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First reported fatalities associated with the 'research chemical' 2'-methoxydiphenidine (MXP)

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Abstract

2'-Methoxydiphenidine, also known as 1-[1-(2-methoxyphenyl)-2-phenylethyl] piperidine and 'MXP' (or 2'-MXP), has been available as a 'research chemical' from 2013 as a purported alternative to the 'dissociative anaesthetics' 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 levels 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 methoxydiphenidine and presents analytical data to assist analytical toxicologists with future casework.

INTRODUCTION

The use of 'dissociative anaesthetics' 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 United Kingdom, 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). Whilst analytical data pertaining to diphenidine and related analogues 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 [^{3}H]-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) analogues 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 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 (20) This paper 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.

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 labelled '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

HPLC-DAD was carried out on a Dionex 3000 Ultimate liquid chromatography 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) protected by a 4 mm x 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 x 2 mm, 5µm) protected by a Phenomenex Synergi Gemini 4 mm x 3 mm guard column (Phenomenex, Cheshire, UK). A mobile phase of 1 mM ammonium formate with 1% formic acid and acetonitrile (column temperature 30 °C) was used.

The UHPLC-high resolution 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 x 2.1 mm, 1.8µm) (Agilent, Cheshire, UK) using mobile

phase of 1% formic acid and acetonitrile (column temperature 40°C). The methodology and parameters have been published previously (21).

Reagents and standards

All solvents and chemicals used, e.g. acetonitrile, 1-chlorobutane, methanol, sodium carbonate, sulphuric acid, formic acid, triethylammonium phosphate 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. (22) followed by full characterization including nuclear magnetic resonance spectroscopy (NMR). 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 mg/L and 2.5 mg/L were also produced. Intra-day and inter-day precision and accuracy was 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 was performed as described elsewhere (21). The chromatographic conditions for qualitative HPLC-DAD, HPLC-MS and UHPLC-QTOF-MS analysis were based on previously published methods involving an acetonitrile gradient (21). 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 minutes.

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) (23). Specifically, 2'-MXP eluted at 8.44 minutes with a 278 nm UV max, 3'-MXP eluted at 8.06 minutes with a 276 nm UV max and 4'-MXP eluted at 8.08 minutes 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 samples. Figure 3 shows that with LC-MS there are numerous fragment ions produced 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 less than 6%, a limit of detection of 0.05 mg/L and a limit of quantitation 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. Prescription drugs (mirtazapine, lamotrigine and citalopram) were also found at therapeutic levels 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 levels 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 level 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 levels 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 concentration levels 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 diphendidine, methoxetamine and ketamine (1, 4, 9, 19, 24). 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 particular 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 towards similar fragments 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 2 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 towards the presence of an unchanged methoxylated diphenyl product ion also present in 2'-MXP (Figure 4A and 4G). Correspondingly, *m/z* 86 and *m/z* 197, implied the presence of the less prominent *O*-desmethyl-2'-MXP metabolite (Figure 4C). With regard the metabolite suggested to be consistent with hydroxy-*O*-desmethyl-2'-MXP, 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 the present study at what appeared to be at trace levels 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'-methoxydiphenidine (2'-MXP) are presented for the first time. In two of the cases 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 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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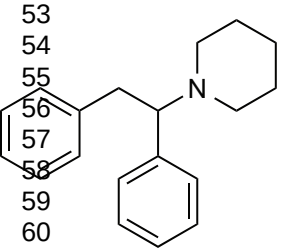
FIGURE LEGENDS

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

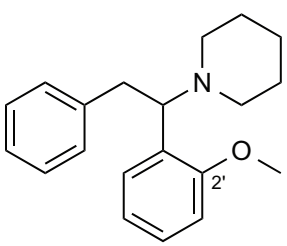
Figure 2. DAD data and HPLC retention times for three synthesized positional isomers of methoxydiphenidine. The differentiation was feasible based on the combination of UV full scan and retention time information. A: UV spectrum obtained from 2'-MXP standard and casework. B: UV full scan trace of 3'-MXP. C: UV full scan trace of 4'-MXP.

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.

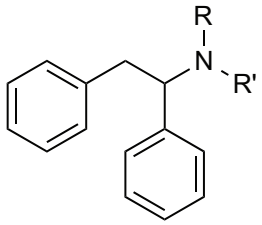
Figure 4. Accurate mass fragmentation of 2'-MXP and purported metabolites detected in case samples.



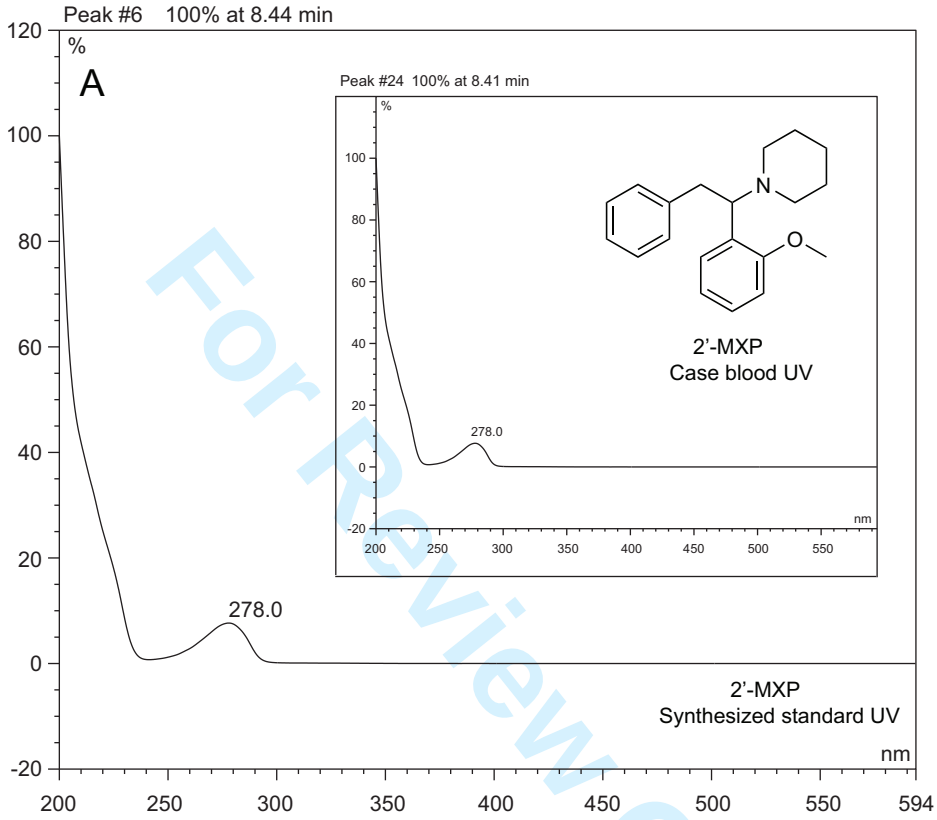
Diphenidine

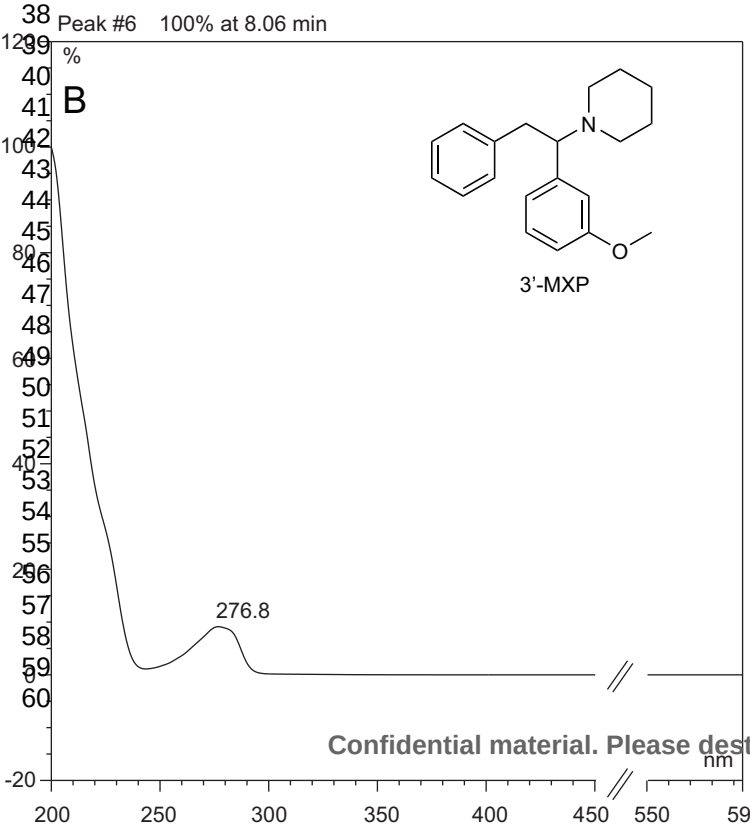


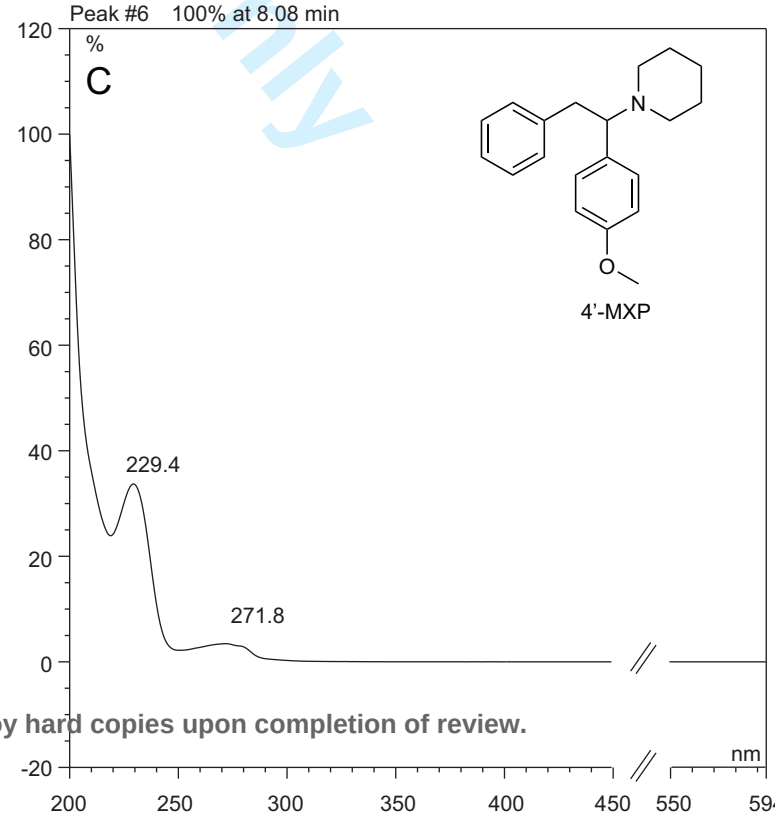
2-MeO-diphenidine (2'-MXP)



R = H; R' = C_2H_5: NE-DPEA
R = H; R' = i-C_3H_7: NIP-DPEA
R = R' = CH_3 (*R*): Lefetamine







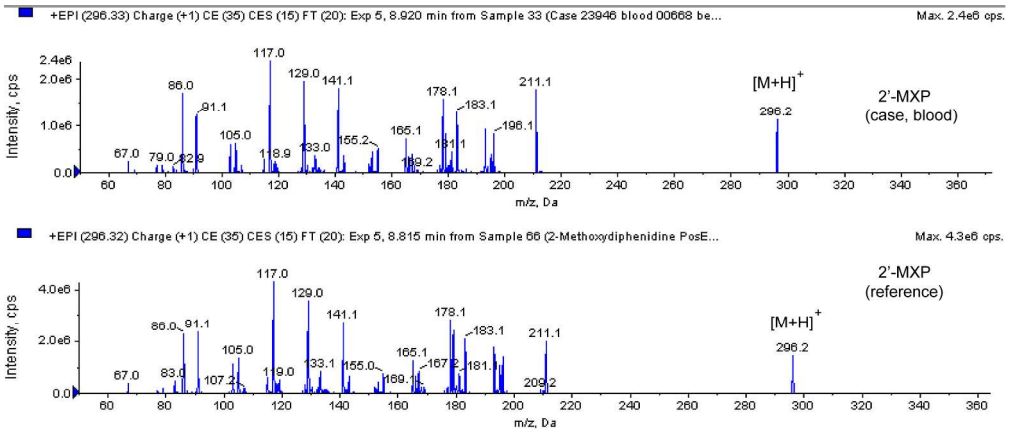
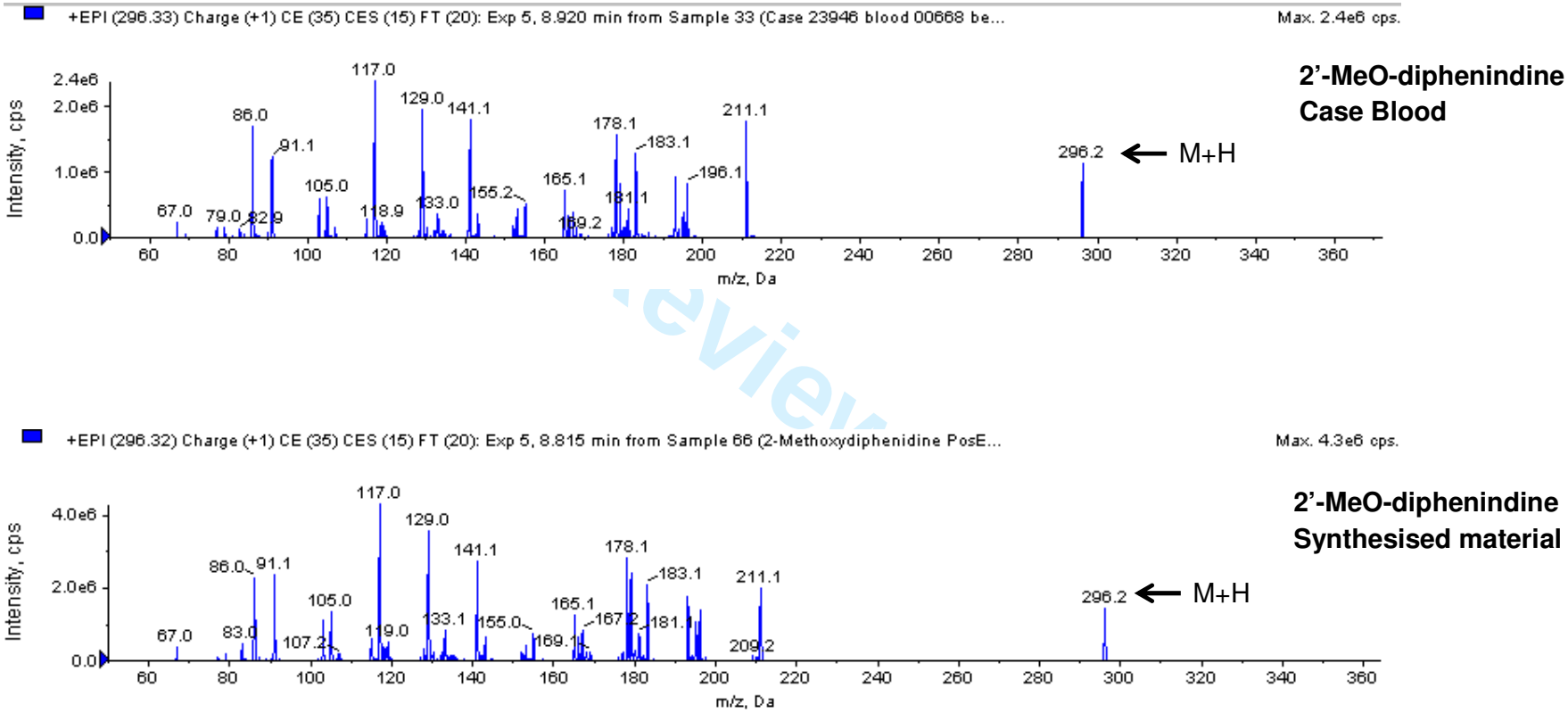
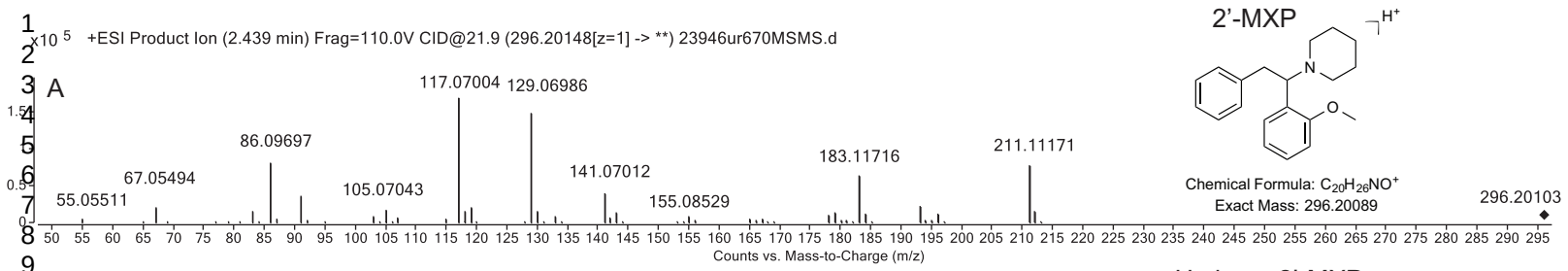
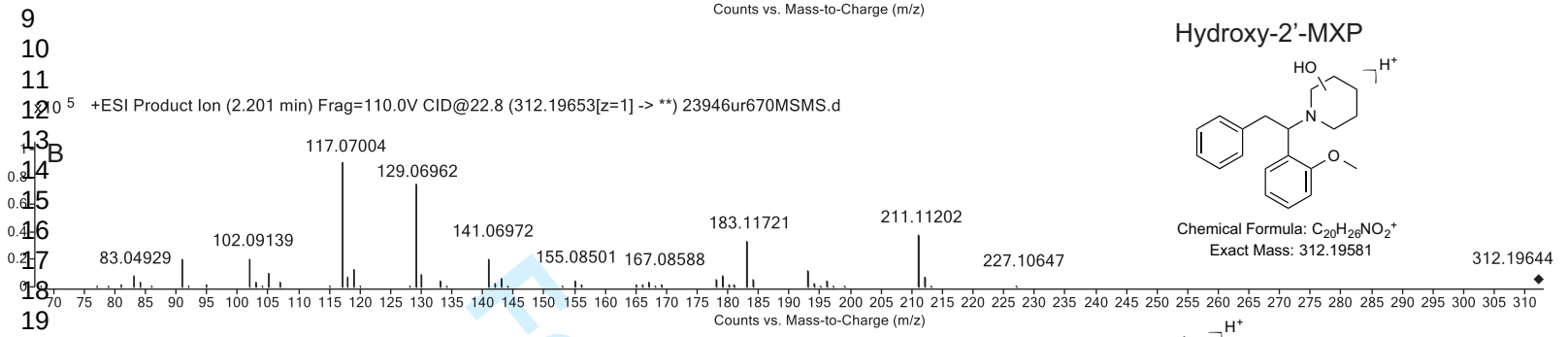


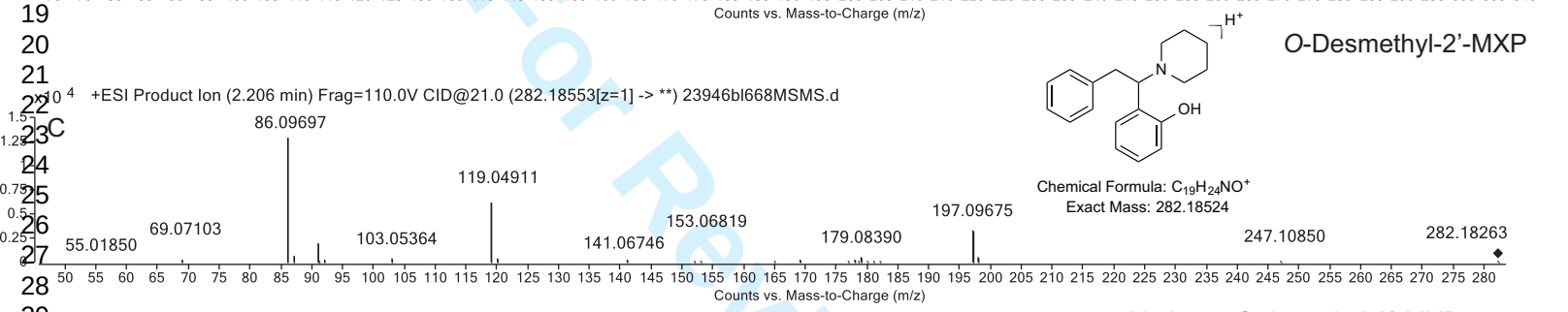
Figure 3. Enhanced product ion (EPI) scan of 2'-MXP (case vs. reference material) at collision energy (CE) spread (35 ± 15 V) using electrospray LC-MS.
220x93mm (300 x 300 DPI)

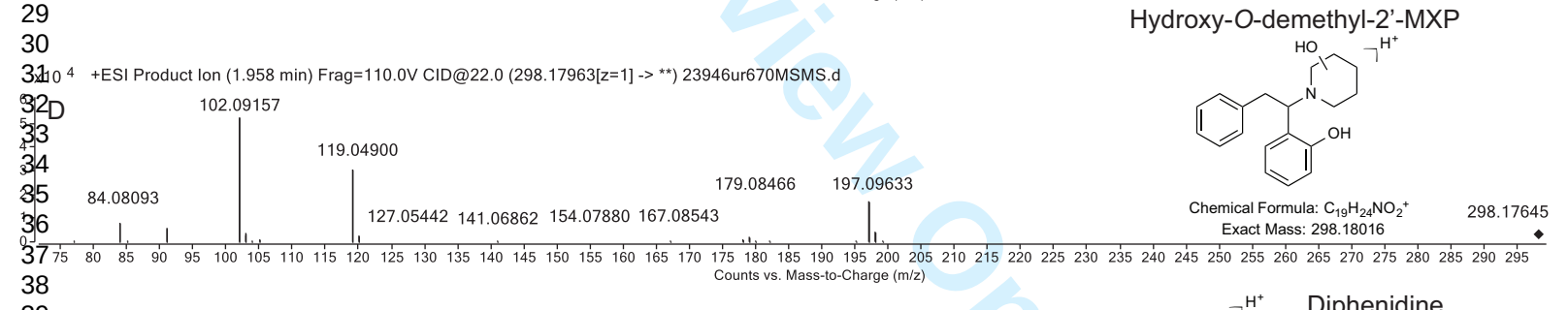
Figure 3. Enhanced Product Ion scan of 2'-Methoxydiphenidine at collision energy (CE) spread (35 +/- 15V) using electrospray LC-MS.

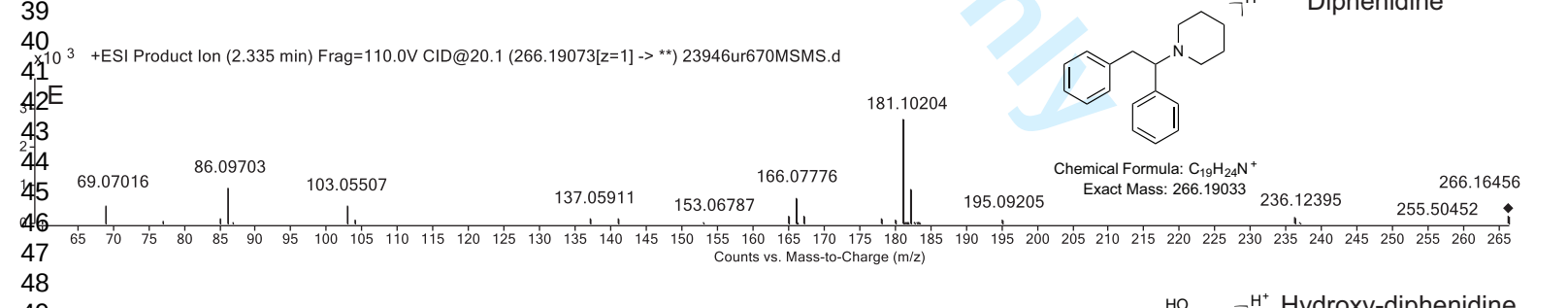


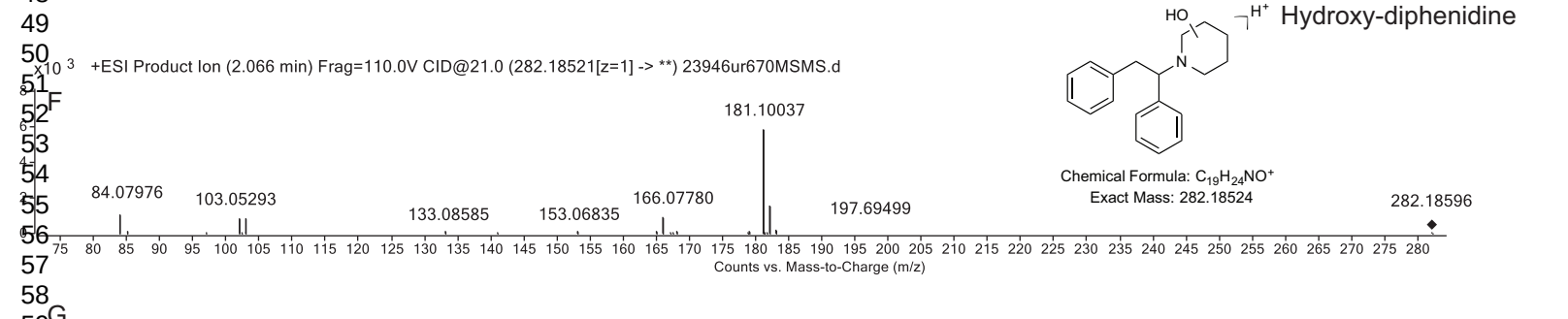












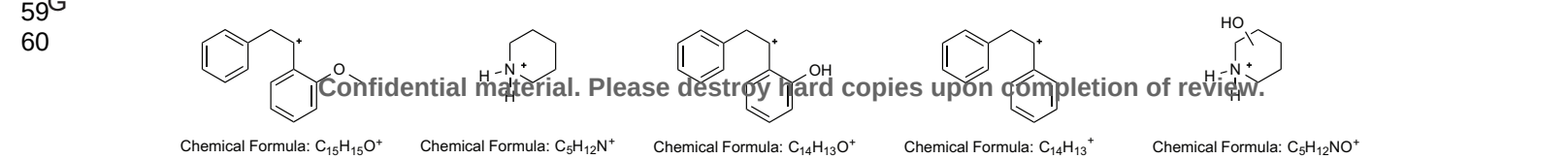


Table 1. Validation data for quantitation of 2'-methoxydiphenidine.

Intra-day (n=10)	Mean conc^{n} mg/L (± SD)	Precision (%)	Accuracy (%)
0.1 mg/L	0.989 ± 0.015	1.5	-1.1
Inter-day (n=6)			
0.5 mg/L	0.52 ± 0.01	1.2	4.6
2.5 mg/L	2.64 ± 0.02	0.9	5.5
LOD	0.05 mg/L		
LOQ	0.10 mg/L	1.5	-1.1

Table 2. 2'-Methoxydiphenidine (2'-MXP) and proposed metabolites found in forensic casework samples using UHPLC-QTOF-MS.

Compound	Molecular formula	Theoretical mass $[M+H]^+$	m/z found
2'-MeO-diphenidine (2'-MXP)	$C_{20}H_{25}NO^+$	296.20089	296.20088
Hydroxy-2'-MXP	$C_{20}H_{25}NO_2^+$	312.19581	312.19556
O-Desmethyl-2'-MXP	$C_{19}H_{23}NO^+$	282.18524	282.18514
Hydroxy-*O*-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