Fully Validated Liquid Chromatographic-Tandem Mass Spectrometric Procedure for Identification and Quantification of Antidepressants and Benzodiazepines in Human Blood Plasma

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Abstract

Aims: Multi-analyte procedures make the analytical process much simpler, faster, and cheaper, and allow monitoring of analytes of different drug classes in a single body sample. The aim of the present study was to validate a multi-analyte procedure for fast target screening, reliable identification, and quantification exemplified here on 62 tested antidepressants (AD) and benzodiazepines (BZ).

Methods: Analyte identification and quantification was performed after liquid-liquid-extraction (Remane et al., ABC, 2010) using a ThermoFisher (TF) TSQ Quantum Access (APCI) with gradient elution on a TF Hypersil GOLD Phenyl column (100 x 2.1 mm, 1.9 µm) in the timed multiple-reaction monitoring mode. The method was validated with respect to selectivity, cross-talk, ion suppression/enhancement of matrix compounds (matrix effects), co-eluting analytes and internal standards, recovery, process efficiency, accuracy and precision, stabilities, and limits of quantification and detection. For accuracy and precision, full as well as one point calibration was performed.

Results and Discussion: During validation, no severe selectivity problems could be detected; cross talk was seen for amitriptyline caused by maprotiline but was distinguishable according to their retention times. Ion suppression/enhancement was already monitored in detail (Remane et al., RCM, 2010 a+b; ABC, 2010). Severe matrix effects could be detected for bupropion and hydroxybupropion. Instability during freeze/thaw cycles was shown for bupropion, hydroxybupropion, and norfluoxetine. The lower limit of quantification was set at the lowest calibrator concentration and was at least at the lower therapeutic concentration with exception for cyclobenzaprine and reboxetine. One point calibration was shown to be an acceptable calibration model for 21 AD and 17 BZ.
This multi-analyte procedure allowed selective detection as well as accurate and precise quantification of 28 AD and 21 BZ, zaleplon, zolpidem, and zopiclon in plasma as part of a multi-analyte method of 136 analytes of different drug classes using the accepted validation criteria.

Conclusion: The method fulfilled the requirements for a fully validated assay and has proved to be efficient in authentic cases for TDM and clinical toxicology, thus allowing drug monitoring and confirmation of diagnosis of an overdose situation caused by ingestion of anti- depressants, benzodiazepines and z-drugs. Furthermore, the time- and cost-saving one-point calibration was shown to be applicable for many analytes for daily routine and especially for emergency cases. This UHPLC-MS/MS method allowed selective target screening as well as accurate and precise quantification of 28 antidepressants and 21 benzodiazepines in plasma. Regarding the fact that a lot of analytes with different chemical structures are implemented in this fast and simple work-up compromises concerning recovery, precision and LLOQ had to be made in some cases.

In the meantime, these studies have been published as original papers:

1. Remane D, Meyer MR, Wissenbach DK, Maurer HH. Full validation and application of an ultra high performance liquid chromatographic-tandem mass spectrometric procedure for target screening and quantification of 34 antidepressants in human blood plasma as part of a comprehensive multi-analyte approach. Anal Bioanal Chem 2011;400:2093-107.
2. Remane D, Meyer MR, Wissenbach DK, Maurer HH. Ultra high performance liquid chromatographic-tandem mass spectrometric multi-analyte procedure for target screening and quantification in human blood plasma: validation and application for 31 neuroleptics, 28 benzodiazepines and Z-drugs. Anal Bioanal Chem 2011; in press.