7th International Workshop on Clinical Pharmacology of HIV Therapy, April 20-22, 2006, Lisbon, Portugal

NOVEL ATAZANAVIR IMMUNOASSAY

JERIKA T. LAM[1], STAN G. LOUIE[1], JOHNNY J. VALDEZ[2], BYUNG S. MOON[2], MICHAEL K. HELMS[2], MICHAEL N. NEELY[1]
[1]University of Southern California, Los Angeles, CA, USA;
[2]ARK Diagnostics, Sunnyvale, CA, USA

Correspondence to:
Stan G. Louie
University of Southern California
1985 Zonal Avenue, PSC 208B
Los Angeles, CA 90033, USA.
Tel: 323-442-3646
Fax: 323-442-1395
Email: slouie@usc.edu

Background

Therapeutic drug monitoring (TDM) in HIV disease may increase antiretroviral (ARV) efficacy by reducing toxicity, preventing drug resistance and managing drug-drug interactions. Measuring ARVs with current techniques (eg. HPLC or LC-MS/MS) is costly, time consuming and requires specialized equipment and skilled technicians. A new rapid automated enzyme immunoassay has been developed for determining plasma atazanavir (ATV) concentrations. Studies were performed to evaluate the performance of the ARK atazanavir assay.

Methods

The ARK ATV-Test is based on competitive binding to antibody between drug in the sample and drug-labeled enzyme (Figure 1). Drug concentration is measured spectrophotometrically (Roche MIRA® bench top analyzer) in terms of enzyme activity. Although each test uses 5 µL of sample, at least 60 µL sample must be added per sample cup due to dead volume. The calibration standards ranged from 0.25 – 8.0 µg/mL. Assay precision of controls (0.375, 0.75, and 3.0 µg/mL), analytical recovery, sensitivity (0.1 µg/mL), endogenous interference (cholesterol 300 – 400 mg/dL, triglycerides 200 -350 mg/dL, and bilirubin total > 25 mg/dL) and crossreactivity of other ARVs were tested. ARK-ATV standards and calibrators were validated on the LC-MS/MS at the concentration ranges of 0.375-8.0 µg/mL (see poster #17).

Results

Inter-assay precision of ATV control levels (CV) is <8% (n = 20) from the COBAS instrument. Analytical recovery was within 15% at all levels tested. Sensitivity was demonstrated at 0.1µg/mL. No interference was noted from other ARV drugs, cholesterol, triglycerides or bilirubin samples. The R^2 is 0.995 for both ARK and LC-MS/MS assay methods for ATV (Figure 2). Mean accuracy for ATV from both instruments was measured at ≥90%, when using ARK-derived ATV standards (Table 1). Mean accuracy for ATV was measured at ≥80%, when using USC-derived ATV standards (Table 2).

Figure 1. ARK's Homogeneous, Competitive Immunoassay Technology

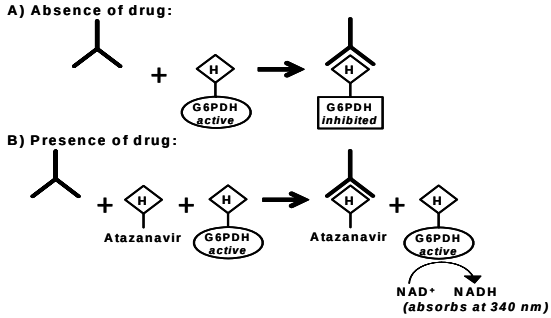

Figure 2. Correlation of ARK Standards with LC-MS/MS

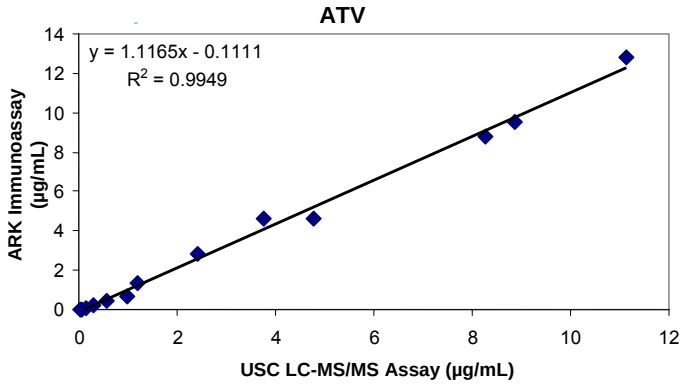

Table 1. ARK Immunoassay: Comparison of Precision & Accuracy

ARK Assay			LC-MS/MS Assay		
Conc (µg/ml)	Precision (%CV)	Mean Accuracy (%)	Conc (µg/ml)	Precision (%CV)	Mean Accuracy (%)
0.25	0	92.00	0.25	0.27	103.00
0.375	3.37	112.00	0.375	0.4	93.33
0.50	1.4	101.00	0.500	0.73	97.50
0.75	1.68	112.00	0.75	0.97	107.40
1.00	0.68	104.50	1.00	0.42	100.40
2.00	0	100.50	2.00	0.03	101.38
3.00	1.31	108.33	3.00	0	105.97
4.00	1.55	91.50	4.00	0.2	96.71
8.00	0	100	8.00	0.69	94.54

Table 2. USC LC-MS/MS Assay: Comparison of Precision & Accuracy

ARK Assay			LC-MS/MS Assay		
Conc (µg/ml)	Precision (%CV)	Mean Accuracy (%)	Conc (µg/ml)	Precision (%CV)	Mean Accuracy (%)
0.25	3.81	118.80	**0.25**	2.43	104.60
0.375	1.05	107.70	**0.375**	3.59	99.87
0.50	5.88	103.50	**0.50**	4.38	100.10
0.75	1.38	102.20	**0.75**	5.06	104.30
1.00	1.05	94.20	**1.00**	5.37	104.10
2.00	1.77	87.85	**2.00**	5.86	102.50
3.00	1.64	94.73	**3.00**	6.02	103.80
4.00	1.65	84.89	**4.00**	6.08	100.50
8.00	0.89	78.56	**8.00**	6.19	94.66

Conclusions

ARK ATV-Test for measuring atazanavir in plasma was evaluated and validated via LC-MS/MS. The test is an automated EIA that requires minimum expertise, small sample volume, no sample pre-treatment and provides the first result within 7.5 minutes. All reagents are supplied ready-to-use. The ARK ATV-Test has great potential for making antiretroviral drug assays more readily available to facilitate therapeutic drug monitoring in pediatric and adult clinical trials and practice.