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Assay of eight tricyclic antidepressants and (nor)clozapine by UPLC-MS/MS

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Introduction

For treatment of psychiatric diseases and psychoses, various drug treatments have been shown to be effective. To prevent toxification, especially of the first generation drugs, therapeutic drug monitoring (TDM) is obligatory. Here, we describe an assay for 10 drugs frequently prescribed in psychiatry using an ultra-performance tandem mass spectrophotometer (UPLC-MS/MS) which stands for rapidity and extremely specific analysis on the basis of atmospheric electrospray ionisation (AP-ESI) focussing on M/Z or MH^+ characteristics for each analyte. A prepurification step to remove or separate ion suppressive material like phospholipids and proteins usually suffices. Accordingly, we evaluated the extraction procedure and ultimately selected one with a good protein precipitating efficiency and optimal electrospray ionization mass sensitivity. In order to achieve the lowest imprecision of this 10 analyte-assay, we also systematically sought for the best match of analyte and one of five internal standards.

Experimental

Solvents and chemicals: Solvents of highest analytical grades were from Biosolve, Valkenswaard, The Netherlands. The TCA standards (from Sigma, summarised in table 1) were dissolved in water, but (nor)clozapine in MeOH. The working standards were made up with 10% MeOH and were then 40- to 160 times further diluted with plasma. For accuracy, we measured the certified reference material (CRM's) as indicated below. Target values as well as our scores with HPLC-fluorescense were used for comparison. To obtain optimal accuracy and precision, we looked for the best match with one of the five IS (trimipramine, doxepine and deuterated clozapine, -amitriptyline, -nortriptyline).
Extraction of samples, controls and standards: Fifty µl of plasma, control or calibrator was pipetted in Eppendorf tubes and extracted by vortex mixing with 800 µl AcN:MeOH 1:1 v/v (including 50 µl IS). After incubation for 10 min, the cups were vortex mixed once more, centrifugated at 13.000*g for 5 min and the extracts were decanted in the autosampler vials from which 10 µL was eluted over a WATERS Acquity UPLC BEH C18 1.7 µM column of 2,1 * 100 mm. Mobile phases A and B consisted of 20 mM ammonium bicarbonate, pH 9.0 and AcN with 0.01% formic acid, respectively. The gradient program switched from 60% to 20% A in 4 min, to 0% in 30 seconds and to 60% A again for 2 min. Retention times (Rt's) for all compounds were between 1.5 and 3.9 min with a total run time of 7 min. Fragments of the various TCA's and (nor)clozapine were detected by MRM using mass-to-charge (*m/z*) transitions as shown in table 1. Instrument parameters were optimized by tuning individual compounds. Note that (nor)clozapine for reasons of uniformity was measured at a capillary voltage of 0.5 V instead of at 3.0 V, which gave 4-fold higher AUC's and similar accuracy.
Ion ratio's for quantitation/recognition were flagged to mark samples with very low concentrations or those essentially free of TCA/(nor)clozapine. System operation and data acquisition were controlled by Masslynx v4.1 software with automated data processing using the Quanlynx Application Manager (Waters).
Evaluation strategy: We experimentally followed the NCCLS Approved Guidelines for Preliminary Evaluation of Clinical Laboratory Methods (EP10-A).

Results

Efficiency: the one-tube extraction procedure was most efficient and cost effective, also compared to a one step SPE. We optimised the different analytical steps. Out of about 30 different pre-purification methods and modifications tested, we finally selected that of Kirchherr and Kühn-Velten (1), but used AcN:MeOH 1:1 instead of

Table 1. MRM file with specific transitions for parents and daughters used for quantitation and recognition

Analyte	MRM for quantitation	MRM for recognition
Clomipramine	315.80> 85.99	315.50>242.10
Imipramine	281.20> 86.05	281.20>208.15
Amitriptyline	278.20> 91.00	278.20>105.20
Nortriptyline	264.14> 90.97	264.15>233.15
Desipramine	267.12> 71.94	267.13>208.00
Norclomipramine	301.11> 72.00	301.15>242.10
Clozapine	327.15> 84.05	327.15>270.10
Norclozapine	313.11> 70.00	313.11>192.10
Trimipramine*	295.11> 99.90	295.25>193.00
Doxepine*	280.15> 84.10	280.15>208.15
Nortriptyline-D_3*	267.15> 91.00	
Clozapine-D_4*	331.10>272.10	
Amitriptyline-D_6*	284.20> 91.00	

* Asterisks indicate Internal Standards (trimipramine and doxepine were not deuterated)

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Abbreviations: TCA: tricyclic antidepressants; UPLC-MS/MS: ultra performance liquid chromatography tandem mass spectrometry; AcN: acetonitrile (methylcyanide); IS: internal standard; AUC: area under the curve; MeOH: methanol; Rt; retention time; TDM: therapeutic drug monitoring; CRM: certified reference material; KKGT: Kwaliteitsbewaking Klinische Geneesmiddelenanalyse en Toxicologie

9:1 v/v. This modification scored similarly as the one in which 50 µl of plasma and 50 µl of IS was extracted with 400 ul AcN, the supernate diluted 1:1 with water to achieve a gradient-like composition. Although the latter procedure resulted in twice higher AUC's, we preferred the AcN:MeOH procedure because of a better protein precipitation and its reported more uniform suitability in the measurement of 48 analytes (1).
Extracting a batch of samples required about 30 min, versus about 4h in the current method with a two step SPE. The chromatographic step required only 7 min instead of 30 min with the HPLC.
Ion suppression: We intensively studied a possible interference by phospholipids through ion-suppression. Using the selective transition 184>184 for phosphocholines (2) we found that SPE removed 90% of these compounds without affecting recovery %. In addition, the highest peak of the phosphocholines cinquet had similar or very near Rt's as norclomipramine (dependent on the gradient modification). Nevertheless recoveries for norclomipramine ranged between 93.7 and 102.6%, which was very similar to the scores for the other analytes.
Imprecision: As mentioned, imprecision scores were calculated using 5 different IS matches. As the imprecision scores on one and the same IS were in a narrow range, results for all 8 TCA and (nor)clozapine variants were averaged. We established that D4-clozapine was optimal for controlling (nor)clozapine, desipramine and nortriptyline; while doxepin was best for controlling norclomipramine; D6-amitriptyline was best for control for imipramine, amitriptyline en clomipramine. Analysing the components of imprecision in sera with low, medium (m) and high (h) concentration of analytes: that of between days contributes somewhat less to the total imprecision (figure 1). Overall total CV% were between 6 and 7%.
The limit of quantification was around the level 'low' (about 20 µg/L for all TCA's and 40 µg/L for (nor)clozapine); these results were excluded from the scores. Imprecision of (nor)clozapine was somewhat higher then of the TCA's, ascribed to the suboptimal capillary voltage used; 0.5 versus 3.0 V.
Linearity: Linearity for the various TCA's was ascertained from 0.1 up to 700 µg/L and for (nor)clozapine up to 1400 µg/L. There was no intercept.

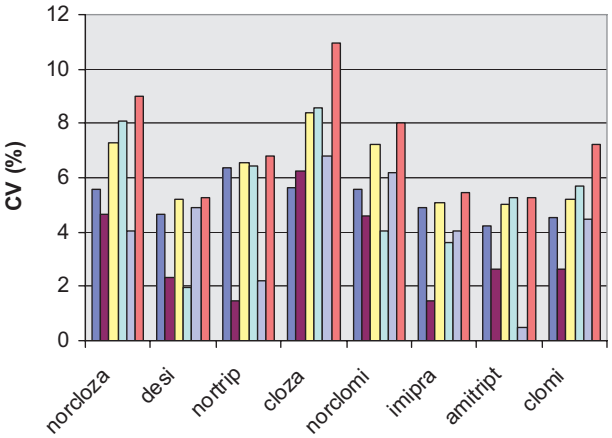

Figure 1. Within run, between run and total imprecision scores for the various TCA's and (nor)clozapine. m = medium conc.; h = high conc. ■ within m; ■ between m; ■ total m; ■ within h; ■ between h; ■ total h.

Accuracy; calibrator equivalency: two efforts to prepare a calibrator (two technicians, different preparations) agreed to within: 99.3; 100.6 en 102.6% for the three highest levels.
Accuracy; recoveries: Averaging the results for all 8 analytes, the recoveries for the additions to obtain the low, medium and high calibrators amounted to 91.05 ± 1.8%; 101.1 ± 3.9% en 102.9 ± 2.0% (mean ± SD and averaged for the 8 analytes). Note that, the small SD's confirm analyte independency.
Accuracy: scores for the CRM's: We evaluated several CRM's from KKGT and Lyphocheck Benza/TCA control. Results for all ranged between 92-104% (mean ± SD: 98,8 ± 3,5%).
Accuracy: correlation UPLC-MS/MS vs HPLC-fluorescense (y): For 40 samples the x-y differences for individual analytes were mostly within 10%, at most to within 20% which is comprehensible giving the measurement errors in both methods.

Conclusion

We here describe a simple, efficient and reliable method for drug monitoring of TCA's and (nor)clozapine in plasma. The extraction step not requiring (automated) SPE and the short chromatography time are hallmarks. We spend much time to optimize the imprecision which we wanted to be of the same low value as for the HPLC-fluorescence method. Finally we obtained a reasonable outcome given the variation in the AP-ESI technique. The differences concerning imprecision between both methods are negligible in the light of the biological/metabolic variation which is estimated to be two to three-fold higher, thus contributing for at least 75% to the total variation in a person or patient.
Reportedly, without TDM, about 6% of patients using TCA's develops CNS toxicity (3); the risk increases 10-fold from <450 ng/l to 734 ± 349 ng/ml (range 438-1200 ng/ml) at a dose of 250 ± 80 mg/day (4); with TDM only 0.4% develop toxicity (4). If the settings are comparable, this is a very important reduction. An important variable in metabolizing TDM's is liver pathology (cirrhosis and infection) sometimes explaining acute large differences in circulating concentrations. This frequently overrules relative stable quantitative differences due to phenotypic differences in the cyt P450 enzymes.

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