AIM

To quantitate 21 benzodiazepines, zolpidem and zopiclone in human serum and plasma using a UPLC/MS/MS method.

RESULTS

Chromatography

Table 1 shows the MRM transitions as well as LOD (S:N >7:1), matrix effects and recoveries for all analytes. This assay shows the benefits of UPLC-based methods in terms of superior chromatographic resolution and shorter run times compared to HPLC-based methods. The new method permitted the analysis of a larger number of samples within a shorter period of time.

INTRODUCTION

Benzodiazepines are the most frequently prescribed drugs in the western world. They are indicated for a variety of disorders including: anxiety; insomnia; agitation; muscle spasm and alcohol withdrawal effects.

They are commonly reported in self-poisonings and are the drug of choice for some forms of alcohol withdrawal. They are also used for drug-facilitated crime due to their sedative properties and amnesia-producing effects.

METHODS

Liquid/liquid extraction (LLE) sample preparation

Spike: 300 µL sample/calibrator with 10 µL internal standard (IS), add 150 µL borate buffer, vortex to mix.

Add 900 µL extraction mixture*, mix and vortex to mix.

Extract: 3000 rpm for 5 min.

Reconstitute and inject: 50 µL (10% water and 90% methanol). (A concentration step), mix and inject.

Authentic serum/plasma sample analysis

48 anonymised samples extracted by LLE and analysed as described.

Table 1 shows the MRM transitions as well as LOD (S:N >7:1), matrix effects and recoveries. Matrix effects ranged from –28% to +6%.

Table 2: MRM transitions for 23 analytes, product 1 is the quantifier ion and product 2 is the qualifier ion. LOD, matrix effect and recoveries refer to the LLE method.

Figure 1 shows the chromatographic distribution of the compounds across the 3.5 minute elution range.

For all the analytes over a period of five days were investigated using triplicate spiked serum calibrators at 0.1, 5, 10, 100 and 1000 ng/mL extracted using the UPLC as described.

The linearity r² values were on average all above 0.99 apart from alpha-hydroxy triazolam which was 0.975 for 1-100 ng/mL, due to interference from the analyte into the deuterated internal standard channel.

The intra- and inter- day variabilities were performed in triplicate at 20 and 400 ng/mL during this five day study and showed precision (CV) values of 15% RSD and accuracy within +/- 11%.

Figure 2 shows the correlation of results from the published method with concentrations in ng/mL.

Figure 3 shows the correlation of results of the 48 anonymised samples extracted by LLE with the published method with concentrations in ng/mL.

Figure 4 shows the correlation of results for diazepam between the published method methodology and the published method with concentrations in ng/mL.

REFERENCES

1. Van Lar MW and Volkerts ER, CNS drugs 2008 32: 491-498

2. Roberts M and Wood M, 720003388en

3. Roberts M and Wood M, €©2010 Waters Corporation

CONCLUSION

This assay shows the benefits of UPLC-based methods in terms of superior chromatographic resolution and shorter run times compared to HPLC-based methods. The new method permitted the analysis of a larger number of samples within a shorter period of time compared to the published method.

The results from 48 authentic serum and plasma samples analysed using UPLC/MS/MS show good correlation with results from the published method with an r² value above 0.95 when comparing results for all analytes found.

Table 1: MRM transitions for 23 analytes, product 1 is the quantifier ion. LOD, matrix effect and recoveries refer to the UPLC method.

Figure 3: Comparison of results for diazepam between the LL with UPLC/MS/MS and the published method with concentrations in ng/mL.

Figure 4: Comparison of results for diazepam between the LL with UPLC/MS/MS and the published method with concentrations in ng/mL.