Identification of Novel Synthetic Benzodiazepines by GC-MS

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INTRODUCTION

With the rise of novel psychoactive substances, there is also an increase in abuse of novel synthetic benzodiazepines. However, detection of these benzodiazepines can be problematic due to lack of sensitivity with immunoassays and routine screens by gas chromatography-mass spectrometry (GC-MS). Numerous screening methods by liquid chromatography (LC) have been developed for synthetic benzodiazepines, but detection by GC-MS has yet to be fully evaluated. Novel synthetic benzodiazepines have been reported in several fatalities, both alone and in combination with other drugs of abuse.

Objective

The purpose of this study was to develop a targeted GC-MS screening method for clonazolam, delorazepam, diclazepam, etizolam, flubromazepam, flubromazolam, and pyrazolam in blood. Studies were also performed to compare the optimized method to a routine, full-scan screening method.

MATERIALS AND METHODS

Preparation of Standards

Standards were purchased from Cayman Chemical (Ann Arbor, MI) and Cerilliant (Round Rock, TX). Dilutions and mixed standards were prepared in methanol as needed. All solutions were stored in amber vials at -20°C.

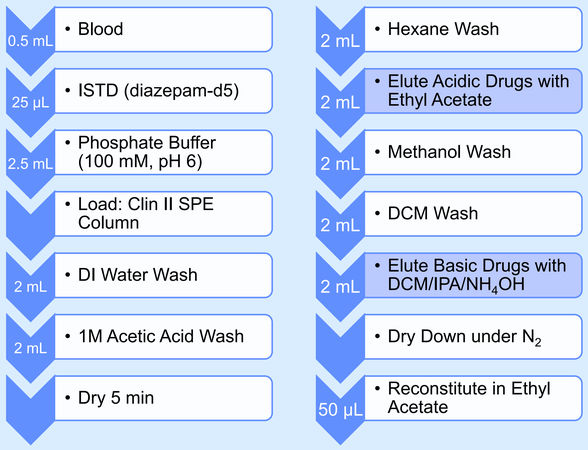

Figure 1: Solid-Phase Extraction Protocol.

RESULTS AND DISCUSSION

Table 1: Elution fraction, pKa, ions selected for the optimized SIM method, and limit of detection (LOD) for the analytes of interest.

Analyte	Benzodiazepine	Elution Fraction	pKa	Ions for SIM (m/z)	LOD (ng/mL)	Recovery (%)
1	Diclazepam	A/N & Basic	2.13	255, 283, 318	50	58.9
2	Flubromazepam	A/N & Basic	1.82, 2.88	223, 305, 333	50	77.0
3	Delorazepam	Basic	2.05, 12.29	177, 275, 304	50	78.2
4	Flubromazolam	Basic	4.01	222, 343, 372	50	59.7
5	Etizolam	Basic	4.55	266, 313, 342	50	62.5
6	Pyrazolam	Basic	2.75	206, 274, 353	100	60.9
7	Clonazolam	Basic	4.12	203, 249, 324	100	43.5
ISTD	Diazepam-d5	A/N & Basic	3.40	226, 261, 287		80.0

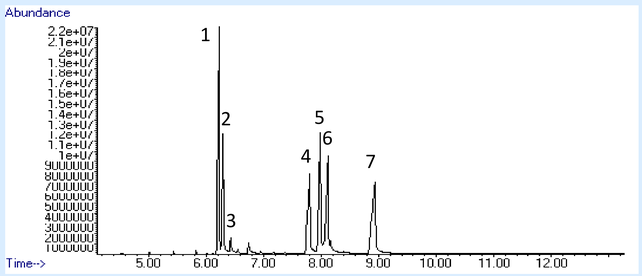

Figure 2: Total Ion Chromatogram of Optimized SIM Method on GC-MS with DB-5MS column.

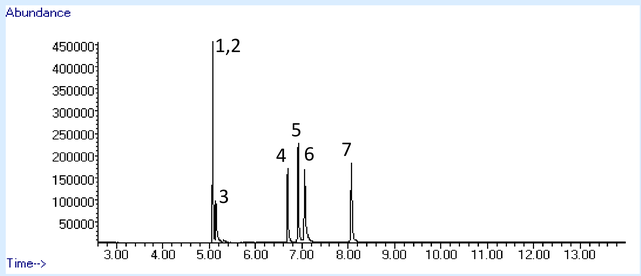

Figure 3: Total Ion Chromatogram of Full-Scan Method on GC-MS with DB-5MS column.

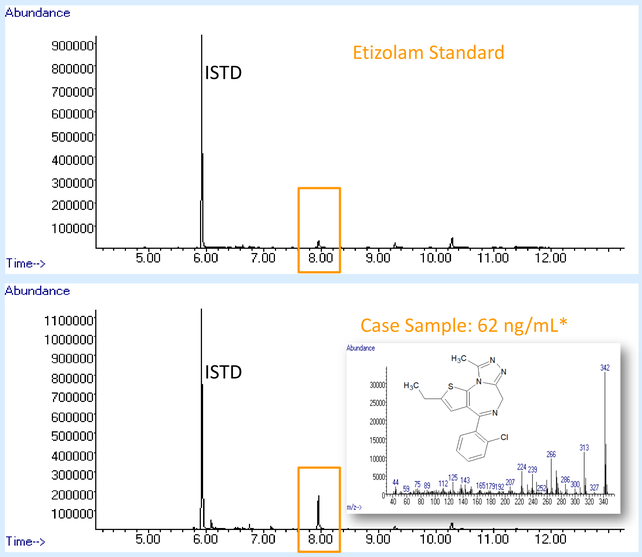

Figure 4: Comparison of the Total Ion Chromatograms between etizolam standard in blood (top) to case sample (bottom) using the Optimized SIM Method where etizolam was detected (boxed). *Reported by submitting laboratory.

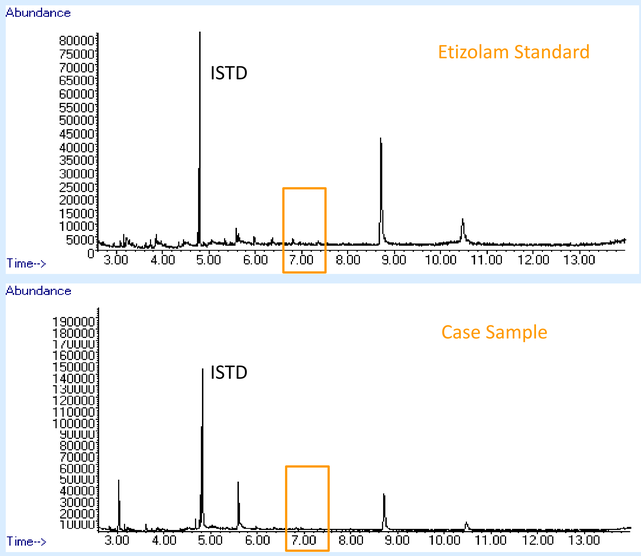

Figure 5: Comparison of the Total Ion Chromatograms between etizolam standard in blood (top) to case sample (bottom) using the Full-Scan Method where etizolam was not detected. A box depicts expected retention time.

ACKNOWLEDGEMENTS

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MATERIALS AND METHODS

Gas Chromatography-Mass Spectrometry

GC-MS analysis was performed on an Agilent 5975C MS/7890A GC (Santa Clara, CA) equipped with an Agilent DB-5MS (30 m x 0.25 mm x 0.10 µm) column. An optimized single ion monitoring (SIM) method was developed and compared to a full-scan screening technique:

Table 2: GC-MS parameters for routine, full-scan screen and optimized synthetic benzodiazepine screen.

Parameter	Optimized SIM Method	Full Scan Screen
Oven Temperature Ramp	160°C for 2 min; 30°C/min to 280°C; 6°C/min to 310°C	160°C for 0.5 min; 30°C/min to 290°C
Inlet Mode	Pulsed Splitless	Split (10:1)
Initial Flow	2 mL/min	1.3 mL/min
Run Time	13.3 min	14 min
Acquisition Method	SIM/Scan	Full Scan

Authentic Sample Analysis

A postmortem blood sample was extracted and analyzed with the targeted SIM and full-scan screening methods. Central blood (0.5 mL) was fortified with ISTD, extracted as described above, and analyzed.

CONCLUSIONS

- Due to low pKa values, some benzodiazepines may elute in the acidic/neutral fraction and could be lost if not collected in SPE.
- Routine, full-scan screening methods may not fully separate all benzodiazepines.
- Targeted SIM methods may be necessary to detect low concentrations of synthetic benzodiazepines missed in full-scan.
- Despite method optimization, detection of synthetic benzodiazepines may warrant more sensitive instrumentation (LC-MS).

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