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SIMULTANEOUS QUANTIFICATION OF PSYCHOTHERAPEUTIC DRUGS IN HUMAN PLASMA BY TANDEM MASS SPECTROMETRY

Michelle Wood and Michael Morris

Clinical Applications Group, Waters Corporation., Manchester, M23 9LZ, UK.

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OVERVIEW

A simple and rapid LC-MS/MS method has been developed which allows the simultaneous quantification of a panel of commonly prescribed psychotherapeutic drugs in human plasma and whole blood.

INTRODUCTION

- Recent figures suggest that approximately a quarter of the world's population will suffer from a diagnosable mental disorder at some point in their lives [1].
- Depression, schizophrenia, anxiety and substance abuse are amongst the most common conditions in the developed countries.
- 78% of affected people suffer from more than one mental disorder at the same time [2].
- First-line treatment generally comprises psychotherapy and psychotherapeutic medication.
- Since the relationship between dose and clinical response is often poorly delineated, as a result of wide inter-individual variations in ADME, therapeutic drug monitoring (TDM) provides a valuable means by which to establish individual target therapeutic concentrations, to determine potential toxicity and to verify compliance.
- Current methods for TDM involve extraction of the drugs from plasma followed by analysis using LC-ECD (or LC-UV). However, these procedures are frequently problematic or insensititve due to the co-elution of contaminants, which frequently persist, even following lengthy sample preparation techniques.
- In order to address this problem we have developed an alternative method. Drugs were isolated from plasma using simple protein precipitation step and subsequently analysed using LC-MS/MS. The procedure requires only 50µL of biological sample and has a total analysis time (including sample preparation) of less than 20 minutes. The method allows the simultaneous quantification of several of the most commonly prescribed psychotherapeutic drugs, in plasma or whole blood. Limits of detection of 1µg/L or better were achieved.

METHODS AND INSTRUMENTATION

LC conditions

HPLC System:	Waters Alliance 2795
Column:	Waters Symmetry 300 C_{18} (2.1mm x 150mm, 5µm) maintained at 30 °C
Mobile phase: (A) = containing	2 mM ammonium acetate 0.1% formic acid
(B) =	Acetonitrile containing 0.1% formic acid
	Isocratic elution (60:40)
Flow rate:	0.35 mL/min
Injection volume:	10 µL

MS conditions

Mass spectrometer:	Micromass Quattro *micro* tandem mass spectrometer (**Figure 1**).
Ionisation mode:	ES positive ion
Capillary voltage:	1kV
MS/MS:	Collision gas: Argon at 4.5 x 10^{-3} mbar

MICROMASS MS TECHNOLOGIES

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Figure 1. The Quattro micro

Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	Cone Voltage (V)	Collision energy (eV)
Amitriptyline	278	91	30	30
Citalopram	325	109	38	25
Clomipramine	315	86	28	18
Dibenzepine	296	251	25	20
Haloperidol	376	165	40	22
Imipramine	281	86	40	30
Imipramine d3	284	89	25	15
Nortriptyline	264	91	30	30
Nortriptyline d3	267	91	25	22
Quetiapine	384	253	35	22
Risperidone	411	191	40	30
Sertraline	306	159	20	25

Table 1: MRM transitions and conditions for the measurement of psychotherapeutic agents. The deuterated analogues of imipramine and nortriptyline were included as an internal standards.

RESULTS AND DISCUSSION

MRM transitions were determined for the psychotherapeutic drugs (**Table 1**). **Figure 2** shows some examples of precursor ion and product ion spectra.

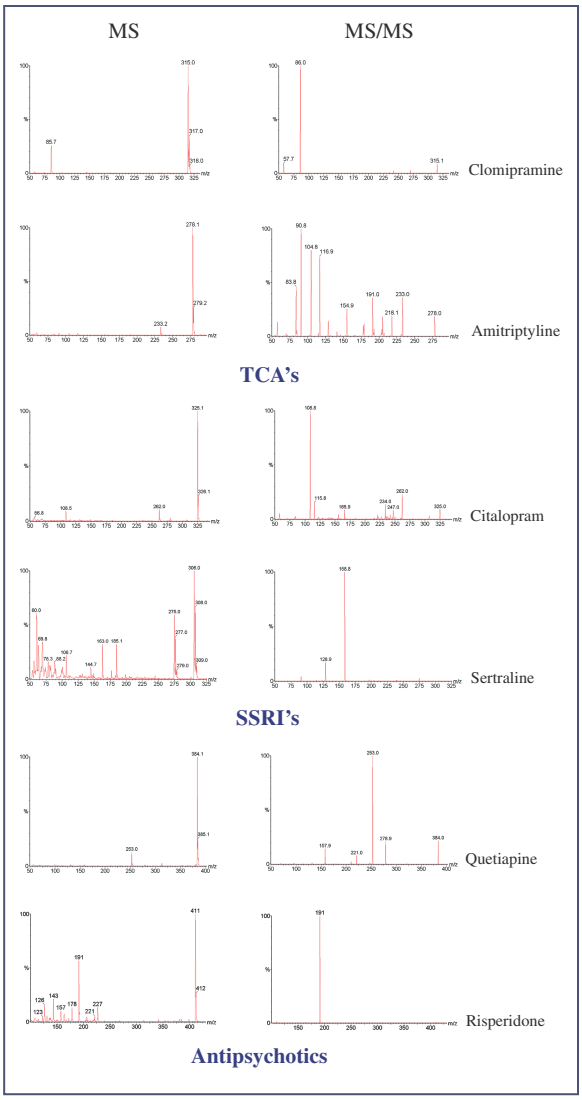

Figure 2. Precursor and product ion spectra for various psychotherapeutic drugs including: tricyclic antidepressants (i.e. clomipramine and amitriptyline), selective serotonin re-uptake inhibitors (i.e. sertraline and citalopram) and antispychotics (i.e. risperidone and quetiapine)

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A series of calibrators (0.1-500µg/L) were prepared by addition of the psychotherapeutic drugs to blank plasma. Following isolation from the matrix using a simple protein precipitation step which also incorporated the addition of internal standards (**Figure 3**), samples were analysed using LC-MS/MS. **Figure 4** shows the MRM chromatograms acquired simultaneously during a single injection of a 50µg/L plasma calibrator. Quantification was achieved by integration of the area under the specific MRM chromatogram. In all cases the responses for the psychotherapeutic agents were calculated in reference to the integrated area of a deuterated internal standard.

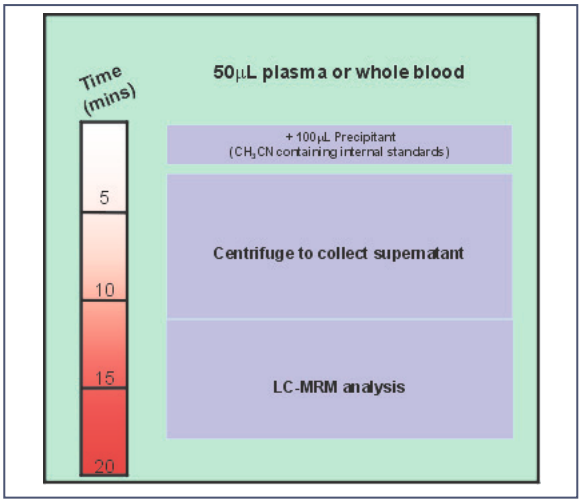

Figure 3. Schematic overview of the LC-MS/MS procedure

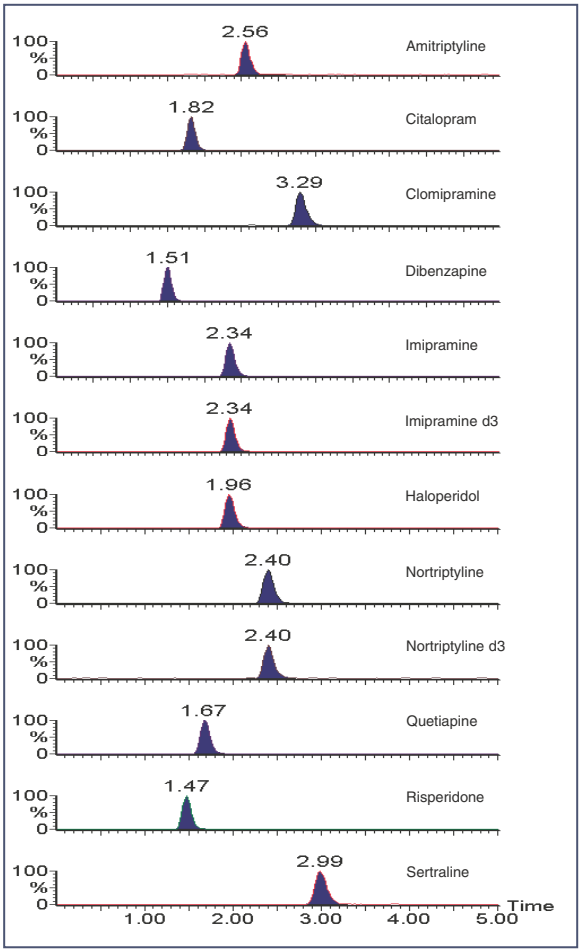

Figure 4. Extracted MRM chromatograms obtained with a single injection of the 50µg/L plasma calibrator

Responses were linear, for all compounds in plasma, over the range investigated ($r^2 > 0.99$). A typical standard curve is shown in **Figure 5a**.

The precision of the assay was assessed by performing replicate ($n = 6$) extractions of plasma samples containing low, medium and high concentrations of the psychotherapeutic compounds (*i.e.* 2, 20 and 200µg/L respectively). Coefficients of variation (%CV's) were found to be highly satisfactory (see **Table 2**).

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The utility of the developed method was assessed by the analysis of actual plasma samples collected from patients currently receiving the various psychotherapeutic drugs. The procedure was demonstrated to be sufficiently sensitive for routine TDM studies. Our initial studies were extended to investigate the quantification of these drugs in whole blood. The described method was found to be suitable for this matrix also. **Figure 5b** shows a typical standard curve for risperidone in whole blood.

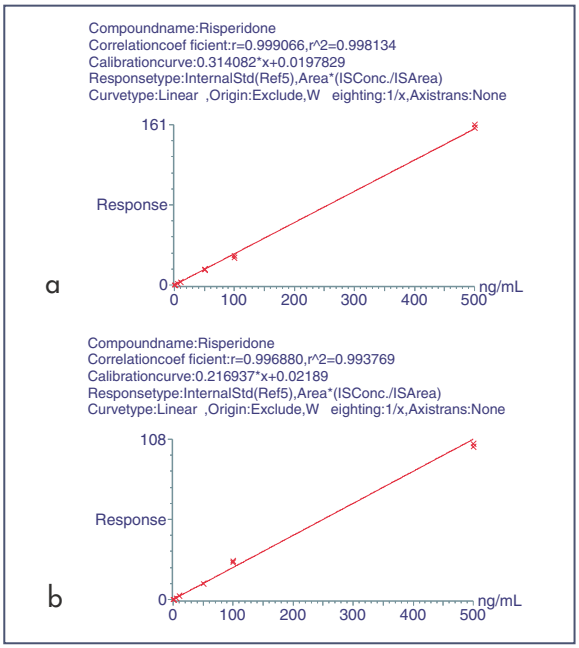


Figure 5. Typical linearity of response for plasma (a) and whole blood (b) containing risperidone. In all cases, drugs were quantified by reference to the internal standard

Compound	CV (%)		
	Low	Med	High
Amitriptyline	13.1	3.5	1.9
Citalopram	8.4	2.9	4.1
Clomipramine	11.9	7.1	4.0
Dibenzepine	6.8	3.1	5.9
Haloperidol	15.9	1.9	3.4
Imipramine	14.0	9.8	5.0
Nortriptyline	15.6	3.3	3.5
Quetiapine	6.0	3.9	3.6
Risperidone	10.8	3.5	5.7
Sertraline	18.6	3.1	3.3

Table 2. Precision of LC-MS/MS method for the analysis of psychotherapeutic agents in plasma.

CONCLUSION

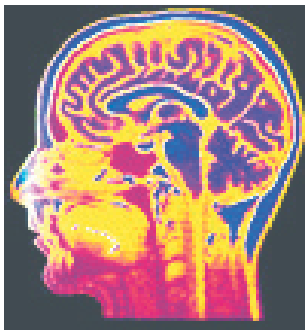
Drug monitoring is advocated for individuals who are receiving psychotherapeutic medication in order to establish target therapeutic concentrations and to evaluate compliance. Thus, we have developed a simple and rapid HPLC-MS/MS method that allows the simultaneous quantification of a several commonly prescribed psychotherapeutic drugs during a single injection. The procedure has been successfully applied to whole blood and plasma samples collected from patients currently receiving treatment with various psychotherapeutic agents and offers several advantages over the existing methods *i.e.* it is more sensitive, faster and less labour-intensive.

FUTURE AIMS

- To continue to assess the utility of this method in routine TDM studies carried out in the clinic.
- To assess the feasibility of using alternative specimens such as saliva.
- To extend the profile of psychotherapeutic agents.

REFERENCES

1. World Health Organisation Annual report 2001.
2. Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S, Wittchen HU, Kendler KS. Arch Gen Psychiatry 1994 Jan; 51(1):8-19



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Author to whom all correspondence should be addressed:
Michelle Wood
Waters Corporation
(Micromass UK Limited)
Floats Road, Wythenshawe
Manchester, M23 9LZ
Tel: + 44 (0) 161 946 2400
Fax: + 44 (0) 161 946 2480
e-mail: michelle.wood@micromass.co.uk

WATERS CORPORATION
34 Maple St.
Milford, MA 01757 U.S.A.
T: 508 478 2000
F: 508 872 1990
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