High-Throughput Quantitative Analysis of Tricyclic Antidepressants and Selective Serotonin Re-uptake Inhibitors in Human Serum Using Ultrafast SPE/MS/MS

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Introduction

Analysis of tricyclic antidepressants (TCAs) could be necessary in forensic cases such as driving under the influence of drugs, cases of violent crime, sexual assault cases and unknown cause of death cases. Selective serotonin reuptake inhibitors (SSRIs) - a class of psychotropic drugs - act by specifically inhibiting the reuptake of serotonin – a neurotransmitter used to communicate between the brain cells – into the presynaptic cell. However, SSRIs are known to have adverse behavioral and mental reactions and drug-drug interactions prompting ongoing research towards improved analytical sensitivity and specificity.

Traditional measurement methods for clinical research in quantitative TCAs and SSRI drugs analysis use HPLC, recently LC/MS/MS and other technologies. The need for greater throughput and faster turn-around times has increased demands on these traditional technologies. The RapidFire High-throughput Mass Spectrometry System is an ultrafast SPE/MS/MS system capable of analyzing samples with cycle times under 13 seconds per sample. In the present study, we developed two ultrafast SPE/MS/MS methods for simultaneous analysis in human serum of 8 TCAs: Amitriptyline, Nortriptyline, Imipramine, Desipramine, Doxepin, Nordoxepin, Clomipramine, and Norclomipramine; and 6 SSRIs: Citalopram, N-desmethyl citalopram, fluoxetine, norfluoxetine, sertraline and paroxetine. These methods are with much faster sample cycle times and similar analytical results compared to HPLC and LC/MS/MS analysis.

Experimental

Online SPE methods for each analyte were optimized using a RapidFire High-throughput Mass Spectrometry system coupled to an Agilent 6460 or 6490 triple quadrupole mass spectrometer. Analysis of all samples was performed at a rate of <13 seconds per sample covering the range of 10-500 ng/mL.

Sample preparation

For the TCAs The samples, calibrators (10, 100, 250, and 500 ng/mL) and QC levels (50, 200, and 400 ng/mL) were prepared using the following procedure. First, 150 ul of sample was added to a 1.5 mL micro centrifuge tube. Next, 150 uL of 0.2M zinc sulfate was added and the sample was gently mixed. Methanol containing the deuterated internal standard (200 ng/mL), 300 uL, was added next, followed by vigorous vortexing for 30 seconds. The samples were then centrifuged at 13,000 rpm for 10 min. A portion of the supernatant from each tube (100 uL) was added into a corresponding well of a deep well plate containing 900 uL of LC/MS grade water. The plate was then sealed with an Agilent PlateLoc Thermal Microplate Sealer and mixed prior to RapidFire/MS/MS analysis.

For the SSRIs Pooled standard calibrators were prepared by spiking 500 ng/mL of each of the above mentioned drugs into drug-free human serum. Serial dilutions were used to achieve the remaining standard calibration concentrations. 100 µL of 0.2M zinc sulphate was added to 100 µL of each sample and vortexed. Next, 200 µL of acetonitrile containing the internal standard citalopram-D6 at 100 ng/ml was added to each sample, vortexed and then centrifuged at 13,000 rpm for 10 min. Samples were then diluted 1:10 with water containing 0.1% formic acid, transferred to 96 well-plates and centrifuged prior to injection on the Agilent RapidFire/MS system.

Experimental

The following MRM transitions were monitored for both panels using Agilent 6460 or 6490 triple quadrupole mass spectrometers.

Compound Name	Precursor Ion	Product Ion	Dwell	Fragmentor	Collision Energy	CAV
Clomipramine-d3	318.2	89.1	5	110	13	4
Clomipramine Q	315.2	86.1	10	80	13	4
Clomipramine	315.2	58.1	10	80	49	4
Norclomipramine Q	301.2	72.1	10	100	13	4
Norclomipramine	301.2	44.1	10	100	49	4
Imipramine-d3	284.2	89.1	5	100	13	4
Doxepin-d3	283.2	107.1	5	115	21	4
Amitriptylene-d3	281.2	91.1	5	110	25	4
Imipramine Q	281.2	86.1	10	75	13	4
Imipramine	281.2	58.1	10	75	45	4
Doxepin	280.2	115	10	115	50	4
Doxepin Q	280.2	107.1	10	115	21	4
Amitriptyline	278.2	117.1	10	115	21	4
Amitriptyline Q	278.2	91	10	115	25	4
Desipramine Q	267.2	72.1	10	90	13	4
Desipramine	267.2	44.1	10	90	50	4
Nordoxepin Q	266.2	235.1	10	100	13	4
Nordoxepin	266.2	107	10	100	21	4
Nortriptyline Q	264.2	233.2	10	100	13	4
Nortriptyline	264.2	91.1	10	100	25	4

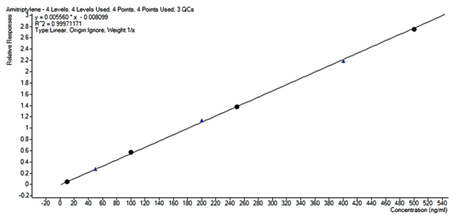
Compound Name	Precursor Ion	Product Ion	Dwell	Fragmentor	Collision Energy	CAV
Citalopram	325.2	109	20	380	25	5
Citalopram	325.2	262	20	380	15	5
N-Desmethyl Citalopram	311.2	262.1	20	380	10	7
N-Desmethyl Citalopram	311.2	234	20	380	10	6
Fluoxetine	310.2	44.1	20	380	8	5
Fluoxetine	310.2	148	20	380	2	2
Norfluoxetine	296.1	134	20	380	0	3
Norfluoxetine	296.1	30*	50	380	10	2
Paroxetine	330.2	192.1	20	380	18	5
Paroxetine	330.2	69.9	20	380	25	4
Sertraline	306.1	158.9	20	380	10	5
Sertraline	306.1	275	20	380	7	3
Citalopram –D_6 (IS)	331.2	109	20	380	25	5
Citalopram –D_6 (IS)	331.2	262	20	380	15	5

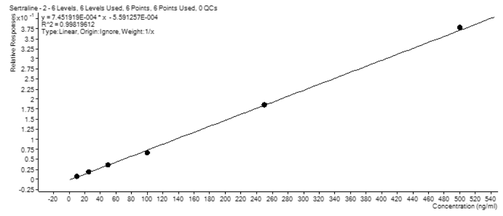
Ionization mode ESI +	Agilent 6460	Agilent 6490
Panels	TCAs	SSRIs
Drying gas temp.	300 °C	250 °C
Drying gas flow	10 L/min	15 L/min
Sheath gas temp.	350 °C	300 °C
Sheath gas flow	11 L/min	12 L/min
Nebulizer pressure	45 psi	45 psi
Nozzle voltage	500 V	0 V
Capillary voltage	3500V	3500 V

RapidFire Method	TCAs	SSRIs
Solvent A	H_2O + 0.1% FA	H_2O + 0.1% FA
Solvent B	Methanol + 0.1% FA	Acetonitrile + 0.1% FA
Solvent C	Methanol + 0.1% FA	Acetonitrile + 0.1% FA
SPE Cartridge	C18	C18
RF State 1: Aspirate	600 ms	1000 ms
RF State 2: Load/Wash	2000 ms	3500 ms
RF State 3: Extra Wash	0 ms	0 ms
RF State 4: Elute	7000 ms	6000 ms
RF State 5: Re-equilibrate	800 ms	2000 ms

Results and Discussion

Linearity: The analytes in both panels had excellent linearity within the measured ranges with R^2 values greater than 0.995.

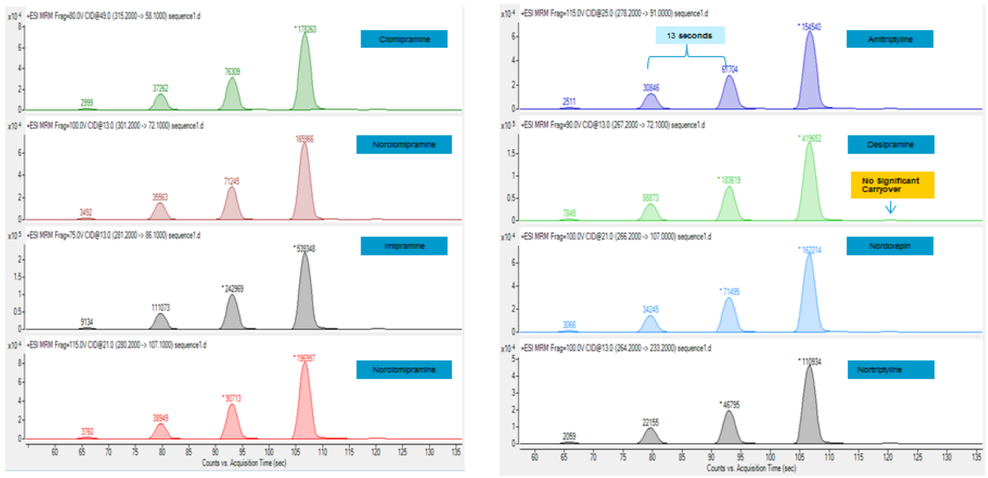




TCAs standard curves in serum had excellent linearity within the measured range of 10-500 ng/mL. The LOQ was determined to be 5 ng/mL for all analytes. Example shown: Amitriptyline.

SSRIs standard curves in serum had excellent linearity within the measured range of 10-500 ng/mL. The LOQ was determined to be 5 ng/mL for all analytes. Example shown: Sertraline.

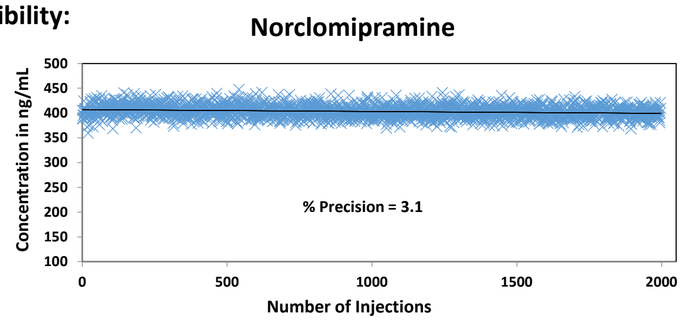
Timing and Carryover: Example of carryover assessment for TCAs



Precision: Inter and intra-day accuracies determined were within 10% and coefficient of variation values were all less than 10% for concentrations within the measured range for both panels. Example : Sertraline

Sertraline	Intraday %	Intraday %	Interday %	Interday %
ng/ml	Accuracy (n=4)	Precision (n=4)	Accuracy (n=4)	Precision (n=4)
10	103.59	5.83	100.41	8.20
25	103.43	6.20	101.35	4.09
50	95.17	4.08	93.73	7.51
100	93.32	4.38	90.08	6.67
250	96.70	6.15	100.52	7.18
500	102.30	4.03	101.96	3.93

Reproducibility:



Repeatability evaluation using >2000 sequential injections of Norclomipramine

Conclusions

TCA and SSRI drug classes were accurately and precisely quantified using an Agilent High-throughput RapidFire Mass Spectrometry System. Samples containing 8 TCAs analytes were simultaneously analyzed at 13 seconds per sample, using a high-throughput method of quantitation for these analytes that is capable of analyzing more than 240 samples per hour. Samples containing 6 SSRIs were similarly analyzed using this same technology. This SPE/MS/MS methodology provides comparable results to LC/MS/MS, but at >10x the speed and efficiency of typical LC/MS/MS methods.