

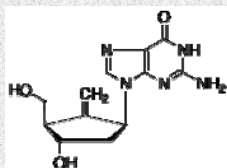
Determination of Entecavir in Human Matrices: Comparison of Dried Blood Spots (DBS) and Plasma LC-MS/MS Methods

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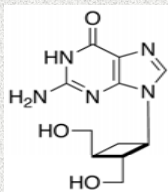
Introduction

Entecavir is a nucleoside analog that inhibits reverse transcription, DNA replication and transcription in the viral replication process. It is more efficacious than previous agents used to treat hepatitis B. Dried blood spot (DBS) sampling is a new sampling approach offering advantages over conventional procedures on sample collection, storage and shipment. In this study, we developed and validated two LC-MS/MS methods for Entecavir in human matrices, one using DBS, and the other using human plasma, over a nominal range of 0.05 to 20 ng/mL. Both methods were rapid, highly sensitive, selective, accurate and rugged.

Figure 1. Chemical Structures of Entecavir and Lobucavir



Entecavir



Lobucavir (IS)

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



Methods

Sample Preparation Procedure

For the DBS method, aliquots of 50 μ L of human blood with Entecavir were spotted in Whatman FTA DMPK C-Cards (no impregnated chemicals). A single 3 mm punch of DBS disk was taken from the center of the card and placed in a glass tube. IS (Lobucavir) and purified H₂O were added and sonicated for ~15 minutes. For the plasma method, add 50 μ L of human plasma with entecavir and IS spike solutions to purified H₂O, vortex for ~30 seconds. The samples were then extracted by SPE using OASIS HLB cartridges. The plasma samples were extracted with the similar SPE procedure. The reconstituted samples were injected onto a Gemini C6-Phenyl column.

Chromatographic Conditions

Column: Gemini C6-Phenyl 50 x 3 mm, 3 micron, 110 A
 Buffered H₂O
 Mobile Phase A: Buffered H₂O
 Mobile Phase B: Buffered ACN
 Needle Wash solvent: MeOH/H₂O (v:v 50:50)
 Flow Rate: 0.55 mL/min
 Injection Volume: 10 μ L (DBS)/ 5 μ L (Plasma)
 Run time: 4.8 min

Mass Spectrometric Conditions

MS: Sciex API 5000 for DBS
 MS: Sciex API 4000 for Plasma
 Ionization interface: TurbolonSpray®
 Detection mode: Positive ion, MRM
 MRM Transitions:
 Entecavir m/z 278 \rightarrow m/z 152
 Lobucavir m/z 266 \rightarrow m/z 152

Results

Linearity

- Validated concentration range: 0.05 to 20 ng/mL for both DBS and plasma
- Coefficient of determination: R² \geq 0.996 for DBS and 0.998 for plasma.

Matrix Effects

- Matrix effects were evaluated by spiking 0.15 ng/mL of Entecavir to 6 lots of human blood/plasma. Average matrix factor was 0.83 for DBS and 0.87 for plasma.

Extraction Recovery

- Overall recovery of Entecavir was 84% for DBS and 89.2% for plasma. Overall recovery of Lobucavir was 99.6% for DBS and 89.2% for plasma.

Table 1. Precision and Accuracy of QC Samples (DBS/Plasma Results)

	Concentration (ng/mL)	0.15	6	15
Intrarun-1	n	6	6	6
	Mean	0.151/0.140	6.022/5.933	14.840/14.750
	%CV	2.6/4.3	2.9/1.0	1.9/1.8
	%Nominal	100.7/93.3	100.4/98.9	98.9/98.3
Intrarun-2	n	6	6	6
	Mean	0.136/0.138	5.833/5.440	14.816/14.010
	%CV	8.8/5.8	1.7/5.6	2.3/4.8
	%Nominal	90.7/92.0	97.2/90.7	98.8/93.4
Intrarun-3	n	6	6	6
	Mean	0.147/0.154	6.236/6.015	15.823/14.394
	%CV	6.1/5.2	4.4/4.1	1.4/2.8
	%Nominal	98.0/102.7	103.9/100.3	105.5/96.0
Inter-run	n	18	18	18
	Mean	0.145/0.144	6.030/5.796	15.160/14.385
	%CV	7.6/6.9	4.1/5.8	3.6/3.8
	%Nominal	96.7/96.0	100.5/96.6	101.1/95.9

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

Conc. (ng/mL)	Volume	n	Mean	%CV	% Diff From 50 μ L
0.15 ng/mL	50 μ L	6	0.151	2.6	NA
	25 μ L	6	0.134	3.7	-11.9
6 ng/mL	50 μ L	6	6.022	2.9	NA
	25 μ L	6	5.307	3.6	-12.6
15 ng/mL	50 μ L	6	14.840	1.9	NA
	25 μ L	6	14.140	3.0	-4.8

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

Conc. (ng/mL)	Punching Location	n	Mean	%CV	% Diff From Center
0.15 ng/mL	Center	6	0.151	2.6	NA
	Edge	6	0.152	9.2	-0.7
6 ng/mL	Center	6	6.022	2.9	NA
	Edge	6	5.545	5.6	-8.2
15 ng/mL	Center	6	14.840	1.9	NA
	Edge	6	13.972	4.3	-6.0

Figure 3. Standard Curve of Entecavir in DBS

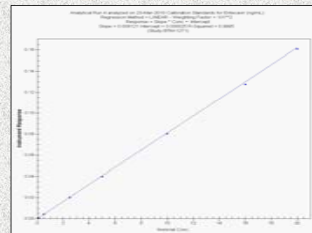


Figure 4. DBS Blank

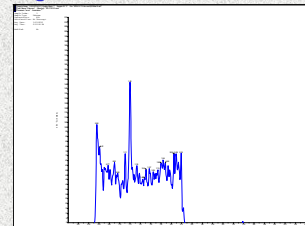


Figure 5. Lower Limit of Quantitation (0.05 ng/mL)

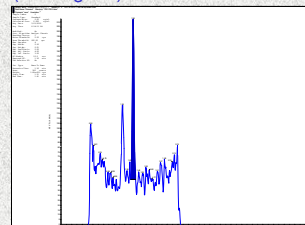


Figure 6. Internal Standard

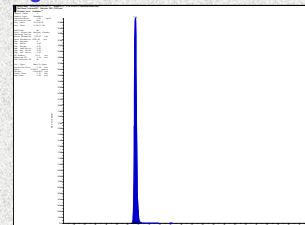


Table 4. Dilution integrity (20 fold dilution)

Sample No.	DBS	Plasma
	Concentration, 150 ng/mL	Concentration, 200 ng/mL
1	141.557	205.195
2	151.175	202.865
3	150.199	206.844
4	143.613	200.255
5	136.518	203.279
6	148.742	202.567
Mean	145.301	203.501
SD	5.733	2.276
CV	3.9	1.1
%Nominal	96.9	101.8

Table 5. Stability Summary

Stability Conditions	Minimum Stability
Processed Sample Stability (DBS)	At least 76 Hours at Room Temperature
Processed Sample Stability (plasma)	At least 88 Hours at Room Temperature
Bench-top Stability in DBS	At least 116 Hours at Room Temperature
Bench-top Stability in plasma	At least 88 Hours at Room Temperature

Conclusions

- The two methods were compared with each other and the results demonstrated that the DBS method was comparable to the plasma method in linearity, selectivity, precision, accuracy, dilution integrity and overall recovery.
- 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 significant impact on the accuracy and precision. However spots with 25 μ L blood resulted in slightly lower concentrations than spots with 50 μ L blood.
- Punching location (center vs. edge) at DBS on FTA DMPK C cards only slightly affect the accuracy and precision.