

A method using an analyte trapping reagent, for the simultaneous analysis of the amino urinary metabolites of flunitrazepam, clonazepam and nitrazepam, by GC-MS.

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INTRODUCTION: Flunitrazepam, clonazepam and nitrazepam are 7-nitrobenzodiazepines. They are used by poly drug abusers for a euphoriant effect. Because they may cause anterograde amnesia, they are used in surreptitious situations to facilitate sexual assault. The literature suggests that analysis of the target metabolites 7-aminoflunitrazepam, 7-aminoclonazepam and 7-aminonitrazepam, by GCMS, may produce aberrant results. A laboratory in 1999 reported the occurrence of irreproducible results when using bis(trimethylsilyl)trifluoroacetamide (BSTFA), and another in 1996 stated the need for extremely clean injection port maintenance.

AIM: To provide a quantitation of 7-aminoflunitrazepam, 7-aminoclonazepam and 7-aminonitrazepam as required within the criteria of the Australia/New Zealand 4308:2001 Standard for evidential drug testing. The positive/negative cut-off concentration for each of the aminometabolites is currently 200 ng/mL. Also to provide a screen for the detection of 7-aminoflunitrazepam, following urgent clinical requests received for the determination of supposed flunitrazepam abuse.

METHOD: Calibrators for each metabolite were at concentrations of 160, 200 and 240 ng/mL. The internal standard for monitoring 7-aminoflunitrazepam was the D7 analogue and for 7-aminoclonazepam and 7-aminonitrazepam was 7-aminoclonazepam-D4. Urine specimens (2mL) were made basic with 4 molar ammonia (0.2mL) and extracted with dichloroethane (2mL). This extract was dried with anhydrous sodium sulphate (0.3g) and 50 microlitres of dimethylformamide (DMF) was added before evaporation of the organic phase. Following evaporation of the dichloroethane, the amino-benzodiazepines were retained in the DMF which was not evaporated to complete dryness. BSTFA with 1% trimethylchlorosilane was added to the DMF solution of the aminometabolites and heated at 65°C for 20 mins. Gas chromatography-mass spectrometry was carried out on a 30m HP-5MS column, in time scheduled SIM mode. The retention times were 8.26, 10.86 and 11.26 for the amino metabolites of nitrazepam, clonazepam and flunitrazepam respectively and the respective quantitation and qualifier ions were m/z 395, 394/396; 429, 394/431; 355, 327/356. Selected quantitation/qualifier ions for aminoclonazepam-D4 and aminoflunitrazepam-D7 were 433/398 and 362/334. The screening procedure for 7-aminoflunitrazepam, does not employ a deuterated standard, but otherwise involves the same extraction/derivatization procedure, with the mass spectrometer functioning in the scan mode. Identification is based on retention time and mass ion library match.

RESULTS: The quantitation method is linear from 150-600 ng/mL. The lower limit of quantitation is 25 ng/mL for both 7-aminoflunitrazepam and 7-aminonitrazepam, but for 7-aminonitrazepam is 75 ng/mL. Within batch precision at 200ng/ml is 4.4, 2.7 and 4.4% for the nitrazepam, clonazepam and flunitrazepam metabolites respectively and between batch precision was similarly, 10.9, 7.8 and 9.4%. The limit of detection in the screening procedure for 7-aminoflunitrazepam is 75 ng/mL.

CONCLUSION: The use of a drying agent, and the retention of the metabolites using a trapping reagent (DMF) rather than evaporation to absolute dryness, have permitted both the simultaneous quantitation of the three 7-amino target metabolites and also the screening for 7-aminoflunitrazepam, without the occurrence of aberrant results.

KEYWORDS: *7-aminobenzodiazepines, Trapping reagent, Flunitrazepam*

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