GC-MS/MS ANALYSIS OF BENZODIAZEPINES USING ANALYTE PROTECTANTS

Jeremy Matthews[1], Alex Chen[2], Flavio Bedini[1]
[1]Thermo Fisher Scientific, Singapore, [2]Alpha Analytical Pte Ltd, Singapore

Overview

Purpose: Assess the feasibility of using analyte protectants to improve the analysis of benzodiazepines.

Methods: Benzodiazepines were analysed using the TSQ 8000 triple-quadrupole GC-MS/MS system. The mass spectrometer was run in EI mode using 3 SRM transitions per analyte.

Results: The calibration linearity of the benzodiazepines varied significantly between compounds; diazepam and nitrazepam showed excellent linearity while lorazepam and lectopam showed very poor response. Sorbitol was used as analyte protectant to significantly improve the calibration behaviour of the more challenging analytes.

Introduction

Benzodiazepines drugs are psychoactives whose structure contains a benzene ring fused with a diazepine ring. One of the most well known benzodiazepines – diazepam – has been marketed under the name “Valium” since 1963. These drugs are effective tranquilizers and as such are commonly used in medication to treat anxiety and sleep disorders amongst other conditions. The relative availability of these drugs combined with their sedative effect has led to their illicit use as either recreational drug and sometimes in suicides. Consequently it is common practice to analyze for benzodiazepines in forensic and toxicology laboratories.

Benzodiazepines compounds are bases and so readily react with active sites in eg. the GC inlet liner causing problems in analysis at low levels and resulting in poor linearity and reproducibility. The use of analyte protectants reduces liner activity and often enables the detection of such ‘active’ compounds at much lower levels. The use of analyte protectants in the GCMS analysis of a group of benzodiazepines was investigated and the results are discussed below.

Methods

Analytes

The following benzodiazepines were analysed in this work: lorazepam, diazepam, lectopam, nitrazepam, clonazepam. Standards of these analytes were spiked into a matrix.

GCMS

All measurements were carried out using the Thermo Scientific™ TSQ 8000™ triple quadrupole GC-MS/MS system equipped with the Thermo Scientific™ TRACE™ 1310 GC with SSL Instant Connect™ SSL module and Thermo Scientific™ TriPlus™ RSH autosampler. The method details are given in Table 1 below.

TABLE 1. Instrument method for benzodiazepine analysis

TRACE 1310 GC	
Injection mode	splitless
Splitless Time	1.0 min
GC Column	Restek™ RTX™-5Sil MS, 15 m × 0.25 mm × 0.25 µm
Carrier gas	He （99.999 %）
Flow	1.2 mL/min, constant flow
Temperature program	50 °C, 2 min 20 °C/min to 300 °C, 2 min
Transfer line temperature	280 °C
Total analysis time	14.6 min
TriPlus RSH Autosampler	
Injection volume	1 µL
TSQ 8000 MS/MS	
Ionization mode	EI, 70 eV
Ion source temperature	250 °C
Scan mode	SRM using timed SRM
SRM transition setup	automatically build-up by AutoSRM software, transitions see Table 2

Results

AutoSRM study

Benzodiazepines were prepared from purchased standards and calibration solutions were prepared between 10 and 1000ppb. The 100ppb solution was then used for an AutoSRM study to determine the optimal SRM transitions and for each compound and thereby create the MS/MS method.

AutoSRM is a unique MS/MS method development tool included in the TSQ 8000 software suite which uses a vial containing a standard solution of the compounds required for the method, in the TriPlus RSH autosampler and automatically optimises retention time, precursor and product masses and collision energy for quan and confirming ions.

The AutoSRM study was completed in several hours with only minimal user interaction required and the optimised SRM transitions are shown in Table 2 below.

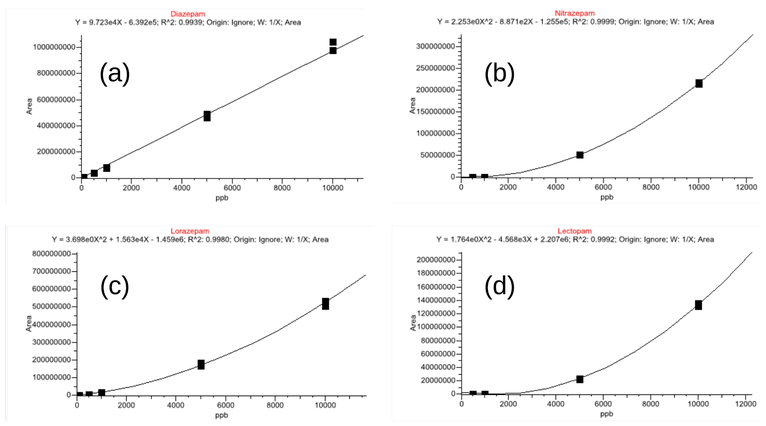
TABLE 1. Instrument method for benzodiazepine analysis

Compound	CAS Number	RT (min)	Precursor Mass (m/z)	Product Mass (m/z)	Collision Energy (eV)
Lorazepam	846-49-1	14.32	239.0	176.7	25
Lorazepam	846-49-1	14.32	274.0	239.1	10
Diazepam	439-14-5	14.45	256.1	165.1	30
Diazepam	439-14-5	14.45	256.1	221.1	10
Lectopam	1812-30-2	15.32	316.7	288.0	20
Lectopam	1812-30-2	15.32	316.7	289.1	10
Nitrazepam	146-22-5	16.02	280.3	205.1	30
Nitrazepam	146-22-5	16.02	280.3	234.1	10
Clonazepam	1622-61-3	16.51	280.0	234.1	10
Clonazepam	1622-61-3	16.51	314.0	268.1	15

Benzodiazepine Calibration Curves

The optimised MRM method created using AutoSRM (described above) was used to record calibration data for the benzodiazepines between 10 and 1000ppb. The linearity of the calibration curves varied widely with the particular analyte as shown in Figures 1 (a) – (d) below.

FIGURE 1. Calibration curves for (a) diazepam, (b) nitrazepam, (c) lorazepam and (d) lectopam at up to 10000 ppb.



The calibration curves for diazepam was very linear (R2 > 0.99) between 5 and 10000 ppb also exhibited excellent peak shape. The other benzodiazepines however strongly deviated from linearity and a quadratic was required to achieve a reasonable fit of the data (also for clonazepam, not shown). The peak areas for these compounds were also fairly low and showed poor signal-to-noise at relatively high concentrations.

The difficulties encountered in analysis of such polar compounds has been previously reported in the analysis of pesticides[1, 2, 3]. The high ‘activity’ of polar functional groups has been observed to lead to poor recoveries and significant loss of analytes at low concentration due to trapping and degradation at active sites in the gas flow path. The degradation of trapped compounds from previous injections can then form further active sites (increased surface area), which may rapidly lead to poor chromatographic response and performance.

It has been previously shown that the use of analyte protectants such as sorbitol is very effective in correcting this behaviour for eg. pesticide analysis whereby the protectant reacts with active sites in the GC flowpath effectively deactivating the system and enhancing analyte recovery, especially at low levels.

This has several advantages including improving peak shape (less tailing) and hence repeatability (RSDs) as well as keeping the inlet liner clean for longer durations as analyte and matrix are no longer trapped and subsequently degraded.

FIGURE 2. Chemical structure of the benzodiazepine compounds analysed in this work.

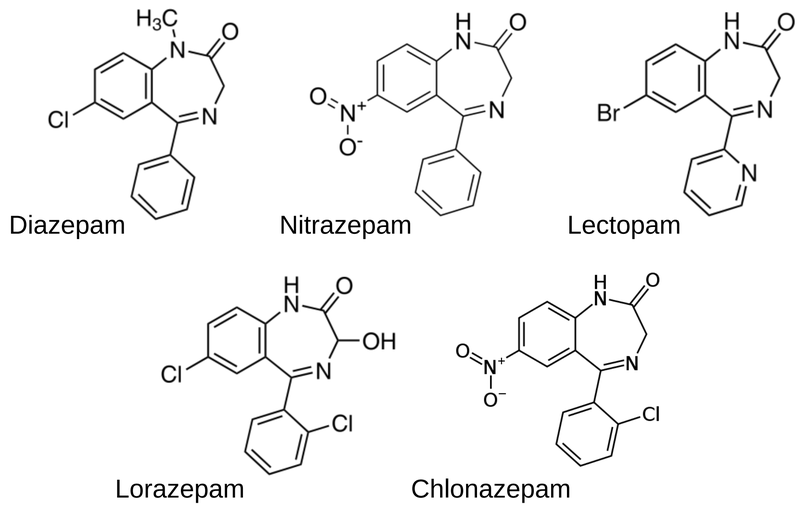
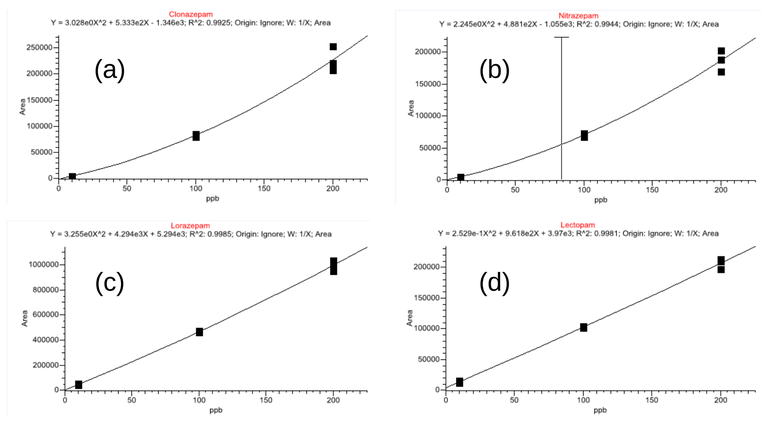


FIGURE 3. Calibration curves for (a) clonazepam, (b) nitrazepam, (c) lorazepam and (d) lectopam between *10 and 200 ppb* using 0.2 % sorbitol as analyte protectant.



Analyte protectant (0.2% sorbitol) was added to the benzodiazepine calibration standards and the calibration curves were measured as shown above in Figure 3. The calibration linearity improved dramatically for lorazepam, lectopam, clonazepam and nitrazepam with the sorbitol addition. The response factors for lectopam and lorazepam increased by roughly 16 and 7 times respectively indicating a dramatic improvement in sensitivity.

Conclusions

- Benzodiazepines are challenging to analyse by GC-MS/MS due to high activity of as a result of polar functional groups.
- Activity of inlet liner and GC column result in adsorption/degradation of some benzodiazepine analytes leading poor linearity of calibration and sensitivity.
- The use of sorbitol as analyte protectant dramatically improves the linearity of the response and signal intensity for these compounds.
- All benzodiazepines could be quantitated at 10ppb using analyte protectant.

References

1. Anastassiades, M., Maštovská, K., Lehotay, S.J., Evaluation of analyte protectants to improve gas chromatographic analysis of pesticides, J. Chromatogr. A 1015 (2003) 163-184.
2. Mastovska, K., Lehotay, S.J., Anastassiades, M., Combination of Analyte Protectants To Overcome Matrix Effects in Routine GC Analysis of Pesticide Residues in Food Matrixes, Anal. Chem. 77(24) (2005) 8129-8137.
3. Li, Y., Chen, X., Fan, C., Pang, G., Compensation for matrix effects in the gas chromatography–mass spectrometry analysis of 186 pesticides in tea matrices using analyte protectants, J. Chrom. A 1266 (2012), 131-142.

Thermo SCIENTIFIC