

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

Joshua Cooper, Ryan Carrell, Ning Chang, Yunming Ying, Greg Kirkovits, Derrell Johnson, Isil Dilek, Uma Sreenivasan

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 PFFA 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-D₈ HCl, Cerilliant Cat# M-150
- Ethylone HCl, Cerilliant Cat# E-071
- Ethylone-D₅ HCl, Cerilliant Cat# E-072
- Butylone HCl, Cerilliant Cat# B-045
- Butylone-D₃ HCl, Cerilliant Cat# B-046
- Mephedrone HCl, Cerilliant Cat# M-138
- Mephedrone-D₃ HCl, Cerilliant Cat# M-139
- Methylone HCl, Cerilliant Cat# M-140
- Methylone-D₃ 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-D₅, Cerilliant Cat# S-039
- JWH-073 3-Hydroxybutyl metabolite, Cerilliant Cat# S-037
- JWH-073 3-Hydroxybutyl metabolite-D₅, Cerilliant Cat# S-040

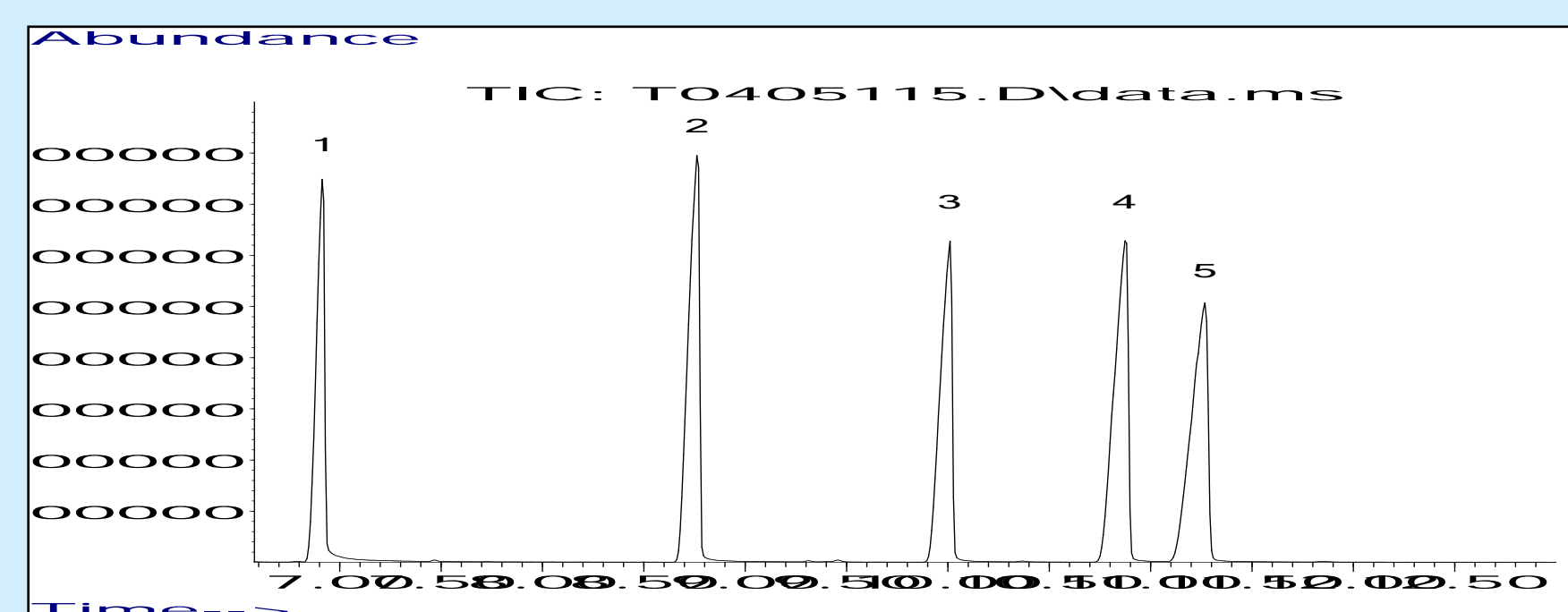
GC/MS Derivatization Method

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.



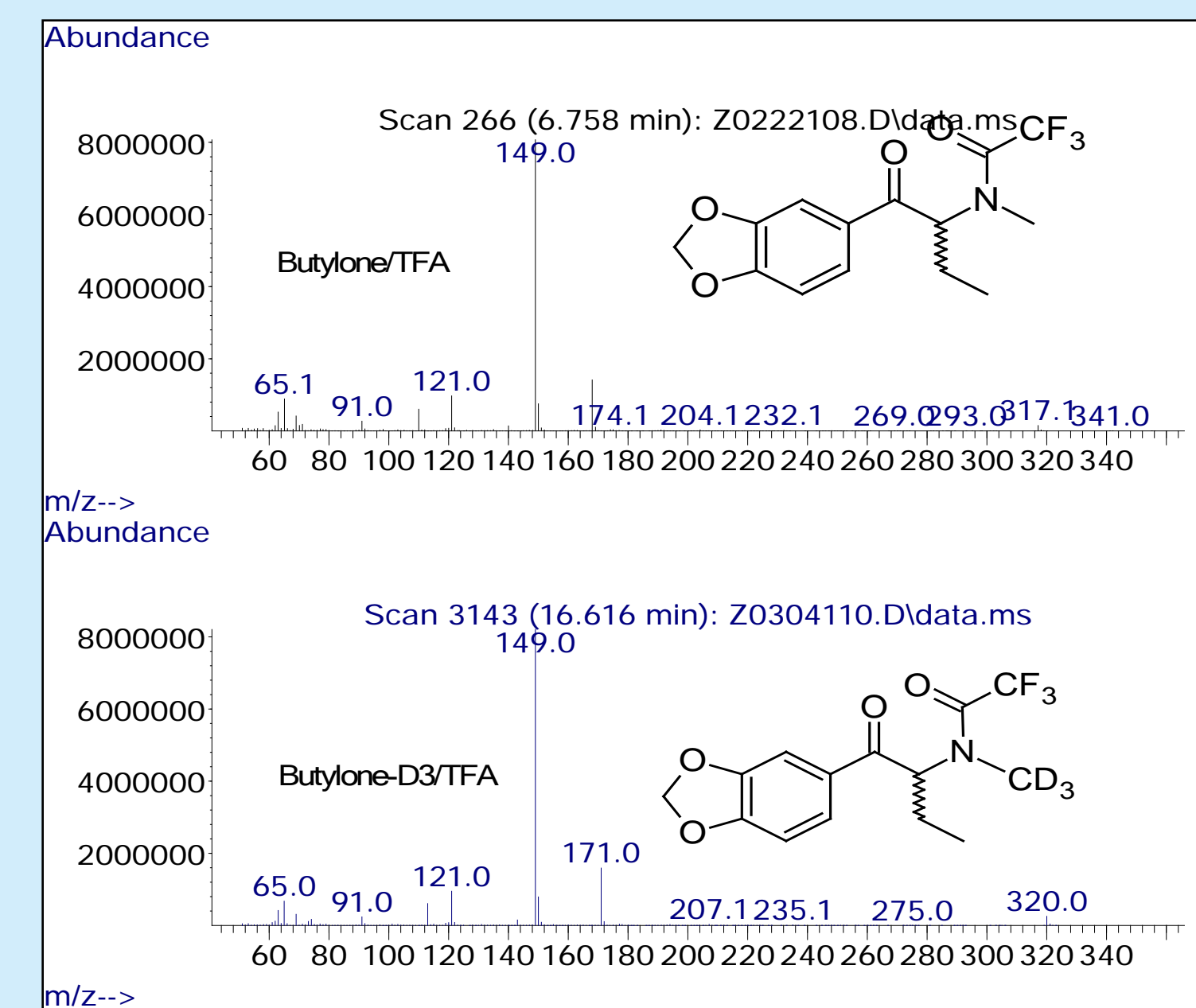
Compound	Peak Width	Resolution	Tailing	RRT vs. Methylone
1 Mephedrone	0.046	NA	0.67	0.691
2 Methedrone	0.063	20.08	0.64	0.876
3 Methylone	0.065	11.50	0.63	1.000
4 Butylone	0.072	7.47	0.63	1.087
5 Ethylone	0.080	3.03	0.63	1.126

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

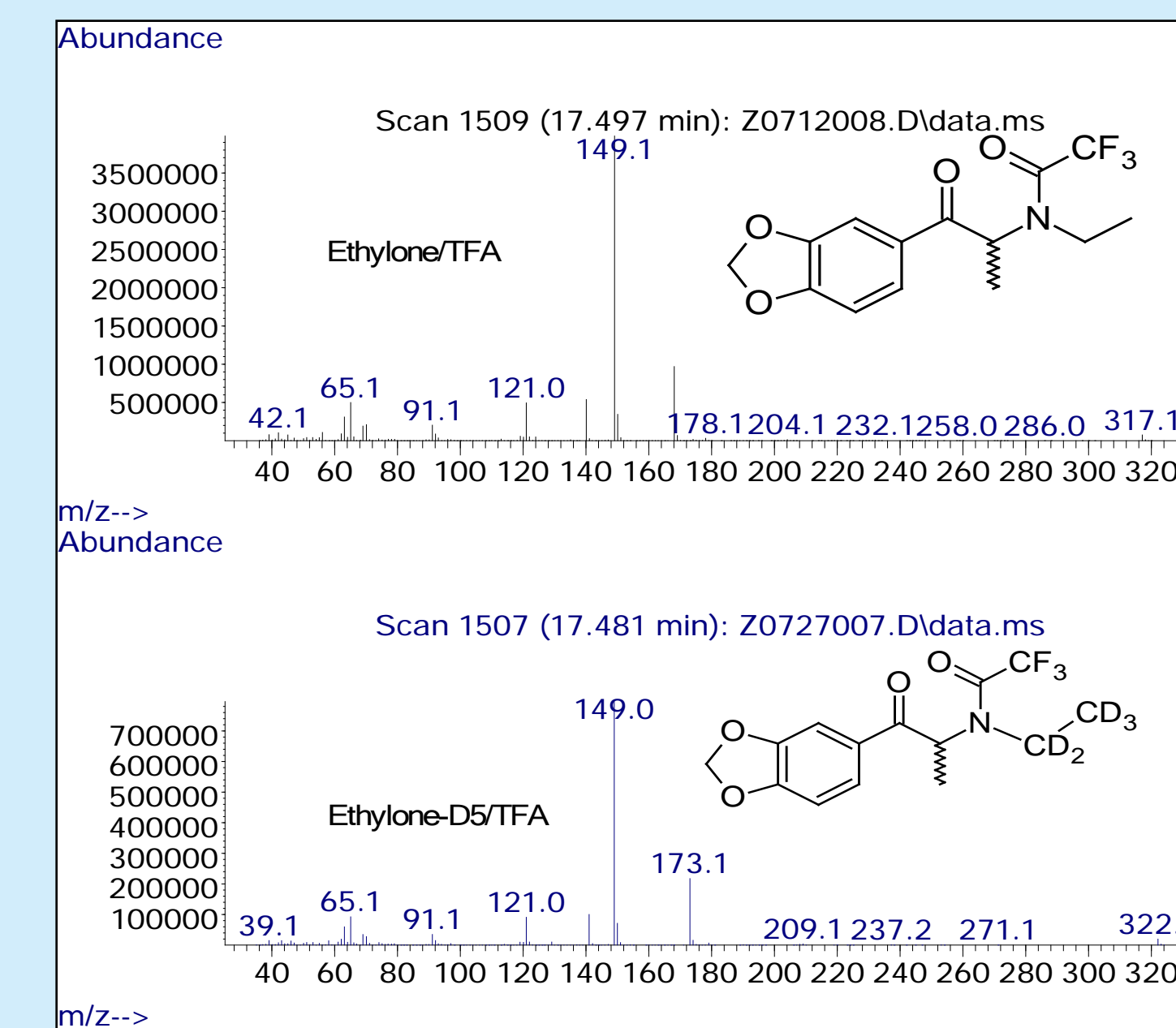
The labeled compounds retain deuterium label from the molecular ion to one or two fragmentations. Quant ion pairs were selected based on ion abundance. Isotopic distribution was evaluated to ensure the majority of the label was on the quant ion.

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

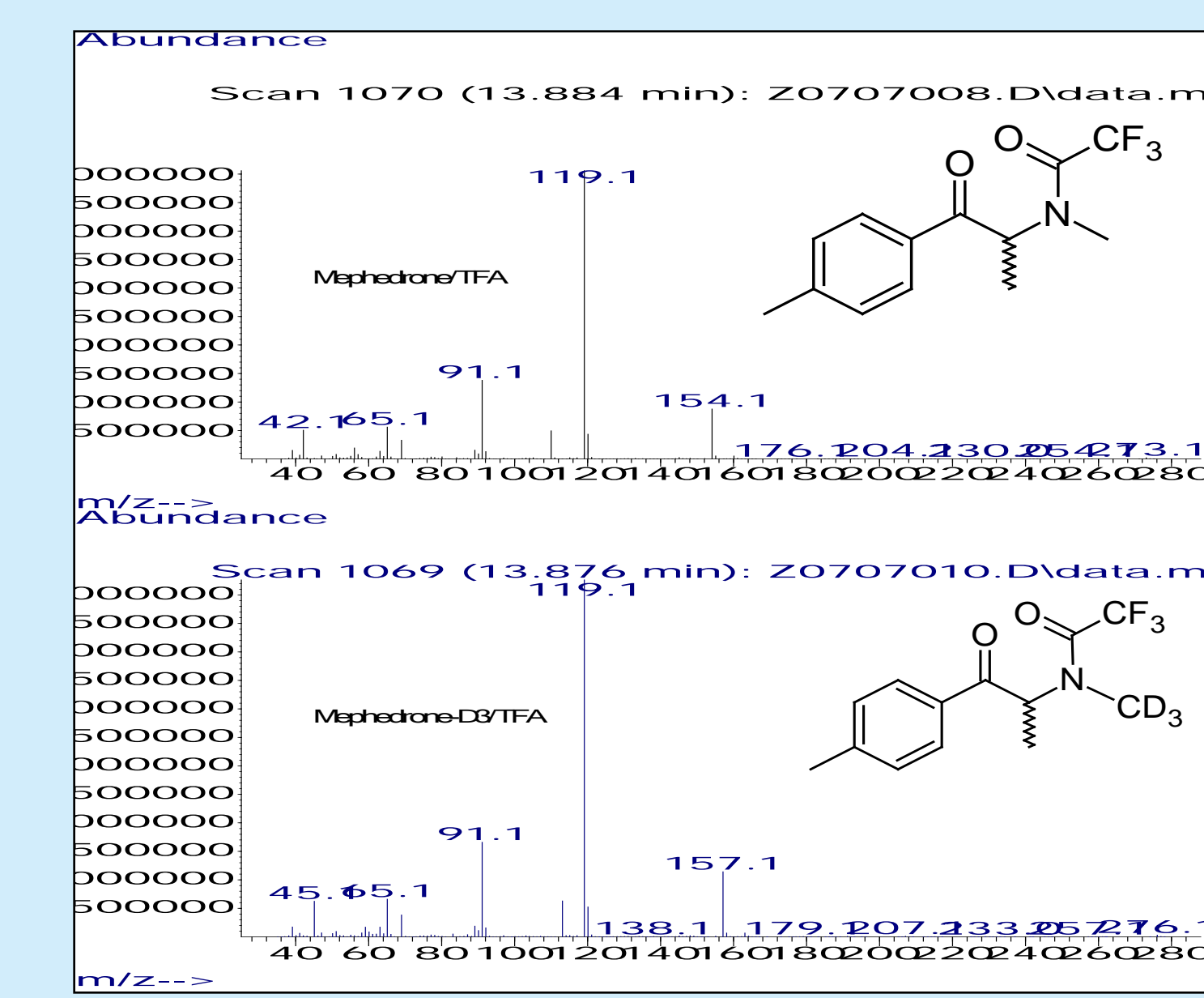
For Methedrone and Mephedrone, the molecular ion abundance is low. The fragment ion is used for quantitation.



MW Pair	Butylone-D ₃ HCl	
	Q1 Pair	Q2 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%



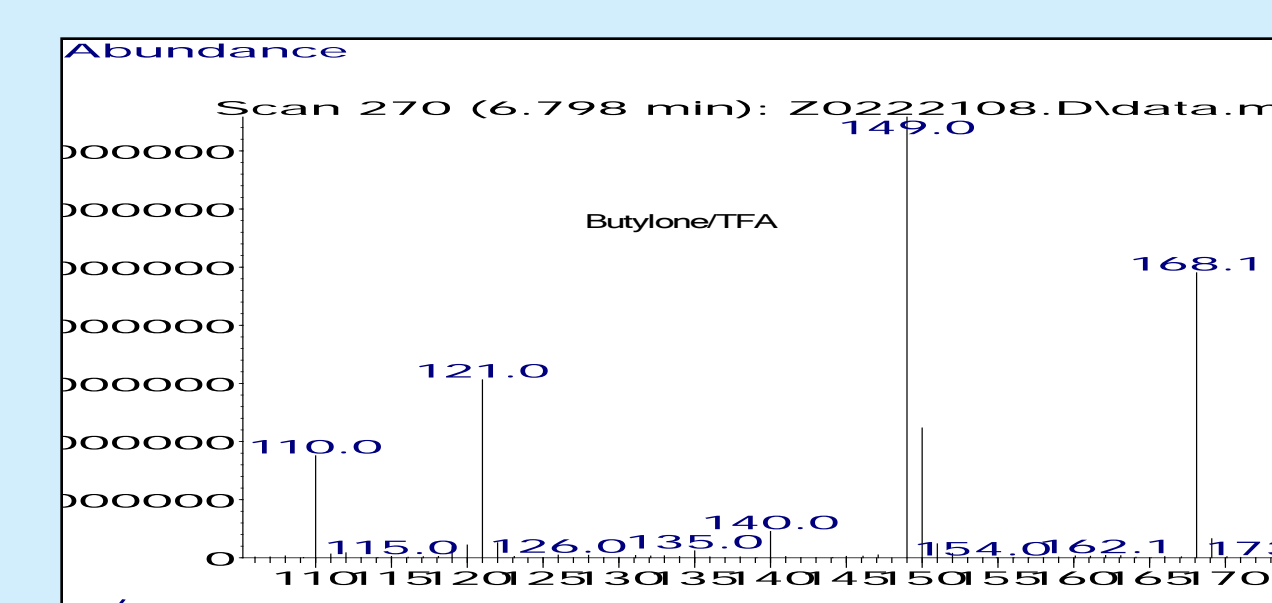
MW Pair	Ethylone-D ₅ HCl	
	Q1 Pair	Q2 Pair
D ₅	96.69%	97.65%
D ₄	3.09%	2.02%
D ₃	0.15%	0.21%
D ₂	0.06%	0.04%
D ₁	0.01%	0.03%
D ₀	0.01%	0.05%
D ₀ /D ₅	0.01%	0.04%



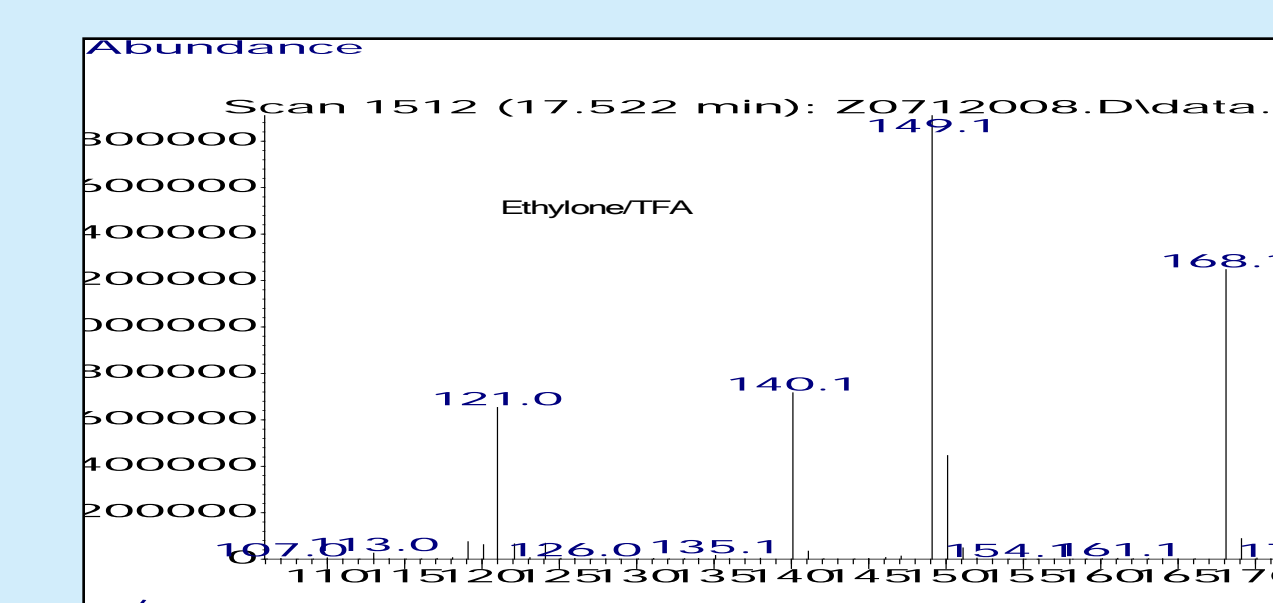
Q1 Pair	Mephedrone-D ₃ HCl	
	Q2 Pair	
D ₃	97.51%	98.74%
D ₂	1.62%	0.81%
D ₁	0.84%	0.21%
D ₀	0.03%	0.24%
D ₀ /D ₃	0.03%	0.24%

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

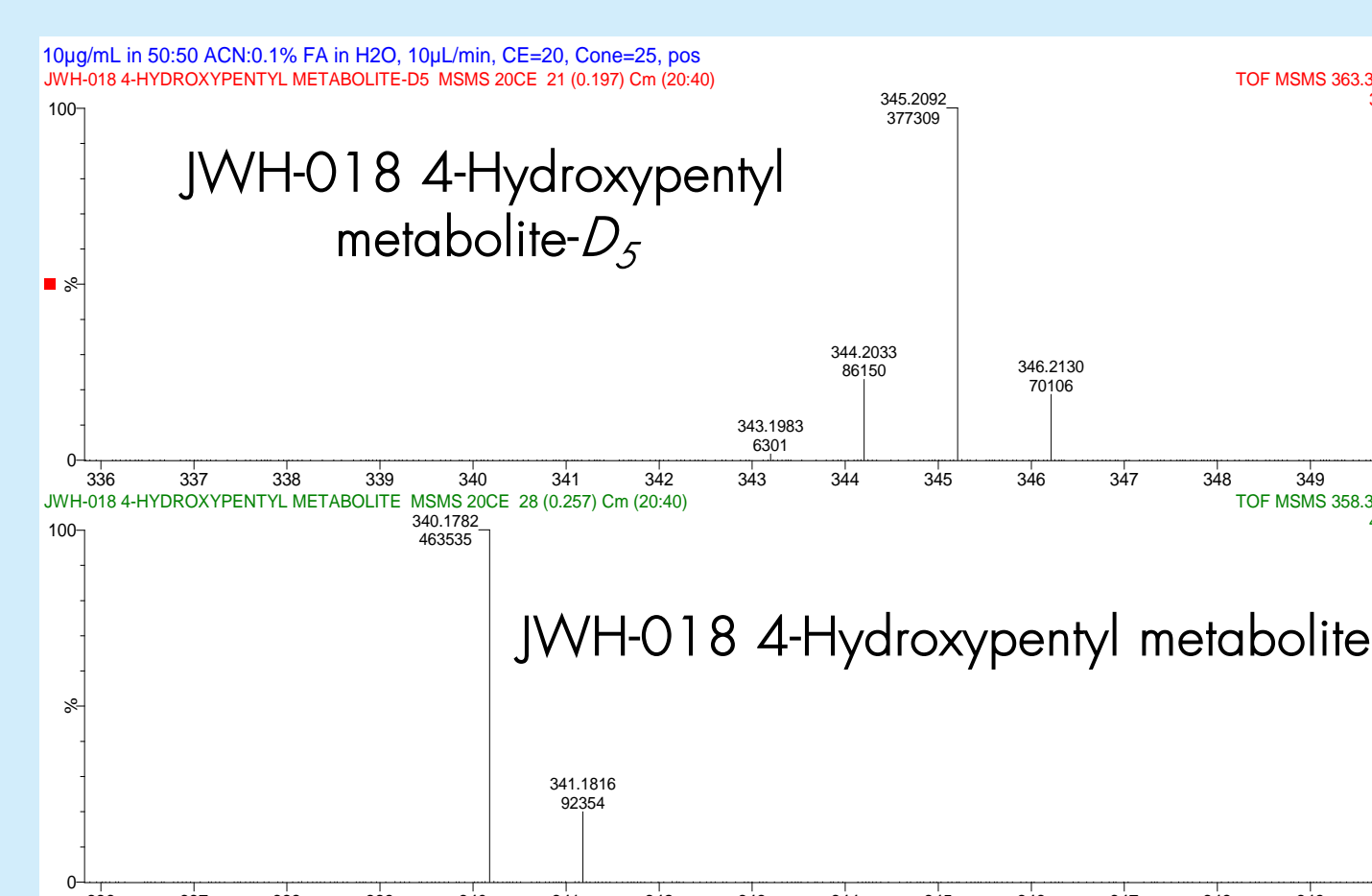
Ethylone and Butylone have identical molecular and primary ion fragmentations of 168 and 149. Differentiation between Ethylone and Butylone is achieved by monitoring the response of fragment ions 121/110 and 140/121.

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

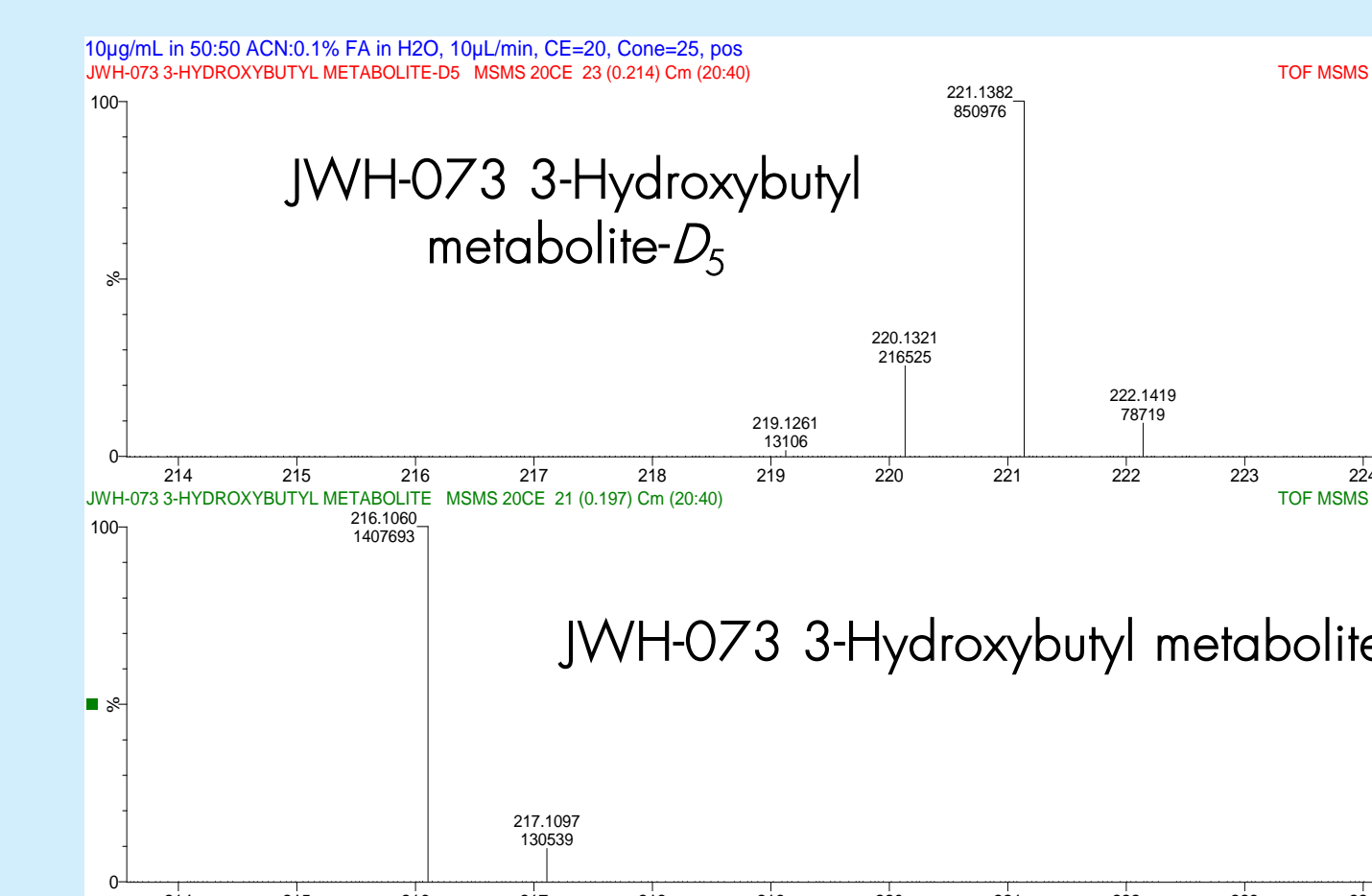
In this study, we investigated several variables that potentially contribute to scrambling in LC-MS/MS applications in order to ascertain reproducibility and impact on scrambling ratios: collision energies and deuterium placement in the internal standard.

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.

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.

