Ultrafast Analysis of Selective Serotonin Reuptake Inhibitors (SSRIs) in Human Serum by the Agilent RapidFire High-Throughput Triple Quadrupole Mass Spectrometry System

Application Note

Authors

Nikunj R. Parikh, Michelle Romm, and Vaughn P. Miller
Agilent Technologies, Inc.
Wakefield, MA USA

Abstract

The need for greater analytical capacity and throughput at clinical research laboratories for the analysis of selective serotonin reuptake inhibitors (SSRIs) in human serum has placed demands on traditional analytical technologies. An ultrafast analytical method for quantifying six SSRIs as a panel in human serum was developed using an Agilent RapidFire High-Throughput Mass Spectrometry system. Citalopram, N-desmethyl citalopram, fluoxetine, norfluoxetine, sertraline, and paroxetine were accurately and precisely measured within a linear range of 10–500 ng/mL. All six analytes, and a common internal standard citalopram-d_6, were simultaneously measured at 13 seconds per sample, providing a throughput of greater than 250 samples per hour.

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Introduction

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, as well as drug-drug interactions, prompting ongoing research towards improved analytical sensitivity and specificity. The steady increase in sample volume for these assays has led to the need for greater analytical capacity, placing a strain on traditional technologies. In this study, we evaluated the ability of the Agilent RapidFire High-throughput Mass Spectrometry System, an ultrafast SPE/MS/MS system, to measure a variety of different drugs within the SSRI class in human serum at low ng/mL concentrations, with sample cycle times of 13 seconds per sample.

Experimental

The RapidFire/MS/MS system consisted of the following modules: an Agilent RapidFire 360 High Throughput Mass Spectrometer, an Agilent 6490 Triple Quadruple Mass Spectrometer, an Agilent MassHunter Qualitative Analysis B.05.00, and an Agilent MassHunter Quantitative Analysis B.05.00. Samples were analyzed at a rate of 13 seconds per sample. Analyte and internal standard ions were monitored simultaneously in all experiments for all six SSRI drugs.

Chemicals and reagents

Citalopram, N-desmethyl citalopram, fluoxetine, sertraline, paroxetine (1.0 mg/mL in methanol), and norfluoxetine (100 µg/mL in methanol), were purchased from Cerilliant, Round Rock, TX. Blank human serum was purchased from UTAK, Valencia, CA. All other solvents and reagents were purchased from Sigma-Aldrich, St. Louis, MO.

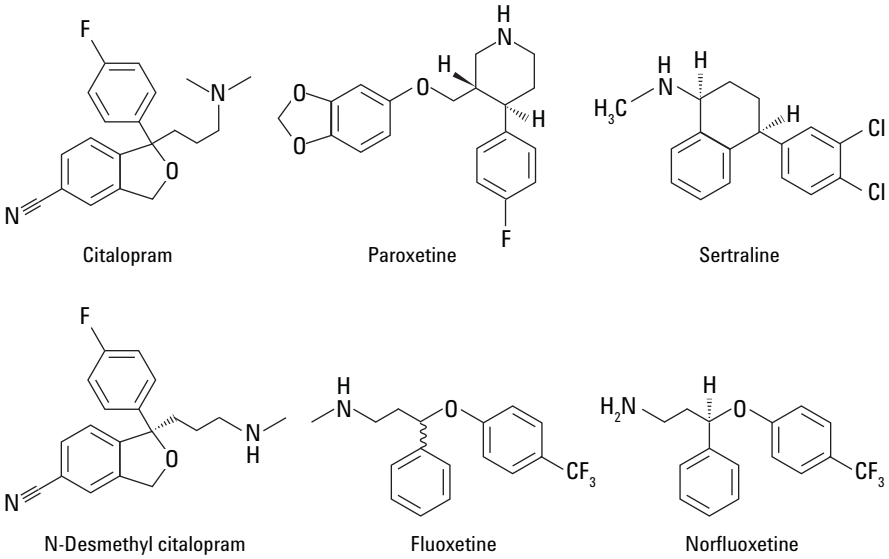


Figure 1. Chemical structures of the six SSRIs in the panel.

Table 1. Agilent RapidFire/MS/MS conditions.

Agilent RapidFire conditions	
Buffer A (Pump 1)	LC/MS grade water + 0.1 % formic acid 1.5 mL/min flow rate
Buffers B and C (Pumps 2 and 3)	100 % LC/MS grade acetonitrile + 0.1 % formic acid 1.25 mL/min flow rate
Back chimney wash	Water (aqueous), methanol (organic)
Injection volume	10 µL
SPE cartridge	Agilent RapidFire cartridge C18 (G9203-80105)
RF State 1	sip sensor
RF State 2	2,500 ms
RF State 3	7,000 ms
RF State 4	500 ms
Agilent 6490 Triple Quadruple conditions	
Gas temperature	250 °C
Gas flow	15 L/min
Nebulizer	45 psi
Sheath gas temperature	300 °C
Sheath gas flow	12 L/min
Nozzle voltage	0 V
Capillary voltage	3,500 V
Peak width	0.03

Sample preparation

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. A 100 µL amount of 0.2 M zinc sulphate was added to 100 µL of each sample and vortexed. Next, 200 µL of acetonitrile containing the internal standard citalopram-D_6 was added to each sample at 100 ng/mL, vortexed, and centrifuged at 2,000 xg for 10 minutes. 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.

Data analysis

The peak integration was performed using the Agilent MassHunter Quantitative Analysis Software. The AUC of each analyte was normalized by the AUC of citalopram-D_6 internal standard. The entire dataset was subjected to linear regression with 1/x weighting.

Table 2. MRM transitions.

Analyte	Q1	Q3	Frag	CE	Dwell	CAV
Citalopram	325.2	109	380	25	20	5
Citalopram-Qual	325.2	262	380	15	20	5
N-Desmethyl citalopram	311.2	262.1	380	10	20	7
N-Desmethyl citalopram-Qual	311.2	234	380	10	20	6
Fluoxetine	310.2	44.1	380	8	20	5
Fluoxetine-Qual	310.2	148	380	2	20	2
Norfluoxetine	296.1	134	380	0	20	3
Norfluoxetine-Qual	296.1	30*	380	10	50	2
Paroxetine	330.2	192.1	380	18	20	5
Paroxetine-Qual	330.2	69.9	380	25	20	4
Sertraline	306.1	158.9	380	10	20	5
Sertraline-Qual	306.1	275	380	7	20	3
Citalopram-D_6 (IS)	331.2	109	380	25	20	5
Citalopram-D_6 (IS)-Qual	331.2	262	380	15	20	5

*MS2 resolution at wide.

Results and Discussion

Samples were prepared by spiking SSRIs into drug-free human serum followed by a crash with zinc sulphate and acetonitrile, then dilution with water and 0.1 % formic acid (1:10). Samples were then analyzed through SPE/MS/MS using the RapidFire/MS/MS system and a C18 cartridge at 13 seconds per sample (Figure 2). This RapidFire/MS/MS methodology is capable of throughputs greater than 250 samples per hour providing a high-throughput and very efficient analysis.

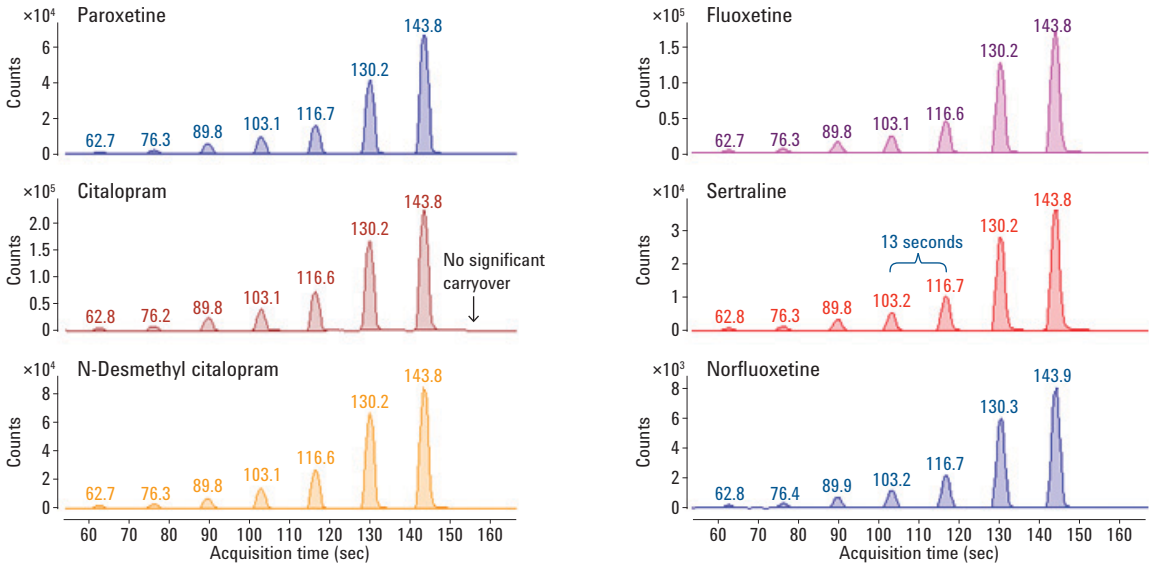


Figure 2. Representative calibration curve data for each of the six SSRI analytes showing the injection to injection interval of 13 seconds. Carryover assessment using a matrix blank immediately after the highest calibrator for all analytes shows no significant carryover for any of the analytes.

Pooled SSRI standard curves containing each of the six SSRIs were analyzed separately to obtain intra- and interday precision and accuracy values. Intra- and interday accuracies determined for the SSRI analytes were within 10 %, and coefficient of variation values were all less than 10 % for concentrations within the measured range (Tables 3–8). The analytes had excellent linearity within the measured ranges with R^2 values greater than 0.99 (Figures 3–8).

SSRIs were quantified between 10–500 ng/mL, and were determined to have limits of detection (LODs) of less than 5 ng/mL. We recommend using a single blank solvent injection for concentrations higher than 500 ng/mL to minimize the potential for carryover. The addition of a blank injection after a highly concentrated sample increases total sample analysis time to approximately 26 seconds for that sample, which is still several-fold faster than traditional LC/MS/MS methods.

This method consisting of a standard crash and dilution for sample preparation followed by quick analysis on RapidFire/MS/MS provides a very efficient mode of quantitatively measuring SSRIs in human serum compared to traditional LC/MS/MS methods.

Table 3. Intraday and interday precision and accuracy for citalopram.

Concentration (ng/mL)	Intraday % accuracy (n = 4)	Intraday % precision (n = 4)	Interday % accuracy (n = 4)	Interday % precision (n = 4)
10	101.55	8.48	103.57	8.51
25	104.87	6.52	101.32	5.52
50	97.94	1.42	97.76	5.34
100	101.23	7.20	100.49	7.12
250	108.09	6.34	105.56	6.86
500	93.72	8.71	97.20	4.50

Table 4. Intraday and interday precision and accuracy for N-desmethyl citalopram.

Concentration (ng/mL)	Intraday % accuracy (n = 4)	Intraday % precision (n = 4)	Interday % accuracy (n = 4)	Interday % precision (n = 4)
10	101.72	3.40	99.66	8.07
25	107.17	5.83	101.26	7.19
50	92.18	4.08	95.35	5.72
100	96.38	3.19	99.03	3.20
250	101.92	8.05	104.20	8.34
500	99.78	4.17	98.21	4.73

Table 5. Intraday and interday precision and accuracy for fluoxetine.

Concentration (ng/mL)	Intraday % accuracy (n = 4)	Intraday % precision (n = 4)	Interday % accuracy (n = 4)	Interday % precision (n = 4)
10	103.05	5.13	99.67	7.69
25	94.98	6.44	99.72	6.44
50	94.23	5.03	94.52	7.87
100	94.25	3.32	95.37	3.99
250	96.64	7.81	101.01	7.13
500	103.07	3.91	100.59	4.37

Table 6. Intraday and interday precision and accuracy for sertraline.

Concentration (ng/mL)	Intraday % accuracy (n = 4)	Intraday % precision (n = 4)	Interday % accuracy (n = 4)	Interday % 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

Conclusions

Analytes from the SSRI class were accurately and precisely quantified using an Agilent High-throughput RapidFire Mass Spectrometry System. Samples containing six SSRI 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 250 samples per hour. This SPE/MS/MS analytical methodology provides comparable results to LC/MS/MS, but at > 10x the speed and efficiency of typical LC/MS/MS methods.

Table 7. Intraday and interday precision and accuracy for norfluoxetine.

Concentration (ng/mL)	Intraday % accuracy (n = 4)	Intraday % precision (n = 4)	Interday % accuracy (n = 4)	Interday % precision (n = 4)
10	99.83	5.08	101.47	8.35
25	104.74	3.73	100.64	6.04
50	99.92	3.63	95.51	7.92
100	96.23	6.75	93.80	4.58
250	100.57	2.48	100.32	5.51
500	99.35	3.38	101.31	3.17

Table 8. Intraday and interday precision and accuracy for paroxetine.

Concentration (ng/mL)	Intraday % accuracy (n = 4)	Intraday % precision (n = 4)	Interday % accuracy (n = 4)	Interday % precision (n = 4)
10	100.85	4.05	99.85	7.39
25	99.21	4.33	101.50	6.05
50	100.08	6.74	95.90	7.88
100	101.90	6.09	98.44	6.52
250	106.81	1.55	103.16	4.04
500	99.52	5.37	99.60	3.33

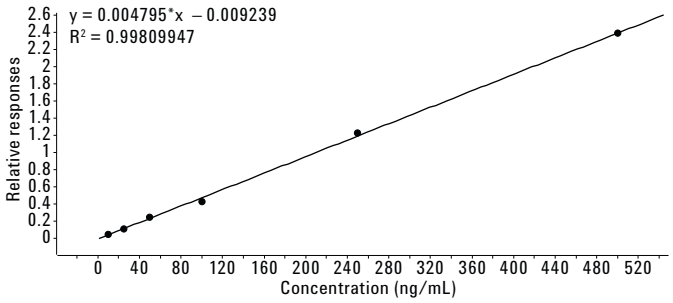


Figure 3. Citalopram representative standard curve in human serum.

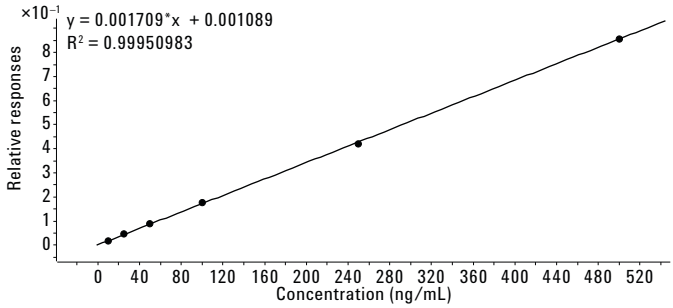


Figure 4. N-Desmethyl citalopram representative standard curve in human serum.

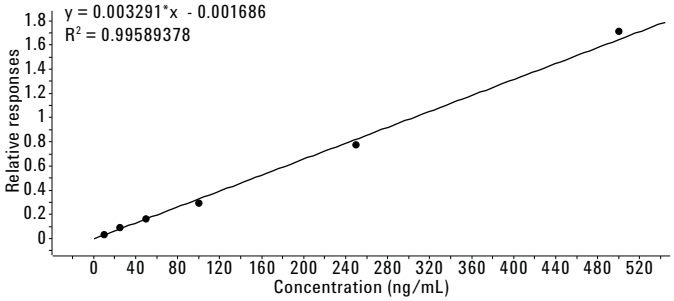


Figure 5. Fluoxetine representative standard curve in human serum.

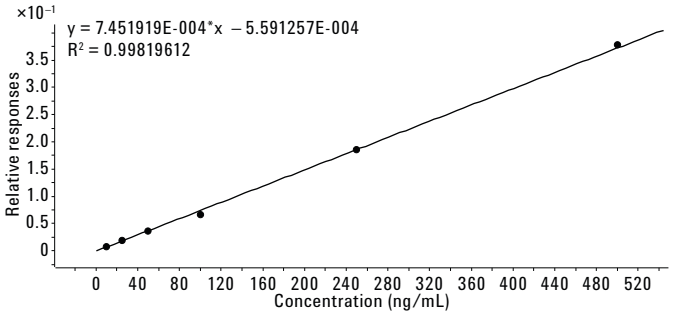


Figure 6. Sertraline representative standard curve in human serum.

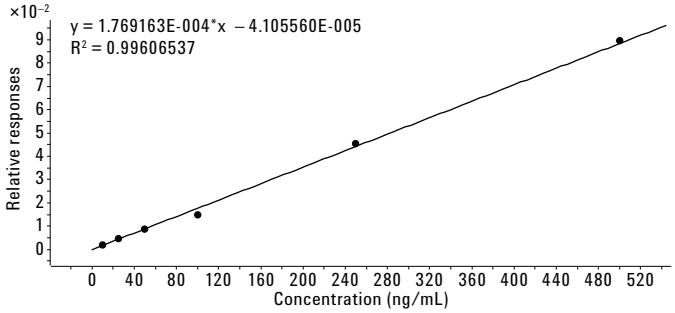


Figure 7. Norfluoxetine representative standard curve in human serum.

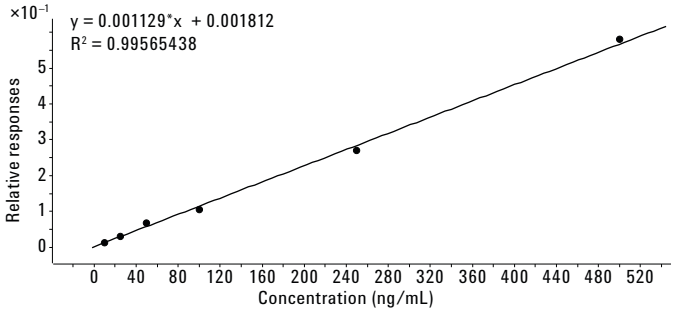


Figure 8. Paroxetine representative standard curve in human serum.

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