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Applikationsbericht

Quantitative Analysis of Multiple Psychotherapeutic Drugs in Human Serum using UPLC-MS/MS

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Abstract

To develop a simple UPLC-MS/MS method for the simultaneous quantitation of multiple psychotherapeutic drugs in human serum and assess its utility with authentic samples. The new method should improve upon the total analysis time, thereby increasing sample throughput and the laboratory capacity for additional samples.

Introduction

- Psychotherapeutic drugs are commonly prescribed for the treatment of depressive, anxiety, bipolar and eating disorders
- The use of antidepressants has increased greatly in recent years
- Analysis of psychotherapeutics can be useful in clinical, post-mortem and forensic toxicology
- Dose optimisation is often required while undergoing treatment therefore therapeutic drug monitoring may be needed

Experimental

Specimens

Human serum samples (n=23) used for validation were obtained from the Medical Toxicology Unit where they were analysed using an established validated HPLC/UV method which involves a liquid/liquid extraction followed by a 20 minute chromatographic run.

Sample Preparation

A simple protein precipitation step was undertaken which comprised the addition of acetonitrile (300 µL) containing 3 deuterated internal standards (Clomipramine-D3, Doxepin-D3 + Imipramine-D3) to the human serum samples (100 µL). The samples were then vortex mixed for 30 seconds before centrifugation at 12000 rpm (~12000 x g) for 10 minutes. The supernatant (200 µL) was added to 5mM Acetic acid, pH 4 (200 µL)

and vortex mixed for 30 seconds.

UPLC-MS/MS

UPLC:	ACQUITY UPLC System(Figure 1)
Column:	Waters ACQUITY UPLC BEH C_{18}(2.1 x 100 mm, 1.7 µm)
Column temp:	40 °C
Mobile phase:	A=5mM Ammonium acetate + 0.05% Formic acid, B=Acetonitrile
Flow rate:	0.35 mL/min
Injection vol:	10 µL
Data processing:	MassLynx v4.1 with TargetLynx Application Manager
Mass spectrometer:	Quattro Premier XE (Figure 1)
Ionisation mode:	Electrospray positive
Capillary voltage:	3kV
Collision gas pressure:	Argon at 3.8 x 10^{-3} mBar

Gradient:

Time(min)	%B	Curve
0.0	10	Initial

Time(min)	%B	Curve
0.5	35	11
2.5	35	6
4.0	40	6
5.0	100	1
8.0	10	1

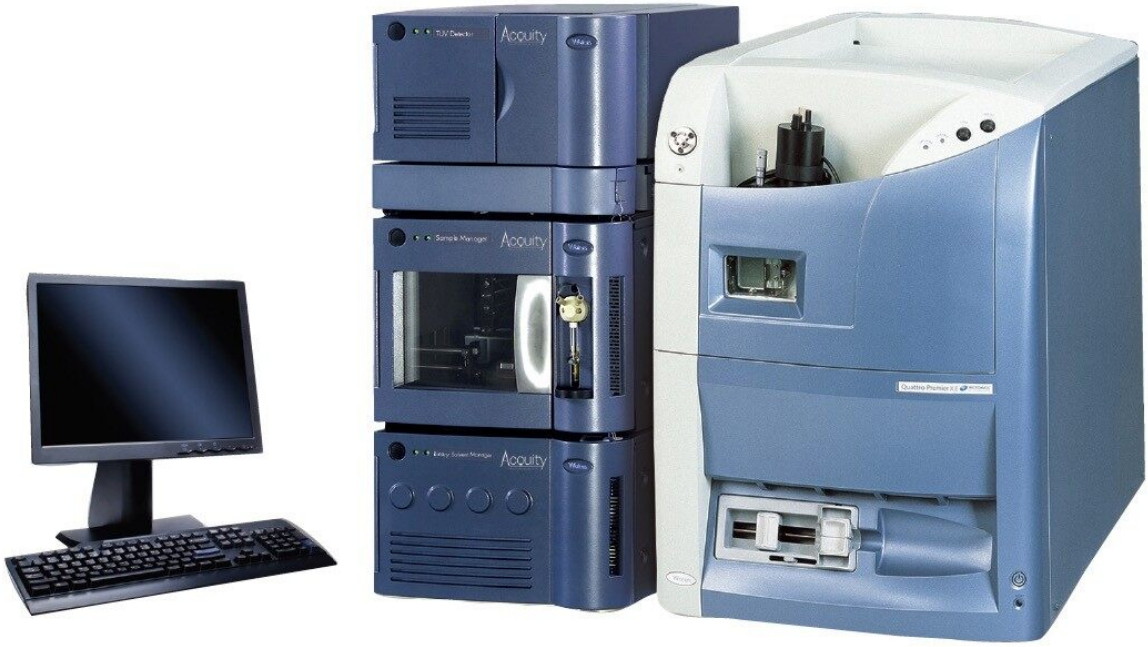

Figure 1. System configuration – ACQUITY UPLC System with a Quattro Premier XE Mass Spectrometer.

Results and Discussion

Figure 2 shows the precursor and product ion spectra for a selection of psychotherapeutic compounds. The MRM conditions used for the measurement of the psychotherapeutic drugs, metabolites and internal standards are summarised in Table 1. A quantifier and qualifier ion was monitored for each compound.

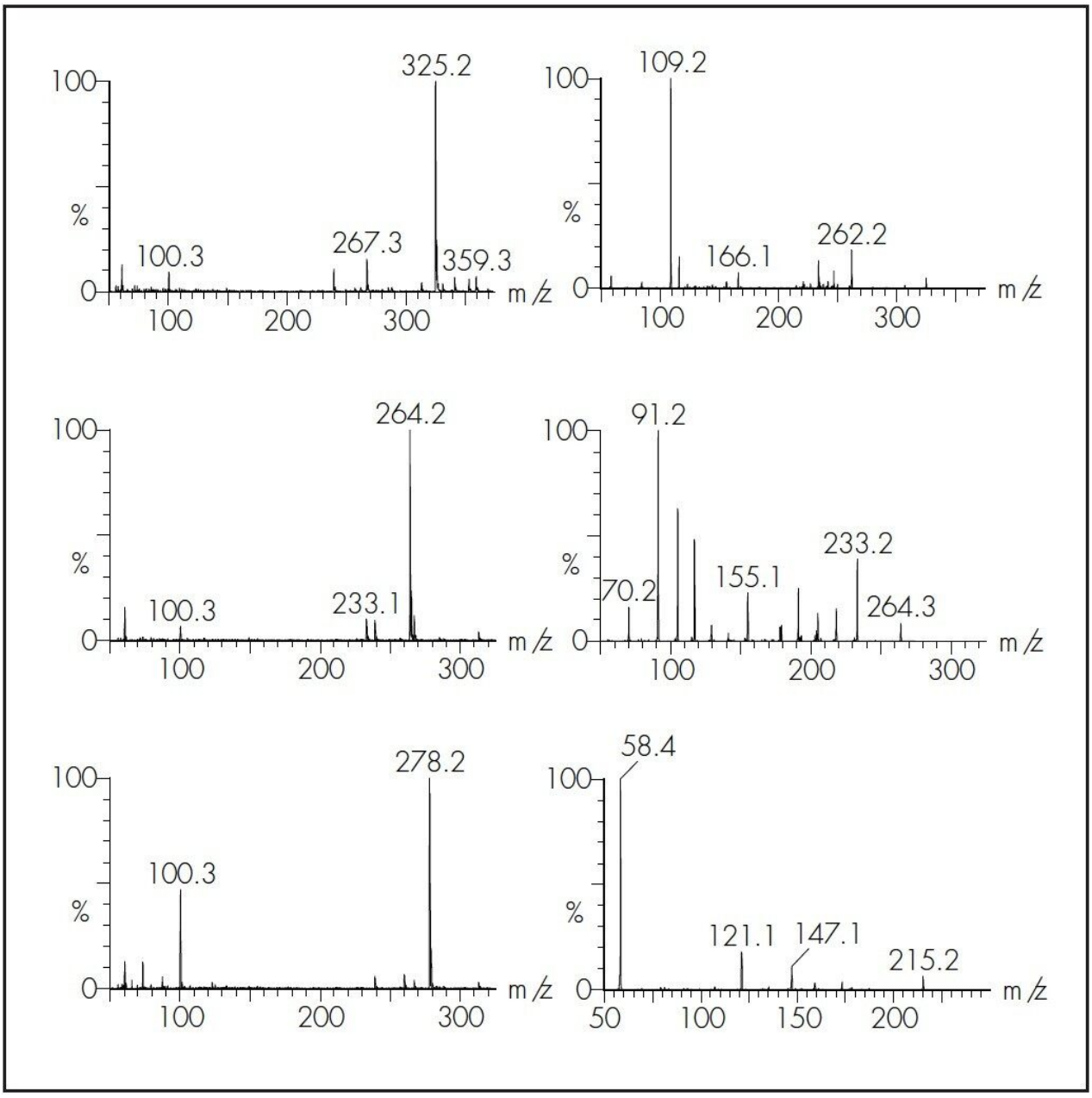

Figure 2. Precursor (left-hand trace) and product (right-hand trace) ion spectra of citalopram, nortriptyline and venlafaxine (top to bottom respectively).

Compound	Precursor ion (m/z)	Product ion (m/z)	Cone voltage (V)	Collision energy (eV)
Amitriptyline	278	91	35	25
Clomipramine	315	86	35	20
Norclomipramine	301	72	35	15
Clomipramine-d3	318	89	35	20
Desipramine	267	72	35	15
Doxepin	280	107	35	25
Doxepin-d3	283	107	40	25
Imipramine	281	86	30	15
Imipramine-d3	284	89	30	15
Nortriptyline	264	91	35	20
Protriptyline	264	155	40	20
Trimipramine	295	100	35	15
Citalopram	325	109	40	25
Fluoxetine	310	148	25	8
Norfluoxetine	296	134	20	5
Fluvoxamine	319	71	30	15
Paroxetine	330	192	45	20
Sertraline	306	159	25	25
Venlafaxine	278	58	30	20
Trazodone	372	148	35	35

Table 1. MRM Conditions used for the psychotherapeutic drug analysis (only quantifier ion conditions shown).

A series of calibrators (1, 2, 5, 10, 50, 100 and 200 µg/L) were prepared by adding the psychotherapeutic drugs to drug-free serum. The drugs were isolated from the matrix by acetonitrile precipitation which incorporated the internal standard addition.

Figure 3 shows the MRM chromatograms obtained from a 10 µL injection of a 2 µg/L serum calibrator.

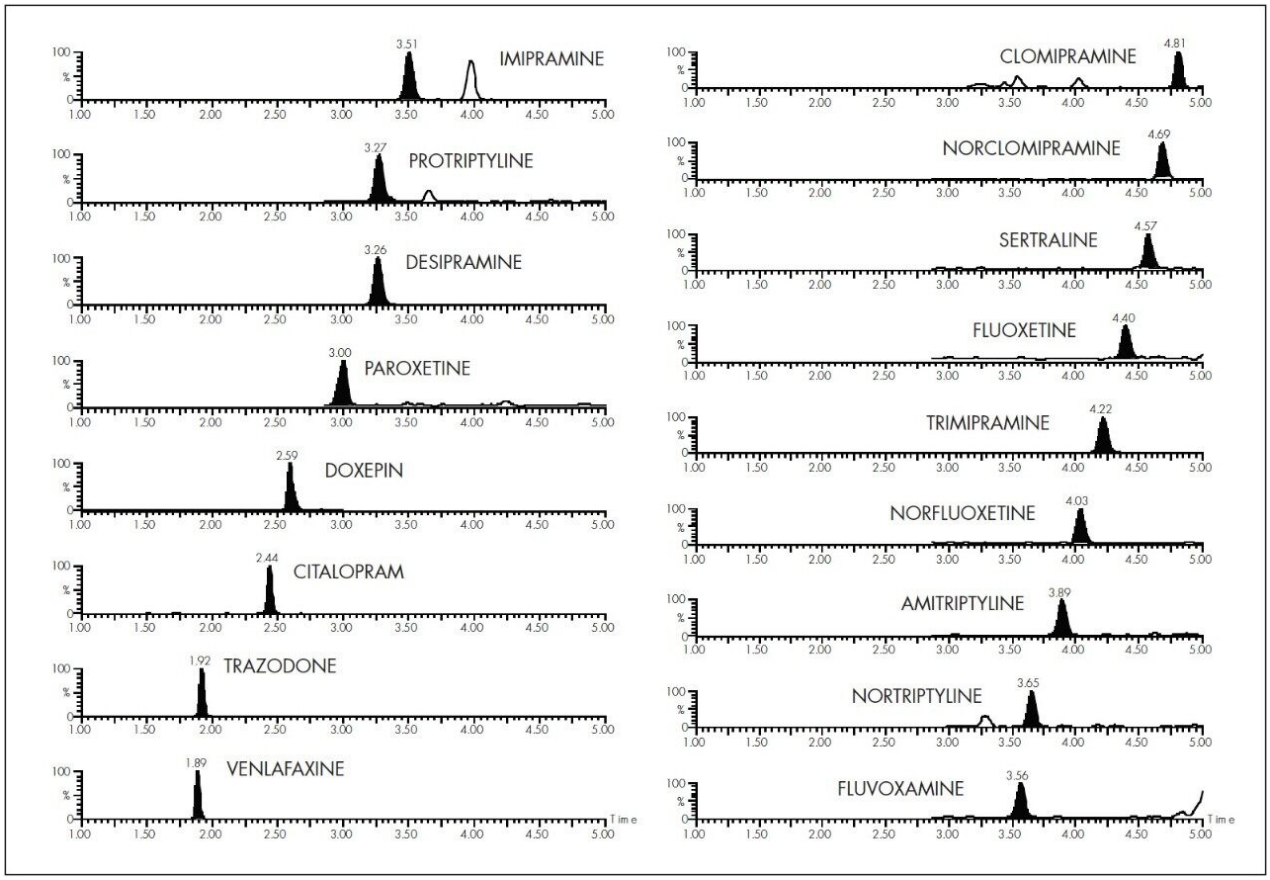

Figure 3. MRM chromatograms for all compounds which were obtained with a 10 µL injection of a 2 µg/L serum calibrator (only quantifier ion chromatograms shown).

Quantitation was performed by the integration of the area under the peak within the specific MRM chromatogram. Figure 4 shows a typical standard curve for venlafaxine in serum. Responses were linear for all compounds, over the investigated range (coefficient of determination > $r^2 = 0.996$).

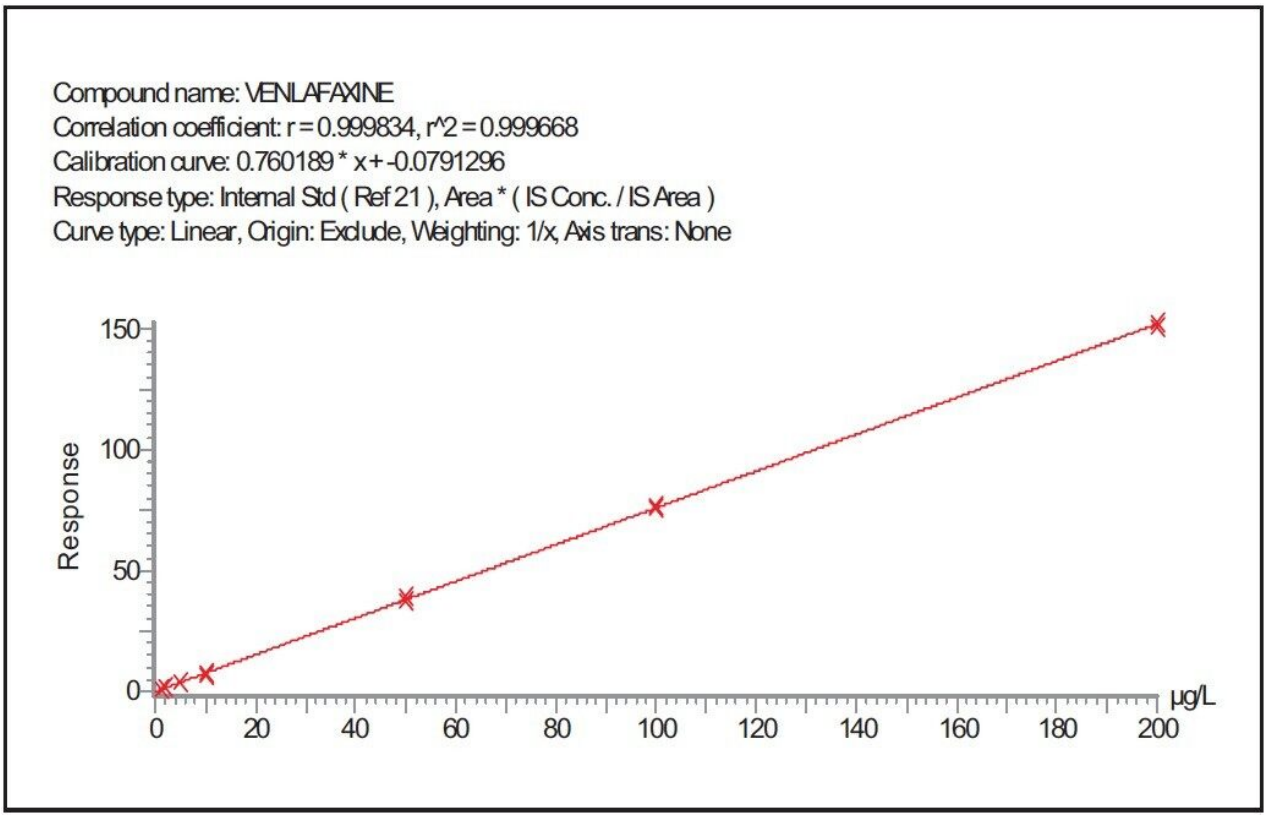

Figure 4. Typical response for venlafaxine extracted from serum. All compounds were quantified by reference to one of the three internal standards

The limits of detection were assessed for all compounds and found to range between 0.1 - 1.0 µg/L, which is below the limits required for this analysis. Figure 5 shows a patient sample containing citalopram at the lower therapeutic range.

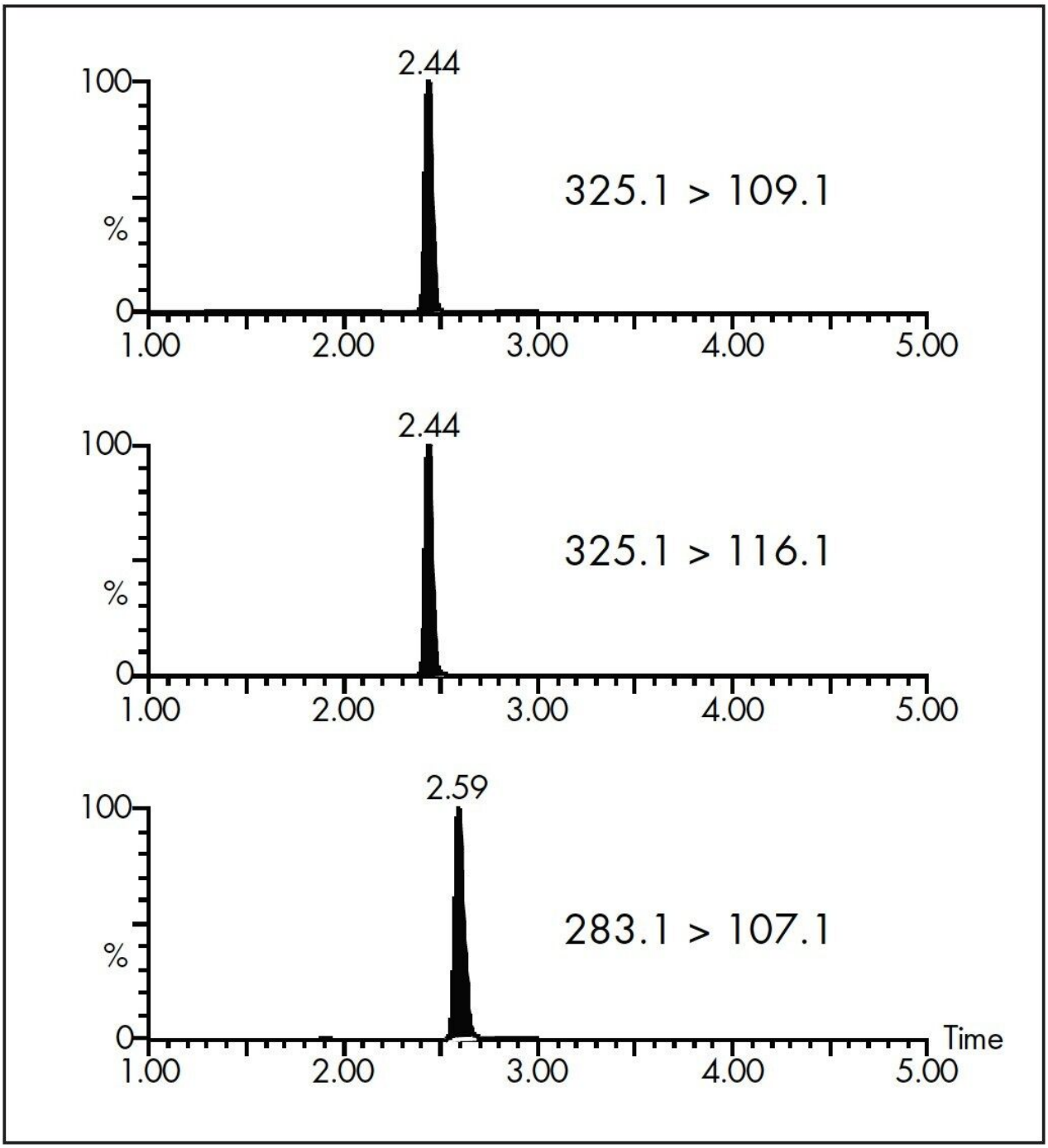

Figure 5. MRM chromatograms for citalopram (quan and qual ion shown in top and middle trace, respectively) in a patient sample at the lower therapeutic range (~20 µg/L). Doxepin-D3 (bottom trace) was used as the internal standard. Concentration of citalopram in the sample was found to be 29.2 µg/L.

Intra-assay precision and accuracy was assessed by adding the psycho-therapeutic compounds to drug-free serum (n=4) at a low, medium and high concentration (10, 50 and 150 µg/L). The spiked QC samples were

extracted as previously described. Intra-assay precision and accuracy results were good with CV's < 15% and > 88%, respectively. The use of protein precipitation was demonstrated to be very efficient and gave reproducible extraction recoveries > 93% for all analytes.

Matrix effects were assessed by the comparison of six different patient serum samples spiked with the psychotherapeutic compounds after extraction against the equivalent concentration solvent standards. Matrix effects were found to be acceptable with the norclomipramine response being most affected (+10%).

Addition of the 5mM acetic acid, pH 4 to the sample in the preparation phase was found to increase the stability of all compounds when assessed over 12 hours by the hourly injection of a 50 µg/L serum calibrator. It was found that was no significant change in response for any compound over the investigated time period.

As part of the testing, patient samples (n=23) were analysed by the newly developed UPLC-MS/MS method and the established HPLC/UV method, the results showed good agreement.

Conclusion

As the use of psychotherapeutic drugs increases the need for their analysis whether it be for therapeutic drug monitoring, clinical or forensic reasons will also grow. Therefore, there is a need for a simple, rapid analytical procedure for the analysis of these drugs.

The developed methodology has been shown to be accurate and precise in the screening and quantitation of psychotherapeutic drugs in a single 8 minute chromatographic run.

This method has been applied successfully to the analysis of clinical samples and the results compared against an established HPLC/UV method.

The objective to reduce the total analysis time was achieved through a combination of a significantly faster sample preparation by the use of protein precipitation and the use of UPLC, which more than halved the chromatographic run time. The new UPLC-MS/MS method resulted in greater laboratory efficiency and therefore sample capacity.

This application is an example of a feasible assay which can be run on Waters Instruments. Complete method validation by users is needed prior to use.

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