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Rapid and Robust Analysis Method for Quantifying Antidepressants and Major Metabolites in Human Serum by UHPLC-MS/MS

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1. Introduction

Therapeutic drug monitoring (TDM) of antidepressants is one of the most widely utilized analysis in clinical labs in Dutch hospitals nowadays. Traditionally quantitation of antidepressants is performed by means of HPLC with diode-array detection (DAD). Obvious disadvantages of the latter method are: sensitivity, selectivity and possibility of false positives as a result of co-elution with other administered drugs, metabolites or endogenic compounds. UHPLC-MS/MS is much more sensitive, more selective, and if robust the perfect replacement of routine HPLC analysis. Due to simplified sample prep, faster analysis and less solvent consumption UHPLC-MS/MS is much more cost effective. UHPLC-MS/MS methods will replace routine HPLC methods in clinical labs.

2. Methods and Materials

Human serum samples were extracted by means of off-line solid phase extraction. The extracts were analyzed with a Shimadzu Nexera UHPLC combined with a LCMS-8040 Tandem Mass Spectrometer. 5 µL of sample was injected with a SIL-30AC autosampler.

2-1. Sample Preparation

Standards both for calibration and control are ready made serum standards. The standards are pretreated in the same way as the samples.

1. Precondition SPE material1 with 1 mL 3% ammonia in acetonitrile
2. Equilibrate with 1 mL citrate buffer pH 3.0
   - Load 2 mL internal standard solution
   - Load 1 mL homogenized serum
3. Wash with 3 mL water (extract A0)
4. Wash with 3 mL acetonitrile (extract A1)
5. Wash with 3 mL 3% ammonia in acetonitrile (extract A2)

1 : Bakerbond Speedisk C18 Polar Plus, 10 µm, 50 mg/3 mL (JT BAKER)

The flow is in all steps adjusted to about 1 to 2 mL/min.
Antidepressants in sample extract A2 are analyzed with UHPLC and a triple quadrupole mass spectrometer using the following conditions. Neutral and acidic compounds are eluted in extract A1.

Fig. 1 LCMS-8040 Triple Quadrupole Mass Spectrometer

High Speed Mass Spectrometer

Ultra Fast Polarity Switching
–15 msec
Ultra Fast MRM
–Min. dwell time 0.8 msec (max. 555 MRM/sec)

2-2. Analytical Conditions

UHPLC conditions (Nexera system)

Column : Phenomenex Kinetex XB-C18 100 mm × 2.1 mm, 2.6 um
Mobile phase A : 10 mM Ammonium fomate
B : Acetonitrile
Flow rate : 0.4 mL/min
Time program : B conc.10%(0 min) -50%(2.5-5 min) - 75%(6 min) – 10% (7-7.5 min)
Injection volume : 5 µL
Column temperature : 30°C

MS conditions (LCMS-8040)

Ionization : ESI, Positive
Scan mode : MRM mode with data dependent product ion scanning

Table 1 MRM transitions

Compound	Quantifier	Qualifiers
10-hydroxynortriptyline	280.20 > 215.10	280.20 > 262.20 ; 280.20 > 231.10
N-desmethylclozapine	313.10 > 192.10	313.10 > 270.05 ; 313.10 > 231.05
clozapine	327.20 > 270.05	327.20 > 192.05 ; 327.20 > 227.05
paroxetine	330.20 > 70.20	330.20 > 192.20
fluvoxamine	319.20 > 71.15	319.20 > 41.15
desipramine	267.15 > 208.10	267.15 > 193.10 ; 267.15 > 236.10
imipramine	281.20 > 208.10	281.20 > 193.05
nortriptyline	264.20 > 233.20	264.20 > 91.15 ; 264.20 > 117.10
N-desmethylfluoxetine	296.20 > 30.30	296.20 > 134.10
Amitriptyline	278.20 > 105.15	278.20 > 233.10 ; 278.20 > 91.10
Fluoxetine	310.15 > 44.25	310.15 > 148.10
N-desmethylclomipramine	301.10 > 242.10	301.10 > 227.00 ; 301.10 > 270.10
Clomipramine	315.15 > 242.10	315.15 > 227.05 ; 315.15 > 270.10
internal standard	353.10 > 280.10	353.10 > 248.10 ; 353.10 > 308.05

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3. Results

3-1. Comparison of HPLC-DAD and UHPLC-MS/MS results

Several hundreds of serum extracts were analyzed with a LC-MS/MS method and three separate HPLC methods with photo diode-array detection to compare results of both techniques.

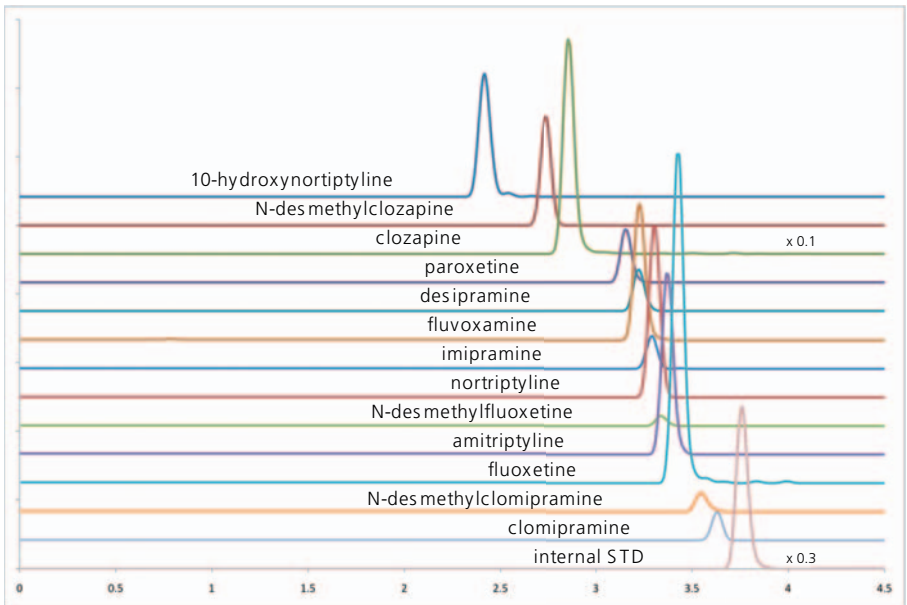

Fig. 2 Mass chromatograms of antidepressants and metabolites in calibration standard (concentration of each compound : 90-100 µg/L in serum, except clozapine: 600 µg/L)

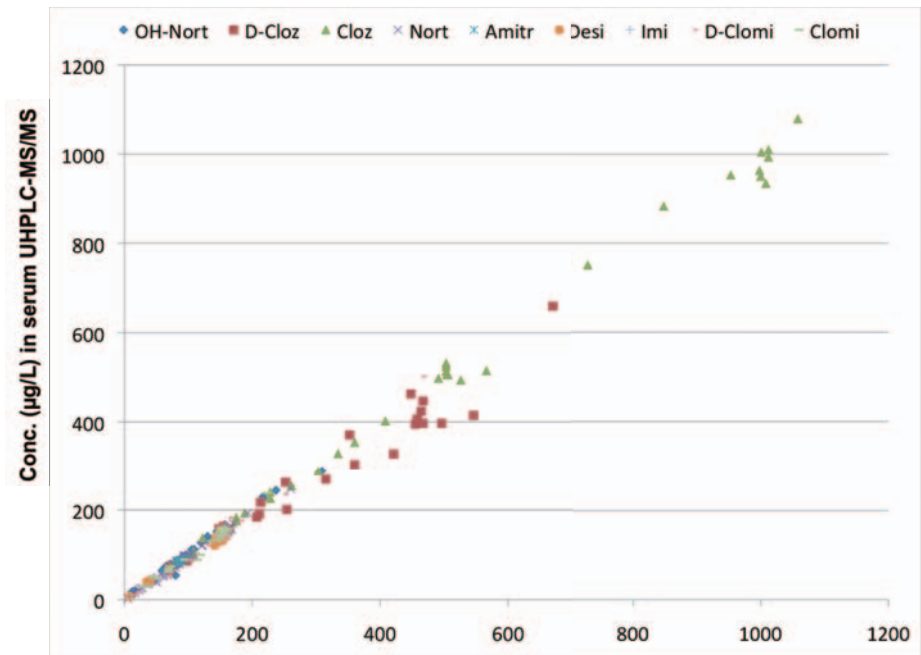

Fig. 3 Comparison of measured results with HPLC-DAD with UHPLC-MS/MS. N-desmethylclozapine shows signs of ion-suppression

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3-2. Automatic Synchronized Survey Scanning (DDA)

The multi-component MRM method was extended with Synchronized Survey Events (DDA), to collect product ion scans for every positive peak above a certain level. The product ion spectra were identified by library search.

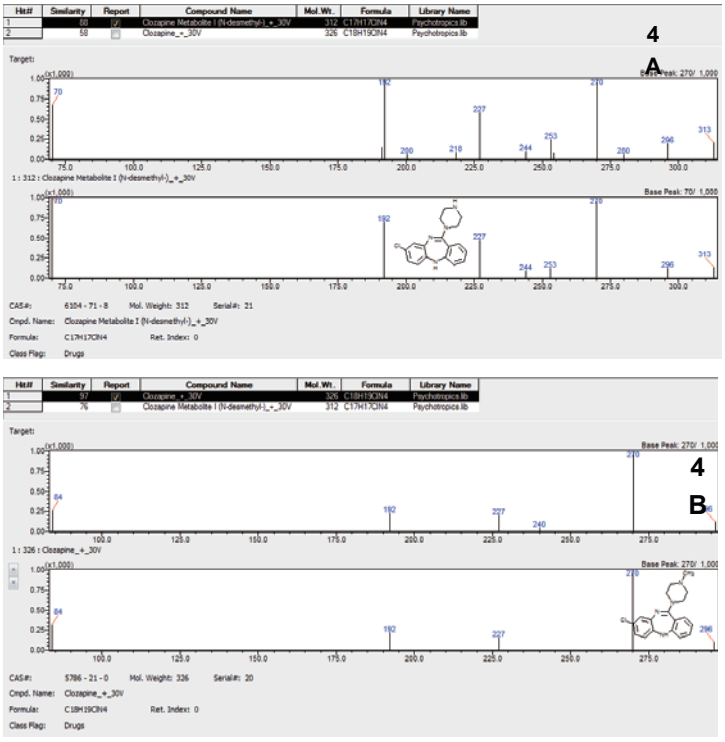

Fig. 4 N-desmethylclozapine (4A) and clozapine (4B) were both positively identified in the same patient serum extract

4. Conclusions

- There is a good correlation between the results from HPLC-DAD and UHPLC-MS/MS. Correlation coefficient is between 0.965 and 0.995 for all compounds. Only N-desmethyl-clozapine deviates due to ion-suppression. Generally can be concluded that the serum extracts obtained after off-line solid phase extraction are clean. There is no need for isotopically labeled internal standards.
- Combining MRM measurements with data dependent product ion scanning provides more reliable results since the identity of compounds can be confirmed by library search. Furthermore it is possible to combine diode-array detection with LCMS/MS to offer even more assurance.
- Co-medication and medication adherence behavior of elderly patients is more easily traced by multi-target compound analysis by UHPLC-MS/MS, as with HPLC-DAD one needs the trained eye of an analyst.
- Reconstitution of the sample extract by solvent evaporation and subsequent dilution in another solvent is mandatory for HPLC-DAD to increase concentration and to eliminate peak broadening of early eluting compounds, but is not necessary for UHPLC-MS/MS. Therefore sample pretreatment could be shortened, reducing the overall sample prep time from 40 to 15 minutes per batch of 15 samples.
- UHPLC-MS/MS method for antidepressants will replace routine HPLC-DAD methods in clinical labs.

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