Overview

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

Methods: Benzodiazepines were analysed using the TSG 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 nitroxetine showed excellent linearity while lorazepam and lectopam showed very poor response. Sorbitol was used as an analyte protectant to significantly improve the calibration behaviour of the more challenging analytes.

Introduction

Benzodiazepines drugs are psychoactive 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 tranquillizers 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 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 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

Analyses

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

GCMS

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

TABLE 1. Instrument method for benzodiazepine analysis

<table>
<thead>
<tr>
<th>Compound</th>
<th>CAS Number</th>
<th>RT (min)</th>
<th>Precursor Mass (amu)</th>
<th>Product Mass (amu)</th>
<th>Collision Energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lorazepam</td>
<td>846-49-1</td>
<td>14.32</td>
<td>239.0</td>
<td>176.7</td>
<td>25</td>
</tr>
<tr>
<td>Diazepam</td>
<td>849-89-5</td>
<td>14.32</td>
<td>274.0</td>
<td>229.1</td>
<td>10</td>
</tr>
<tr>
<td>Dazepam</td>
<td>450-14-5</td>
<td>14.46</td>
<td>250.1</td>
<td>181.9</td>
<td>10</td>
</tr>
<tr>
<td>Nitroxetine</td>
<td>430-14-5</td>
<td>14.46</td>
<td>251.6</td>
<td>231.1</td>
<td>10</td>
</tr>
<tr>
<td>Lectopam</td>
<td>1812-30-2</td>
<td>15.32</td>
<td>310.7</td>
<td>289.1</td>
<td>10</td>
</tr>
<tr>
<td>Nitrinex</td>
<td>1812-30-3</td>
<td>15.32</td>
<td>310.7</td>
<td>289.1</td>
<td>10</td>
</tr>
<tr>
<td>Diazepam</td>
<td>146-22-5</td>
<td>16.02</td>
<td>280.3</td>
<td>259.1</td>
<td>10</td>
</tr>
<tr>
<td>Lectopam</td>
<td>1622-81-3</td>
<td>16.55</td>
<td>280.0</td>
<td>254.1</td>
<td>10</td>
</tr>
<tr>
<td>Lectopam</td>
<td>1622-81-3</td>
<td>16.55</td>
<td>314.0</td>
<td>296.1</td>
<td>15</td>
</tr>
</tbody>
</table>

Results

AutoSRM study

Benzodiazepines were prepared from purchased standards and as such were prepared between 10 and 1000 ppb. The 100 ppb 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 TSG 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

Protectant

Benzodiazepine Calibration Curves

The optimised MRM method created using AutoSRM (described above) was used to record calibration data for the benzodiazepines between 10 and 1000 ppb. 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) nitroxetine, (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 polar compounds like steroids. The high polarity 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 fowpath 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 3. Calibration curves for (a) clonazepam, (b) nitroxetine, (c) lorazepam and (d) lectopam between 10 and 200 ppb using 0.2 % sorbitol as analyte protectant.

Conclusions

• Benzodiazepines are challenging to analyse by GC-MS/MS due to high activity of 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 10 ppb using analyte protectant.