LC/MS/MS Research Method for 14 Antidepressants Utilizing Dried Blood Spots

Kerry M. Hassell, Sarah J. Fair, and Joseph L. Herman
Thermo Fisher Scientific, Franklin, MA, USA

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Overview

Purpose: To develop an LC-MS/MS research method for measuring the concentration of antidepressants from dried blood spots (DBS).

Methods: An LC-MS/MS method was developed to extract 14 antidepressants from dried blood spots for quantitation.

Results: The analytes of interest were extracted successful from dried blood spots showing good linearity, accuracy, and precision.

Introduction

Important factors in the analysis of drugs in whole blood are accurate measurements, storage capabilities, small sample volume, and easy extraction. Dried blood spots are becoming an adopted clinical research technique for the analysis of drugs in biological matrices. Due to the complexity of the solution resulting from dissolving the blood spot, the sample must undergo further cleanup by chromatographic separation before introduction into the mass spectrometer. A research application is demonstrated using the Thermo Scientific™ Prelude SPLC™ system and the Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer (Figure 1) to quantitatively analyze 14 antidepressant drugs collected from dried blood spots.

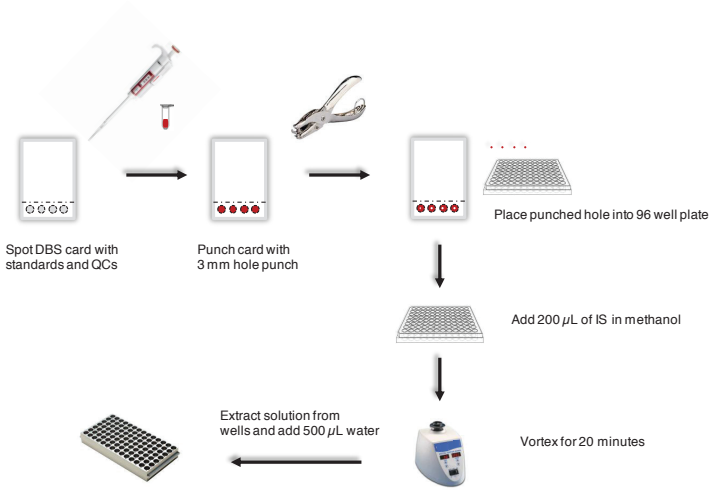
FIGURE 1. Prelude SPLC system with TSQ Endura MS

Methods

Sample Preparation

The analytes of interest were spiked into human whole blood at various concentrations to make calibrators and controls. The samples were spotted at a fixed volume onto Whatman® paper. Then, the analyte was extracted by solvent containing isotopically labeled internal standards and transferred into clean vials for LC-MS/MS analysis. The entire workflow is depicted in Figure 2.

FIGURE 2. Workflow of preparing the dried blood spot for LC/MS



Instrumentation

A Prelude SPLC system was used in TX mode and equipped with a Thermo Scientific™ TurboFlow™ Fluoro XL 0.5 x 50 mm cleanup column and a 2.1 x 50 mm, 2.6 µm particle size Thermo Scientific™ Accucore™ aQ analytical column. The detector for the system was a TSQ Endura triple quadrupole mass spectrometer with a heated electrospray ionization (HESI-II) probe in positive mode. Thermo Scientific™ TraceFinder™ software version 3.1 was used for quantitation. The liquid chromatography flow path is found in Figure 3.

Method Parameters

Mobile phases were (A) 10 mM ammonium formate, 0.05% formic acid in water; (B) 10 mM ammonium formate, 0.05% formic acid in methanol; and (C) 45/45/10 acetonitrile/isopropanol/acetone. The LC method is shown in Table 1. The mass spectrometer quantifier and qualifier selected-reaction monitoring (SRM) transitions are shown in Table 2. The method range for all the analytes was 10–750 ng/mL.

FIGURE 3. Online sample cleanup and analytic separation flow path

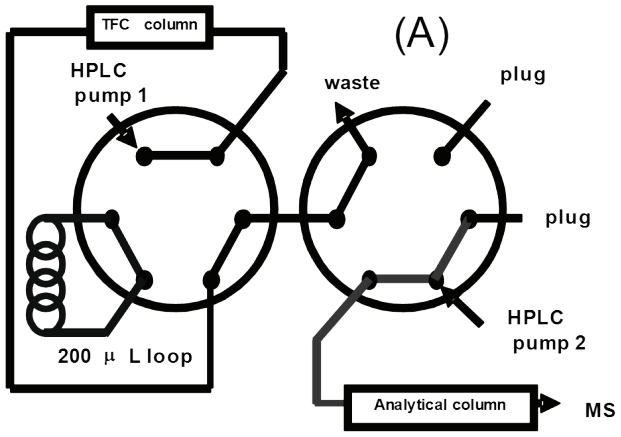


TABLE 1. LC method parameters

Step	Start	Sec	Flow	%A	%B	%C	Tee	Loop	Flow	Grad	%A	%B
1	0.00	40	1.5	100.0	-	-	====	out	0.50		100	-
2	0.67	65	0.20	-	100	-	T	in	0.50		100	-
3	1.75	5	0.20	-	100	-	====	in	0.50		40	60
4	1.83	120	1.20	-	-	100	====	out	0.50		25	75
5	3.83	30	1.10	-	-	100	====	out	0.50		-	100
6	4.33	60	1.50	100	-	-	====	out	0.50		100	-

TABLE 2. SRM transitions

Analyte	Precursor Ion (Q1)	Product Ions (Q3)	Collision Energy	S-lens
Amitriptyline	278.1	233.2	15	61
		191.1	15	61
Doxepin	280.2	235.0	26	61
Imipramine	281.0	86.3	25	88
		208.0	25	88
Fluvoxamine	319.1	200.0	19	70
		228.0	19	70
Clomipramine	314.9	86.2	20	55
		242.1	20	55
Fluoxetine	310.1	117.1	10	83
		148.0	10	83
Paroxetine	330.1	192.0	28	94
		151.0	28	94
Citalopram	325.1	109.1	25	120
		262.0	25	120
Nortiptyline	264.1	233.0	20	50
		191.0	20	50
Desipramine	267.1	208.1	20	48
		236.1	20	48
Venlafaxine	278.2	147.1	15	61
		121.1	15	61
Sertraline	306.0	275.0	13	80
		158.9	13	80
Duloxetine	298.0	154.0	8	55
		-		
Bupropion	241.0	167.0	15	61
		185.0	15	61
Amitriptyline-d_3	281.1	233.2	24	90
Doxepin-d_3	283.2	235.1	24	106
Fluoxetine-d_6	316.1	154.0	16	79
Paroxetine-d_6	336.1	198.1	20	125
Nortiptyline-d_3	267.1	233.1	18	97
Sertraline-d_3	309.0	275.0	12	69

Results

One day of accuracy and precision measurements were performed for system verification on each of the following analytes: amitriptyline, doxepin, imipramine, fluvoxamine, clomipramine, fluoxetine, paroxetine, citalopram, nortriptyline, desipramine, venlafaxine, sertraline, duloxeine, and bupropion. The inter-day and intra-day accuracy and precision were tested from 10–750 ng/mL for each analyte. A summary of the results is shown in Table 3. The assay precision had RSD values that were less than 15.0% for all compounds tested; LOQ compounds had values less than 20.0%. Additionally, accuracy was ±15.0% of the theoretical value for all assays. The correlation coefficient values for all compounds ranged from 0.9900 to 0.9950, showing linearity throughout all concentrations and analytes. All analytes passed carryover, recovery, and selectivity criteria, as well as benchtop and autosampler stability criteria. Recoveries were all above 90.0% including matrix effects. Example SRM chromatograms at the LOQ are shown in Figure 4. Examples of the calibration curves are shown in Figure 5.

TABLE 3. Quality control accuracy and precision summary

Analyte		Expected Conc		Amitriptylin	Doxepin	Imipramine	Fluvoxamine	Clomipramine	Fluoxetine	Paroxetine	Citalopram	Nortriptyline	Desipramine	Venlafaxine	Sertaline	Duloxetine	Bupropion
Level	Low	20	AVG	17.6	18.0	19.9	19.6	19.3	17.6	18.2	18.9	18.4	19.4	21.4	19.1	18.9	17.3
			%RSD	12.25	10.25	0.20	2.00	3.70	12.25	9.28	5.38	7.88	3.20	7.00	4.75	5.70	13.70
	Mid	110	AVG	113	98	115	125	116	118	115	115	116	111	114	17	117	118
			%RSD	3.14	11.18	4.29	8.87	5.82	7.09	4.18	4.73	5.64	0.91	4.00	6.00	1.91	2.44
	High	380	AVG	399	336	409	423	411	418	394	391	409	391	401	415	389	375
			%RSD	4.95	11.36	7.58	11.26	8.05	10.05	3.68	2.89	7.58	2.95	5.42	9.16	2.42	1.27

FIGURE 4. Representative chromatograms at the LOQ

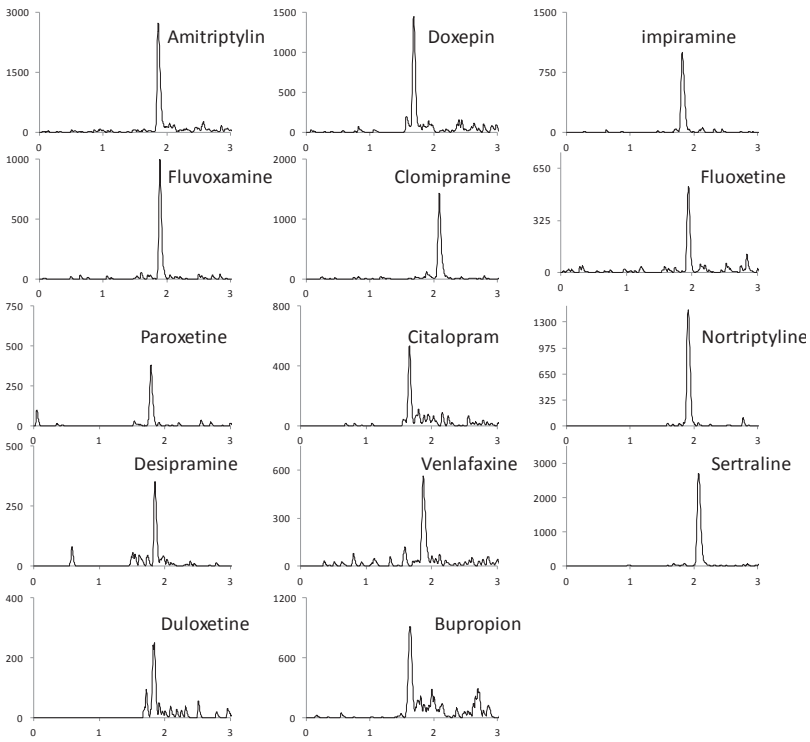
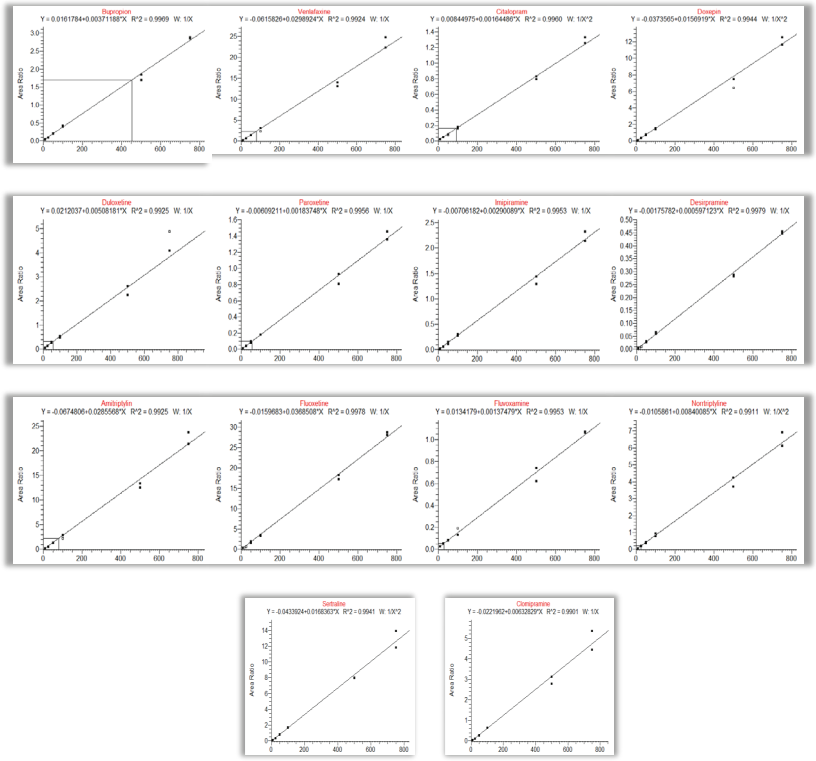


FIGURE 5. Representative calibration curves



Conclusion

- An LC-MC/MS research method has been successfully developed and verified for the quantification of 14 antidepressant drugs collected from dried blood spots using a Prelude SPLC system and a TSQ Endura triple quadrupole mass spectrometer.
- Online sample cleanup of the matrix resulting from dried blood spot collection reduced the complexity of the LC-MS/MS workflow.
- Due to the use of online sample preparation, this research method is more accurate, easier to perform, takes less time, is more robust, and is less costly then traditional offline sample preparation such as SPE plates.

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