP directly from the tablet surface following addition of 3-nitrobenzonitrile as the matrix.

Keywords: 1,2-diphenylethylamines; diphenidine; 'research chemicals'; new psychoactive substances; methoxyphenidine

Introduction

The 1,2-diphenylethylamine analog 1-[1-(2-methoxyphenyl)-2-phenylethyl]piperidine (2-MeO-diphenidine, MXP, methoxyphenidine, 2-MXP) (Figure 1A) has attracted attention as a 'research chemical' and is suspected to share some psychopharmacological features that are also associated with 'dissociative anaesthetics', such as ketamine, 1-(1-phenylcyclohexyl)piperidine (PCP) and other uncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonists.[1] The preparation of 2-MXP and a number of analogs originated from medicinal chemistry research into the association between NDMA receptor antagonism and neuroprotection[2] The availability of substances originally explored during drug discovery related research efforts from Internet retailers as 'research chemicals' has moved this substance into the arena of new psychoactive substances (NPS).[3] In addition, recently published case reports described the detection of 2-MXP and its association with acute PCP-type toxicity[4] and deaths.[5]

Detailed information on pharmacodynamic properties of 2-MXP remains to be uncovered. Binding experiments, based on crude whole rat brain membrane preparations using 1 nM [^{3}H]-TCP as the radioligand, revealed involvement of the NMDA receptor and indicated distinct differences in affinity under the conditions studied. For example, whereas 2-MXP and 3-MXP (Figure 1B) yielded apparent K_i values of 32 nM and 26 nM, respectively, a significant loss in affinity was observed with the *para*-substituted 4-MXP isomer (K_i = 8100 nM). In comparison, 1-[1-(thiophen-2-yl)cyclohexyl]piperidine (TCP), PCP and 2-chlorodiphendine gave K_i values of 20 nM, 96 nM and 0.19 nM, respectively.[2] The closely related 1-(1,2-diphenylethyl)piperidine (diphenidine) (Figure 1A) also appeared on the 'research chemicals' market before 2-MXP was introduced and both appear to be psychoactive in humans.[1] There are indications that diphenidine displays stereoselective differences in a number of assays[2,6] but similar data on the 2-MXP enantiomers appear to be unavailable. Diphenidine has recently been shown to reduce NMDA-mediated field excitatory postsynaptic potentials in rat hippocampal slices, which confirmed the impact of diphenidine on synaptic transmission.[7] Although it seems conceivable that 2-MXP may have similar properties, further work is warranted to explore the extent of this possibility.

The 1,2-diphenylethylamine structural template provides access to a range of substances with various properties including bronchodilation[8] and analgesic activity.[9,10] The synthetic opioid analgesic 1-cyclohexyl-4-(1,2-diphenylethyl)piperazines (MT-45, Figure 1A) has also been encountered as a 'research chemical' and 'legal opioid'. It has recently been associated with a range of fatal and non-fatal intoxications, which led to a risk assessment procedure that was carried out under the auspices of European Monitoring Centre for Drugs and Drug Addiction (EMCDDA).[10,11] Interestingly, (*R*)-1-(2-(2-ethoxyphenyl)-1-phenylethyl)piperazine (PF-526014, Figure 1A) and closely related analogs were shown to behave as dual serotonin and noradrenaline reuptake inhibitors with good selectivity over dopamine inhibition. The lipophilic nature associated with some particular analogs also demonstrated a range of off-target effects associated with other biological targets.[12-16] Whether these particular analogs are available as

'research chemicals', however, is not known.

In situations where reference material is not always available when new psychoactive substances are encountered for the first time, challenges can arise when approaching the ability to correctly identify these analogs. The availability of 2-MXP as a 'research chemical' and the presence of three positional isomers associated with the phenyl ring raised questions about the ability to differentiate between them. The present study reports on the analysis of three powdered 2-MXP samples sold on the Internet in the United Kingdom. This was supported by the analytical characterization of all three isomers (2-, 3- and 4-MXP) that were obtained from two independent synthetic routes (Figures 1B/1C). In addition, the analysis of a 2-MXP tablet, also obtained from a UK-based vendor, was carried out using matrix assisted inlet ionization Orbitrap mass spectrometry to assess the applicability of this technique with minimal sample preparation.

Experimental

Materials

For synthesis procedure 1, starting materials, reagents and HPLC-grade solvents were obtained from Sigma-Aldrich (St. Louis, MO, USA) except for benzyl bromide 99% (Lancaster synthesis, NH, USA) and $CDCl_3$ 100%, 99.96 atom % D with 0.03 % (v/v) TMS Aldrich (St. Louis, MO, USA). Flash column chromatography was conducted on silica gel 230-400 Mesh, 60 Å, obtained from Sigma Aldrich. Melting point range were obtained using a DigiMelt A160 SRS melting point apparatus (Stanford Research Systems, Sunnyvale, CA, USA) at a ramp rate of 2 °C/min and are uncorrected. For synthesis procedure 2, starting materials, reagents and solvents were mostly obtained from Sigma Aldrich (Arklow, Co. Wicklow, Ireland) except for 2- and 4-methoxybenzoyl chloride and *N*,O-dimethylhydroxylamine, which were obtained from TCI – Tokyo Chemical Industry Co. Ltd, Tokyo, Japan. LC-MS grade solvents were obtained from Fisher Scientific (Dublin, Ireland). 3-Nitrobenzonitrile (3-NBN), 99% purity, was purchased from TCI America (Portland, OR). Microscope cover glasses were purchased from VWR International (Suwanee, GA, USA). Three samples, advertised as 2-methoxydiphenidine (2-MXP), were purchased from three different online vendors based in the United Kingdom. A photograph of one representative product is shown in the supplemental information. A tablet advertised to contain 2-MXP was also obtained from a UK based 'research chemical' vendor. The oval-shaped tablet (259.1 mg, see supplemental data for picture) had a single score across the center. Quantitative information was not provided by the supplier.

Syntheses

Synthesis procedure 1 (Figure 1B)

1-[1-(2-Methoxyphenyl)-2-phenylethyl]piperidine (2-MeO-diphenidine, 2-MXP)

To a dry round bottom flask containing 40 mL dry acetonitrile (4 Å molecular sieves) was added zinc powder (61.2 mmol, 4.0 g), benzyl bromide (6.72 mol, 0.8 mL), and trifluoroacetic acid (5.38 mmol, 0.625 mL). The solution was stirred under argon at room temperature for 15 minutes. The remaining benzyl bromide (49.9 mmol, 5.94 mL), piperidine (23.5 mol, 2.39 mL) and 2-methoxybenzaldehyde (22.0 mmol, 3.0 g) were added with vigorous stirring under argon. After approximately 1 hour the reaction was found to be complete. The reaction was quenched with a 2 N potassium hydroxide solution (200 mL) and extracted with dichloromethane (3 x 100 mL). The pooled organic extracts were gravity filtered through a small silica plug to remove insoluble material. The organic phase was washed with saline, dried with anhydrous magnesium sulfate and concentrated under vacuum to give an amber oil. This crude base was purified via flash column chromatography over silica gel with a mobile phase of hexanes to give 4.1 g of colorless oil (63.2 % yield). For the formation of the hydrochloride salt, the purified freebase was dissolved in 20 mL acetone and titrated to pH 1 with concentrated HCl solution. The solvent was then reduced under a stream of warm air. Acetone was added and evaporation continued until all residual acid and H_2O were removed. Following a number of repeated cycles, a white crystalline solid was obtained. The solids were washed with ethyl acetate and dried in an oven at 60 °C. The crystals were then recrystallized by dissolving in 3 mL warm EtOH and diluting to 50 mL Et_2O and storing at 0 °C overnight. Solids were collected, via decanting of the solvent, washed with ethyl acetate (2 x 5 mL) and dried in an oven at 60 °C. This was repeated for a total of 3 times to give a white crystalline solid (m.p. 171.5–172.6 °C). HR-ESIMS: observed *m/z* 296.2005 (theory $[M + H]^+$: $C_{20}H_{26}NO^+$ *m/z* 296.2009). ^1H and ^{13}C NMR data are shown in Table 1.

1-[1-(3-Methoxyphenyl)-2-phenylethyl]piperidine (3-MeO-diphenidine, 3-MXP)

The reaction was carried out as described above using 3-methoxybenzaldehyde instead. Melting point of hydrochloride salt: 169.0–170.5 °C. HR-ESIMS: observed *m/z* 296.2005 (theory $[M + H]^+$: $C_{20}H_{26}NO^+$ *m/z* 296.2009). ^1H and ^{13}C NMR data are shown in Table 1.

1-[1-(4-Methoxyphenyl)-2-phenylethyl]piperidine (4-MeO-diphenidine, 4-MXP)

The reaction was carried out as described above using 4-methoxybenzaldehyde instead. Melting point of hydrochloride salt: 93.0–94.0 °C (MeOH/Et_2O) (lit. 132 – 135 °C (toluene)[17]). HR-ESIMS: observed *m/z* 296.2004 (theory $[M + H]^+$: $C_{20}H_{26}NO^+$ *m/z* 296.2009). ^1H and ^{13}C NMR data are shown in Table 1.

Synthesis procedure 2 (Figure 1C)

1-[1-(2-Methoxyphenyl)-2-phenylethyl]piperidine (2-MeO-diphenidine, 2-MXP)

Triethylamine (Et_3N) (21.2 g, 30 mL, 210 mmol) was added to a mixture of 2-methoxybenzoyl chloride (50.0 mmol, 8.53 g), *N*,*O*-dimethylhydroxylamine (65.5 mmol, 4.0 g) and catalytic amounts of 4-dimethylaminopyridine (DMAP) (100 mg) in dichloromethane (100 mL) and the resulting mixture was stirred for 14 hours. Water was added and the resulting mixture was extracted with ethyl acetate (3 x 200 mL).

The organic fractions were combined, dried with $MgSO_4$, filtered and concentrated to give the desired Weinreb amide (7.74 g). To a solution of this amide (20.5 mmol, 4 g) in tetrahydrofuran (30 mL) at 0 ^oC was added benzylmagnesium chloride (20 mL) and the resulting mixture was stirred overnight. The reaction was quenched with 5% HCl and extracted with diethyl ether (3 x 200 mL). The organic fractions were combined, dried with $MgSO_4$, filtered and concentrated to give the desired ketone (10.42 g). A mixture of this ketone (26.5 mmol, 6 g), ammonium acetate (700 mmol, 56 g), powdered 3 Å molecular sieves (16.8 g) in methanol (360 mL) was stirred at room temperature for one hour. To this mixture, 1.6 g of sodium cyanoborohydride (25 mmol) in 24 mL THF was added and the mixture was stirred overnight. The reaction was then filtered over a pad of celite and rinsed with 200 mL methanol and 200 mL dichloromethane. The combined filtrates were concentrated and the residue dissolved in water. This was made alkaline with 5 M NaOH and extracted with dichloromethane (3 x 100 mL). The organic fractions were combined, dried with $MgSO_4$, filtered and concentrated to give desired primary amine (5.7 g). 1,5-Dibromopentane (71 mmol, 16.25 g, 9.6 mL) was added dropwise to a solution of the primary amine (21 mmol, 4.7g), potassium carbonate (0.14 mol, 19.5 g) in acetonitrile (182 mL) and stirred under nitrogen for 3 days at room temp. The potassium salts were removed by filtration, and the filtrate was washed with acetonitrile and concentrated yielding crude 2-methoxydiphenidine (MXP) (17.7 g). For the formation of the hydrochloride salt, 30 mL of ethereal HCl was added to the crude 2-MXP (20 mmol, 6.0 g). The addition of *tert*.-butyl methyl ether and an overnight stirring period followed. The stirring process (trituration) allowed the 2-MXP HCl salt to fall out of solution. The mixture was centrifuged, supernatant removed, and the solid washed with hexane and dried under vacuum yielding 2-methoxydiphenidine HCl salt as a white powder (0.434 g, 45% total yield calculated from 2-methoxybenzoyl chloride) m.p. 171.0–172.0 ^oC. HR-ESIMS: observed *m/z* 296.1999 (theory $[M + H]^+$: $C_{20}H_{26}NO^+$ *m/z* 296.2009). ^1H and ^{13}C NMR data are shown in Table 1.

1-[1-(3-Methoxyphenyl)-2-phenylethyl]piperidine (3-MeO-diphenidine, 3-MXP)

The reaction was carried out as described above using 3-methoxybenzoyl chloride instead yielding 3-methoxydiphenidine HCl salt as a white powder (0.534g, 55% yield calculated from 3-methoxybenzoyl chloride) m.p. 142.0-145.5 °C. HR-ESIMS: observed *m/z* 296.1998 (theory $[M + H]^+$: $C_{20}H_{26}NO^+$ *m/z* 296.2009). ^1H and ^{13}C NMR data are shown in Table 1.

1-[1-(4-Methoxyphenyl)-2-phenylethyl]piperidine (4-MeO-diphenidine, 4-MXP)

The reaction was carried out as described above using 4-methoxybenzoyl chloride instead yielding 4-methoxydiphenidine HCl salt as a white powder (0.497g, 24% yield calculated from 4-methoxybenzoyl chloride) m.p. 88.5-91.0 °C. HR-ESIMS: observed *m/z* 296.1996 (theory $[M + H]^+$: $C_{20}H_{26}NO^+$ *m/z* 296.2009). ^1H and ^{13}C NMR data are shown in Table 1.

Instrumentation

Nuclear magnetic resonance spectroscopy

^{1}H (400 MHz) and ^{13}C NMR spectra (100 MHz) were recorded on a Bruker Ultrashield 400 plus spectrometer with a 5 mm BBO S1 (Z gradient plus) probe at 24 °C. The freebase samples were dissolved in $CDCl_3$ (100% and 99.96% D, 0.03% (v/v) TMS) to give approximately 20 mg/mL concentrations. Aliphatic chemical shifts were assigned using 1-D and 2-D heteronuclear experiments. Internal chemical shift references were TMS (δ = 0.00 ppm) and solvent (δ = 77.0 ppm). The MXP standards synthesized using procedure 2 were prepared in deuterated dimethyl sulfoxide (DMSO-d_6) (20 mg/mL). ^{1}H (600 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker AV600 NMR spectrometer using a 5 mm TCI cryoprobe. ^{1}H NMR spectra were referenced to an external TMS reference at δ= 0 ppm.

Gas chromatography ion trap mass spectrometry

GC-MS data were acquired on a Varian 450-GC gas chromatograph coupled to a Varian 220-MS ion trap mass spectrometer (Walnut Creek, CA, USA) and in electron (EI) and chemical ionization (CI) full scan mode. Samples (0.5 mg/mL) were introduced into a Varian CP-1177 injector (275 ºC) in split mode (1:50) using a CP-8400 autosampler. The MS Data Review function of the Workstation software, version 6.91 was employed for data acquisition. Transfer line, manifold and ion trap temperatures were set at 310, 80 and 220 ºC, respectively. Helium was the carrier gas (1 mL/min, EFC constant flow mode) and 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. An Agilent J&W VF-5ms GC column (30 m × 0.25 mm, 0.25 µm film thickness) was used to obtain separation. (Agilent, Cheadle, UK). The temperature profile was as follows: start at 130 ºC and held for 1 min followed by an increase to 280 ºC at 20 ºC/min. This was then held constant for 11.50 min to give a total run time of 20.00 min.

Liquid chromatography electrospray mass spectrometry

LC-MS analyses were performed on an Agilent 1100 system. Separation was obtained on a Kinetex phenyl-hexyl column (2.6 µm, 100 x 2.10 mm) Phenomenex (Cheshire, United Kingdom). Mobile phase A consisted of 0.1% formic acid in water, whereas, mobile phase B consisted of 0.1% formic acid in acetonitrile. The Aligent LC-MSD settings were as follows: positive electrospray mode, capillary voltage 3500 V, drying gas (N_2) 12 L/min at 350 °C, nebulizer gas (N_2) pressure 50 psi, SIM *m/z* 296 and *m/z* 211, fragmentor voltage 50 V and 110 V. Samples for LC-MS analysis were dissolved in acetonitrile/water (1:1, containing 0.1% formic acid) at a concentration of 10 µg/mL. The injection volume was 1 µL, flow rate was 0.2 mL/min and the column temperature was 30 °C. The total run time was 35 min. The following gradient elution program was used: 0–2 min 15% B, followed by an increase to 20% within 20 min, returning to 15% within 35 min.

High-resolution electrospray ionization mass spectrometry

HR-ESI mass spectra were recorded by direct injection into a LTQ Orbitrap Discovery (Thermo Fisher, Loughborough, UK). Samples were dissolved in acetonitrile/water (1:1, containing 0.1% formic acid) and infused at a rate of 5 µL/min. Full accurate high-resolution (30000) mass scans were performed in positive electrospray mode. Measured accurate masses were within ± 5 ppm of the theoretical masses. The following conditions were used: drying gas (N_2) 10 L/min, capillary temperature 310 ºC, spray voltage 4 V, capillary voltage 22 V and tube lens 77 V. The mass calibration procedure was performed in both positive and negative mode using solutions containing caffeine, L-methionyl-arginyl-phenylalanyl-alanine acetate × H2O (MRFA), Ultramark 1621®, sodium docecyl sulfate and sodium taurocholate.

Thin layer chromatography

Analysis was conducted using TLC silica gel 60 F_{254} 20 x 20 cm aluminium sheets (Merck, Germany). The mobile phase used was dichloromethane/methanol (9:1) and 0.8% ammonia (7N in methanol). All standards and vendor samples were dissolved in the mobile phase and vortex mixed before spotting onto the TLC plate. Both UV light and modified Dragendorff Ludy-Tenger reagent was used for detection. For this purpose, bismuth subcarbonate (1 g), potassium iodide (6 g) and concentrated hydrochloric acid (15 mL) were diluted with water to give a final volume of 100 mL.

High-resolution matrix assisted inlet ionization mass spectrometry (MAII-MS)

A Thermo Scientific Exactive™ mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) was modified by removing the Ion Max source to expose the inlet capillary for sample introduction using glass slides. The trap fill time was set at 1000 ms to correspond with the 1 s required to achieve a resolution of 100000 (50% FWHH, *m/z* 200). The sheath, auxillary, and sweep gas flow rates, as well as the electrospray ionization spray voltage, were set to zero. The inlet capillary temperature was set at 70 °C. The capillary, tube lens and skimmer voltages were optimized at 30, 60, and 18 V, respectively, and acquisition time was set to continuous mode.

Tablet preparation for MAII-MS and NMR analysis

The 3-nitrobenzonitrile (3-NBN) matrix was prepared at a 3 mg/mL concentration with acetonitrile/water (1:1) with 0.1% formic acid. For matrix assisted inlet ionization experiments on cover glass, 100 ppb of a synthesized 2-MXP standard solution was prepared in conjuction with the 3-NBN matrix solution. Onto the cover glass, 1 µL of analyte/matrix solution was and allowed to air-dry for 2 minutes. For direct MAII-MS analysis, 1 µL of matrix solution was added onto the surface of the 2-MXP tablet and allowed to air-dry. In order to obtain structural confirmation, the tablet was split in half. One half of the tablet (137.6 mg) was extracted by dissolution in 2 N (3 mL) aqueous HCl solution, washed with ethyl acetate (3 x 5 mL), gravity filtered to remove insoluble components, made basic with concentrated KOH solution and extracted

with ethyl acetate (3 x 5 mL). Organic extractions were pooled, dried with $MgSO_4$ and concentrated under a stream of warm air to yield about 15 mg of colorless oil. The oil was dried and dissolved in 1 mL $CDCl_3$ for ^{1}H and ^{13}C NMR analysis.

Results and discussion

The appearance of new psychoactive substances on the market can cause difficulties in the ability to identify these materials. Furthermore, increasing challenges are encountered when facing the potential presence of various positional isomers. The three MXP isomers were synthesized using two alternative routes and the implementation of techniques commonly used in a forensic science laboratory revealed that differentiation between isomers was possible. It was also confirmed that 2-MXP was present in three powdered and one tablet sample obtained from online retailers in the United Kingdom.

The preparation of 2-, 3-, and 4-MXP isomers was compared using two synthetic routes. Procedure 1 (Figure 1B) was based on a three-component procedure published by Le Gall *et al.* who employed this approach for the preparation of diarylmethylamines.[18] The application of this one-step procedure provided a convenient route of synthesis of the desired isomers. An alternative synthesis procedure 2 (Figure 1C), however, was also explored for its applicability to the synthesis of 2-, 3-, and 4-MXP. In this particular case, the Grignard reagent was added to the Weinreb amide[19] to give the corresponding ketone intermediate. Conversion to the primary amine followed by reaction with 1,5-dibromopentane yielded the MXP isomers. The final step of the second procedure, i.e. not requiring the use of piperidine as a reagent, has also been successfully implemented for the preparation of diphenidine and PCP derivatives.[2,7,20] The purification process used in procedure 2 was rapid and thus deemed well suited for the purification step in a high throughput forensic science laboratory. It involved the direct conversion of the crude freebase to the hydrochloride salt. Addition of ethereal HCl and *tert*.-butyl methyl ether to the crude freebase and stirring overnight afforded the purified HCl salt following precipitation from the solution. All three isomers could be distinguished by ^{1}H and ^{13}C NMR (Table 1).

Analytical features

Three samples labeled to contain 2-MXP were obtained from three different Internet providers and analyzed by gas chromatography (GC) ion trap mass (IT) mass spectrometry (MS) in electron- (EI) and chemical (CI) ionization modes. A comparison with the synthesized reference material is shown in Figure 2 and confirmed that all test purchase samples were consistent with the identity of the 2-MXP isomer as indicated on the product label. The employed GC method did allow for the separation of all three isomers as well. Mass spectral data obtained from all three isomers are shown in Figure 3. EI-IT mass spectra (Figure 3A–C) displayed a comparatively weak abundance of the molecular ion while the corresponding CI-IT mass spectra (Figure 3D–F) confirmed the presence of the $[M + H]^+$ without difficulty. The structural suggestions for the key ions observed under these conditions are

shown in Figure 3 and the mechanisms of formation may be comparable to diphenidine. In this case (e.g. under EI conditions), a *m/z* 174 base peak is observed rather than *m/z* 204 due to the absence of the methoxyl group.[7]

The samples labeled to contain 2-MXP and the MXP standards (procedure 2) were also analyzed using an alternative GC-EI quadrupole MS method. The GC method used was able to distinguish between all three isomers and baseline separation was achieved between each isomer. The retention times were recorded at 19.15 min, 19.54 min and 19.86 min for 2-MXP, 3-MXP and 4-MXP isomers, respectively. The EI mass spectra obtained for each MXP isomer were similar as expected (supplemental data). Under EI quadrupole MS conditions, the abundance of the molecular ion was also low (supplemental data). All test purchases were found to be consistent with the identity of 2-MXP using this alternative method.

HPLC-MS was also utilized for the discrimination of the MXP isomers. Early attempts using an Allure® PFP Propyl column failed to resolve the isomers. However, switching to a phenyl hexyl column successfully permitted differentiation between isomers. The HPLC method achieved baseline separation for the 2-MXP isomer but the 3- and 4-MXP isomers provided partial separation. Although this appeared suitable for identification purposes, distinctive differences in the stability of the protonated molecules were also observed. For example, implementation of in-source collision-induced dissociation (110 V fragmentor voltage) under electrospray ionization single quadrupole MS conditions (Figure 4A) revealed that 4-MXP displayed the lowest stability, which resulted in the disappearance of $[M + H]^+$ via elimination of piperidine and formation of *m/z* 211. Inspection of the relative abundance values related to the product ions also indicated that the 3-MXP isomer appeared to yield the most stable protonated molecule. In comparison, 2-MXP was noticed to exhibit a lower stability than 3-MXP. Figure 4B depicts the suggested mechanisms of dissociation that may account for the differences in stability of the protonated molecules. Elimination of piperidine from 4-MXP may give rise to a resonance-stabilized cation that could have been the driving force behind the extensive dissociation of $[M + H]^+$. Resonance-stabilization following dissociation of 3-MXP was considered impossible, in addition to the possible formation of a less thermodynamically favored bicyclo[4.1.0]heptadienylidene oxonium species (Figure 4B). 2-MXP appeared to be less stable than 3-MXP because of the ability to form a resonance-stabilized product ion at *m/z* 211 while the possibility of an *ortho*-effect, i.e. hydrogen bond interaction with the piperidine hydrogen, might have resulted in higher stability than 4-MXP

This differential feature provided the opportunity to obtain further unambiguous information when switching the fragmentor voltage settings between 50 V and 110 V, respectively. The *m/z* 296 ($[M+H]^+$) and *m/z* 211 product ions were investigated using the selected ion-monitoring (SIM) mode. At the lower voltage of 50 V, the SIM chromatogram at *m/z* 296 revealed the detection of all three isomers (Figure 5A). However, 4-MXP was detected when the *m/z* 211 product ion was chosen while 3-MXP remained undetectable and 2-MXP showed low detectability (Figure 5B). At higher energies of 110 V, the SIM trace at *m/z* 296, revealed the detection of 2-MXP and 3-MXP isomers only (Figure 5C), which confirmed reduced stability of the 2-MXP isomer compared to its 3-MXP counterpart. Consistent with extensive dissociation, 4-

MXP was not visible in the SIM chromatogram of the protonated molecule. However, all three isomers were detected when the dissociated *m/z* 211 was used instead (Figure 5D). For comparison, electrospray ionization triple quadrupole tandem mass spectra and high mass accuracy quadrupole time-of-flight tandem mass spectra of all three isomers are shown as supplemental information. All three synthesized MXP isomers were also separable using thin layer chromatography (TLC) and confirmation was obtained that 2-MXP was present in the commercially available samples. The values obtained for the retardation factors of 2-, 3-, and 4-MXP were 0.57, 0.77, and 0.60, respectively (supplemental data). It was encouraging to observe the facile separation under TLC conditions, which illustrated the value of employing a seemingly simplistic method of analysis. This TLC method could be advantageous in situations where pressures on time and sampling conditions might place limitations on the ability to implement instrumental analysis.

Recent casework involving the detection of 2-MXP in three deaths revealed that the differentiation between the three MXP isomers could present challenges on HPLC retention time differences alone. It was reported that 2-MXP was separated from the 3-MXP and 4-MXP but the latter two were observed to co-elute under the conditions used. Implementation of the diode array detection system (DAD), however, allowed for the differentiation between 3-MXP and 4-MXP due to distinct ultraviolet fullscan spectra, which provided evidence that HPLC-DAD was considered suitable to confirm the capability for differentiation.[5]

The implementation of matrix assisted inlet ionization mass spectrometry (MAII-MS)[21-23] (Figure 6) established that it was possible to detect the protonated molecule of 2-MXP directly from the tablet surface (supplemental data) following addition of the 3-nitrobenzonitrile matrix. The MAI ionization process occurs in the heated inlet capillary of the instrument without the assistance of a laser, as, for example required for the matrix-assisted laser desorption ionization (MALDI) approach. NMR analysis of the extracted material then confirmed the presence of the 2-MXP isomer (supplemental data).

The appearance of diphenidine and 2-MXP as commercially available 'research chemicals' was considered a replacement for methoxetamine. In the UK, market introduction of both substances were closely linked to the introduction of legislative control of methoxetamine.[1] Closely related compounds have also been detected in 2008 where a drug seizure obtained from a clandestine laboratory operating in Germany revealed the presence of 1,2-diphenylethylamine and the two *N*-ethyl and *N*-isopropyl analogs. Mass spectral investigations and micro synthesis of several additional derivatives have been reported, which illustrated the importance to identify these newly emerging substances.[24] A comprehensive investigation of phase I and phase II metabolism using both *N*-alkylated substances in rats has been published recently.[25] The need for additional analytical investigations of products suspected to contain 1,2-diphenylethylamine derivatives might increase as more information about their prevalence of use and availability become accessible.

Conclusion

The appearance of newly emerging psychoactive 'research chemicals' and their recreational use outside controlled settings creates analytical challenges for scientists in the forensic, clinical and toxicology fields. The present study demonstrated the ability to differentiate between 2-methoxydiphenidine and its two positional isomers. The combination of test purchases from online vendors, analytical characterization and confirmation by organic synthesis was found to be a useful approach for the generation of analytical data that may be of interest to a range of stakeholders. The analysis of a 2-MXP tablet by matrix assisted inlet ionization Orbitrap mass spectrometry provided the indication that surface analysis from solid sample material may be a viable option for forensic analysis, which warrants further investigation.

Acknowledgements

The authors are thankful for the support received from Dr. Nicola M. Dempster (LJMU), Dr. Adeboye Adejare (USC) and Dr. Charles McEwen (USC). A grateful appreciation is also extended to the anonymous reviewers who provided very helpful comments.

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Figure captions:

Figure 1. A: Chemical structures of 1,2-diphenylethylamine representatives. 2-Methoxydiphenidine, diphenidine and MT-45 been encountered as 'research chemicals'. B and C: Synthesis of three MXP isomers via two different routes.

Figure 2. Implementation of gas chromatography ion trap mass spectrometry for the analysis of three powdered samples obtained from three UK-based vendors.

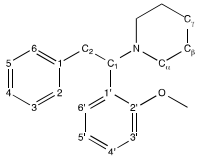
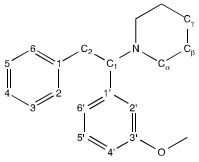
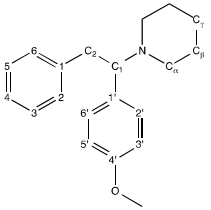
Figure 3. Electron (EI) and chemical (CI) ionization ion trap mass spectra obtained from introduction by gas chromatography.

Figure 4. A: Electrospray ionization single quadrupole mass spectrum following in-source collision-induced dissociation at 110 V. B: Suggested mechanisms of dissociation that may account for the differences in stability of the protonated molecule (3-MXP > 2-MXP > 4-MXP).

Figure 5. Analysis of synthesized MXP isomers using high performance liquid chromatography selected ion monitoring (SIM) mass spectrometry with varying fragmentor voltages.

Figure 6. Top left: Tablet obtained from an UK-based vendor and sold as 2-MXP. Direct analysis was carried out by matrix assisted inlet ionization Orbitrap mass spectrometry using 3-nitrobenzonitrile (3-NBN) as the matrix. Top right: mixed matrix and 2-MXP reference droplets after air-dry on glass plate. Bottom left: MAII operating with samples on cover glass. Bottom right: Direct analysis of 2-MXP tablet and confirmation of the protonated molecule by high resolution Orbitrap MS.

Table 1. NMR data for the MXP standards freebase (synthesis procedure 1) and hydrochloride salts (synthesis procedure 2)

2-Methoxydiphenidine				3-Methoxydiphenidine				4-Methoxydiphenidine			
											
Freebase		HCl Salt		Freebase		HCl Salt		Freebase		HCl Salt	
^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
157.99 (Ar-C; 2')	7.34 (dd; *J* = 7.6 Hz, 1.8 Hz; 1 Ar-H; 6')	157.98 (Ar-C; 2')	7.63 (m; 1 Ar-H; 6')	159.13 (Ar-C; 3')	7.09–7.20 (m; 4 Ar-H; 3, 4, 5, 5')	159.53 (Ar-C; 3')	7.28 (t; *J* = 7.9 Hz; 1 Ar-H, 4)	158.37 (Ar-C; 4')	7.19–7.11(m; 3 Ar-H, 3, 4, 5)	160.24 (Ar-C; 4')	7.47 (d; *J* = 8.7 Hz; 2 Ar-H; 2', 6')
140.23 (Ar-C; 1)	7.18–7.08 (m; 3 Ar-H; 3, 5, 4')	136.28 (Ar-C; 1)	7.36 (dt; *J* = 7.4, 2.1 Hz; 1 Ar-H; 4')	141.19 (Ar-C; 1')	7.04 (dd; *J* = 8.1, 1.3 Hz; 2 Ar-H; 2, 6)	136.77 (Ar-C; 1')	7.15–7.20 (m; 3 Ar-H; 2, 6, 2')	140.06 (Ar-C; 1)	7.08 (d; *J* = 8.6 Hz; 2 Ar-H; 2', 6')	136.82 (Ar-C; 1)	7.16-7.19 (m; 2 Ar-H; 3, 5)
129.35 (Ar-C; 2,6)	7.08–7.01 (m; 3 Ar-H; 2, 6, 4)	130.98 (Ar-C; 4')	7.15 (tr; *J* = 7.4 Hz; 2 Ar-H; 3, 5)	139.89 (Ar-C; 1)	6.74–6.79 (m; 2 Ar-H; 4', 6')	133.36 (Ar C; 1)	7.06–7.12 (m; 4 Ar-H; 3, 5, 4', 5')	131.17 (Ar-C; 1')	7.02 (dd; *J* = 7.6, 1.1 Hz; 2 Ar-H; 2, 6)	132.35 (Ar-C; 4)	7.07–7.13 (m; 3 Ar-H, 2, 4, 6)
128.80 (Ar-C; 6')	6.92 (dt; *J* = 7.5 Hz; 1.2 Hz; 1 Ar-H; 5')	130.47 (Ar-C; 6')	7.09 (tr; *J* = 7.4 Hz; 1 Ar-H; 4)	129.37 (Ar-C; 2, 6)	6.72 (tr; *J* = 1.6 Hz; 1 Ar-H; 2')	129.98 (Ar-C; 5')	6.93 (dd; *J* = 8.2, 2.1 Hz; 1 Ar-H; 6')	129.95 (Ar-C; 2', 6')	6.81 (d; *J* = 8.6 Hz; 2 Ar-H; 3', 5')	129.37 (Ar-C; 2', 6')	6.94 (d; *J* = 8.7 Hz; 2 Ar-H; 3', 5')
128.50 (Ar-C;1')	6.74 (dd; *J* = 8.3, 1.2 Hz; 1 Ar-H; 3')	128.91 (Ar-C; 2,6)	6.97-7.05 (m; 4 Ar-H; 2, 6, 3', 5')	128.47 (Ar-C; 5')	3.78 (s; 3 H; OCH_3)	129.41 (Ar-C; 2, 6)	4.65–4.69 (m; 1 H; C_1)	129.37 (Ar-C; 2, 6)	3.80 (s; 3 H; OCH_3)	128.57(Ar-C; 2, 6)	4.68–4.72 (m; 1 H; C_1)
127.58 (Ar-C; 3, 5)	4.31 (dd; *J* = 9.2, 5.7 Hz; 1 H; C_1)	128.44 (Ar-C; 3,5)	4.91-4.96 (m; 1 H; C_1)	127.79 (Ar-C; 3, 5)	3.59 (dd; *J* = 9.2, 5.3 Hz; 1 H; C_1)	128.53 (Ar-C; 3, 5)	3.75 (s; 3 H, OCH_3)	127.86 (Ar-C; 3, 5)	3.59 (dd; *J* = 10.0, 5.9 Hz; 1 H; C_1)	126.72 (Ar-C; 3, 5)	3.75 (s; 3 H, OCH_3)
127.47 (Ar-C; 4')	3.56 (s; 3 H; OCH_3)	126.44 (Ar-C; 5')	3.71 (s; 3 H; OCH_3)	125.69 (Ar-C; 4)	3.31 (dd; *J* = 13.3, 5.3 Hz; 1 H; C_2)	126.76 (Ar-C; 4)	3.72 (dd; *J* = 13.2, 3.7 Hz; 1 H; C_2)	125.61 (Ar-C; 4)	3.32 (dd; *J* = 13.0, 5.9 Hz; 1 H; C_2)	122.92 (Ar-C, 1')	3.60–3.67 and 3.51-3.56 (2 x m; 2 H, C_2)
125.39 (Ar-C; 4)	3.26 (dd; *J* = 13.4, 5.7 Hz; 1 H; C_2)	120.54 (Ar-C; 4)	3.66 (dd; *J* = 13.2; 3.3 Hz; 1 H; C_2)	121.49 (Ar-C; 6')	3.03 (dd; *J* = 13.3, 9.2 Hz; 1 H; C_2)	122.97 (Ar-C; 6')	3.51 (dd; *J* = 13.2, 12.2 Hz; 1 H; C_2)	113.00 (Ar-C; 3', 5')	3.02 (dd; *J* = 13.0, 10.0 Hz; 1 H; C_2)	114.28 (Ar-C; 3', 5')	3.68-3.73, 3.44-3.49 and 2.57-2.65 (3 x m; 4 H; C_α)
119.94 (Ar-C; 5')	2.94 (dd; *J* = 13.4, 9.2 Hz; 1 H; C_2)	119.26 (Ar-C; 1')	3.48 (dd; *J* = 13.2, 10.7 Hz; 1 H; C_2)	114.75 (Ar-C; 2')	2.39–2.53 (m; 4 H; C_α)	116.61 (Ar-C; 2')	3.74–3.79, 3.36-3.42 and 2.61–2.71 (3 x m; 4 H; C_α)	71.64 (CH; C_1)	2.34–2.55 (m; 4 H; C_α)	69.69 (CH; C_1)	1.64–1.92 (m; 4 H; C_β)
110.86 (Ar-C; 3')	2.45 (t; *J* = 5.3 Hz; 4 H; C_α)	111.90 (Ar-C; 3')	3.68–3.71, 3.36–3.42, 2.74–2.83 and 2.63–2.71 (4 x m; 4 H; C_α)	112.04 (Ar-C; 4')	1.50–1.71 (m; 4 H; C_β)	115.02 (Ar-C; 4')	1.93–2.02 and 1.77–1.86 (2 x m, 3 H; C_β)	54.64 (CH_3; C_c)	1.50–1.71(m; 4 H; C_β)	55.44 (CH_3; C_c)	1.48–1.54 and 1.24-1.34 (m; 2 H; C_γ)
62.48 (CH; C_1)	1.63–1.46 (m; 4 H, C_β)	59.13 (CH; C_1)	1.91–1.99, 1.73–1.85 and 1.64–1.69 (3 x m; 3 H; C_β)	72.26 (CH; C_1)	1.40 (quint; *J* = 5.7 Hz; 2 H; C_γ)	70.13 (CH; C_1)	1.64–1.70 and 1.27–1.35 (m; 2 H; C_γ)	51.28 (2xCH_2; C_α)	1.38 (quint; *J* = 5.6 Hz; 2 H; C_γ)	52.01 and 48.46 (2 x CH_2; C_α)	
55.49 (CH_3; C_c)	1.35 (quint; *J* = 5.8 Hz; 2 H; C_γ)	55.78 (CH_3; C_c)	1.29–1.38 (m; 2 H; C_γ)	55.14 (CH_3; C_c)		55.54 (CH_3; C_c)		39.21 (CH_2; C_2)		35.10 (CH_2; C_2)	
51.33 (2 x CH_2; C_α)		51.89 and 48.81 (2xCH_2; C_α)		51.44 (2xCH_2; C_α)		52.15 and 49.07 (2xCH_2; C_α)		26.30 (2xCH_2; C_β)		22.95 (2 x CH_2; C_β)	
39.24 (CH_2; C_2)		34.52 (CH_2; C_2)		39.19 (CH_2; C_2)		35.29 (CH_2; C_2)		24.62 (CH_2; C_γ)		21.74 (CH_2; C_γ)	
26.46 (2 x CH_2; C_β)		22.52 and 22.48 (2xCH_2; C_β)		26.36 (2xCH_2; C_β)		22.78 (2xCH_2; C_β)					
24.72 (CH_2; C_γ)		21.28 (CH_2; C_γ)		24.64 (CH_2; C_γ)		21.83 (CH_2; C_γ)					

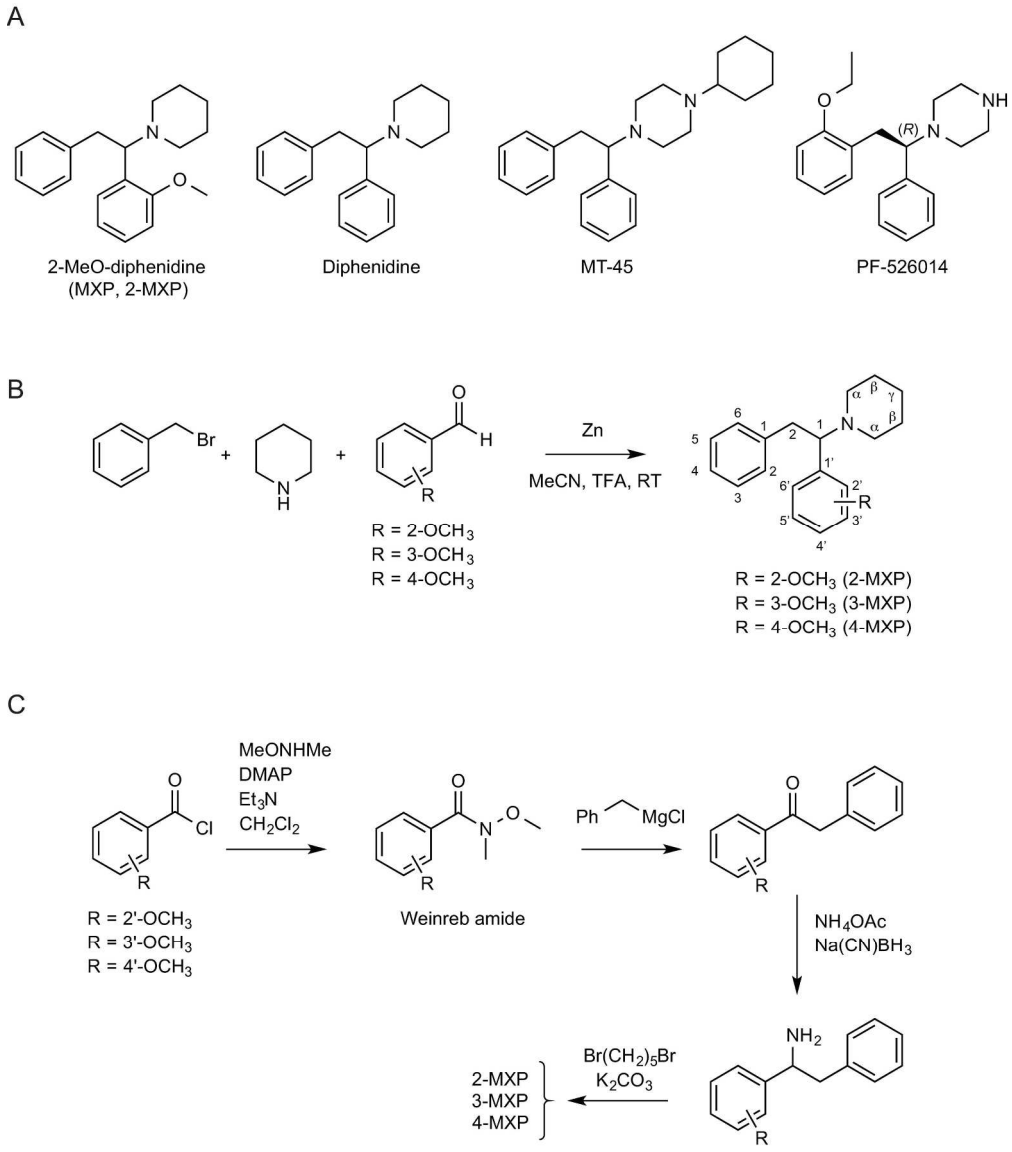


Figure 1. A: Chemical structures of 1,2-diphenylethylamine representatives. 2-Methoxydiphenidine, diphenidine and MT-45 been encountered as 'research chemicals'. B and C: Synthesis of three MXP isomers via two different routes.
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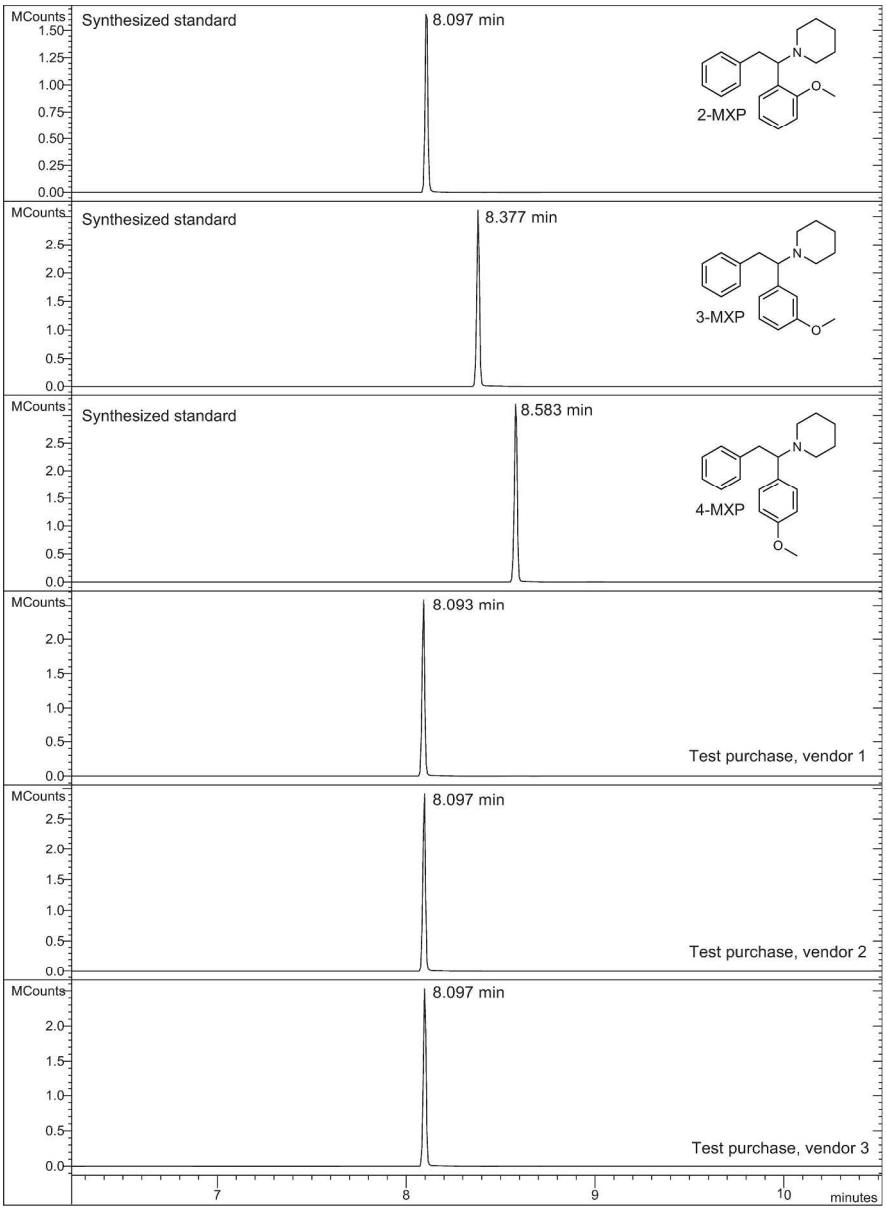

Figure 2. Implementation of gas chromatography ion trap mass spectrometry for the analysis of three powdered samples obtained from three UK-based vendors.
262x358mm (300 x 300 DPI)

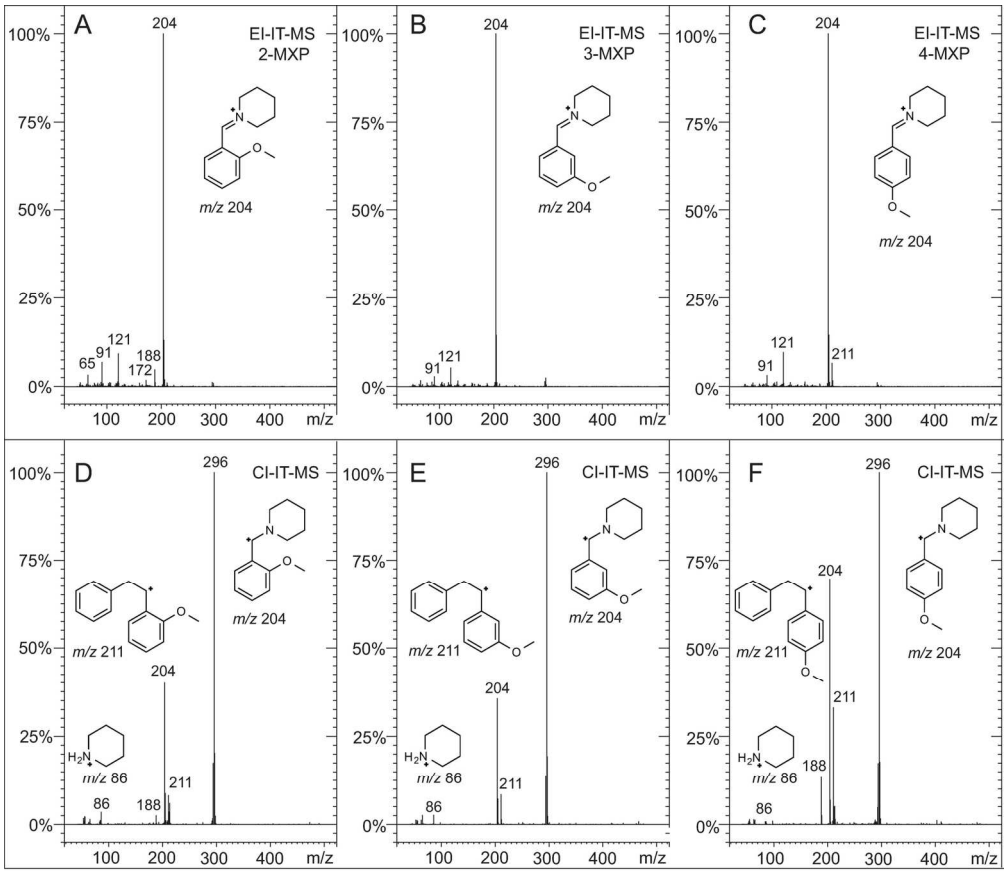

Figure 3. Electron (EI) and chemical (CI) ionization ion trap mass spectra obtained from introduction by gas chromatography.
163x141mm (300 x 300 DPI)

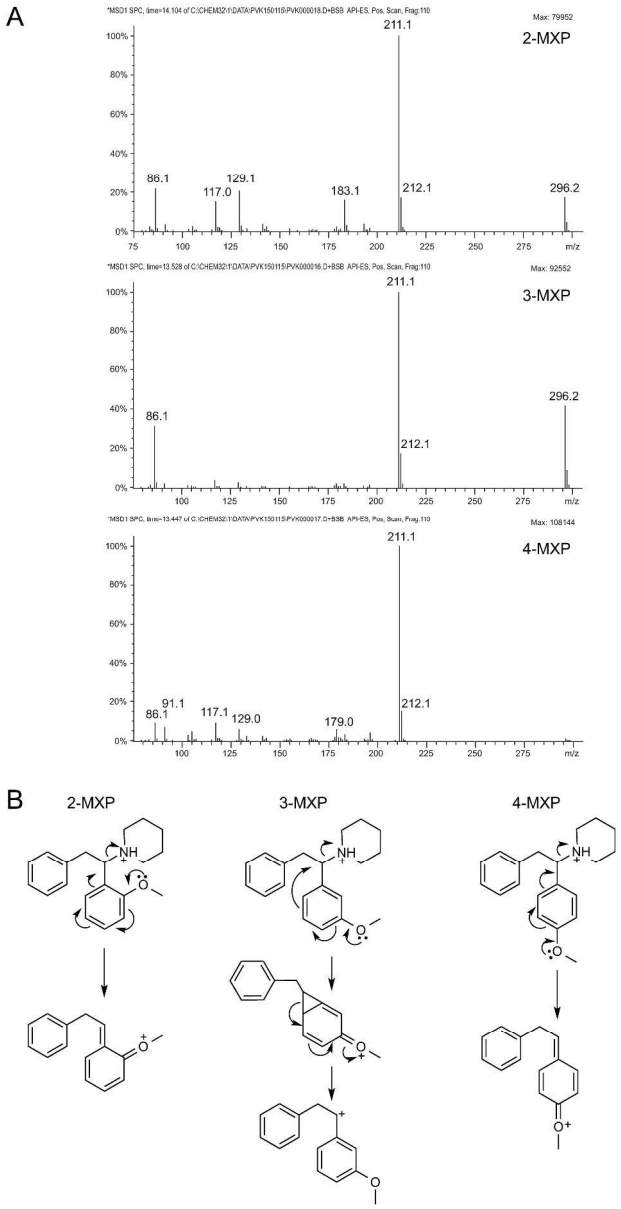

Figure 4. A: Electrospray ionization single quadrupole mass spectrum following in-source collision-induced dissociation at 110 V. B: Suggested mechanisms of dissociation that may account for the differences in stability of the protonated molecule (3-MXP > 2-MXP > 4-MXP).
291x574mm (300 x 300 DPI)

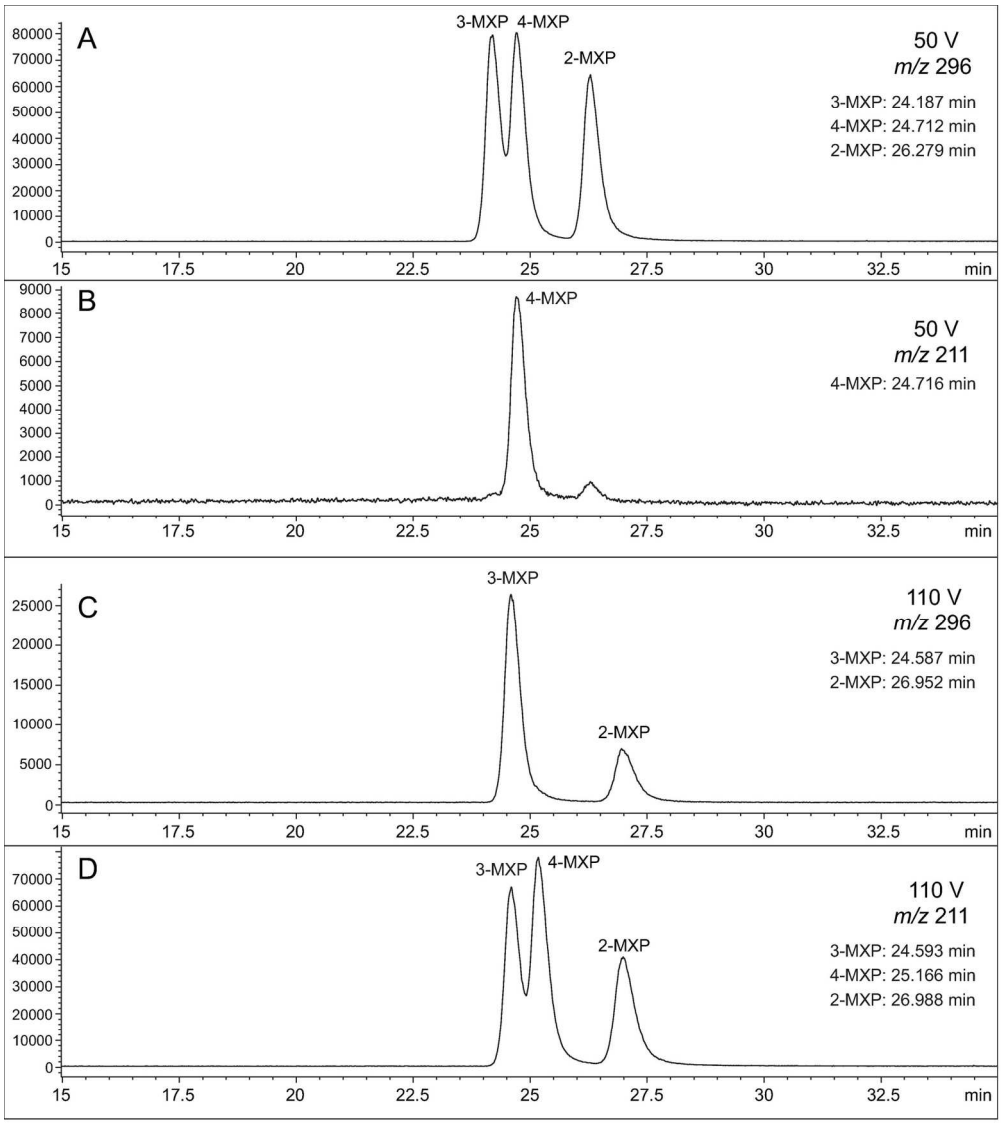

Figure 5. Analysis of synthesized MXP isomers using high performance liquid chromatography selected ion monitoring (SIM) mass spectrometry with varying fragmentor voltages.
163x183mm (300 x 300 DPI)

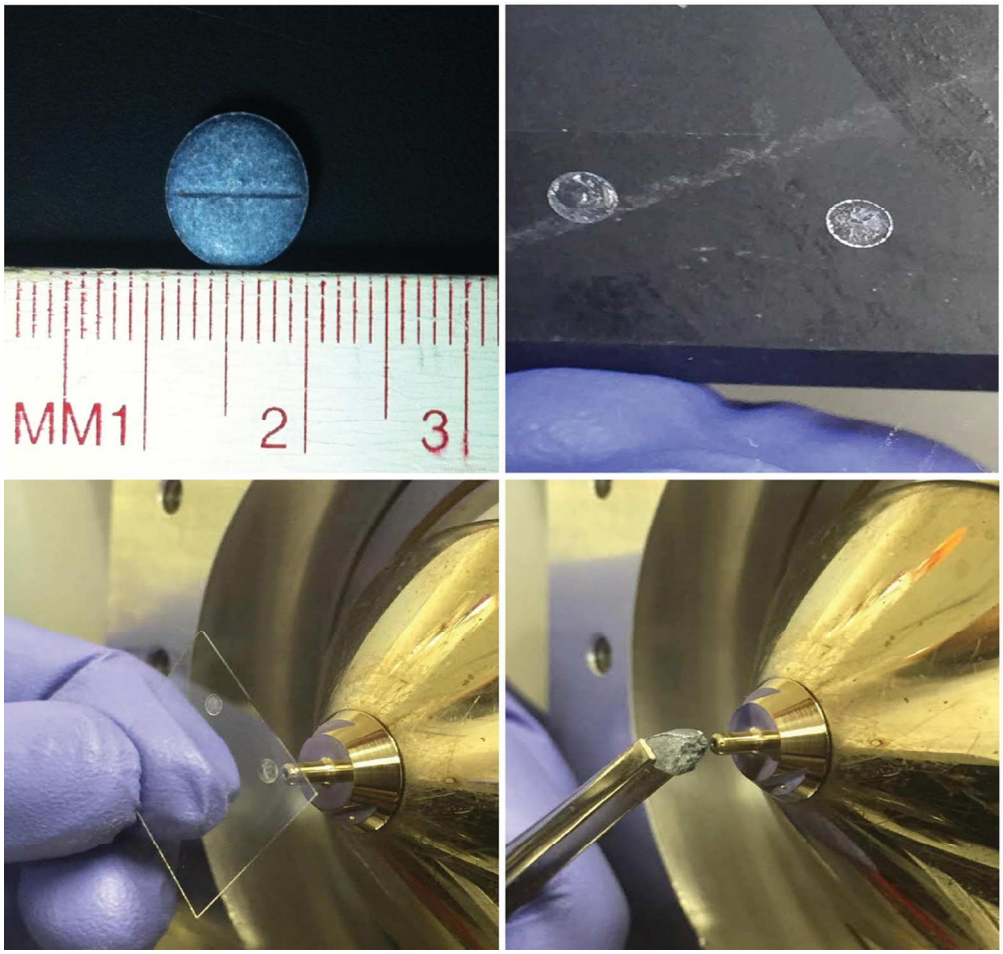

Figure 6. Top left: Tablet obtained from an UK-based vendor and sold as 2-MXP. Direct analysis was carried out by matrix assisted inlet ionization Orbitrap mass spectrometry using 3-nitrobenzonitrile (3-NBN) as the matrix. Top right: mixed matrix and 2-MXP reference droplets after air-dry on glass plate. Bottom left: MAII operating with samples on cover glass. Bottom right: Direct analysis of 2-MXP tablet and confirmation of the protonated molecule by high resolution Orbitrap MS.
106x101mm (300 x 300 DPI)