

Rapid, Automated Immunoassay for Atazanavir in Plasma and Cerebrospinal Fluid (CSF) Using the ARK ATV-Test™ : Methods Validation, Application and Comparison with HPLC Plasma Method

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Abstract

Background: ARV assays for pharmacokinetics, adherence or therapeutic drug monitoring with current techniques are costly, time consuming, requiring specialized equipment and technologists. To measure ARVs in CSF requires mass spectrometry for sensitivity. A rapid automated enzyme immunoassay system for ARV assays has been developed for several drugs and we have applied the atazanavir (ATV) test to plasma (ARK ATV plasma Test™) and CSF (ARK ATV-CSF Test™). Here we report results of the validation and the comparisons to the HPLC method.

Methods: The ARK ATV-Tests™ are based on competitive binding to antibody between drug in the sample and drug-labeled enzyme. All reagents are supplied ready-to-use, the test is automated and is read with the Roche MIRA® bench top analyzer. For the plasma assay, 50µl of spun sample was required and each test used 5µl in duplicate. Calibration standards ranged from 0.25 to 8µg/ml with a sensitivity of 0.05 µg/ml. Patient and proficiency testing (PT) samples were run and compared to HPLC results. The CSF test required 100µl of sample and each test used 36µl in duplicate. The CSF test's curve range was 5-80ng/ml with a sensitivity of 5ng/ml. These validated ARK ATV-Tests™ were used to measure ATV concentrations in 26 study subject samples.

Results: Plasma validation inter-assay precision was <9.2% CV and accuracy was within 11% deviation (n=36). There was no interference from other ARV drugs or blank plasma. All PT samples tested (n=13) were within 15.2% of target. Patient samples analyzed by HPLC and the ARK ATV-Test™ yielded the following results: $y = 0.95x + 0.13$, $R^2 = 0.96$, $n = 53$. CSF validation, inter-assay precision and accuracy were within 18% at 5ng/ml, and within 15% for the remaining controls.

Conclusions: The plasma test showed good correlation with a validated HPLC method; it requires no specialized laboratory training, small sample volume, no sample pre-treatment and provides first results within 7.5 minutes. The CSF test provides correlation between plasma and CSF exposures. Work load volume for routine reporting of ARV concentrations among patients on standard therapy can be dramatically increased using this method, which would be of benefit in hospitals and especially in field work.

COBAS MIRA Parameters

Table 1. Parameters have been established for the ARK atazanavir assays on the COBAS MIRA System:

	Plasma Assay	CSF Assay
Sample Volume (µL)	5	36
Reagent 1 (Antibody) Volume (µL)	150	150
Reagent 2 (Enzyme) Volume (µL)	75	75
Assay Temperature (°C)	37	37
Wavelength (nm)	340	340
Throughput (tests/hour)	72	72

HPLC Procedure

Plasma atazanavir (ATV) levels were determined by a validated reverse-phase high-performance liquid chromatography (RP-HPLC) using UV detection. Briefly, plasma proteins were removed using acetonitrile. After centrifugation, the supernatant was injected directly onto a C-18 RP column and ATV was separated using a buffer of pH 3.1 which included 49% acetonitrile. UV detection was at 245nm. The preferred sample size for HPLC is 300-500µl.

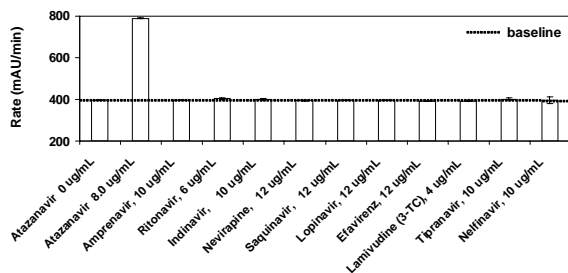
Inter-Assay Precision and Accuracy Studies

Table 2. Five QC samples (for the plasma assay) and four QC samples (for the CSF assay) were tested using the ARK Atazanavir Assay for plasma on the COBAS MIRA analyzer. For the plasma assay, data are derived from 6-7 days: 1 run per day, 6 replicates per run with a total of 36-42 replicates of each control level. For the CSF assay, data are derived from 3 days: 1 run per day, up to 6 replicates per run with a total of 21 replicates of each control level.

Conc. (µg/mL or ng/mL)	Assayed (Mean ± SD)	Precision (CV%)	Accuracy (Deviation %)
Plasma Assay:			
0.13 µg/mL	0.12 ± 0.01	9.1	-9.7
0.54 µg/mL	0.54 ± 0.02	4.4	-1.5
0.75 µg/mL	0.83 ± 0.03	4.1	10.8
3.0 µg/mL	3.14 ± 0.10	3.2	4.6
6.0 µg/mL	5.78 ± 0.39	6.7	-3.6
CSF Assay:			
5 ng/mL	4.3 ± 0.8	18.6	-14.3
10 ng/mL	11.5 ± 0.8	7.0	15.2
30 ng/mL	31.7 ± 2.1	6.5	5.7
60 ng/mL	58.9 ± 3.7	6.3	-1.8

Specificity

Figure 1. Antiretrovirals whose chemical structure or concurrent therapeutic use would suggest possible cross-reactivity were tested at the levels indicated. None of the compounds tested gave an apparent atazanavir concentration as indicated by rates of change of absorbance within 2% of the baseline value.



Lower Limit of Quantitation

Table 3. Pooled human serum samples were supplemented with known amounts of atazanavir at the concentrations shown below. Each sample was then assayed 20 times (for the plasma assay) or 21 times (for the CSF assay). The lowest concentration measured with acceptable accuracy and precision was 50 ng/mL for the plasma assay and 5 ng/mL for the CSF assay.

	Conc. (ng/mL)	Assayed (Mean ± SD)	Precision (CV %)	Accuracy (Deviation %)
Plasma assay	50 ng/mL	55 ± 8	15	10
CSF assay	5 ng/mL	4.3 ± 0.8	19	-14

Note: This device has not yet been approved by the US FDA. The clinical utility of this device has not yet been established.

Analytical Recovery

Table 4. Pooled human serum samples were supplemented with known amounts of atazanavir. Each sample was then assayed 10 times. The amount of atazanavir recovered from nominal ranged from 104.2% to 110%.

Conc. Tested (µg/mL)	Recovery	
	(Mean ± SD)	(%)
0.100	0.11 ± 0.01	110.0
0.250	0.27 ± 0.01	107.6
0.375	0.41 ± 0.01	108.3
0.75	0.79 ± 0.02	105.6
1.50	1.65 ± 0.07	109.7
3.00	3.13 ± 0.08	104.2
6.00	6.6 ± 0.4	109.4

AIDS Clinical Trials Group Proficiency Testing

Table 5. Proficiency testing samples were prepared by the AIDS Clinical Trials Group. High, medium, and low concentrations of protease inhibitors and non-nucleoside reverse transcriptase inhibitors were added to drug-free EDTA plasma. The samples were assayed for atazanavir in duplicate by the ARK Atazanavir Assay and the mean compared to target values.

I.D.	Target (µg/mL)	ARK Mean (µg/mL)	I.D.	Target (µg/mL)	ARK Mean (µg/mL)
UCSD-RD15-A	1.02	0.90	UCSD-RD14-C	0.92	0.99
UCSD-RD15-B	6.46	5.48	JHU-RD14-A	3.50	3.52
UCSD-RD15-C	0.16	0.15	JHU-RD14-B	7.42	7.44
JHU-RD-15-A	0.23	0.24	JHU-RD14-C	0.41	0.40
JHU-RD15-B	4.70	4.52	UCSD-RD13-K	0.29	0.27
UCSD-RD14-A	0.15	0.16	JHU-RD15-C-2	8.78	7.60
UCSD-RD14-B	6.00	6.08			

Endogenous Interference

Table 6. Five (5) hyperlipidemic, 5 hyperbilirubinemic, and 2 normal plasma samples obtained from individual patients were supplemented with 2.0 µg/mL of atazanavir and tested in triplicate. The endogenous substances tested did not interfere significantly with the ARK Atazanavir Assay.

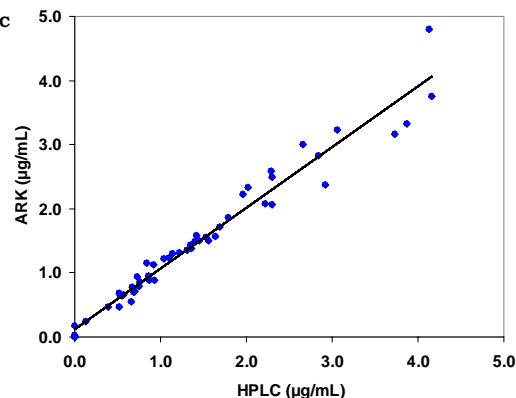
Endogenous Substance	Endogenous Substance Concentration Range	Atazanavir Recovery (% of expected value)
Cholesterol	304 - 327 mg/dL	105 - 111
Triglyceride	266 - 312 mg/dL	105 - 113
Total Bilirubin	26 - 32 mg/dL	110 - 116
Normal Human Serum	-	110 - 111

Comparative Analysis

Figure 2. Patient samples dosed with atazanavir were analyzed using the ARK Atazanavir Assay on the COBAS MIRA chemistry analyzer and HPLC, and the results are compared below.

ARK Atazanavir Assay vs HPLC

Slope: 0.95
Intercept (µg/mL): 0.13
Correlation: 0.96
N: 53



CSF Assay Results

- 76 CSF samples from 57 patients were measured with the ARK Atazanavir Test
- Results:
 - Median ATV = 10.3 ng/mL
 - Range = BQL to 38.4 ng/mL
 - CSF ATV concentrations were ~1% that of plasma

- Data are from Best, B. et al. (2006) Low Atazanavir Concentrations in Cerebrospinal Fluid. Poster #576 presented at CROI 2006.

Conclusions

The ARK Atazanavir Assay is an accurate and precise method to conveniently measure atazanavir in plasma or cerebrospinal fluid (CSF). These assays offer the following advantages to laboratories:

- No sample extraction or pretreatment required
- High specificity and good sensitivity
- Small sample size
- Excellent correlation to an HPLC method for ATV
- Ready-to-use liquid reagents and calibrators
- Rapid turn-around time