A Dried Blood Spot Method for Therapeutic Drug Monitoring of Rifampin to Optimize Tuberculosis Treatment in Children Using LC-MS/MS

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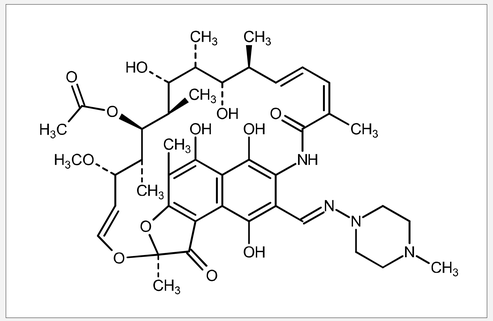
INTRODUCTION

An LC-MS/MS method has been developed for the determination of Rifampin, the major first-line anti-tuberculosis drug, in dried blood spots (DBS). This method will be utilized for an NIH funded project for therapeutic drug monitoring (TDM) and genetic analysis of Rifampin in combinational therapies to optimize treatment in children. The pharmacokinetics of antitubercular agents are unrepresented in children and unlike many other pathogenic bacteria, the predominant mechanism of resistance for tuberculosis is spontaneous mutation. With the prevalence of subtherapeutic doses, drug-resistant strains predominate. DBS has the distinct advantage to facilitate pharmacokinetic/ pharmacogenetic analyses in global trials involving children with tuberculosis and provide information on dose-exposure relationships necessary to guide dosing in TB subpopulations.



Rifampin (MW 822.94)

METHOD

Rifampin was validated in DBS with K_2EDTA whole blood over a concentration range of 200 to 20,000 ng/mL. Blood containing Rifampin was spotted onto DBS cards (Perkin Elmer 226, untreated paper) and 7 mm punches were made. Each punch was placed in extraction solvent (90/10 Methanol/Water, 300 µL) containing Rifampin-d_8 (I.S.) and sonicated for 60 minutes. The solution (100 µL) was transferred to injection vials with 500 µL of 10 mM Ammonium Formate pH 3.0.

Liquid Chromatography

HPLC : Shimadzu 10ADvp
Autosampler : Perkin Elmer 200
Column: Prodigy ODS3 (50x2 mm, 5µ) by Phenomenex
MP A : 0.1% Formic acid in DI H_2O
MP B : 0.1% Formic acid in MeOH

Mass Spectrometry

AB Sciex API 3000
Polarity : Positive
Scan type : Multiple Reaction Monitoring
Rifampin: m/z 823.6 → 791.6
Rifampin-d_8: m/z 831.6 → 799.6 (I.S.)

RESULTS

The method showed a linear range of 200 to 20,000 ng/mL with weighted linear regression ($1/x^2$). The correlation coefficient was 0.996 or better (Figure 1).

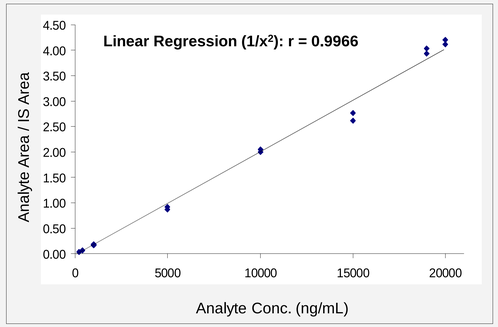


Figure 1. A Typical Calibration Curve for Rifampin Using DBS Method

Blood spots of varying volumes (10, 30, 50 µL) were evaluated at all four QC levels and a 30 µL spot gave the best results. Rifampin was assayed in the presence of high concentrations of three other first-line anti-tuberculosis drugs, Isoniazid, Ethambutol, and Pyrazinamide. None of these drugs at high concentrations interfered chromatographically or with quantitation of Rifampin.

Figures 2 through 4 show typical chromatograms for selected samples. The S/N ratio for Rifampin peak at LLOQ level was greater than 70.

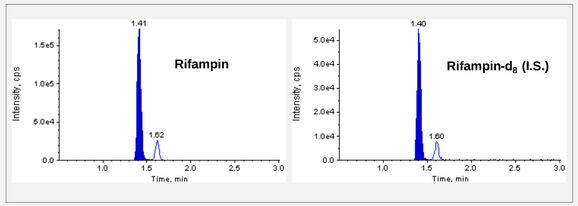


Figure 2. Chromatogram of an Extracted ULOQ Sample (20,000 ng/mL).

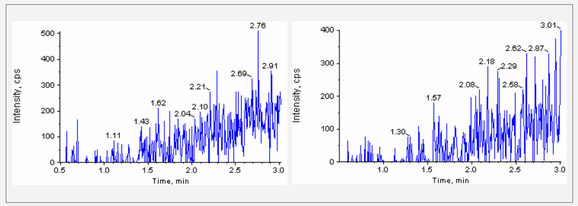


Figure 3. Chromatogram of a Recon Blank (No I.S.) Injected after ULOQ

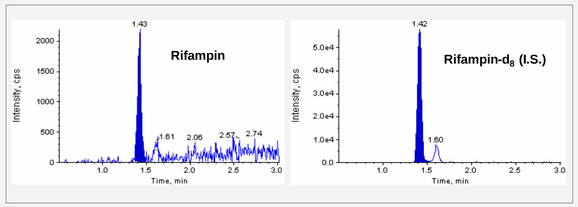


Figure 4. Chromatogram of an Extracted LLOQ sample (200 ng/mL).

Stability: Rifampin in whole blood was stable for 74 days at room temperature on DBS paper.

Hematocrit Evaluation: Rifampin showed consistent results at hematocrit levels between 33 and 67, but the recovery was low at low levels (24). Average hematocrit is usually around 45 for men and 40 for women.

Inter-assay precision and accuracy was evaluated with a % Bias and % CV of less than or equal to 8.1% and 13.6% respectively. **Intra-assay precision and accuracy** had a % Bias and % CV of less than or equal to 5.2% and 8.7% respectively (**Table 1**). Matrix effect was evaluated over six different lots of K_2EDTA whole blood and overall % CV was 2.3% (**Table 2**).

Batch		**200** ng/mL (LLOQ)	**600** ng/mL (Low)	**7,500** ng/mL (Mid)	**17,500** ng/mL (High)
1	**Mean**	199	581	7305	18170
	% CV	8.9	2.2	6.4	4.9
	% Bias	-0.8	-3.1	-2.6	3.8
2	**Mean**	184	580	7280	18130
	% CV	6.5	13.6	12.2	10.1
	% Bias	-8.1	-3.3	-3.0	3.6
3	**Mean**	187	570	7295	17730
	% CV	9.6	7.4	3.0	4.5
	% Bias	-6.8	-5.0	-2.7	1.4
Overall	**Mean**	190	577	7290	18000
	n	18	18	18	18
	% CV	8.7	8.5	7.6	6.7
	% Bias	-5.2	-3.8	-2.8	2.9

Table 1. Precision and Accuracy Results for Rifampin Quality Control Samples (n = 6 replicates per batch).

	Avg drug area	Avg I.S. area	Avg area ratio
Lot 1	17541	139140	0.1261
Lot 2	16749	137906	0.1215
Lot 3	17957	141960	0.1265
Lot 4	17659	140944	0.1253
Lot 5	17984	138072	0.1302
Lot 6	17812	139326	0.1278
mean	**17617**	**139558**	**0.1262**
n	**6**	**6**	**6**
SD	**458**	**1603**	**0.0029**
%CV	**2.6**	**1.1**	**2.3**

Table 2. Matrix Effect for Rifampin in Whole Blood on DBS Cards.

CONCLUSION

An LC-MS/MS method for quantitation of Rifampin in DBS has been developed for clinically relevant concentrations of Rifampin in children. The use of DBS in this method will allow for ease of collection from children in pediatric studies as well as for transport in remote locations.

ACKNOWLEDGEMENTS

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