

First Reported Fatalities Associated with the ‘Research Chemical’ 2-Methoxydiphenidine

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2-Methoxydiphenidine, i.e. 1-[1-(2-methoxyphenyl)-2-phenylethyl]piperidine, also known as ‘MXP’ or ‘2-MeO-diphenidine’ (or 2-MXP), has been available as a ‘research chemical’ since 2013 as a purported alternative to the ‘dissociative anesthetics’ methoxetamine and ketamine. Three deaths which involved the detection of 2-MXP in post-mortem blood and urine were encountered in forensic casework. The 2-, 3- and 4-methoxyphenyl positional isomers were synthesized to confirm the identity and concentration of 2-MXP. The 2-MXP femoral blood concentrations in the cases were found to be 24.0, 2.0 and 1.36 mg/L (the latter with an alternative cause of death). Some additional prescription drugs were encountered at therapeutic concentrations in all three cases. Analysis of the biofluids allowed the detection and characterization of various metabolites, including the suggested presence of hydroxy-2-MXP as the main metabolite with the hydroxyl group located on the piperidine rather than the phenyl or benzyl moiety. Additional metabolites included *O*-desmethyl-2-MXP and hydroxylated *O*-desmethyl-2-MXP. Diphenidine and hydroxy-diphenidine, also showing the presence of the hydroxyl group on the piperidine ring, were also detected. It was not possible to identify whether these arose from 2-MXP biotransformation or whether they represented the presence of diphenidine as a separate substance. These are the first published fatalities involving 2-MXP and presents analytical data to assist analytical toxicologists with future casework.

Introduction

The use of ‘dissociative anesthetics’ for non-medical purposes has been observed since the development of the prototypical 1-(1-phenylcyclohexyl)piperidine (PCP) and 2-(2-chlorophenyl)-2-(methylamino)cyclohexanone (ketamine). An important feature linked with some of the psychoactive properties of these agents includes uncompetitive antagonism at the *N*-methyl-D-aspartate (NMDA) receptor (1–3). A number of arylcyclohexylamines and aryl-amino-cyclohexan-2-ones are some of the more commonly encountered structural templates associated with dissociative ‘research chemicals’ (4, 5).

A more recent development in the field of dissociative ‘research chemicals’ involves the availability of substances that display the 1,2-diarylethylamine backbone. Two current examples are 1-(1,2-diphenylethyl)piperidine (diphenidine) and 1-[1-(2-methoxyphenyl)-2-phenylethyl]piperidine (2-MeO-diphenidine, methoxphenidine, MXP, 2-MXP) (Figure 1). From the perspective of the UK, it appears that their appearance dates back to 2013 following the control of 2-(ethylamino)-2-(3-methoxyphenyl)cyclohexanone (methoxetamine, MXE), which attracted attention in

the ‘research chemicals’ community (4) but also in clinical and forensic situations due to the observation of adverse effects (6, 7). While analytical data pertaining to diphenidine and related analogs and isomers have recently been published (8–10), data for methoxy derivatives (especially in biological fluids) are currently less abundant. A recently published case report described the qualitative detection of 2-MXP in plasma and urine following acute intoxication (11).

Detailed data on the pharmacodynamics of 2-MXP are not yet available but it has been previously synthesized as part of a series of 1,2-diarylethylamines that were investigated for their potential neuroprotective properties. Racemic 2-MXP, when compared against PCP and 1-[1-(thiophen-2-yl)cyclohexyl]piperidine (TCP), was reported to bind to crude membrane preparations obtained from whole rat brains (1 nM [³H]-TCP as radioligand), which implicated involvement of the NMDA receptor ($K_i = 170$ nM for 2-MXP, 96 nM for PCP, 20 nM for TCP and 39 nM for racemic diphenidine, respectively) (12). In 2008, German authorities seized two 1,2-diphenylethylamines that were subsequently identified as the monoalkylated *N*-ethyl (NE-DPEA) and *N*-isopropyl (NIP-DPEA) analogs of lefetamine (Figure 1) (13) and recent investigations of their detection in rat urine have also been reported (14). The 1,2-diarylethylamine class and their associated pharmacological properties appear to be diverse in nature and difficult to predict based on structural features alone (15–19). With regard to the dissociative effects of diphenidine and 2-MXP, anecdotal reports suggest that 2-MXP may be more potent than diphenidine but further research is necessary.

The presence of the methoxy group on the phenyl ring gives rise to three methoxyphenyl positional isomers (2-MXP, 3-MXP and 4-MXP). It is important in forensic chemistry in particular to be able to distinguish between these isomers given that they might display different pharmacological properties (12). This article presents analytical data pertaining to these isomers and was used to identify the form found in three fatalities involving 2-MXP with subsequent quantification for the first time.

Case histories

Case 1

A 34-year-old male was found dead at home. There was evidence of drug paraphernalia including white powder in clear bags that was later identified to be ‘methoxphenidine’ (isomer not distinguished) by the police drug laboratory. The drug was found to have been purchased over the Internet from a ‘research chemical’ company. At autopsy, he was found to have an enlarged heart and hypertensive heart disease with no other contributory findings.

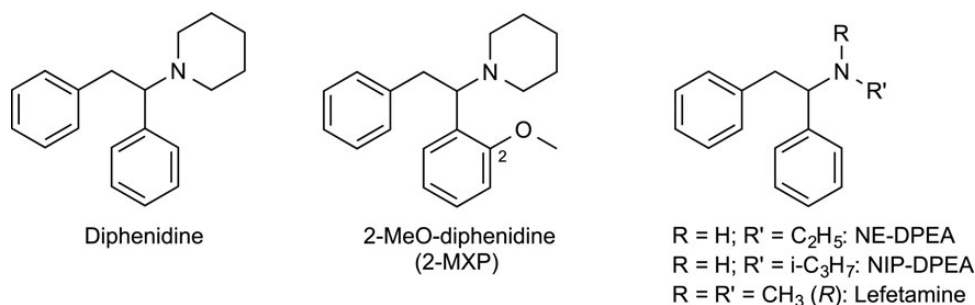


Figure 1. Chemical structures of diphenidine, 2-MXP and closely related derivatives of forensic interest.

Case 2

A 34-year-old male was found dead at home. He had a medical history of epilepsy, attention deficit hyperactivity disorder and social anxiety. He had been prescribed levetiracetam, dexamphetamine and diazepam. A sachet labeled 'methoxphenidine 2g' was found in his pocket. At autopsy, he was found to have a moderately enlarged heart and mild atheroma with no other contributory findings.

Case 3

A 38-year-old male was found dead on a road having jumped or fallen from a road bridge suffering fatal injuries. He had a medical history of schizophrenia. No other information was available.

Experimental

Instrumentation

Analysis by high-performance liquid chromatography diode array detection (HPLC-DAD) was carried out on a Dionex 3000 Ultimate liquid chromatography system coupled to a UV DAD (Thermo Fisher, St Albans, UK), using a Phenomenex Synergi Fusion column (150 mm × 2 mm, 4 μm) protected by a 4 mm × 3 mm Phenomenex Synergi Fusion guard column (Phenomenex, Cheshire, UK). The mobile phase consisted of 25 mM triethylammonium phosphate (TEAP) buffer and acetonitrile and the column temperature was maintained at 30°C.

An ABSciex 3200 QTRAP mass spectrometer coupled to an Agilent 1200 HPLC-DAD system (ABSciex, Cheshire, UK) was used for LC-MS-MS analysis. Chromatographic separation was based on a Phenomenex Gemini column (150 mm × 2 mm, 5 μm) protected by a Phenomenex Synergi Gemini 4 mm × 3 mm guard column (Phenomenex). A mobile phase of 1 mM ammonium formate with 1% formic acid and acetonitrile (column temperature 30°C) was used.

The ultra-high performance liquid chromatography (UHPLC)-high-resolution quadrupole time of flight mass spectrometry (QTOF-MS) system was the Agilent 6540 UHD Accurate-Mass QTOF LC-MS coupled to an Agilent 1290 Infinity UHPLC system (Agilent, Cheshire, UK). Chromatographic separation was based on an Agilent Zorbax Eclipse Plus C18 column (100 mm × 2.1 mm, 1.8 μm) (Agilent, Cheshire, UK) using mobile phase of 1% formic acid and acetonitrile (column temperature 40°C). The methodology and parameters have been published previously (20).

Reagents and standards

All solvents and chemicals used, e.g., acetonitrile, 1-chlorobutane, methanol, sodium carbonate, sulfuric acid, formic acid, TEAP

buffer and ammonium formate, were of analytical grade or equivalent from Sigma Aldrich (Dorset, UK) and/or Rathburn Chemicals Ltd (Walkerburn, Scotland, UK). Due to the unavailability of certified reference materials, all three positional isomers, i.e., 2-, 3- and 4-MXP hydrochloride were synthesized utilizing a method published by Le Gall *et al.* (21) followed by full characterization including nuclear magnetic resonance (NMR) spectroscopy. These were used to prepare fresh reference and calibration standards for the formal identification and quantitation in the specimens analyzed. Following determination of limit of detection (LOD) and limit of quantitation (LOQ) using an extended calibration range (from 0.025 and 0.078 mg/L, respectively), a calibration range of 0.3125, 0.625, 1.25, 2.5 and 5 mg/L was produced for 2-MXP using blank equine plasma. Internal quality control standards of 0.5 and 2.5 mg/L were also produced. Intra-day and inter-day precision and accuracy were determined. Where appropriate, post-mortem blood case samples were diluted in equine plasma (e.g., 3-, 5- or 10-fold) for matrix matching and to be within the linear calibration range.

Extraction and analysis

Basic back extraction using sodium carbonate buffer (with internal standards) and 1-chlorobutane solvent extraction of the calibration and case samples were performed as described elsewhere (20). The chromatographic conditions for qualitative HPLC-DAD, Analysis by high-performance liquid chromatography mass spectrometry (HPLC-MS) and UHPLC-QTOF-MS analyses were based on previously published methods involving an acetonitrile gradient (20). Quantitative HPLC-DAD analysis was based on 30% acetonitrile (with 25 mM TEAP buffer) under isocratic elution conditions at a flow rate of 2 mL/min. 2-MXP eluted at 4.0 min.

Results and discussion

Analysis of the 2-, 3- and 4-methoxydiphenidine isomers showed that the positional isomer could be differentiated by HPLC-DAD retention time and UV spectrum which was consistent with the ability to differentiate between substituted piperazines, such as trifluoromethylphenylpiperazine (TFMPP) and chlorophenylpiperazine (CPP) (22). Specifically, 2-MXP eluted at 8.44 min with a 278 nm UV max, 3-MXP eluted at 8.06 min with a 276 nm UV max and 4-MXP eluted at 8.08 min with UV maxima at 229 and 272 nm (Figure 2). Based on these analytical data as well as LC-MS and QTOF-MS comparison with the synthesized standards, only the 2-MXP isomer was detected in the case

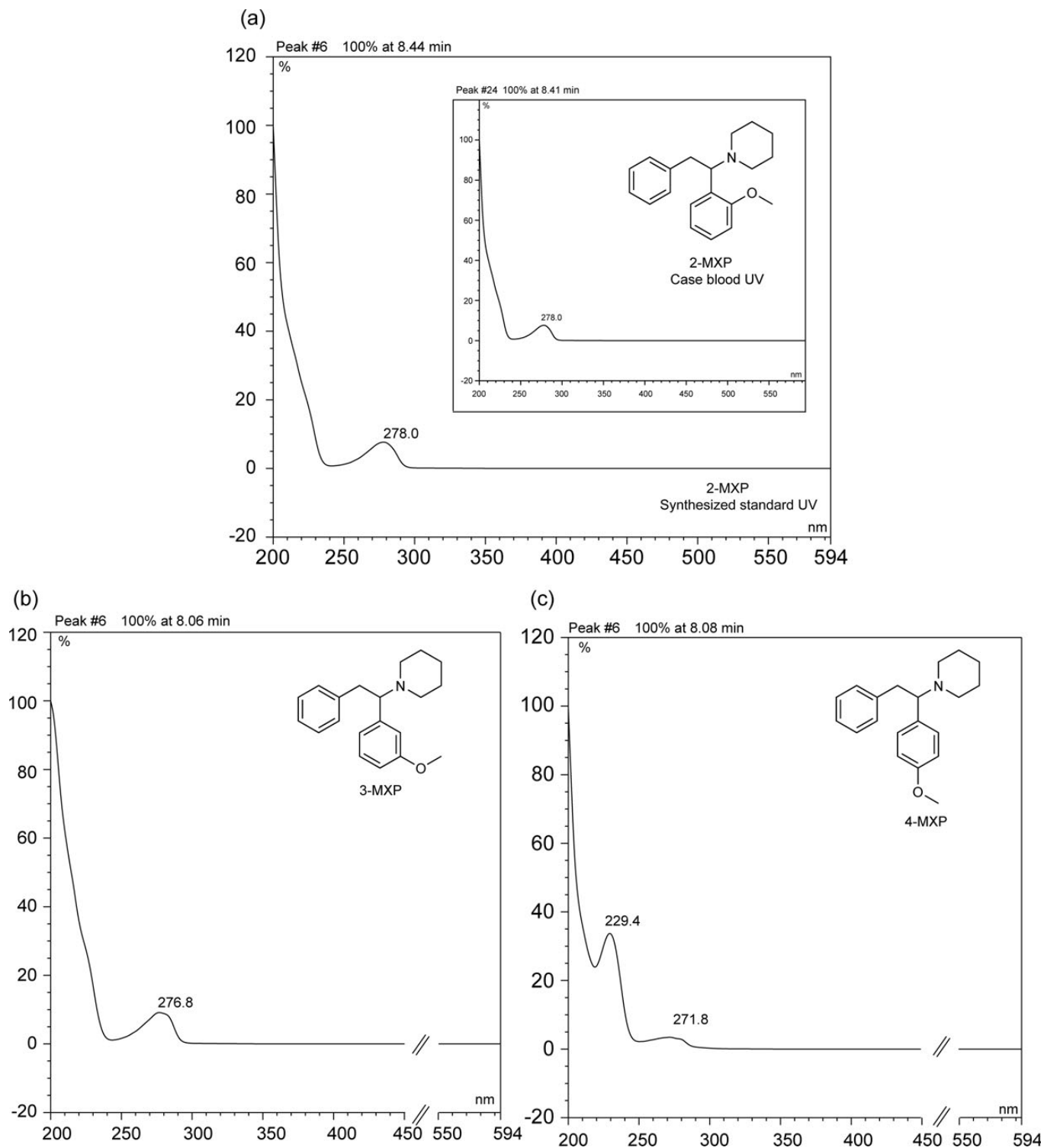


Figure 2. DAD data and HPLC retention times for three synthesized positional isomers of methoxydiphenidine. The differentiation was feasible based on the combination of UV full scan and retention time information. (a) UV spectrum obtained from 2-MXP standard and casework. (b) UV full scan trace of 3-MXP. (c) UV full scan trace of 4-MXP.

samples. Figure 3 shows that with LC-MS numerous product ions were observed to assist with identification and selection of appropriate ion transitions for targeted screening analysis. For quantitative analysis by HPLC-DAD, validation of the method

showed accuracy and precision values of <6%, a LOD of 0.05 mg/L and a LOQ of 0.10 mg/L (Table 1).

For case 1, 2-MXP was found to be present at 24.0 mg/L in post-mortem femoral blood and was also detected in urine.

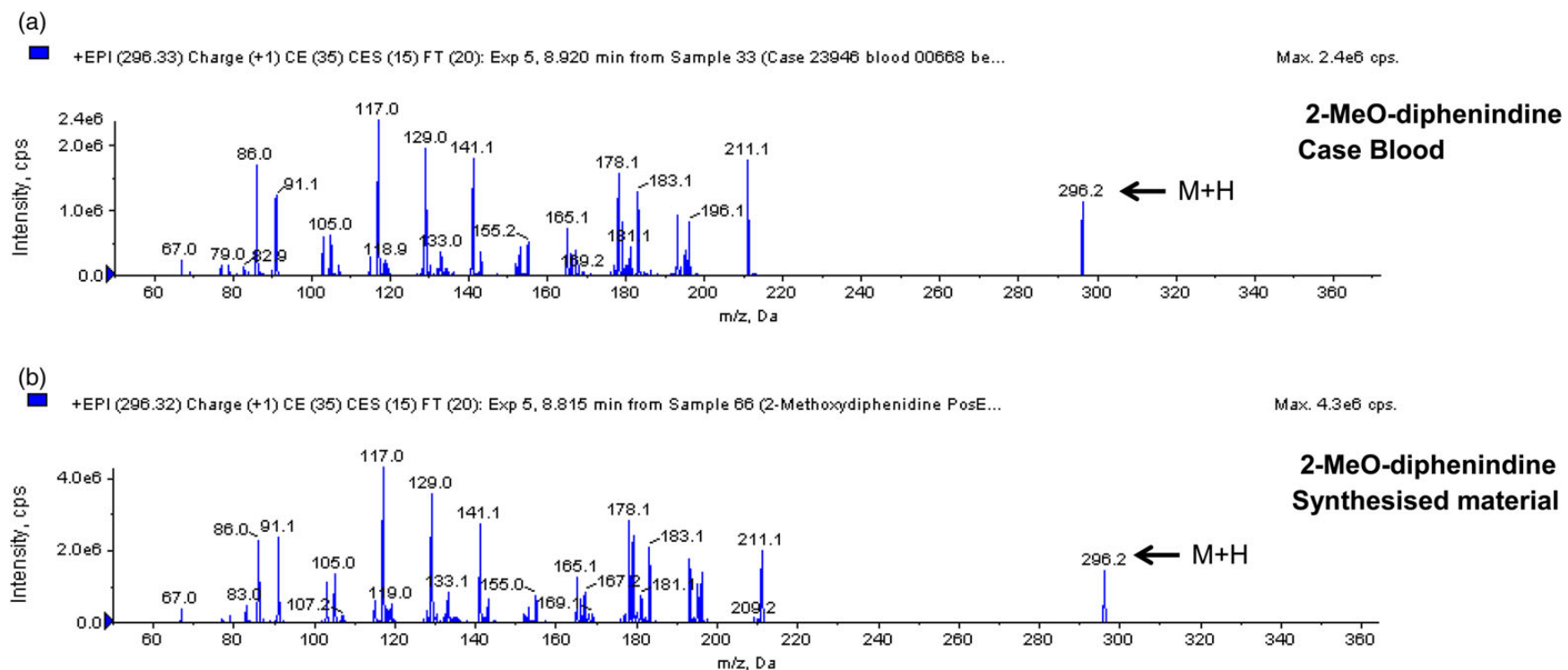


Figure 3. Enhanced product ion (EPI) scan of 2-MXP (case vs. reference material) at collision energy (CE) spread (35 ± 15 V) using electrospray LC-MS.

Table I
Validation Data for Quantitation of 2-MXP

	Mean concentration, mg/L (\pm SD)	Precision (%)	Accuracy (%)
Intra-day ($n = 10$)			
0.1 mg/L	0.989 \pm 0.015	1.5	-1.1
Inter-day ($n = 6$)			
0.5 mg/L	0.52 \pm 0.01	1.2	4.6
2.5 mg/L	2.64 \pm 0.02	0.9	5.5
LOD	0.05 mg/L		
LOQ	0.10 mg/L	1.5	-1.1

Table II
2-MXP and Proposed Metabolites Found in Forensic Casework Samples Using UHPLC-QTOF-MS

Compound	Molecular formula	Theoretical mass [M + H] ⁺	<i>m/z</i> found
2-MXP	C ₂₀ H ₂₃ NO ⁺	296.20089	296.20088
Hydroxy-2-MXP	C ₂₀ H ₂₃ NO ₂ ⁺	312.19581	312.19556
<i>O</i> -Desmethyl-2-MXP	C ₁₉ H ₂₃ NO ⁺	282.18524	282.18514
Hydroxy- <i>O</i> -desmethyl-MXP	C ₁₉ H ₂₃ NO ₂ ⁺	298.18016	298.18007
Diphenidine	C ₁₉ H ₂₃ N ⁺	266.19033	266.19076
Hydroxy-diphenidine	C ₁₉ H ₂₄ NO ⁺	282.18524	282.18521

Prescription drugs (mirtazapine, lamotrigine and citalopram) were also found at therapeutic concentrations and no ethanol was detected. The cause of death was given as 'methoxyphenidine use and hypertensive heart disease'. For case 2, 2-MXP was found to be present at 2.0 mg/L in post-mortem femoral blood and was also detected in urine. Prescription drugs (diazepam and quinine) were found at therapeutic concentrations and no ethanol was detected. Levetiracetam or dexamphetamine were not detected. The cause of death was given as 'probable methoxyphenidine toxicity' due to the absence of any other pathological findings. For case 3, 2-MXP was found to be present at 1.36 mg/L in post-mortem femoral blood and was also detected in urine. The prescription antipsychotic drug risperidone was present at a therapeutic concentration and no ethanol was detected. The cause of death was due to multiple injuries following the fall and the inquest conclusion was 'suicide whilst suffering from a mental illness'. Based on only three cases it is not possible to determine what concentrations may constitute excessive use, recreational or otherwise. The variation in concentrations fundamentally depends on a range of factors, such as routes of administration and dosage used but also the extent to which individuals have been aware of the drug actually being taken (as evidenced in two of these cases). Nevertheless, users may still not be aware of what dose would likely result in desired or toxic effects. Therefore, the concentrations reported here are presented for future case reference only.

Apart from a number of anecdotal reports (4), detailed information about the pharmacodynamic features of 2-MXP is currently not available. It is tempting to consider the potential involvement of the NMDA receptor when attempting to make comparisons with what has been described for diphenidine, MXE and ketamine (1, 4, 9, 19, 23). Such effects may include drowsiness, dissociative and catatonic states, hallucinations, confusion and cardiovascular effects including hypertension and tachycardia. These potential effects were important in the circumstances of the three cases described here and were

considered in determining the manner of death, irrespective of the concentration measured. However, further research into the pharmacological properties of these diarylethylamines seems warranted in order to elucidate additional mechanisms of actions that may be involved.

During the analysis of the case samples, apparent metabolites of 2-MXP were also detected. Initially, this was observed by HPLC-DAD analysis through UV spectral comparison with the parent molecule 2-MXP and further confirmation was obtained from LC-MS investigations, which pointed toward similar productions compared to 2-MXP under full scan mass spectrometry conditions. When high-resolution QTOF-MS analysis was carried out, further details about the associated empirical formulae emerged as summarized in Table II and Figure 4. These metabolites were observed in all three cases in both the post-mortem blood and urine samples. The suggested structural features associated with the detected analytes were based on previous analytical studies on diphenidine isomers (9). In addition to the parent molecule 2-MXP (Figure 4a), the primary metabolite (based on observed LC peak abundance) appeared to be hydroxylated 2-MXP (Figure 4b) with a relative abundance of around 25% to that of 2-MXP in blood and 50% to similar abundance to 2-MXP in urine. Other hydroxylated metabolites were observed (based on the predicted empirical formula) but were present at significantly lower concentrations based on signal intensity (relative abundance of around 5% to that of 2-MXP in blood and urine). The exact position of the hydroxyl group present in the main hydroxylated metabolite (Figure 4b) remains to be confirmed but tandem mass spectral data indicated that hydroxylation might have occurred on the piperidine ring instead of the phenyl and benzyl ring. Key indicators included the presence of product ions at *m/z* 102 (hydroxylated and protonated piperidine) and *m/z* 211 which pointed toward the presence of an unchanged methoxylated diphenyl product ion also present in 2-MXP (Figure 4a and g). Correspondingly, *m/z* 86 and *m/z* 197 implied the presence of the less prominent *O*-desmethyl-2-MXP metabolite (Figure 4c). Consistent with the suggested hydroxy-*O*-desmethyl-2-MXP metabolite, the product ions at *m/z* 102 and *m/z* 197 suggested hydroxylation of the piperidine ring (Figure 4d). In a previously published case report, the detection of various hydroxylation products has also been mentioned (11). Furthermore, diphenidine (Figure 4e) was also detected in this study at what appeared to be at trace concentrations in both blood and urine. Whether this was formed as a result of desmethoxylation, and therefore indicating a potential metabolite, or detected due to the presence of diphenidine as a separate compound, was unclear. However, as far as case 1 was concerned, the presence of diphenidine in the product was not reported. Figure 4f displays the QTOF-MS-MS data associated with what would have been consistent with a hydroxylated diphenidine metabolite where hydroxylation, as observed with the main metabolite of 2-MXP described above, might have occurred on the piperidine ring.

Conclusion

Three fatalities associated with the 'research chemical' 2-MXP are presented for the first time. In two of the cases 2-MXP was given as the cause of death and in one case, death was due to a fall. The combination of HPLC-based separation with UV full scan

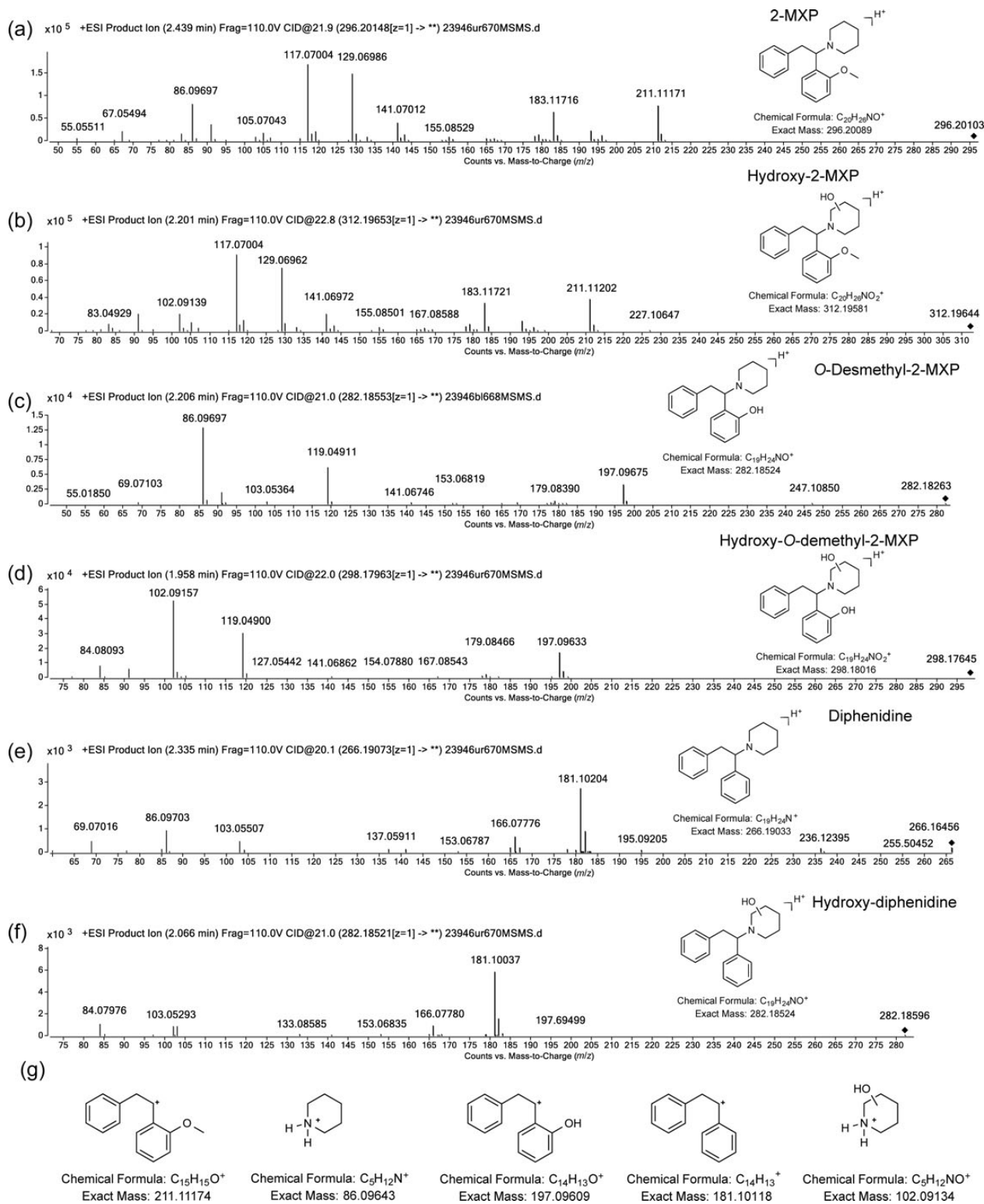


Figure 4. Analysis of case samples by high-performance liquid chromatography high accuracy quadrupole time-of-flight tandem mass spectrometry. (a) 2-MXP. (b)–(d) 2-MXP metabolites. (e) Diphenidine. (f) Diphenidine metabolite. It was unclear whether diphenidine represented a potential metabolite of 2-MXP or whether it was detected following co-ingestion. (g) Suggested structural representations of selected product ions.

and high-resolution mass spectrometry allowed for the detection of 2-MXP and metabolites in both post-mortem blood and urine. The high abundance of the main metabolite, interpreted as

hydroxy-2-MXP (on the piperidine ring), may be of specific analytical importance when investigating clinical and forensic case samples.

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