

The Analysis of Tricyclic Antidepressant Drugs in Plasma for Clinical Research

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Background: The accurate measurement of tricyclic antidepressants (TCAs) in plasma for clinical research, is vital in order to undertake pharmacokinetic studies and monitor therapy efficiently. Waters has developed a clinical research method using LC-MS/MS which allows for efficient measurement of 15 TCAs: amitriptyline, clomipramine, clozapine desipramine, doxepin, imipramine, maprotiline, norclomipramine, norclozapine, nordoxepin, normaprotiline, nortrimipramine, nortriptyline, protriptyline and trimipramine in human plasma.

Methods: Calibrators and quality control samples were prepared using pooled human plasma. Samples (50 µL) were extracted with internal standard (150 µL) in acetonitrile, vortex mixed, centrifuged (2 minutes) and the supernatant was diluted in water and mixed. Using an ACQUITY™ UPLC™ I-Class FTN System, samples were injected onto a XSelect™ Premier HSS T3 Column using a water/methanol/formic acid/ammonium formate gradient elution profile, with detection using a Xevo™ TQ-S micro Mass Spectrometer.

Results: Chromatographic separation was achieved for isobaric compounds nortriptyline and protriptyline (precursor m/z 264.1) and the interfering qualifier transitions of imipramine and nortrimipramine (m/z 281.2>86.1). There was no significant carryover observed from a high concentration plasma sample into a subsequent blank injection. A 1:5 dilution of a high concentration sample was performed that gave a mean bias of less than 10 % for all analytes. Analytical sensitivity results demonstrated precise quantification (<6.9 % bias and <10 % CV) at concentrations equal to or lower than the lowest calibrator. Total precision and repeatability were assessed (3 pools, 5 replicates, 5 days; n=25) and determined to be ≤8.0 % CV. Matrix effects studies evaluated at low and high concentration levels, demonstrated compensation by the internal standard for any signal enhancement or suppression observed. The method also demonstrated excellent linearity for all analytes, with calibration lines exhibiting coefficient of determination (r^2) of > 0.995.

Conclusion: The analysis of 15 tricyclic antidepressants with different polarities was achieved by a selective and robust UHPLC-MS/MS clinical research method and used Waters XSelect Premier HSS Technology to separate isobaric compounds. The method required only 50µL of sample with an analysis time less than 4.5 minutes.

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