

# Homogeneous Enzyme Immunoassay for HIV-1 Antiretroviral Drugs: Darunavir and Maraviroc

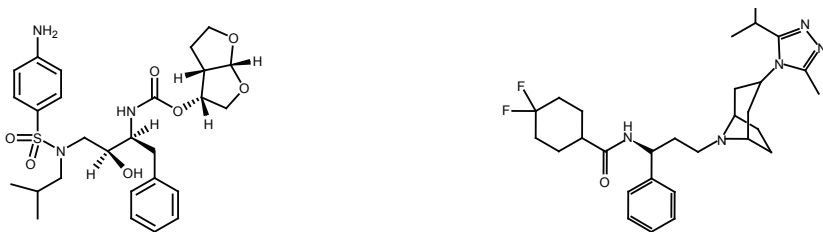
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## Introduction

### ABSTRACT

#### Background

Homogeneous enzyme immunoassays for measurement of darunavir (DRV, protease inhibitor, PREZISTA™, Tibotec) and maraviroc (MVC, entry inhibitor, SELZENTRY™, Pfizer) in human serum. Median trough concentrations in HIV-infected persons receiving the recommended dose of DRV was 3.30 µg/mL [1]. MVC specifically blocks the chemokine receptor CCR5 on T cells used by CCR5-tropic human immunodeficiency virus (HIV-1). Trough MVC concentrations have been shown to be an important predictor of virologic success in studies conducted in antiretroviral therapy-experienced persons [2-3]. Also, guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents indicate that multiple scenarios exist in which both antiretroviral concentration data and expert opinion may be useful in patient management [4].



Darunavir [(1S,2R)-3-[[[4-aminophenyl]sulfonyl](2-methylpropyl)amino]-2-hydroxy-1-(phenylmethyl)propyl]-carbamic acid (3R,3a5,6aR)-hexahydrofuro[2,3-b]furan-3-yl ester monoethanolate]

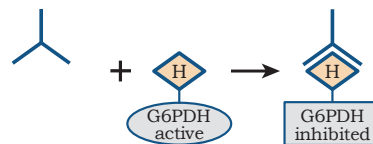
Maraviroc [4,4-difluoro-N-[(1S)-3-[3-(3-isopropyl-5-methyl-4H-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]oct-8-yl]-1-phenylpropyl]cyclohexanecarboxamide]

#### Methods

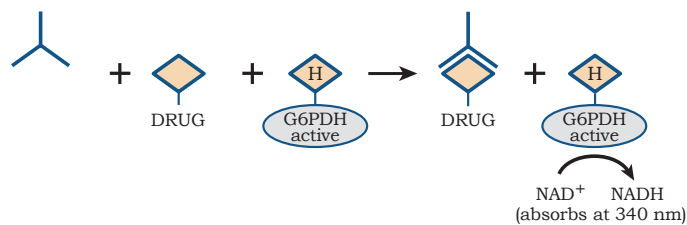
ARK assays were optimized on the Roche/Hitachi 917 analyzer. Calibrator levels ranged from 0 to 12 µg/mL (DRV; plasma/serum), 0 to 100 ng/mL (DRV; cerebrospinal fluid) and 0 to 350 ng/mL (MVC), respectively. Performance testing included precision, limits of quantification, analytical recovery, and specificity.

The assay principle is shown in the following figure. Increasing reaction rate correlates to increasing drug concentration.

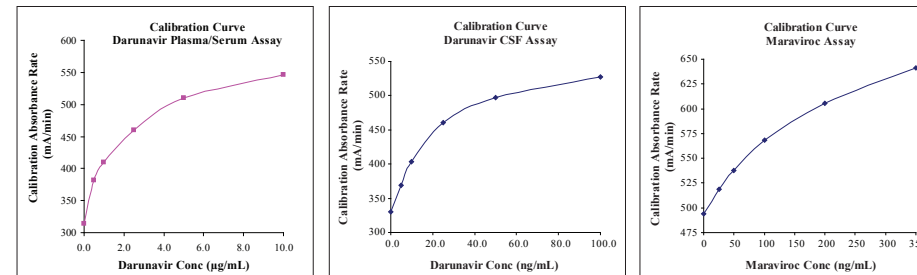
#### A) Absence of drug



#### B) Presence of drug



## Calibration



## Lower Limit of Quantitation

The LOQ of the ARK Darunavir Assay ( $\leq 20\%$  CV with  $\pm 15\%$  recovery) was evaluated as 0.20 µg/mL in serum (six replicates; 2 runs of 3 replicates) and 2.5 ng/mL in cerebrospinal fluid (eight replicates; 2 runs of 4 replicates).

ARK Darunavir Plasma/Serum Assay				
Target (µg/mL)	Mean (µg/mL)	SD	CV (%)	Recovery (%)
0.20	0.19	0.01	5.1	95.8

ARK Darunavir CSF Assay				
Target (ng/mL)	Mean (ng/mL)	SD	CV (%)	Recovery (%)
2.5	2.8	0.21	7.5	113.2

The LOQ of the ARK Maraviroc Assay ( $\leq 20\%$  CV with  $\pm 15\%$  recovery) was evaluated as approximately 25 ng/mL in serum.

ARK Maraviroc Plasma/Serum Assay					
Target (ng/mL)	Mean (ng/mL)	SD	CV (%)	Recovery (%)	N
15	17.8	2.1	11.6	118.8	60

## Precision Verification

Five-day precision experiments involved two runs per day and four replicates of each control level per run. The within run, between day, total SD, and percent CVs were calculated.

ARK Darunavir Plasma/Serum Assay								
QC Sample (µg/mL)	N	Mean (µg/mL)	Within Run		Between Day		Total	
			SD	CV(%)	SD	CV (%)	SD	CV (%)
Low (0.35)	40	0.36	0.02	4.7	0.01	1.7	0.02	6.0
Mid (3.50)	40	3.70	0.12	3.7	0.12	3.1	0.18	5.0
High (7.50)	40	7.76	0.35	4.4	0.19	2.5	0.42	5.6

ARK Darunavir CSF Assay								
QC Sample (ng/mL)	N	Mean (ng/mL)	Within Run		Between Day		Total	
			SD	CV(%)	SD	CV (%)	SD	CV (%)
Low (7.5)	40	7.7	0.26	3.5	0.27	3.5	0.50	6.6
Mid (15.0)	40	15.0	0.62	4.1	0.33	2.2	0.72	4.8
High (35.0)	40	35.5	0.83	2.4	0.74	2.1	2.04	5.8

ARK Maraviroc Serum Assay								
QC Sample (ng/mL)	N	Mean (ng/mL)	Within Run		Between Day		Total	
			SD	CV(%)	SD	CV (%)	SD	CV (%)
35	40	35.59	2.35	6.6	0.76	2.1	2.55	7.2
75	40	77.93	2.06	2.6	1.73	2.2	2.70	3.5
150	40	146.69	3.36	2.3	2.70	1.8	5.21	3.5

## Analytical Recovery

Analytical spike recovery for darunavir was determined in serum (average 102.3%) and cerebrospinal fluid (average 100.3%) based on the mean recovery for six replicates of each level tested.

ARK Darunavir Plasma/Serum Assay				
Target (µg/mL)	Mean (µg/mL)	SD	CV (%)	Recovery (%)
0.75	0.80	0.01	1.8	106.4
2.00	1.84	0.08	4.6	92.1
3.50	4.28	0.31	7.2	107.1
6.50	6.66	0.41	6.2	102.4
8.50	8.81	0.46	5.3	103.6

Mean percent recovery: 102.3

ARK Darunavir CSF Assay				
Target (ng/mL)	Mean (ng/mL)	SD	CV (%)	Recovery (%)
8.0	7.1	0.41	5.7	89.2
20.0	19.3	0.55	2.8	96.4
40.0	42.4	1.04	2.5	105.9
60.0	63.7	3.88	6.1	106.2
75.0	77.8	3.82	4.9	103.7

Mean percent recovery: 100.3

Analytical spike recovery for maraviroc was determined in serum (average 103.8%) based on the mean recovery for eight replicates of each level tested.

ARK Maraviroc Plasma/Serum Assay				
Target (ng/mL)	Mean (ng/mL)	SD	CV (%)	Recovery (%)
20	20.55	1.46	7.12	102.7
40	40.01	1.44	3.42	104.9
90	94.18	2.09	2.22	104.7
175	180.05	5.02	2.79	102.9
250	259.89	6.19	2.38	104.0

Mean percent recovery: 103.8

## Specificity

The following other antiretroviral drugs listed were tested at 30 µg/mL in serum and did not crossreact.

Abacavir	Emtricitabine	Nevirapine	Terfavir
Amprenavir	Indinavir	Rotinavir	Tipranavir
Atazanavir	Lamivudine	Saquinavir	Zalcitabine
Didanosine	Lopinavir	Stavudine	Zidovudine
Efavirenz	Nelfinavir	Tenofovir	

## Conclusions

Available For Research Use Only.

Sufficient precision and accuracy was obtained, and feasibility was shown for general clinical laboratory performance to verify target levels of DRV and MVC in human serum and DRV in cerebrospinal fluid. Such methods have potential for Therapeutic Drug Monitoring (TDM). Also, the short turn-around time afforded by immunoassay may benefit the clinical validity of TDM.

- Food and Drug Administration (FDA). Prezista (package insert). 2010. [http://www.accessdata.fda.gov/drugsatfda\\_docs/label/2010/021976s016lbl.pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/021976s016lbl.pdf).
- Pfizer Inc. Selzentry (maraviroc) tablets prescribing information NY. 2007.
- McFayden L, Jacqmin P, Wade J, et al. Maraviroc exposure response analysis: phase 3 antiviral efficacy in treatment-experienced HIV+ patients. Paper presented at: 16th Population Approach Group in Europe Meeting; June 2007, 2007; Kobenhavn, Denmark. Abstract P4-13
- Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents January 10, 2011. Developed by the DHHS Panel on Antiretroviral Guidelines for Adults and Adolescents – A Working Group of the Office of AIDS Research Advisory Council (OARAC).