Analysis of MT-45, A Novel Synthetic Opioid in Human Whole Blood by LC-MS/MS

Donna Papsun[1] and Barry K. Logan[1,2]

[1]NMS Labs, Willow Grove, PA, [2]The Center for Forensic Research and Education, Willow Grove, PA

Introduction

The rise of novel psychoactive substance (NPS) creates ongoing challenges for the forensic toxicology laboratory. Compounds classified as NPS are attractive to drug users due to widespread availability aided by the internet, their ambiguous legal status, and the fact they are not detected in most routine drug tests such as those used for pre-employment or random workplace testing. Although the first classes of drugs to appear on the NPS market included synthetic cannabinoids (K2, Spice) and synthetic cathinones ("bath salts" such as MDPV & methylone), other drug classes with different mechanisms of actions followed suit. Examples include the NBOMe suite of designer hallucinogens, phenazepam and etizolam as designer benzodiazepines, and most recently designer opioids AH-7921 and MT-45, This presentation describes the analysis of MT-45 recently identified in a death investigation case in the United States.

MT-45 is an *N*,*N*-disubstituted piperazine compound, and acts is an analgesic substance developed by the Dainippon Pharmaceutical Co in the 1970's as an alternative to morphine, to which it is structurally unrelated. The pharmacology of MT-45 is complex, and involves opioid and other non-opioid receptors that have not been fully characterized. MT-45 has been reported to have 80% the potency of morphine. See Figure 1 for the structures of MT-45 compared to morphine.

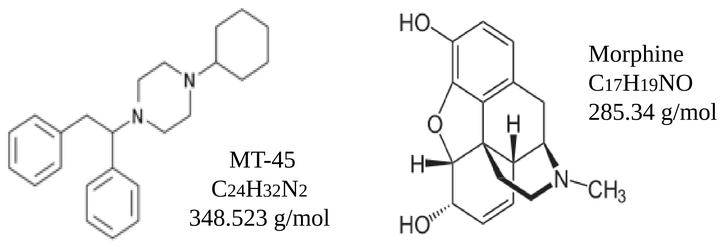


Figure 1: Designer opioid MT-45 compared to Morphine

Internet suppliers and retailers sell MT-45 in its dihydrochloride salt form. It has typically been seized as a white or brown powder, and occasionally in tablet form. It has also been seized in combination with other drugs, including heroin, synthetic cannabinoids, pyrrolidinophenones such as alpha-PBP, benzofurans such as 6-APDB, and synthetic cathinones such as methylone. Typical routes of administration include oral, insufflation, intravenous, and intramuscular, with varying reported doses.

NPS use had been detected in all types of forensic cases, including postmortem investigations. MT-45 has been identified in both postmortem and human performance toxicology casework in Sweden and has been linked to 33 adverse events, including 21 deaths. In these fatalities occurring in 2013 and 2014, the concentration of MT-45 in post-mortem femoral blood ranged from 60 to 1900 ng/mL. In 17 of the reported cases, MT-45 was found in combination with at least one other psychoactive substance.

This poster describes an analytical method for the identification and quantitation of MT-45 in human whole blood. Using the described methods, the analysis was used on human whole blood samples obtained in a death which was ultimately attributed to combined toxicity of MT-45 and the designer benzodiazepine, etizolam.

Resources

1. EMCDDA-Europol Joint Report on a new psychoactive substance: 1-cyclohexyl-4-(1,2-diphenylethyl)piperazine (MT-45). Luxembourg: Publications Office of the European Union. 2014.
2. Vorce S, Knittel J, et al. A Fatality Involving AH-7921. J Anal Tox. 2014; 38:226-230.
3. Natsuka K, Nakarama H, et al. Studies on 1-substitued 4-(1,2-diphenylethyl)piperazine derivatives and their analgesic activities. J of Med Chem. 18(12):1240-4.
4. Uchiyama N, Matsuda S, Kawamura M, Kikura-Hanajiri R, Goda Y Identification of two new-type designer drugs, piperazine derivative MT-45 (I-C6) and synthetic peptide Noopept (GVS-111), with synthetic cannabinoid A-834735, cathinone derivative 4-methoxy-α-PVP, and phenethylamine derivative 4-methylbuphedrine from illegal products. Forensic Toxicology, January 2014, 32(1):9-18

Acknowledgments

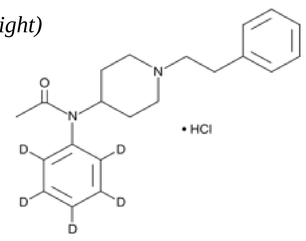
The authors are grateful to Dr. Elizabeth Bundock of the OCME in Burlington, VT for providing investigative information in this case.

Methods

Sample Preparation. Blood (0.5mL) was made alkaline with ammonium hydroxide and extracted with 4:1 n-butylchloride/ethyl acetate. After transfer and solvent evaporation, the residue was reconstituted in 80:20 mobile phase A:B for LCMSMS analysis.

Analysis. The method was validated on a Waters® TQD Tandem Mass Spectrometer coupled to a Waters ® Acquity Ultra Performance LC system. The instrument was operated in positive electrospray, multiple reaction monitoring (MRM) mode. Separation was achieved on an Acquity UPLC BEH C18 column, 2.1 x 5.0 mm, 1.7 µn. Mobile phases consisted of 0.1% formic acid in deionized water (A) and 0.1% formic acid in methanol (B).

ISTD. D5-acetyl fentanyl *(structure to the right)*



Retention Times and Transitions:
Acetyl fentanyl d5 = 1.72 min
MT-45 = 2.26 min
See Table 1 for transitions.

LC-MS/MS Transitions

Analyte	Quant Ion	Qual Ion
Acetyl-fentanyl d5	328.3 > 105.1	328.3 > 188.1
MT-45	349.3 > 181.1	349.3 > 169.2

Table 1: LC-MS/MS transitions for analytes

Ion Traces

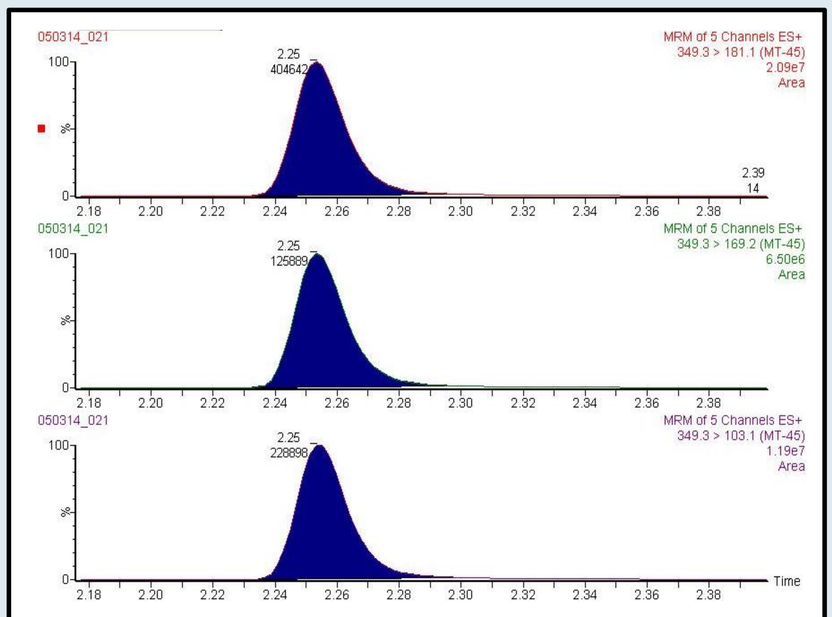


Figure 2: MRM Ion Traces for MT-45

EI Mass Spectrum

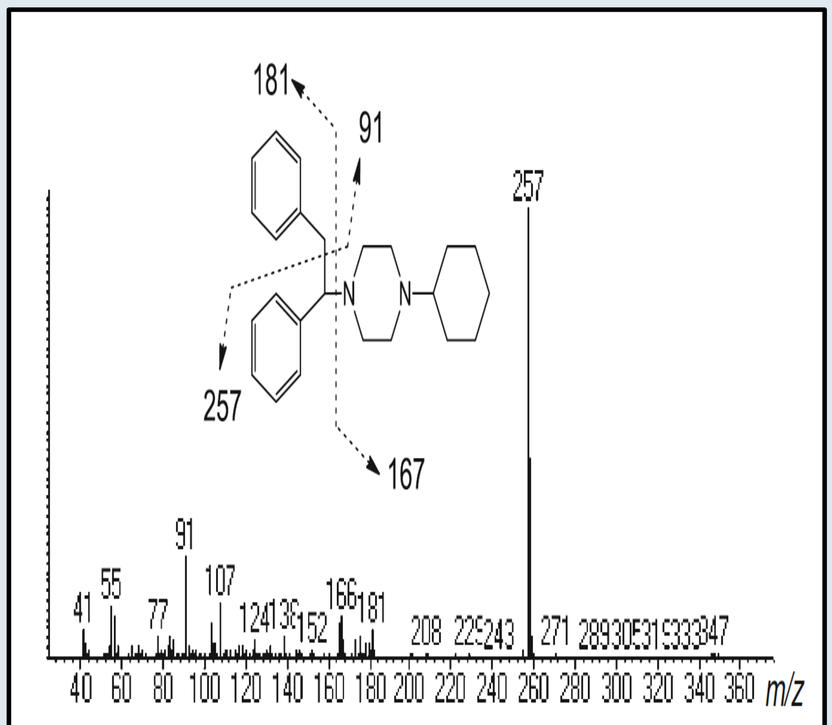


Figure 3: MT-45 Mass Spectrum
(from Uchiyama et al, 2014)

Results

Validation
A preliminary validation of the method consisted of running three days of matrix-matched calibration curves and controls in replicate. The analytical range was specified at 1.0-100 ng/mL and verified analytically. Analysis of calibration statistics over three days demonstrated a slope of 0.999 and a bias of 0.05%. High, mid, and low controls were spiked at 80, 30, and 3 ng/mL. See Table 2 for precision and accuracy.

Sample		Precision (%CV)		Accuracy (% Difference)	
Level	Concentration	Between Run	Total	Between Run	Total
Reporting Limit	1 ng/mL	4.5	8.1	5	5
Low QC	3 ng/mL	5.2	6.9	2	2
Mid QC	30 ng/mL	3.6	4.3	16.7	16.7
High QC	80 ng/mL	3.8	6	3.8	3.8

Table 2: Precision and Accuracy for Reporting Limits and Controls

See Figure 4 for an example calibration curve and Figure 5 for an excerpt of the raw data showing chromatography.

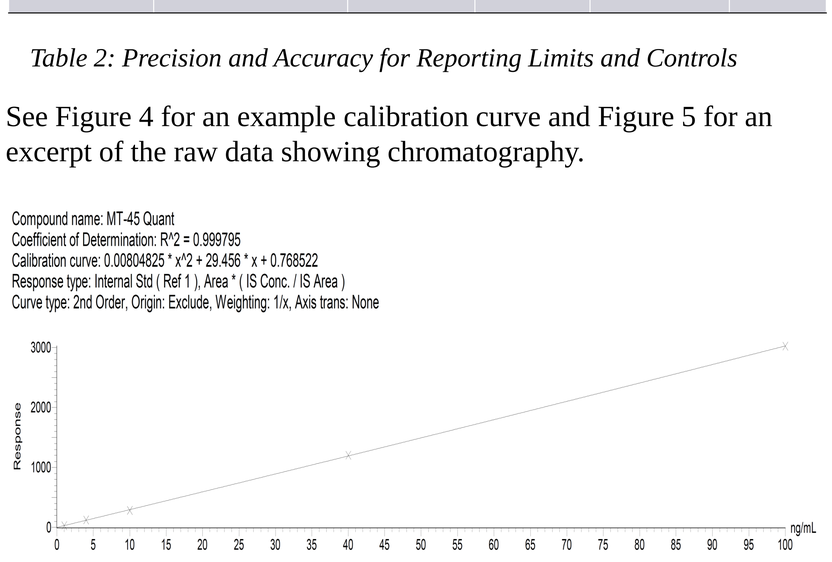
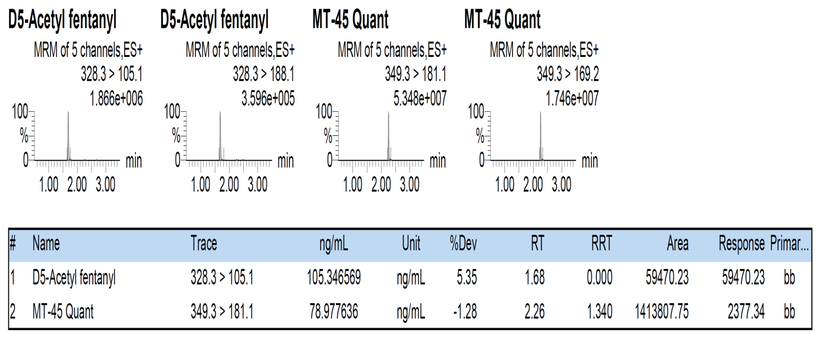


Figure 4: MT-45 Calibration Curve



#	Name	Trace	ng/mL	Unit	%Dev	RT	RRT	Area	Response	Primar...
1	D5-Acetyl fentanyl	328.3 > 105.1	105.346569	ng/mL	5.35	1.68	0.000	59470.23	59470.23	bb
2	MT-45 Quant	349.3 > 181.1	78.977636	ng/mL	-1.28	2.26	1.340	1413807.75	2377.34	bb

Figure 5: MT-45 High Control data

Conclusion

The extraction and analytical method described were used to analyze a human whole blood sample sent for targeted toxicological analysis for MT-45, since both MT-45 and etizolam were identified in powders located in the residence of a decedent during an investigation. Quantitation was performed using the method of standard addition, and was performed on dilution due to the high concentration. A whole blood from the suspected overdose contained MT-45 at a concentration of 520 ng/mL, consistent with the concentrations reported in other deaths. Etizolam was also present in this case at a concentration of 35 ng/mL.

Due to the novel nature of these new synthetic substances, there is often very little available data regarding effects, toxicity, and pharmacodynamic profile, especially when compounds first appear on the drug market. Most information regarding these new substances derive from case reports, such as this case. This case also highlights the importance of thorough investigation, as the identification of the solid material allowed a targeted analysis to be performed for these novel substances which are not included in the routine scope of testing for death investigation casework.