

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

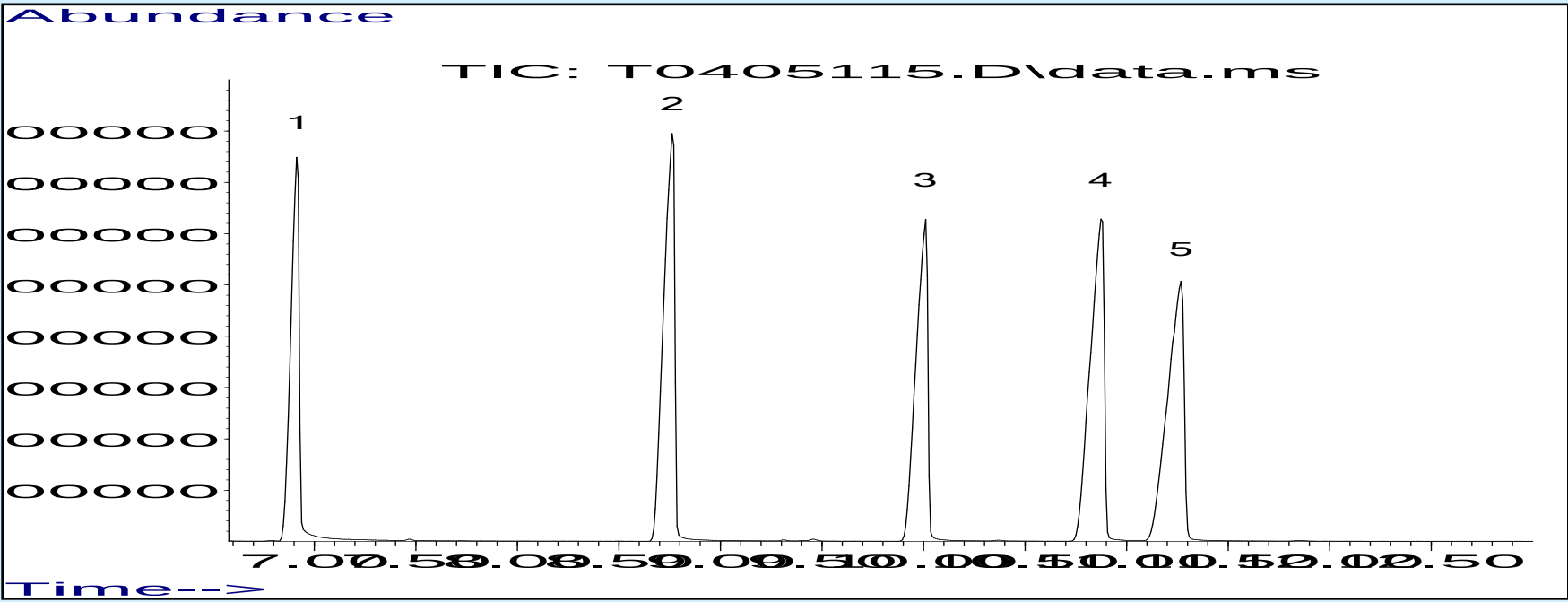
- Materials:**
3,4-MDPV HCl, Cerilliant Cat# M-146
3,4-MDPV-D₈ HCl, Cerilliant Cat# M-150
Ethylone HCl, Cerilliant Cat# E-071
Ethylone-D₅ HCl, Cerilliant Cat# E-072
Butylone HCl, Cerilliant Cat# B-045
Butylone-D₃ HCl, Cerilliant Cat# B-046
Mephedrone HCl, Cerilliant Cat# M-138
Mephedrone-D₃ HCl, Cerilliant 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.

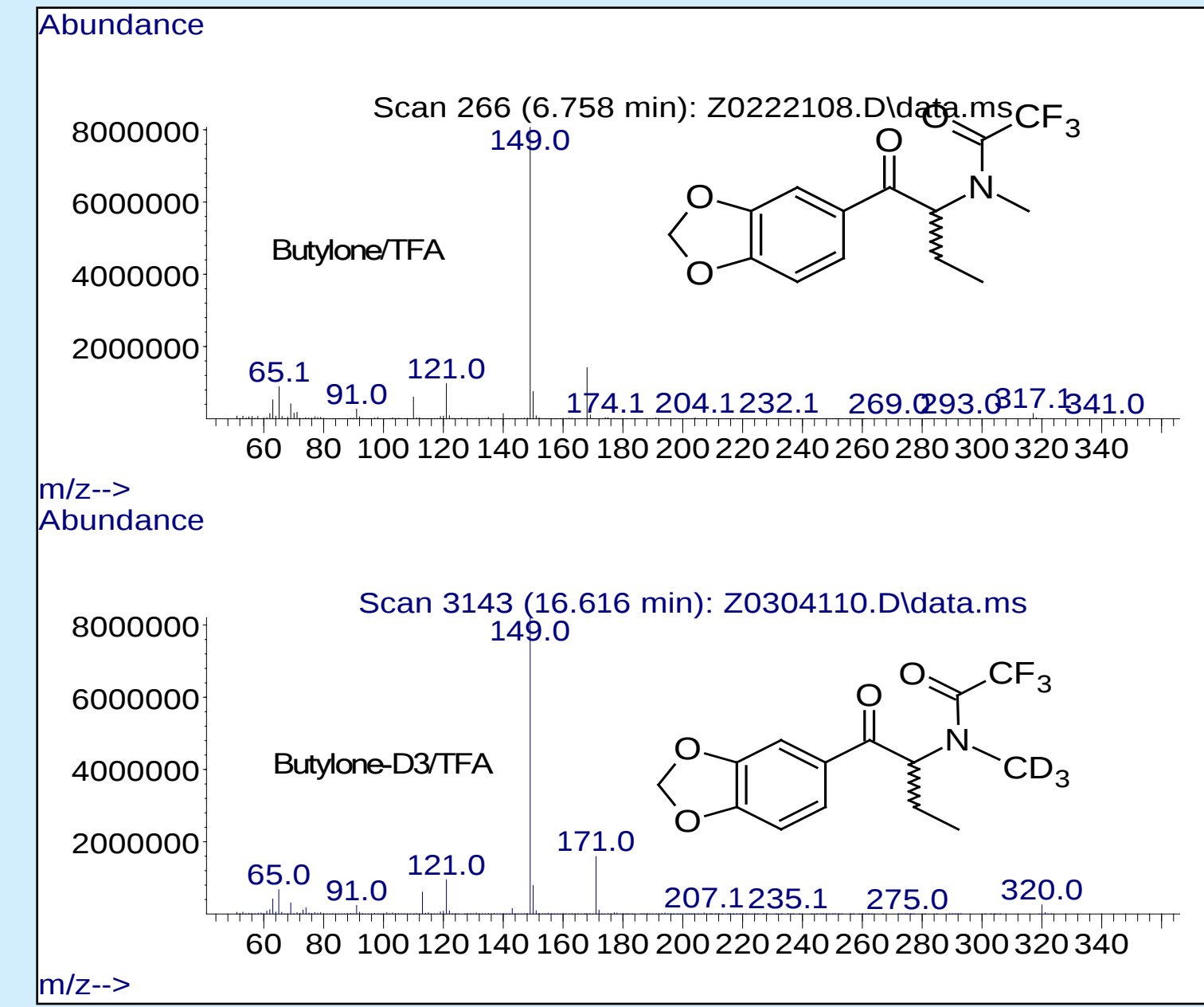
Temperature ramp: 3 min at 150°C, 150°C to 200°C at 10°C/min, 200°C to 210°C at 2°C/min.



	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

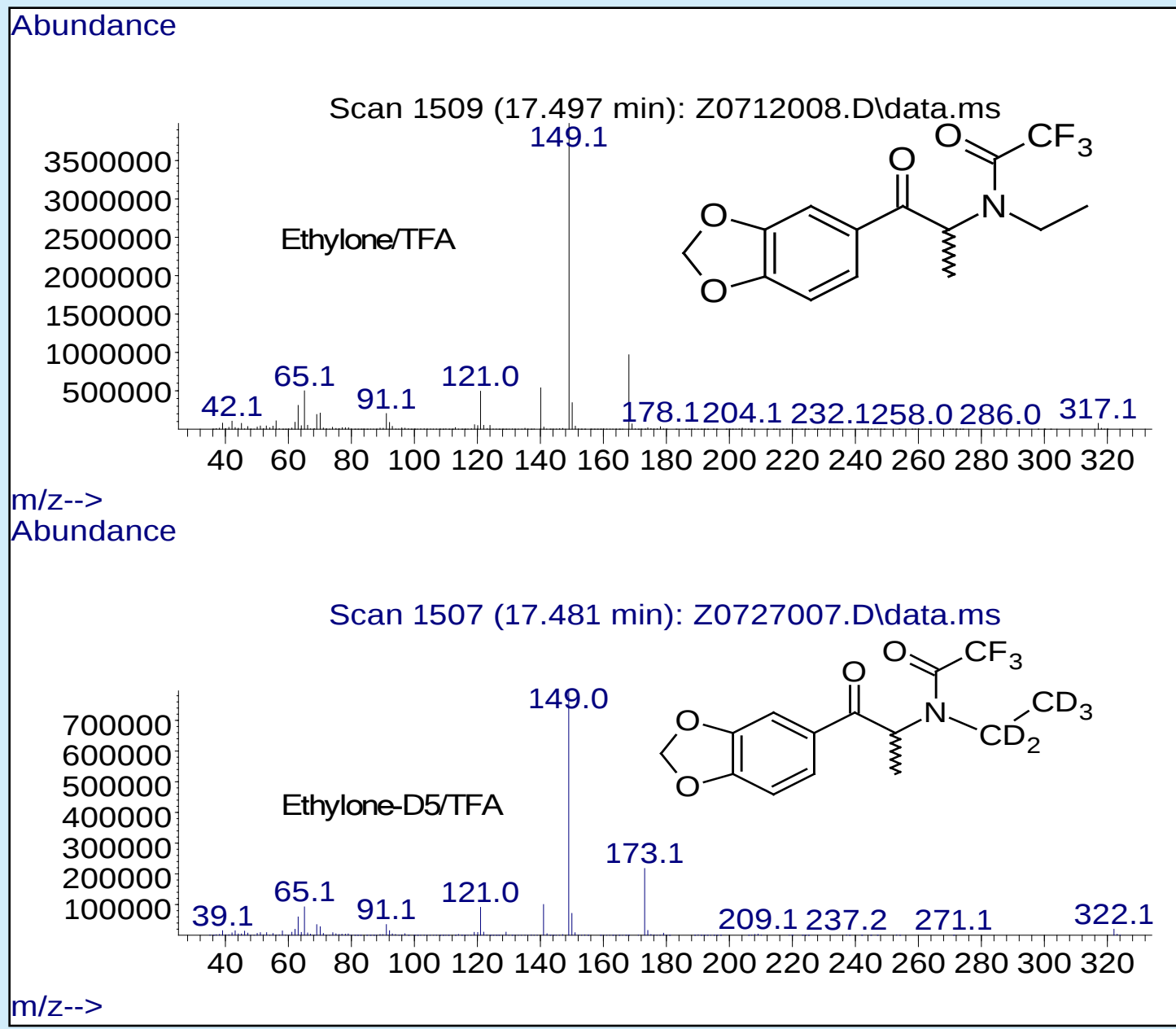
GC MASS SPECTRA AND ISOTOPIC DISTRIBUTION OF TFA-DERIVATIZED BATH SALTS

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.



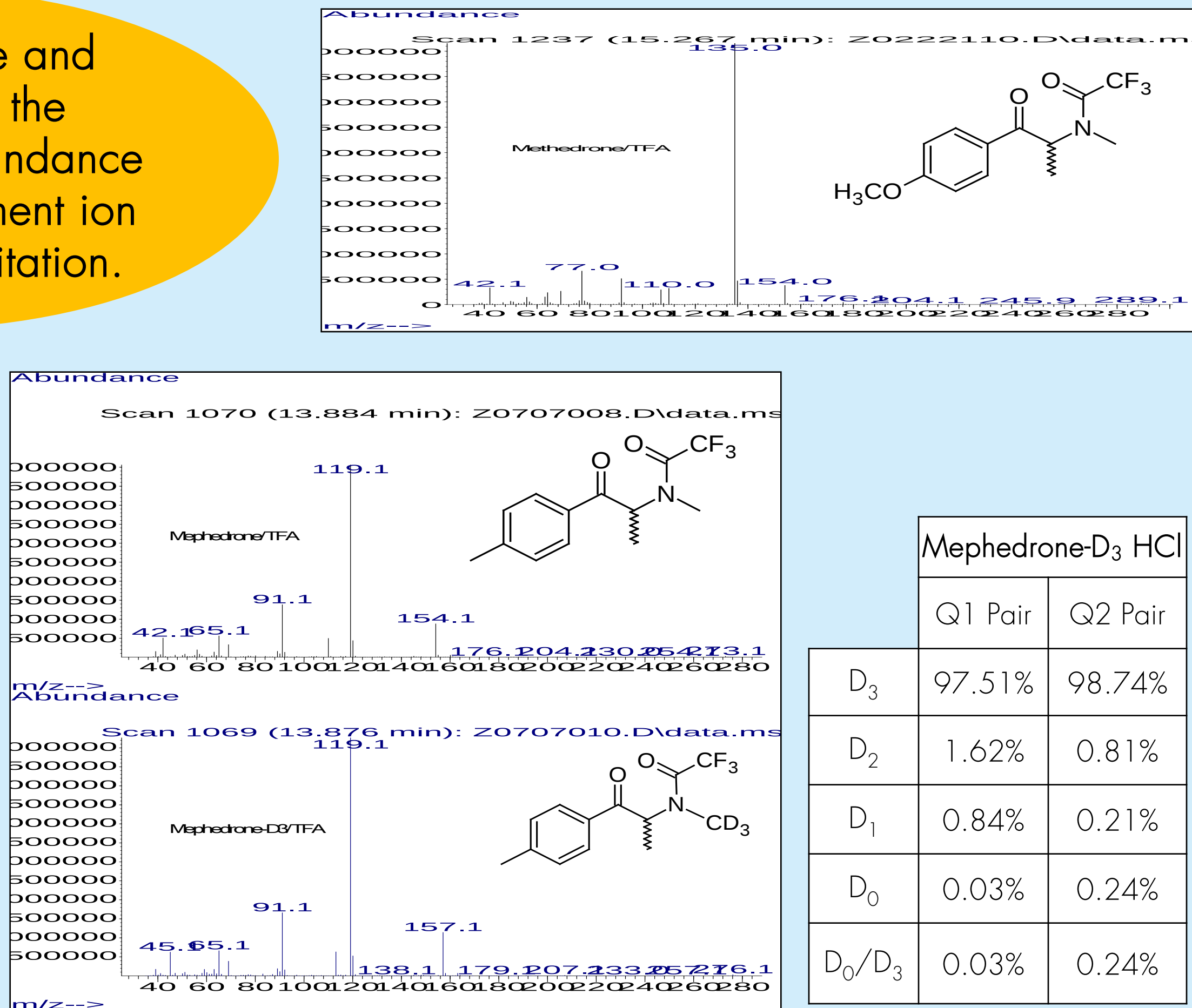
Compound (TFA)	MW Pair	Q1 Pair	Q2 Pair
Mephedrone / Mephedrone-D ₃ HCl	273.1 / 276.1	154.1 / 157.1	110.1 / 113.1
Methylone / Methylone-D ₃ HCl	303.1 / 306.1	154.1 / 157.1	NA
Butylone / Butylone-D ₃ HCl	317.1 / 320.1	168.0 / 171.0	110.0 / 113.0
Ethylone / Ethylone-D ₅ HCl	317.1 / 322.1	168.0 / 173.0	NA

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%



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

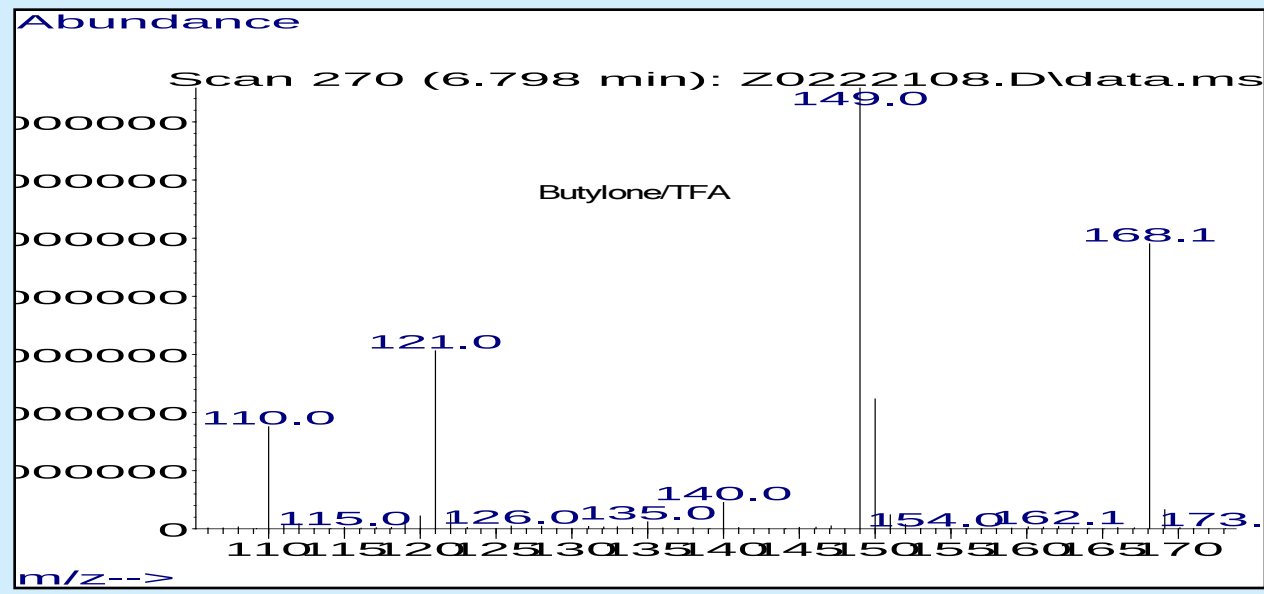
Ethylone-D ₅ HCl	
MW Pair	Q1 Pair
D ₅	96.69% / 97.65%
D ₄	3.09% / 2.02%
D ₃	0.15% / 0.21%
D ₂	0.06% / 0.04%
D ₁	0.01% / 0.03%
D ₀	0.01% / 0.05%
D ₀ /D ₅	0.01% / 0.04%



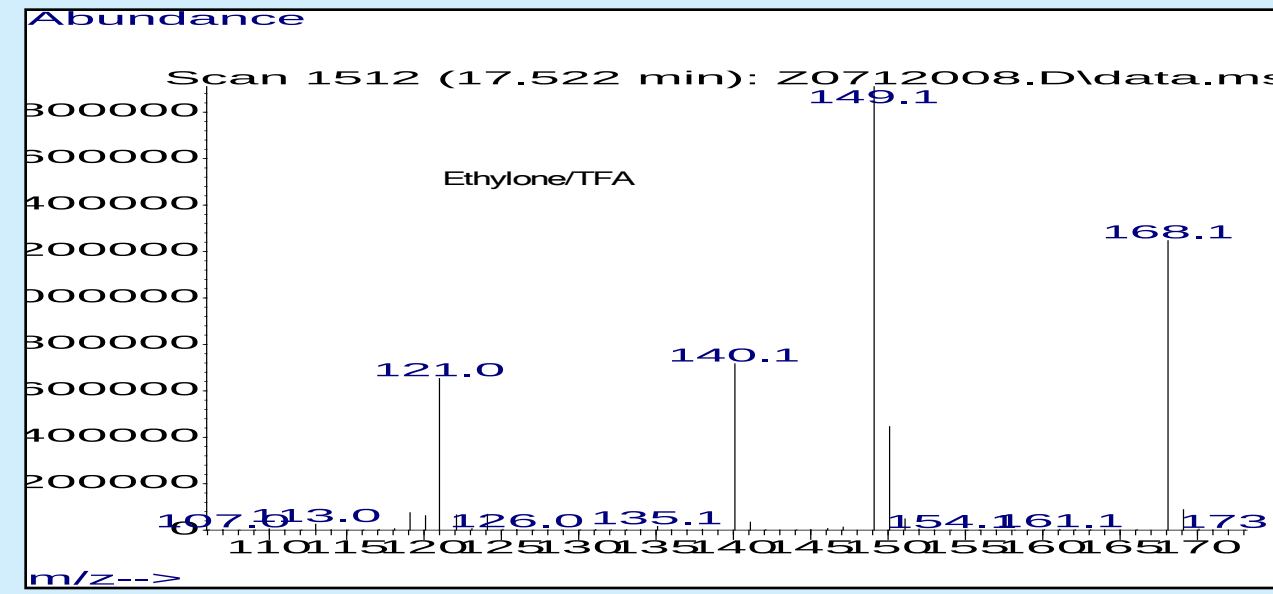
Mephedrone-D ₃ HCl	
Q1 Pair	Q2 Pair
D ₃	97.51% / 98.74%
D ₂	1.62% / 0.81%
D ₁	0.84% / 0.21%
D ₀	0.03% / 0.24%
D ₀ /D ₃	0.03% / 0.24%

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

