SIMULTANEOUS QUANTIFICATION OF BENZNIDAZOLE, ATAZANAVIR AND EFAVIRENZ BY UHPLC-MS/MS IN PLASMA OF PATIENTS COINFECTED WITH CHAGAS' DISEASE AND HIV/AIDS

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INTRODUCTION

Chagas disease is a parasite disease endemic to Latina America. It can produce a chronic silent disease that affects organs such as the heart and gastrointestinal tract many years after initial infection. Chagas disease can produce severe central nervous system infections, such as meningoencephalitis, in immuno-compromised patients, such as uncontrolled HIV patients. Chagas meningoencephalitis has a high mortality if untreated. The main treatment is the parasiticidal drug Benznidazole (BNZ) , but we know very little about its pharmacokinetics especially in HIV patients, and its potential interactions with anti-HIV drugs. We developed a rapid and accurate HPLC-MS/MS method to measure Benznidazole and relevant anti-HIV drugs like Atazanavir (ATZ) and Efavirenz (EFV) in plasma, and its simplicity would allow it to be used in other biological fluids as well.

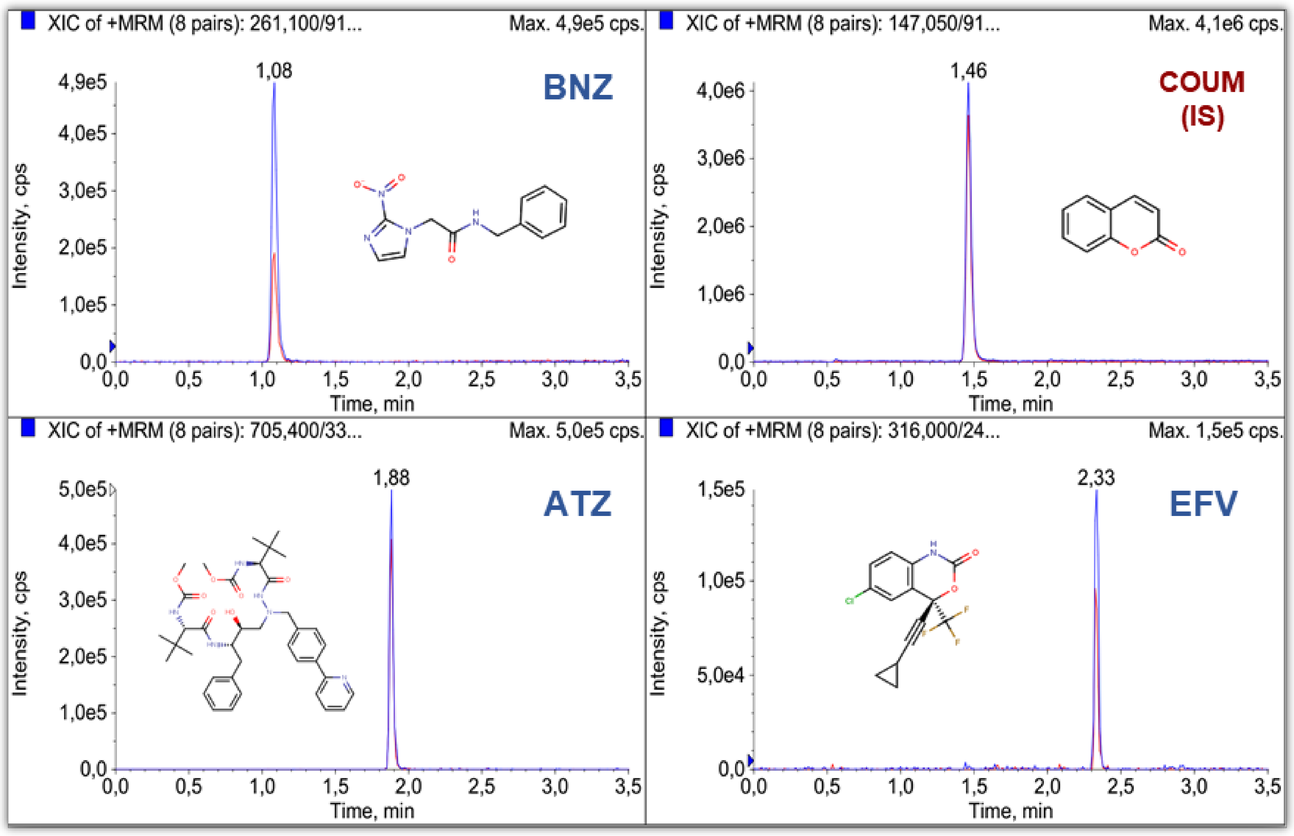
METHODOLOGY

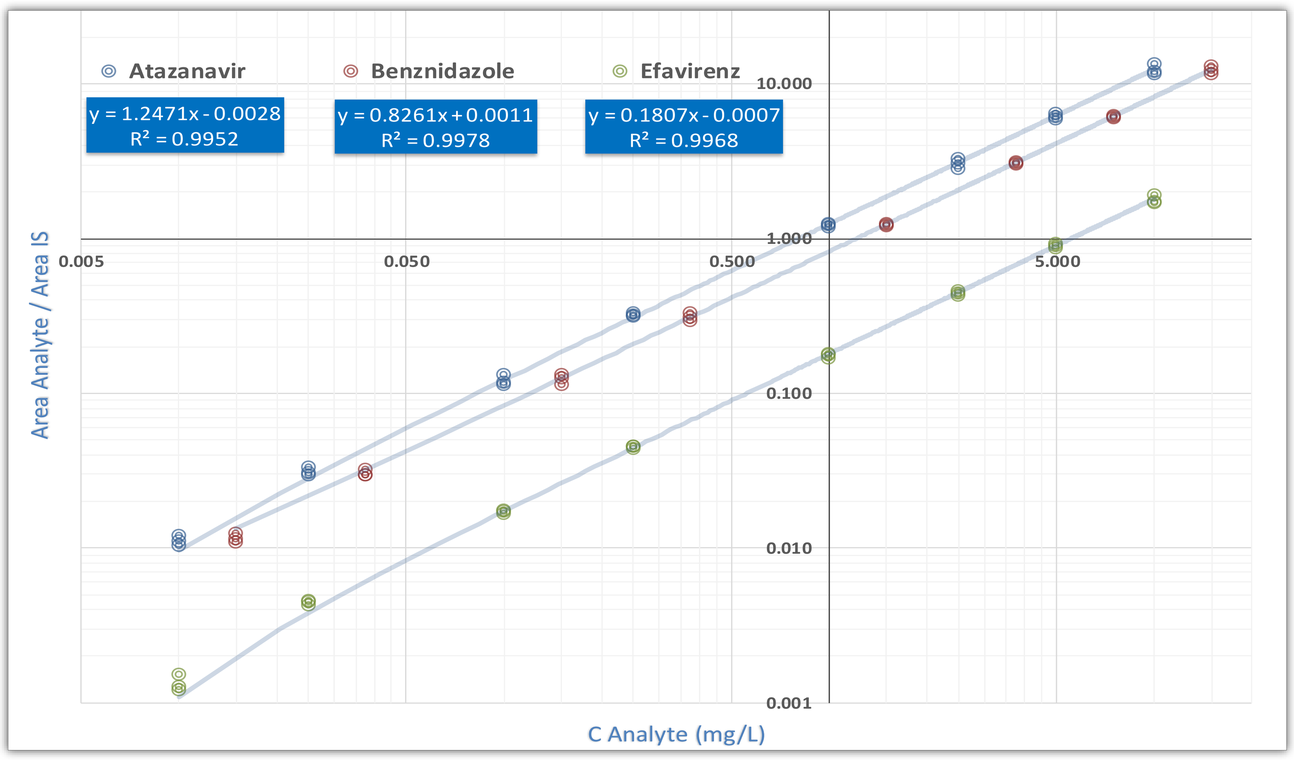
Instruments: UHPLC *Shimadzu Nexera X2* with *Restek Force* C18 (1.8 µm, 100 x 2.1 mm) column. *AB Sciex QTRAP 6500* mass spectrometer with ESI(+) ionization.
Sample preparation: 2 µL of a mixture of Benznidazole, Atazanavir and Efavirenz standards was added to 60 µL plasma (from healthy donors), then precipitated with 120 µL cold ACN, and centrifuged for 10 min at 13200 g at 5°C. 2 µL supernatant was injected into the UHPLC system.
Chromatography: Water (A) and ACN (B) were used as mobile phases (with 0.1% formic acid), flow of 0.35 mL/min, in a gradient of 50-95% (0-0.5 min), 95% (0.5-2.6 min), 95-50% (2.6-2.7 min), 50% (2.7-3.0 min), with 0.5 min for column equilibration. Column temperature was 45 °C. Total run was 3.5 min.
Mass spectrometry: Standard solutions of BNZ, ATZ and EFV were prepared (500 ng/mL in ACN) and their ion fragmentation profile $[M+H]^+$ was studied by direct infusion in the mass spectrometer. The optimization of ESI source parameters and compounds led to the quantifying MRM transitions: m/z 261→91 (BNZ), 705→335 (ATZ), 316→244 (EFV) and 147→103 (IS).

RESULTS

Analyte	Q1/Q3	DP (V)	EP (V)	CE (V)	CXP (V)	DT (ms)
BNZ	261.1/91.1	40	4	30	6	60
	261.1/107.1	30	5	25	8	60
EFV	316.0/244.0	40	4	20	18	60
	316.0/232.0	30	4	20	16	60
ATZ	705.4/335.2	20	9	40	12	60
	705.4/534.2	20	8	40	16	60
COUM (IS)	147.1/91.1	20	7	30	10	60
	147.1/103.1	20	5	20	12	60

C Teo. (mg/L)	C Calc. (mg/L)	SD (mg/L)	RSD (%)	Accuracy (%)
BNZ				
0.015	0.013	1.0E-03	7.8	85.6
0.038	0.036	1.7E-03	4.7	95.9
0.15	0.15	1.2E-02	8.2	99.9
0.38	0.38	2.1E-02	5.7	100.7
1.50	1.50	2.4E-02	1.6	100.3
3.75	3.76	6.7E-02	1.8	100.4
7.50	7.48	8.1E-02	1.1	99.7
15.00	15.01	7.9E-01	5.3	100.1
EFV				
0.010	0.011	9.1E-04	7.9	114.1
0.025	0.029	6.6E-04	2.3	115.1
0.10	0.10	2.6E-03	2.6	100.3
0.25	0.26	5.0E-03	2.0	102.1
1.00	0.98	2.9E-02	3.0	98.3
2.50	2.48	8.1E-02	3.3	99.0
5.00	5.05	1.8E-01	3.5	100.9
10.00	9.98	6.2E-01	6.2	99.8
ATZ				
0.010	0.011	7.4E-04	6.5	113.0
0.025	0.027	1.5E-03	5.3	109.7
0.10	0.10	7.5E-03	7.5	99.8
0.25	0.26	5.7E-03	2.2	104.9
1.00	0.99	1.8E-02	1.8	99.1
2.50	2.49	1.9E-01	7.4	99.7
5.00	5.00	2.1E-01	4.1	99.9
10.00	10.00	7.4E-01	7.4	100.0





PARAMETERS	BNZ	EFV	ATZ	CRITERIA
Selectivity	No interferences were observed in RT	No interferences were observed in RT	No interferences were observed in RT	Identification of interferences in RT
Linear range	15 – 15,000 ng/mL r^2 = 0.9978	10 – 10,000 ng/mL r^2 = 0.9952	10 – 10,000 ng/mL r^2 = 0.9968	$r^2 \geq 0.985$
Matrix Effect	102.3 - 110.1 %	96.5 - 99.5 %	101.2 - 108.8%	100 ± 15%
Recovery	95.4 - 98.6 %	86.2 - 91.7 %	89.5 - 94.0 %	Consistent. Reproducible
LOD	6.4 ng/mL	8.3 ng/mL	2.5 ng/mL	S/N ≥ 3
LOQ	21.4 ng/mL	27.8 ng/mL	8.3 ng/mL	S/N ≥ 10
Accuracy	85.6 – 100.7 %	98.3 – 115.1 %	99.1 – 113.0 %	100 ± 15% (± 20% LLOQ)
Precision (%RSD)	1.1 – 8.2 %	2.0 –7.9 %	1.8 – 87.5 %	≤ ±15% (≤ ±20% LLOQ)

CONCLUSIONS

We developed a rapid and effective UHPLC-MS/MS for therapeutic monitoring of BNZ, EFA and ATZ in plasma with high sensitivity in few minutes. It was validated and is a simple method capable of quantifying multiple drugs in a single run. We hope it could contribute to the PK/PD studies to define a safe and effective concomitant dosage.