Ion Trap GC-MS/MS Analysis of "Bath Salts" in Biological Samples

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Introduction

The new Drug Problem: "Snorting Chemical Salts"

All over the U.S., chemical salts are being sold with names like "Ivory Wave," White Lightning," and "Hurricane Charlie."

These so-called "bath salts" are chemicals intended to be snorted, smoked or injected-and users are getting high off them. The DEA does not regulate these substances, but they are under federal scrutiny, as the effects of these salts are comparable to cocaine and methamphetamine abuse.

The powders often contain mephedrone and methylenedioxypyrovalerone, also known as MDPV.

Objectives:

Confident identification and quantitation of Methylenedioxypyrovalerone (MDPV) and Naphthylpyrovalerone (Naphyrone) in blood, urine, vitreous, gastric, and tissue matrices using GC-MS/MS methodology.

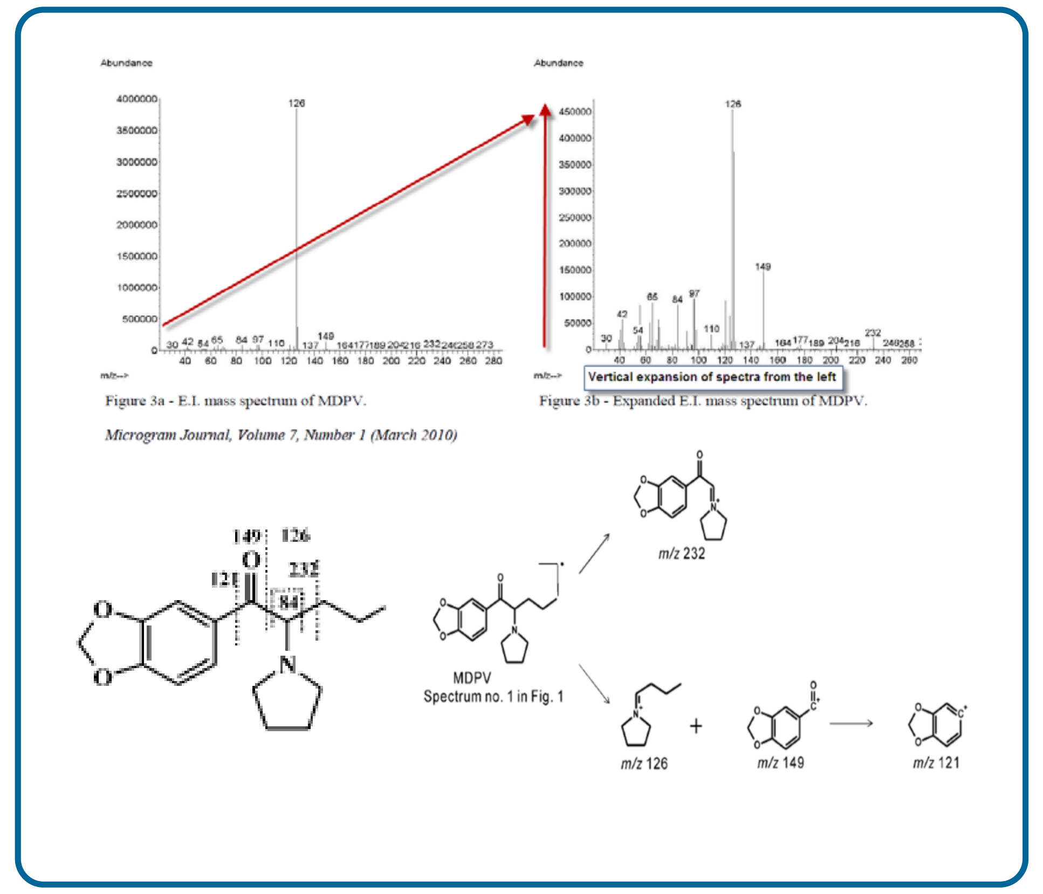

Figure 3a - E.I. mass spectrum of MDPV.

Figure 3b - Expanded E.I. mass spectrum of MDPV.

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Experimental

Materials and Methods

An Ion Trap Mass Spectrometer with internal ionization EI/MS/MS was used with a GC configured in a traditional capillary column mode to facilitate efficient, reproducible chromatographic separations and characteristic clean spectra to gain definite qualitative and quantitative results.

GC-MS/MS Conditions
Injector 250 C
GC-Column – DB-5 phase , 30 m x 0.25 x 0.5
Oven Program
  70 °C/min. hold for 1.0 min.
  25 °C/min to 310 °C hold for 4.4 min.
Injection Volume 0.50 uL, splitless
Column flow 1.3 mL/min.
Transferline 310 °C
Trap 210 °C

EI MS/MS Mode: MRM Transitions

Name	Precursor	Product	Collision Energy
MDPV	126	84,124	0.50 Volts
Naphyrone	126	84, 124	0.50 Volts
Ropivacaine (IS)	126	84, 98	0.50 Volts

All compounds were found to have a predominate ion at m/z 126 to use as the Precursor ion. The MS/MS CID voltages were optimized using the Automated Method Development (AMD) tool in the acquisition software.

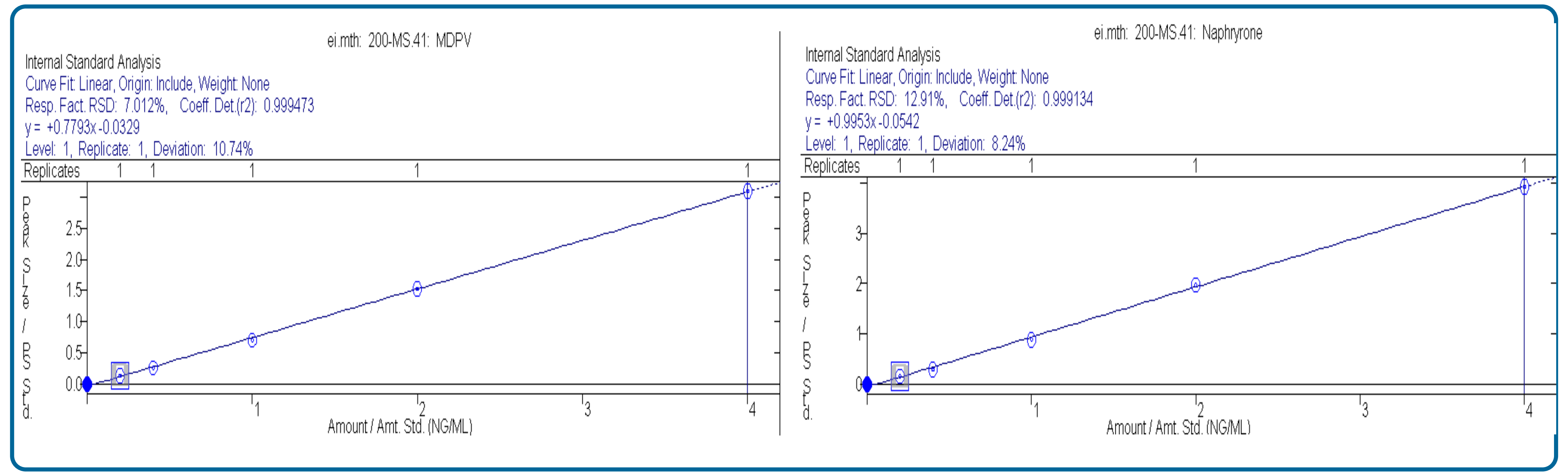
Calibration samples were prepared in comparable matrix with MDPV, Naphyrone, an internal standard (Ropivacaine) and extracted with a basic liquid-liquid extraction along with unknown samples and spiked controls. (blood and urine matrix)

Calibrator	Working Standard	Negative Matrix
50 ng/mL	0.010 mL	3 mL
100 ng/mL	0.020 mL	3 mL
250 ng/mL	0.050 mL	3 mL
500 ng/mL	0.100 mL	3 mL
1000 ng/mL	0.200 mL	3 mL

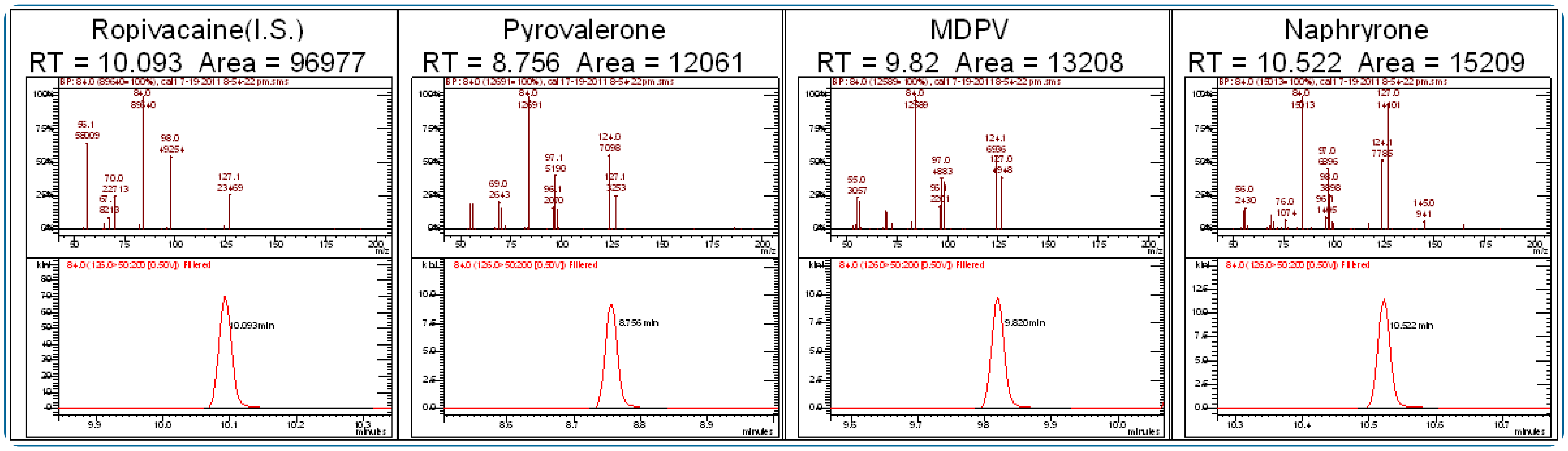
1. In culture tubes pipet 3.0 mL of each sample and control. Add 50 uL of the working internal standard and 2 mL of pH 9.8 Carbonate Buffer. Add 2 drops of NH4OH and vortex gently to mix.
2. Add 7.0 mL of n-butyl chloride to each tube and rotate all tubes for at least 10 minutes.
3.Centrifuge all tubes at 3000 RPM for 10 minutes.
4. Transfer organic layer to a second culture tube and add 2 drops of 0.1% methanolic HCL and evaporate to dryness at 37 C with nitrogen.
5. Reconstitute dried extracts with 200 uL of ethyl acetate and transfer to ALS vial.
6. Inject 0.5 uL into GC/MS/MS for analysis.

Results and Discussion

Calibration Curves for MDPV and Naphyrone from 50.0 ng/mL to the ULOL at 1000 ng/mL.



Low calibration level 50.0 ng/mL with quantitation ion chromatogram and spectra. Pyrovalerone added to compound table.



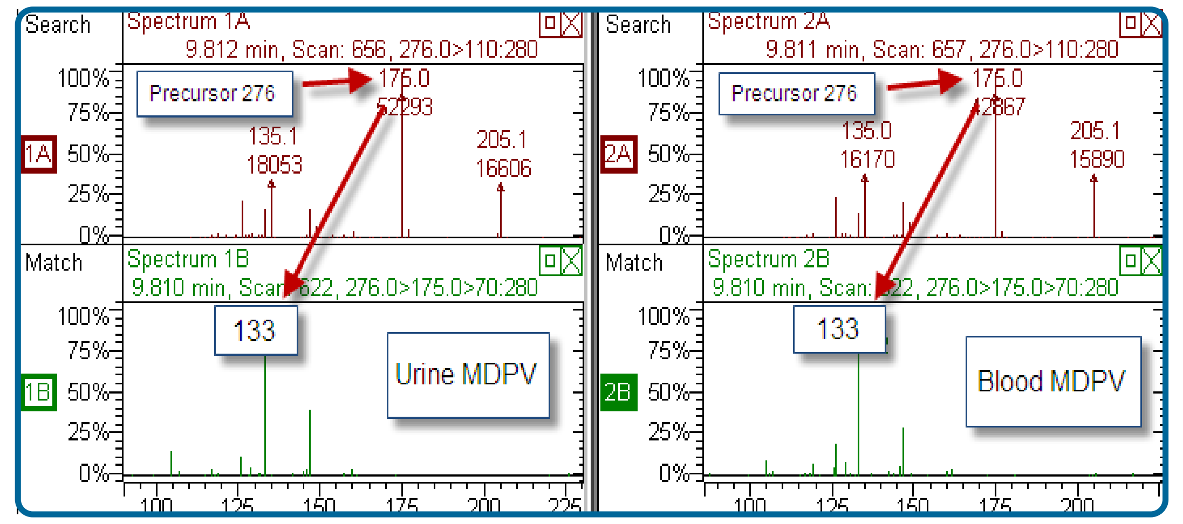
The imprecision for both intra and inter-assay analysis was determined to be less than 5 % for all sample types.
The statistical LOQ values for all matrices:

	std dev	std dev		
MDPV	**Inter**	**Intra**		**LOQ ng/mL**
number	**25**	**5**		
blood	2.825	1.073		0.2369
brain	4.63	0.72927		0.2558
liver	2.494	2.583		0.9263
Naphyrone	**Inter**	**Intra**		**LOQ ng/mL**
number	**25**	**5**		
blood	1.943	2.45086		0.2291
brain	2.79107	0.85757		0.0557
liver	1.04588	0.71814		0.6768

Results and Discussion

Confirmation techniques

Use of Chemical Ionization for molecular ion confirmation
In the case of MDPV, MW 275.3 (CI ion 276 M+1)
Precursor 276>175 (CI/MS/MS) - 276>175>133 (CI/MS/MS/MS)



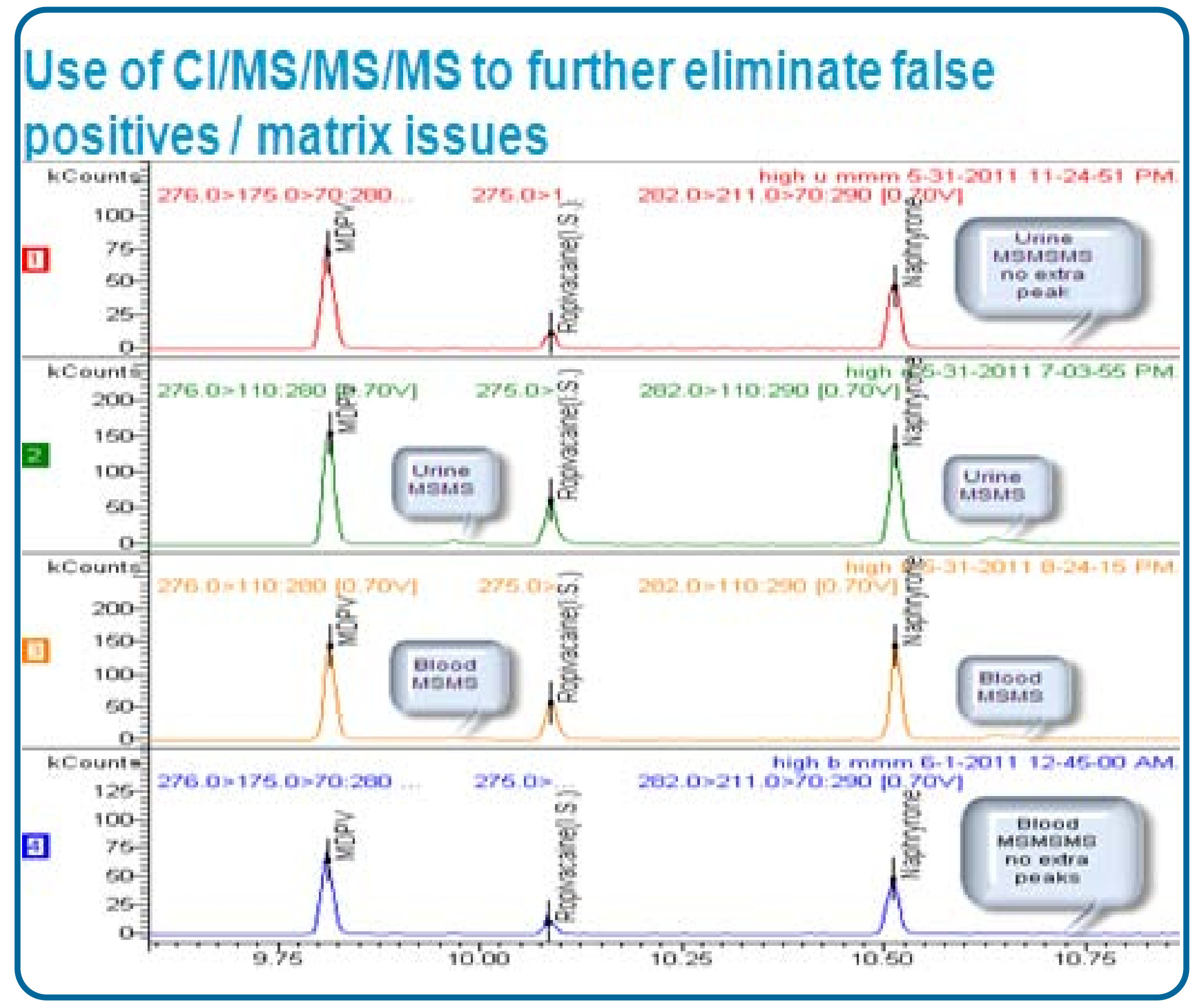
Shown here is a high control - extracted in both Urine and Blood, comparing the selectivity of using one more step in the MS/MS function available only to Ion Traps to give more selective information

The selection of the M+ ion for the precursor and observing the full scan product ions gives the MS/MS step.

We then select a significant higher mass from the product ions and use this as a new precursor to produce again full scan product ions.

In this case we used the M+ ion 276 for MDPV which gave a significant 175 ion to use as the precursor in the MS/MS/MS step.

Use of CI/MS/MS/MS to further eliminate false positives / matrix issues



Chemical Ionization (CI)I offers more selective ionization.

The sensitivity of EI and CI is comparable in internal ionization ion traps, so using this more selective ionization technique does not reduce sensitivity.

Switching between EI and CI also simple, automated step and can be executed even in the same run.

CI delivers unique, intense precursor ions, the M+1 ions which is 276 for MDVP and 282 for Naphyrone.

The product ion spectrum generated from the M+1 precursor ion also carries the full qualitative information of the analytes.

Conclusions

Herein is presented a sensitive, selective, and robust analytical method to determine MDPV and Naphyrone using Ropivacaine as an internal standard. The method allows for both designer drugs to be identified in blood, urine, vitreous, gastric, brain and liver tissue matrix by their unique precursor product ion spectrum and retention times. Matrix interference was mitigated through the MS/MS processes. Two levels of positive controls were used in conjunction with negative controls to assure accurate quantification and rule out false negatives in the unknown biological samples. Low nanogram/mL detection limits were observed for both MDPV and Naphyrone in the various sample matrices. Seven replicates were run at 50 ng/mL with CV(method precision) of 5.4% and 4.2% respectively in urine extracts.

For Forensic Use.