Analytical Challenges with MS Analysis of Bath Salts and Spice Cannabinoid Metabolites

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INTRODUCTION

Mass spectrometry (MS) is the tool of choice for detection and quantitation of new illicit drugs like Spice cannabinoids and bath salt cathinones. While GC/MS and LC/MS provide numerous benefits for these purposes, they also offer challenges unique to the particular technique.

GC/MS methods, for example, require use of derivatization reagents for analysis of cathinone-based analogs like bath salts. The use of PFPA and BSTFA derivatives with deuterium-labeled internal standards have been reported to cause loss of label in the GC/MS fragmentation.

Analysis of matrix-based samples by LC/MS can suffer from interferences or lower ionization efficiency due to matrix effects. While deuterium-labeled internal standards are most commonly used to compensate for matrix effects in LC-MS/MS applications, some labeled compounds may exhibit hydrogen-deuterium scrambling/exchange in the collision cell which necessitates careful selection of MS/MS transitions.

MATERIALS & GC/MS DERIVATIZATION METHOD

Materials:

3,4-MDPV HCl, Cerilliant Cat# M-146
3,4-MDPV-D_8 HCl, Cerilliant Cat# M-150
Ethylone HCl, Cerilliant Cat# E-071
Ethylone-D_5 HCl, Cerilliant Cat# E-072
Butylone HCl, Cerilliant Cat# B-045
Butylone-D_3 HCl, Cerilliant Cat# B-046
Mephedrone HCl, Cerilliant Cat# M-138
Mephedrone-D_3 HCl, Cerilliant Cat# M-139
Methylone HCl, Cerilliant Cat# M-140
Methylone-D_3 HCl, Cerilliant Cat# M-141
Methedrone HCl, Cerilliant Cat# M-147
JWH-018 4-Hydroxypentyl metabolite, Cerilliant Cat# S-035
JWH-018 4-Hydroxypentyl metabolite-D_5, Cerilliant Cat# S-039
JWH-073 3-Hydroxybutyl metabolite, Cerilliant Cat# S-037
JWH-073 3-Hydroxybutyl metabolite-D_5, Cerilliant Cat# S-040

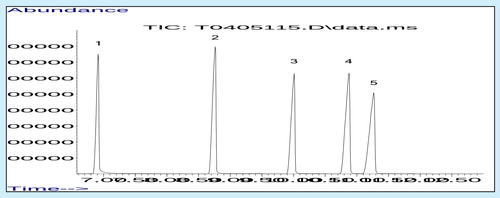
GC/MS Derivatization Method

Native and deuterated reference materials of the above bath salts were used to develop the derivatization method with trifluoroacetic anhydride (TFAA). The HCl salts were converted to free base with 0.1M sodium bicarbonate and heated to 60°C for five minutes with TFAA and ethyl acetate to acylate the amino group. The free up procedure is sensitive to choice of base due to instability of α-amino ketones. Optimization of derivatization time is critical, as decomposition occurs with excessive heating.

GC/MS CHROMATOGRAPHIC DATA

Derivatives were analyzed directly by GC/MS with cool-on-column injection on a DB-5ms narrow-bore (30m x 0.25mm x 0.25µm) column.

Temperature ramp: 3 min at 150°C, 150°C to 200°C at 10°C/min, 200°C to 210°C at 2°C/min.



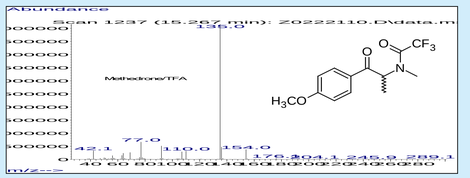
	Compound	Peak Width	Resolution	Tailing	RRT vs. Methylone
1	Mephedrone	0.046	NA	0.67	0.691
2	Methedrone	0.063	20.08	0.64	0.876
3	Methylone	0.065	11.50	0.63	1.000
4	Butylone	0.072	7.47	0.63	1.087
5	Ethylone	0.080	3.03	0.63	1.126

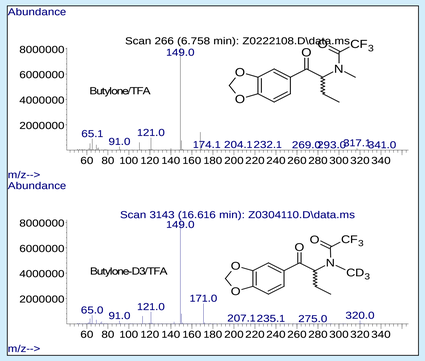
GC MASS SPECTRA AND ISOTOPIC DISTRIBUTION OF TFA-DERIVATIZED BATH SALTS

The labeled compounds retain deuterium label from the molecular ion to one or two fragmentations. Quant ion pairs were selected based on ion abundance. Isotopic distribution was evaluated to ensure the majority of the label was on the quant ion.

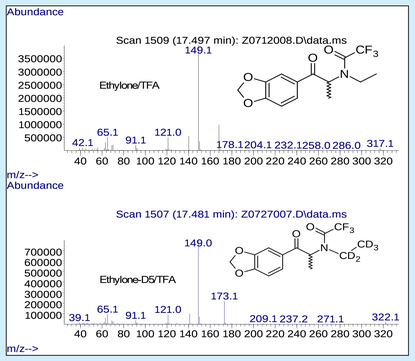
Compound (TFA)	MW Pair	Q1 Pair	Q2 Pair
Mephedrone / Mephedrone-D_3 HCl	273.1 / 276.1	154.1 / 157.1	110.1 / 113.1
Methylone / Methylone-D_3 HCl	303.1 / 306.1	154.1 / 157.1	NA
Butylone / Butylone-D_3 HCl	317.1 / 320.1	168.0 / 171.0	110.0 / 113.0
Ethylone / Ethylone-D_5 HCl	317.1 / 322.1	168.0 / 173.0	NA

For Methedrone and Mephedrone, the molecular ion abundance is low. The fragment ion is used for quantitation.

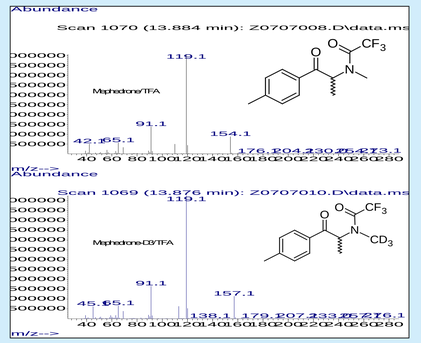




	Butylone-D_3 HCl	
	MW Pair	Q1 Pair
D_3	98.96%	99.16%
D_2	0.97%	0.39%
D_1	0.04%	0.42%
D_0	0.02%	0.02%
D_0/D_3	0.03%	0.02%



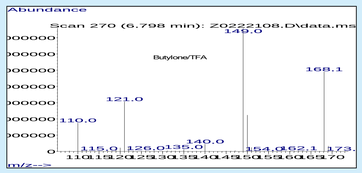
	Ethylone-D_5 HCl	
	MW Pair	Q1 Pair
D_5	96.69%	97.65%
D_4	3.09%	2.02%
D_3	0.15%	0.21%
D_2	0.06%	0.04%
D_1	0.01%	0.03%
D_0	0.01%	0.05%
D_0/D_5	0.01%	0.04%



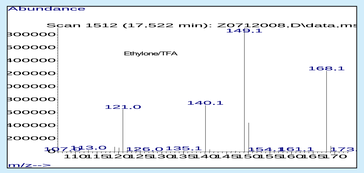
	Mephedrone-D_3 HCl	
	Q1 Pair	Q2 Pair
D_3	97.51%	98.74%
D_2	1.62%	0.81%
D_1	0.84%	0.21%
D_0	0.03%	0.24%
D_0/D_3	0.03%	0.24%

BUTYLONE & ETHYLONE COMPARISON BY GC/MS

Ratio	Butylone/TFA	Ethylone/TFA
121/110	58.11%	0.27%
140/121	14.99%	34.05%



Butylone Expansion



Ethylone Expansion

Ethylone and Butylone have identical molecular and primary ion fragmentations of 168 and 149. Differentiation between Ethylone and Butylone is achieved by monitoring the response of fragment ions 121/110 and 140/121.

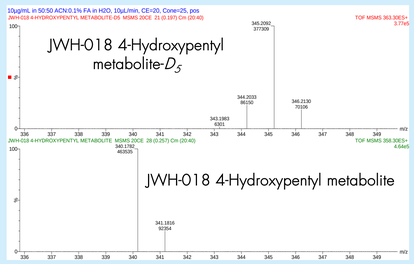
INVESTIGATION OF SCRAMBLING IN LC-MS/MS ANALYSIS OF BATH SALTS & SPICE CANNABINOID METABOLITES

In this study, we investigated several variables that potentially contribute to scrambling in LC-MS/MS applications in order to ascertain reproducibility and impact on scrambling ratios: collision energies and deuterium placement in the internal standard.

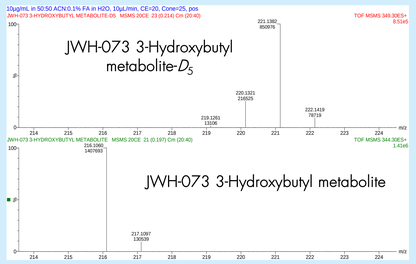
LCMS System:

Waters Alliance UPLC-Xevo G2 Q-Tof

JWH-018 4-Hydroxypentyl Metabolite
Scrambling at m/z 340



JWH-073 3-Hydroxybutyl Metabolite
Scrambling at m/z 216



Scrambling was observed for the deuterium-labeled Spice metabolites but not for the bath salts. Scrambling was eliminated with selection of a different transition.

Scrambling Comparison using Xevo G2 Q-Tof

Compound	Label	Polarity	Collision Energy	Transition(s) d_n	Scrambling % d_{n-1}/d_n
JWH-018 4-Hydroxypentyl metabolite	D_5	pos	20	363→345	22.83
	Native	pos	20	358→340	0
JWH-018 4-Hydroxypentyl metabolite	D_5	pos	20	363→155	0
	Native	pos	20	358→155	0
JWH-073 3-Hydroxybutyl metabolite	D_5	pos	20	349→221	25.44
	Native	pos	20	344→216	0
JWH-073 3-Hydroxybutyl metabolite	D_5	pos	20	363→155	0
	Native	pos	20	358→155	0
3,4-MDPV HCl	D_8	pos	15	284→134	0
	Native	pos	15	284→126	0
Ethylone HCl	D_5	pos	15	227→209	0
	Native	pos	15	222→204	0
Butylone HCl	D_3	pos	15	225→209	0
	Native	pos	15	222→204	0
Mephedrone HCl	D_3	pos	10	181→163	0
	Native	pos	10	178→160	0
Methylone HCl	D_3	pos	10	211→163	0
	Native	pos	10	208→160	0
Methylone HCl	D_3	pos	10	211→135	0
	Native	pos	10	208→132	0

CONCLUSIONS

- TFAA derivatization is an effective method for analysis of bath salt cathinones by GC/MS. The derivatization method using TFAA prevented loss of label in the GC/MS fragmentation of deuterium-labeled bath salts.
- Increased mass spectral abundances for the molecular and fragment ions were also observed using TFAA derivatization. Butylone and ethylone are readily distinguished by the different relative abundance of two common fragment ions.
- Scrambling was observed at select transitions in the LC-MS/MS analysis of deuterium-labeled omega-1 hydroxy Spice metabolites. In each case, scrambling was eliminated by optimizing instrument conditions and transition selection.
- Scrambling may be mitigated or eliminated by altering instrument conditions and transition selection. The impact of scrambling must be considered in choice of transitions for optimal selectivity.
- Direct infusion can provide rapid and accurate determination of scrambling ratios.
- Awareness of potential scrambling is important for proper internal standard selection.

Deuterium-labeled internal standards of bath salt cathinones and Spice cannabinoid metabolites are suitable for GC/MS and LC/MS applications when consideration is given to choice of derivatization reagent and transition selection.

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Analytical Reference Standards
a SIGMA-ALDRICH company