Supplemental Materials

Assay Validation Methods

Accuracy and Precision. Intra- and inter-assay accuracy and precision for both rifapentine (RPT) and desacetyl-rifapentine (desRPT) were determined through the analysis of quality control (QC) levels in plasma and whole blood as dried blood spots prepared at lower limit of quantification (LLOQ), low, mid and high levels. Intra-assay studies were evaluated via the analysis of 5-6 QC specimens tested within a single analytical run, which are flanked by two calibration curves. Inter-assay studies were carried out through the extraction and testing of QC samples across three independent runs. Accuracy was determined as the percent deviation (% DEV) from the target concentration, calculated by the difference between the experimental value and the target value divided by the target value and then multiplied by 100. Precision was defined as the coefficient of variation (%CV), calculated by using the standard deviation of a QC level divided by the mean observed concentration and multiplied by 100.

Calibration Curve Analysis. For calibration curve analysis, standards (10 levels) were analyzed at the beginning and end of each analytical method, with the first set of standards run in ascending order, and the latter in descending order. Calibrators were calculated by the analysis of the peak area of the target drug divided by the peak area of the internal standard to yield a peak area ratio. Calibration curve analysis was assessed from the r^2 of a $1/x^2$ weighted quadratic regression analysis for both rifapentine and its metabolite, which provided the best fit for the 3-log dynamic range of this assay. Calibration curve analysis was assessed by the analysis of three calibration curves in either plasma or whole blood as dried blood spots. Each standard curve was evaluated by duplicate analyses of matrix-specific calibrators.

Stability Challenges. QC levels prepared in plasma and whole blood as dried blood spots were challenged under a variety of conditions to assess stability. Sample matrix stability was assessed by incubating plasma or whole blood as a dried blood spot at room temperature and unprotected from light for 24 h-72 h. Injection matrix stability was tested by leaving extracted samples at 4-7°C for 72 h or 120 h for whole blood as dried blood spots or plasma, respectively. Additionally, whole blood controls as dried blood spots were tested at high heat (45°C) and 100% humidity to mimic real world conditions. Alternatively, plasma samples were subjected to 3 freeze-thaw cycles at -80°C. Stability was determined by comparing stability-challenges QC samples to specimens freshly prepared or extracted. In all cases, stability was analyzed through the determination of a percent difference (% difference), which is calculated as the difference between challenged and unchallenged samples divided by unchallenged sample concentrations, and multiplied by 100. Specimens were considered stable if the % difference was ≤ 15% from unchallenged samples.

Matrix Effect Characterization. Matrix effects, extraction efficiency and processing efficiency were assessed following the approach described by Matuszewski and colleagues to quantitatively assess the impact of matrix interferents on ion suppression or enhancement (1). A quantitative assessment of matrix effects, as well as extraction and overall processing efficiency, was performed by preparing low, mid and high QC levels across 6 independent plasma or whole blood lots. Three sets of conditions were tested. An un-extracted set was prepared by spiking rifapentine, desacetyl-rifapentine and isotopically-labeled internal standard into 500 ml of acetonitrile with 0.5 mg/ml ascorbic acid and 500 ml of 5mM ammonium formate with 0.5

mg/ml ascorbic acid for plasma and 450 µL of extraction solvent (90:10 methanol: 50mM ammonium formate buffer with 0.50 mg/mL ascorbic acid) for whole blood as dried blood spots, to achieve final concentrations of 150, 2,500 and 70,000 ng/ml. Following extraction of multiple lots of plasma or whole blood as dried blood spots was performed as previously described, post-extracted samples were spiked with stock solutions of rifapentine and its metabolite, as well as the internal standard. A pre-extracted set was analyzed by processing QC levels prepared in plasma or whole blood as dried blood spots as previously described. Raw peak area for rifapentine, desacetyl-rifapentine and its internal standard were observed and peak area ratios of each analyte of interest were determined to assess matrix effects (post-extracted samples compared to un-extracted samples, multiplied by 100), extraction efficiency (pre-extracted samples compared to post-extracted samples, multiplied by 100) and overall processing efficiency (pre-extracted samples compared to un-extracted samples, multiplied by 100).

Supplemental Table A1. UPLC schematic for detection of rifapentine and desacetyl-rifapentine in human heparinized plasma and whole blood as dried blood spots.

Step	Start Time (min)	Flow (ml/min)	%A[a]	%B[b]
1	0.00	0.3	80	20
2	1.50	0.3	1.0	99
3	3.50	1.0	1.0	99
4	4.00	0.3	80	20

[a] 5mM ammonium formate

[b] 3% DMSO in acetonitrile

Supplemental Table A2. Extraction conditions attempted for RPT, desRPT and the deuterated internal standard d3-rifampin from whole blood as DBS on Whatman 903 Protein Saver Cards and Whatman FTA DMPK-C Cards.

Extraction Solvent	RPT	desRPT	d3-RIF	RPT	desRPT	d3-RIF
	% Recovery			% Recovery		
	Whatman 903 Paper			DMPK-C Paper		
70:30 ACN:H_2O	79	87	89	58	66	73
50:50 ACN:H_2O	75	68	86	58	53	73
50:50 ACN:H_2O with 0.1% Formic Acid	46	26	35	47	23	27
90:10 ACN:5mM Ammonium formate	55	69	86	40	56	70
50:50 MeOH:H_2O	77	86	105	55	70	81
50:50 MeOH:H_2O with 0.1% Formic Acid	53	35	55	44	37	47
90:10 MeOH:5mM Ammonium formate	77	73	100	51	72	65

Supplemental Table A3. Punch sizes attempted for RPT, desRPT and the deuterated internal standard d3-rifampin from whole blood as DBS on Whatman 903 Protein Saver Cards and Whatman FTA DMPK-C Cards.

Punch Size and Paper	RPT Peak Area (counts)	desRPT Peak Area (counts)	d3-RIF Peak Area (counts)
3mm DMPK-C	2576	274	18075
3mm Whatman 903	1700	457	17204
1/8in DMPK-C	1441	254	17110
1/8in Whatman 903	1552	109	15574
6mm DMPK-C	4583	675	16574
6mm Whatman 903	4213	647	15623

REFERENCES

1. **Matuszewski, B. K., M. L. Constanzer, and C. M. Chavez-Eng.** 1998. Matrix effect in quantitative LC/MS/MS analyses of biological fluids: a method for determination of finasteride in human plasma at picogram per milliliter concentrations. Anal. Chem. **70:**882-889.