Improved GC/MS Derivatization Techniques for Analysis of New Designer Drugs: Methylone, Ethylone, Butylone, Mephedrone, and Methedrone

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INTRODUCTION

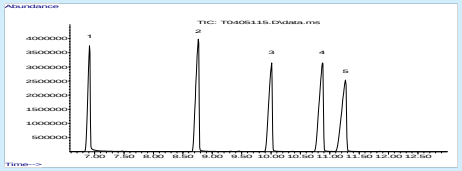
As new "bath salt" drugs such as methylone, ethylone, mephedrone, butylone and other methcathinone analogs increase in popularity, toxicologists require native and labeled certified reference materials to accurately identify and quantify the new compounds in patient samples. Internal standards must have quantitation ions that do not interfere with native quantitation ions. There have been problems with use of PFPA and BSTFA derivatives of deuterated internal standards of these methcathinones due to loss of label in the GC/MS fragmentation. An alternate derivatization method is presented in this study.

DERIVATIVE METHOD

Native and deuterated reference materials of methylone, ethylone, butylone, mephedrone, and methedrone were synthesized at Cerilliant as HCl salts and 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 at 60°C for five minutes with TFAA and ethyl acetate to acyalte the amino group. The free up procedure is sensitive to choice of base due to instability of α-amino ketones. Derivatization time is critical, as decomposition occurs with excessive heating.

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.

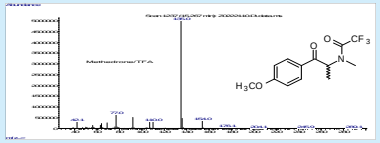


	Compound	Peak Width	Resolution	Tailing	RRT vs. Methylone
1	Mephedrone	0.046	NA	0.67	0.691
2	Methedrone	0.063	20.08	0.64	0.876
3	Methylone	0.065	11.50	0.63	1.000
4	Butylone	0.072	7.47	0.63	1.087
5	Ethylone	0.080	3.03	0.63	1.126

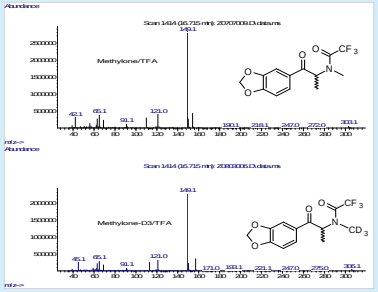
MASS SPECTRA AND ISOTOPIC DISTRIBUTION

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.

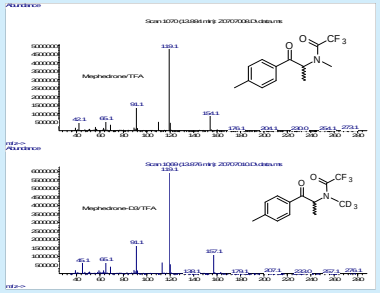
Compound	MW Pair	Q1 Pair	Q2 Pair
Mephedrone / Mephedrone-D_3 HCl	273.1 / 276.1	154.1 / 157.1	110.1 / 113.1
Methylone / Methylone-D_3 HCl	303.1 / 306.1	154.1 / 157.1	NA
Butylone / Butylone-D_3 HCl	317.1 / 320.1	168.0 / 171.0	110.0 / 113.0
Ethylone / Ethylone-D_5 HCl	317.1 / 322.1	168.0 / 173.0	NA



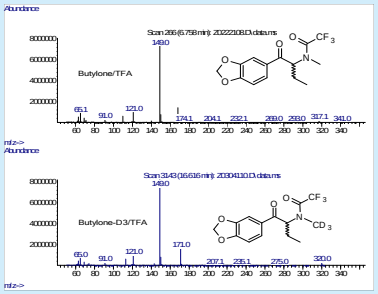
For Methedrone and Mephedrone, the molecular ion abundance is low. The fragment ion is used for quantiation.



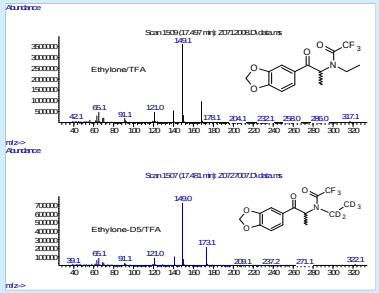
	Methylone-D_3 HCl	
	MW Pair	Q1 Pair
D_3	98.01%	98.42%
D_2	1.84%	1.04%
D_1	0.10%	0.51%
D_0	0.05%	0.03%
D_0/D_3	0.05%	0.03%



	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%



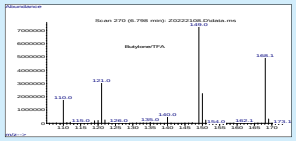
	Butylone-D_3 HCl	
	MW Pair	Q1 Pair
D_3	98.96%	99.16%
D_2	0.97%	0.39%
D_1	0.04%	0.42%
D_0	0.02%	0.02%
D_0/D_3	0.03%	0.02%



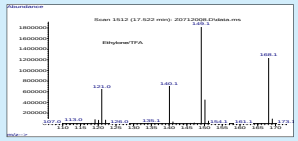
	Ethylone-D_5 HCl	
	MW Pair	Q1 Pair
D_5	96.69%	97.65%
D_4	3.09%	2.02%
D_3	0.15%	0.21%
D_2	0.06%	0.04%
D_1	0.01%	0.03%
D_0	0.01%	0.05%
D_0/D_5	0.01%	0.04%

BUTYLONE /ETHYLONE COMPARISON

Ethylone and butylone are distinguished by the response of fragment ions 110 and 140 relative to 121 amu.



Butylone Expansion



Ethylone Expansion

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

ADVANTAGES OF TFAA AS DERIVATIZING REAGENT

- Thermally stable, repeatable derivatization.
- Increased mass spectral abundances for molecular and fragment ions.
- Labeled analogs retain deuterium label in the fragment ions.
- The deuterium labeled analogs are suitable for use as internal standards to quantitate the native compounds.
- Butylone and ethylone are readily distinguished by the different relative abundance of two common fragment ions.

CONCLUSIONS

- Deuterium labeled internal standards were developed for quantitation of new methcathinone analogs, "bath salts". These standards are suitable for both GC/MS and LC/MS applications.
- A method of derivatization of these analogs with TFAA was successfully developed with retention of label in the GC/MS fragment ions.

Cerilliant
Analytical Reference Standards
a SIGMA-ALDRICH company