

The Use of Human Dried Blood Spot (DBS) Samples for the Quantification of Clarithromycin by LC-MS/MS Using Core-Shell HPLC Column

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Introduction

Clarithromycin (Figure 1) is a macrolide antibiotic used to treat pharyngitis, tonsillitis, acute maxillary sinusitis, acute bacterial exacerbation of chronic bronchitis, pneumonia, skin and skin structure infections. Dried blood spot (DBS) sampling is a new sampling approach which offers advantages over the conventional procedures for sample collection, storage, and shipment. In this study, a comprehensive evaluation of DBS sampling technique was performed with clarithromycin in human dried blood spot. A novel, sensitive and specific LC-MS/MS method was developed and validated to determine clarithromycin in human DBS in combination with the use of core-shell PFP column technology for LC separation.

Figure 1. Chemical Structures of Clarithromycin and Roxithromycin

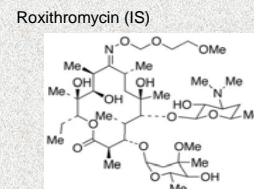
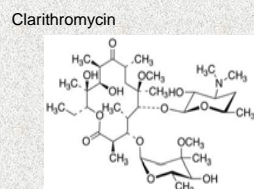


Figure 2. DBS on FTA DMPK Card and 3 mm Micro-Punch



Methods

Sample Preparation Procedure

Aliquots of 15, 25, and 50 μ L of human blood spiked with clarithromycin were spotted on FTA DMPK A, B (chemically impregnated), and C Cards (no impregnated chemicals). Punches of 3 mm DBS disks were taken (Figure 2) and placed in glass tubes for sample extraction. A structure analog (roxithromycin) was used as the internal standard (IS). Methanol was added to DBS samples in the tubes followed by sonication and centrifugation. The supernatant was diluted and analyzed.

Chromatographic Conditions

Column: Kinetex PFP, 50 X 3 mm, 2.6 μ m
 Mobile Phase: Buffer ACN/water
 Flow Rate: 1 mL/min, isocratic
 Inj. Volume: 7 μ L
 Run Time: 3.8 minutes

Mass Spectrometric Conditions

Mass Spectrometer: Sciex API 4000
 Interface: TurbolonSpray®
 Detection Mode: SRM in positive mode
 MRM Transitions: Clarithromycin m/z 748 \rightarrow 158
 Roxithromycin (IS) m/z 837 \rightarrow 679

Results

Linearity

- Validated concentration range: 10 to 5000 ng/mL
- Coefficient of determination: $r^2 \geq 0.994$.

Matrix Effects

- Matrix effects were evaluated by spiking 30 ng/mL of clarithromycin to 6 lots of human blood. Average matrix factor was 0.92, %CV was 3.3.

Extraction Recovery

- Overall recovery of clarithromycin in DBS was 78%.

Table 1. Precision and Accuracy of QC Samples

	Concentration (ng/mL)	30	1500	3750
Intrarun-1	n	6	6	6
	Mean	31.9	1643	4041
	%CV	3.3	2.3	3.9
	%Nominal	106.3	109.5	107.8
Intrarun-2	n	6	6	6
	Mean	30.6	1644	4064
	%CV	4.0	1.9	2.5
	%Nominal	101.9	109.6	108.4
Intrarun-3	n	6	6	6
	Mean	30.8	1660	3914
	%CV	4.2	2.7	2.9
	%Nominal	102.6	110.7	104.4
Interrun	n	18	18	18
	Mean	31.1	1649	4006
	%CV	4.1	2.3	3.4
	%Nominal	103.6	109.9	106.8

Table 2. Comparison of Blood Volume Spotted on DMPK C Cards

Conc. (ng/mL)	Volume	n	Mean	%CV	% Diff From 50 μ L
30	50 μ L	6	30.8	4.2	NA
	25 μ L	6	30.8	4.0	0
	15 μ L	6	28.4	7.0	-7.8
1500	50 μ L	6	1660	2.7	NA
	25 μ L	6	1547	3.9	-6.8
	15 μ L	6	1494	3.3	-10.0
3750	50 μ L	6	3914	2.9	NA
	25 μ L	6	3707	4.3	-5.3
	15 μ L	6	3503	3.7	-10.5

Table 3. Comparison of Punching Location at DBS on DMPK C Cards

Conc. (ng/mL)	Punching Location	n	Mean	%CV	% Diff From Center
30	Center	6	31.9	3.3	NA
	Edge	6	31.8	7.1	-0.3
1500	Center	6	1643	2.3	NA
	Edge	6	1703	3.8	+3.7
3750	Center	6	4041	3.9	NA
	Edge	6	3997	2.7	-1.1

Figure 3. Standard Curve of Clarithromycin in DBS

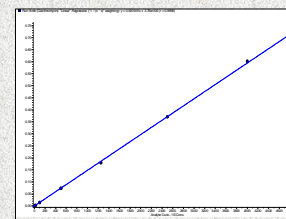


Figure 4. DBS Blank

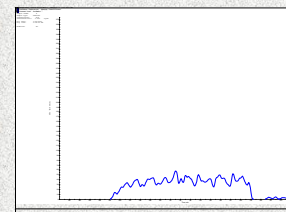


Figure 5. Lower Limit of Quantitation (10 ng/mL)

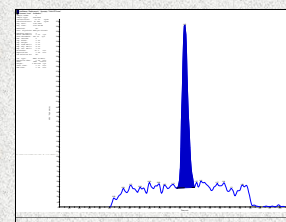


Figure 6. Internal Standard

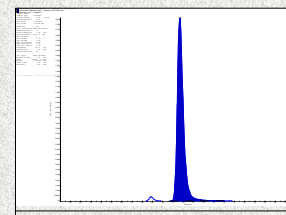


Table 4. Comparison of FTA DMPK A, B, and C Cards

Conc. (ng/mL)	Type of Card	n	Mean	%CV	% Diff From C Card
30	C	6	30.8	4.2	NA
	B	6	34.3	3.5	+11.4
	A	6	29.8	6.7	-3.2
1500	C	6	1660	2.7	NA
	B	6	1887	6.0	+13.7
	A	6	1650	4.3	-0.6
3750	C	6	3914	2.9	NA
	B	6	4417	4.9	+12.9
	A	6	3923	2.9	+0.2

Table 5. Stability Summary

Stability Conditions	Minimum Stability
Processed Sample Stability	72 Hours at Room Temperature
Bench-top Stability in DBS	At Least 11 Days at Room Temperature
Refrigerator Stability in DBS	At Least 11 Days at 4 $^{\circ}$ C

Conclusions

- The LC-MS/MS method using a Core-Shell Technology PFP column for LC separation was highly sensitive, selective, rapid, and rugged. The matrix suppression arising from the whole blood was minimized to insignificant by protein precipitation for sample clean up and >1000-fold sample dilution.
- DBS is a novel approach to reduce blood collection volume, stabilize the analyte at room temperature, and facilitate easy storage and shipping.
- The volume spotted to FTA DMPK C cards did not have a significant impact on the accuracy and precision. However, spots with a volume of 15 μ L blood resulted in slightly lower concentrations.
- Punching location (center vs. edge) of the DBS on FTA DMPK C cards did not affect the accuracy and precision.
- There was no significant difference among the three FTA DMPK cards tested. However, the chemically impregnated B cards resulted in slightly higher concentrations. The results suggested that one type of card should be used for an entire study.