Bioanalytical Method Transfer from the API 3200™ System to the SCIEX Triple Quad™ 3500 System and the Quantitative Bioanalysis of Efavirenz.

SCIEX Triple Quad™ 3500 LC/MS/MS System

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Key Features of the SCIEX Triple Quad™ 3500 System

- The SCIEX Triple Quad™ 3500 system takes the best features of the API 3200 system and enhances them with modern engineering and electronics.
- The robustness and ruggedness you expect from SCIEX instruments featuring the Turbo V™ source and Curtain Gas™ interface
- High pressure Q0 and QJet® ion guide and Fast eQ™ electronics gives you enhanced sensitivity through more efficient ion focusing and improved support of fast LC with ultra-low MRM dwell times and polarity switching experiments.

Challenges in Bioanalytical Method Transfer

- Crossvalidation – Transferring a method from one instrument type to another can be time consuming. Choosing a replacement system that does not require extensive re-tuning of method parameters saves valuable project time.
- Robustness – Selecting a system with similar or better sensitivity, precision and accuracy will reduce variability during method transfer.
- Training – Choosing a replacement system with the same software control and data format eliminates the need for personnel training programs.

The SCIEX Triple Quad™ 3500 system. Powerful, Modern Hardware for Ultimate Reliability and Productivity

Introduction

Efavirenz is a HIV-1 specific, non-nucleoside, reverse transcriptase inhibitor (Figure 1). Efavirenz, in combination with other antiretroviral agents, is indicated for the treatment of HIV-1 infection at a dose of 600 mg once a day[1]. The main objective of this work was to evaluate the method transferability from the API 3200™ System to the SCIEX Triple Quad™ 3500 System and its applicability into regulated bioanalysis.

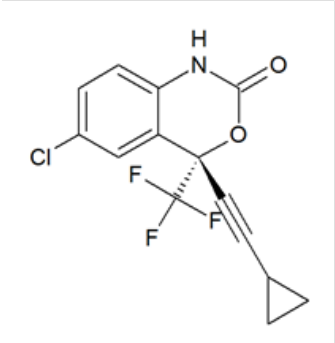

Figure 1. The chemical structure of Efavirenz ($C_{14}H_{19}ClF_3NO_2$).

Materials and Methods

Sample Preparation

Human plasma calibration and QC samples were prepared by spiking with Efavirenz working solutions (2% v/v) and then the labeled internal standard (Efavirenz-$^{13}C_6$).

Table 1. Standard and QC sample concentrations

Sample Name	Concentration (ng/ml)	Working Solution Concentration (ng/ml)
Std A	10.2	510.0
Std B	27.5	1375.0
Std C	172.0	8600.0
Std D	429.9	21495.0
Std E	1074.8	53740.0
Std F	2149.7	107485.0
Std G	4299.4	214970.0
Std H	5512.0	275600.0
LQC	25.7	1285.0
MQC	2185.6	109280.0
HQC	4371.5	218575.0

Solid phase extraction was employed for sample preparation using the following procedure: samples were pretreated with 2% v/v formic acid solution in water then loaded onto Oasis® HLB 30mg/1mL cartridges that were conditioned and equilibrated with methanol followed by Milli-Q water. The cartridges were washed with 0.1% v/v formic acid solution and then with 20% v/v methanol. The analyte was eluted with 100% methanol, evaporated under a stream of nitrogen and reconstituted with mobile phase in preparation for injection.

HPLC Conditions

Table 2. Isocratic conditions for sample analysis.

System	Shimadzu Nexera 30 AD
Column	Poroshell 120 EC-C18, 100 x 4.6 mm, 2.7µm
Mobile Phase A	1mM ammonium acetate
Mobile Phase B	Methanol
Isocratic Conditions	20:80, A:B
Flow rate	600 µL/min
Column temperature	30°C
Injection volume	10 µL
Run Time	5 minutes
Rinsing Solution	50:50 Methanol:Water

MS/MS Conditions:

The SCIEX Triple Quad™ 3500 LC/MS/MS system with Turbo V™ source and electrospray ionization (ESI) probe operating in positive mode was used. The optimized compound dependent parameters for the analyte and internal standard are summarized in Table 3. These optimized parameters were taken from an API 3200 system and used without further optimization. Quadrupole mass analyzers (Q1 and Q3) were set at unit resolution for quantitative analysis. Multiple reaction monitoring was done by selecting the singly charged molecular ions (for Efavirenz and Efavirenz $^{13}C_6$ (IS). The mass spectrometer was operated with electrospray voltage +2500 V and source temperature of 550°C. Nitrogen was used as nebulizing gas (GS1), drying gas (GS2) and curtain gas at 55, 60 and 40 units respectively. The source probe and electrode were set to the same position on both systems.

Table 3. Optimized MS parameters for MRM transitions of Efavirenz and Efavirenz $^{13}C_6$

Analyte	MRM	DP (V)	EP (V)	CE (V)	CXP (V)
Efavirenz	316.0/244.1	76	10	21	10
Efavirenz $^{13}C_6$	322.0/250.1	76	10	23	10

Results and Discussion

An accuracy and precision batch consisting of eight calibration standards and four QC samples was processed and analyzed to evaluate method transferability. Samples were first analyzed on the API 3200 system, then on the SCIEX Triple Quad™ 3500 System.

The calibration curve for Efavirenz measured on the SCIEX Triple Quad™ 3500 System was linear over a range of 10.20 - 5512.0 ng/mL in plasma (Figure 2) with an r value 0.9995. A linear calibration curve was constructed using $1/X^2$ weighting.

The statistics of the Efavirenz accuracy and precision batch are given in Table 4 and the results of each injection are given in Table 6. Efavirenz and its IS eluted at 3.33 min with minimumal background noise (Figure 3). The signal to noise ratio of Efavirenz at the LLOQ (10.2 ng/mL) in plasma was 246.4 calculated using 1-sigma standard deviation of the baseline (Figure 4). The signal to noise is 2 times higher in comparison to the data obtained using the API 3200 system with the same LC-MS/MS method. The area response for Efavirenz at the LOQ level was ~12000 using the SCIEX Triple Quad™ 3500 System while ~6000 was obtained using the API 3200 system. The details are given in Table 4.

Table 5. Sensitivity comparison API 3200 vs SCIEX TripleQuad™ 3500 System from six replicate injections of the LLOQ standard.

Parameter	API 3200	SCIEX Triple Quad 3500 System	Gain
Signal to Noise	125	246	1.9
Area Response	6000	12000	2.0

Conclusions

The SCIEX Triple Quad™ 3500 LC/MS/MS System offers powerful modern hardware and new electronics for ultimate performance, reliability and productivity.

In transferring a previously developed method from the API 3200 system to the SCIEX Triple Quad™ 3500 system we achieved higher sensitivity of area response and signal to noise for Efavirenz with generic positioning of the ion source and no further optimization of mass dependent parameters. Results obtained on the SCIEX Triple Quad™ 3500 system for Efavirenz showed twice the sensitivity for both signal to noise and peak area.

The SCIEX Triple Quad™ 3500 system offers quick and easy method transferability for bioanalytical quantitation from the API 3200 system and therefore reduces the time, expense and man power required for cross-validation and training in high throughput environments.

References

1. Supriya N Halde; Ashish A; Mungantiwar; Meena Chintamaneni; Tandem mass spectrometric estimation of Efavirenz from human plasma and comparison of various statistical approaches for evaluation of incurred sample reanalysis. Annals of Biological Research, 2011, 2 (4): 126-135
2. Praveen Srivastava, Ganesh S. Moorthy, Robert Gross, Jeffrey S. Barrett; A Sensitive and Selective Liquid Chromatography/ Tandem Mass Spectrometry Method for Quantitative Analysis of Efavirenz in Human Plasma. PLOS ONE 2013, 8 (6): 1-9

Table 4: Statistical analysis of Quality Control samples for Efavirenz in human plasma.

Expected Concentration	Sample Name	Number Of Values Used	Mean	Standard Deviation	%CV	Accuracy
25.7	LQC	15 of 15	24.73	0.319	1.29	96.24
612.0	M1QC	3 of 3	594.21	5.12	0.86	97.09
2185.6	MQC	3 of 3	2169.41	6.91	0.32	99.25
4371.5	HQC	3 of 3	4507.68	27.58	0.61	103.11

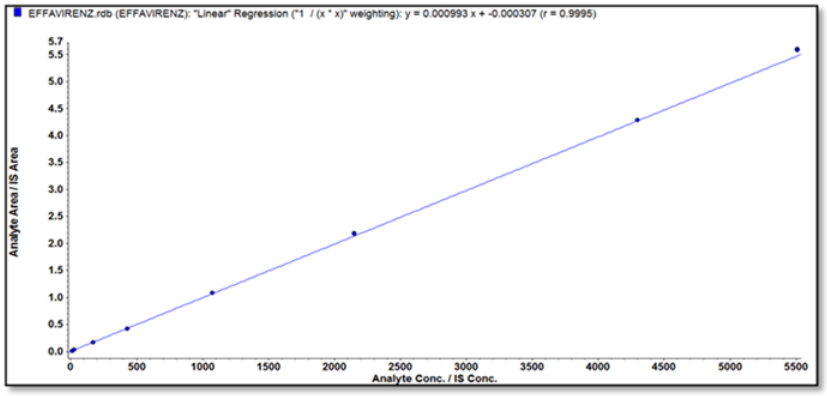

Figure 2: Calibration curve of Ffavirenz in plasma from 10.2 ng/mL to 5512.0 ng/mL run on the SCIEX Triple Quad™ 3500 System. The method has shown excellent linearity over the concentration range with r = 0.9995

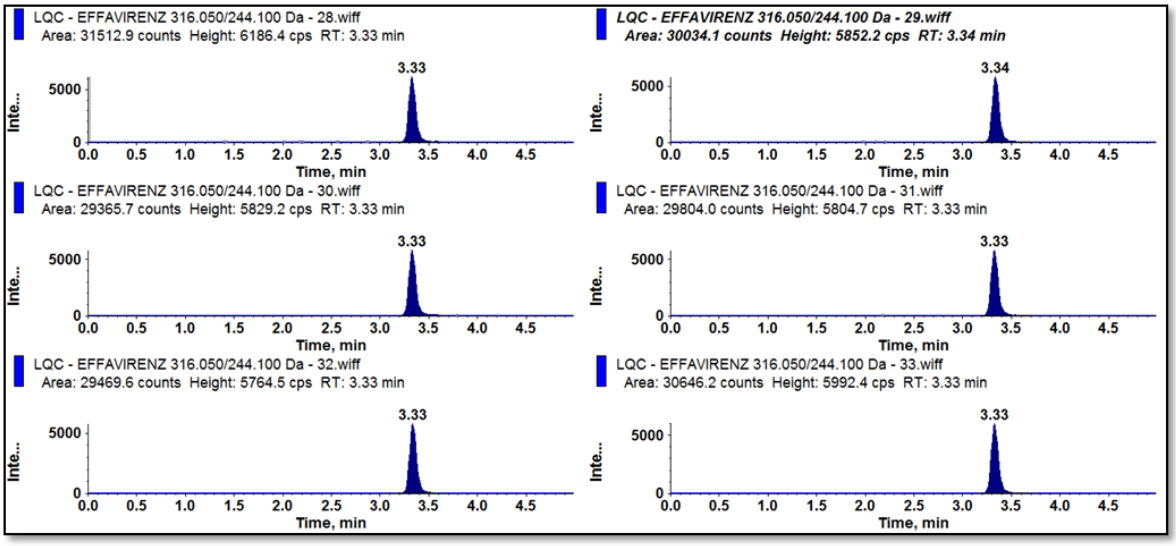

Figure 3. Chromatograms of 6 LQC QC samples from Precision and Accuracy Batch

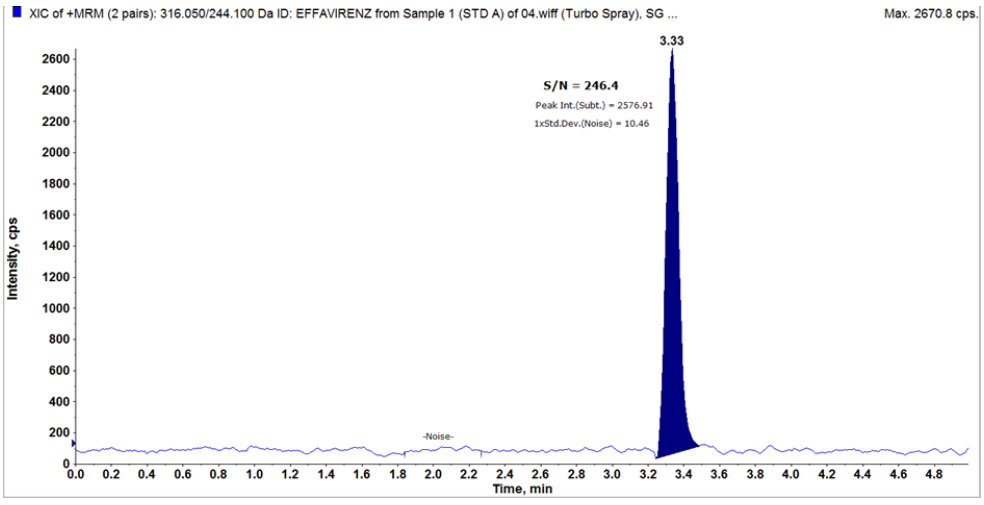

Figure 4. Signal to noise calculation for Efavirenz in extracted plasma sample at LLOQ level (10.20 ng/mL).

Table 6: Results of the full accuracy and precision batch data for Efavirenz in human plasma sample measured on the SCIEX Triple Quad™ 3500 system.

Sample ID	Sample Type	Retention Time (Min)	Analyte Peak Area	IS Peak Area	Area Ratio	Analyte Con. (ng/mL)	Calculated Con. (pg/mL)	% Accuracy
RFS	Unknown	3.33	5492800.2	990759.9	5.544	N/A	N/A	N/A
BLANK	Blank	N/A	79.2	54.5	1.4541	N/A	N/A	N/A
BLANK+IS	Unknown	0	70.1	1093464.9	0.0001	N/A	N/A	N/A
STD A	Standard	3.33	12182.6	1270193.8	0.0096	10.2	10	97.71
STD A DUP	Standard	3.33	13567.7	1324668.6	0.0102	10.2	10.6	104.14
STD B	Standard	3.33	31586.6	1223557.3	0.0258	27.5	26.3	95.64
STD C	Standard	3.33	190695.5	1161182.8	0.1642	172	165.7	96.31
STD D	Standard	3.33	527468.8	1256458.6	0.4198	429.9	423	98.39
STD E	Standard	3.33	1015753.2	940566.6	1.0799	1074.8	1087.6	101.19
STD F	Standard	3.33	2155136.4	988223.7	2.1808	2149.7	2196	102.16
STD G	Standard	3.33	4354107.0	1016071.3	4.2852	4299.4	4314.8	100.36
STD H	Standard	3.33	5065130.2	905452.5	5.594	5512	5632.6	102.19
STD H DUP	Standard	3.33	5877039.1	1053368.8	5.5793	5512	5617.7	101.92
LQC -01	Quality Control	3.33	31067.4	1311185.4	0.0237	25.7	24.2	94.03
LQC-02	Quality Control	3.33	30051.5	1214691.5	0.0247	25.7	25.2	98.13
LQC-03	Quality Control	3.33	31387.0	1290668.0	0.0243	25.7	24.8	96.48
M1QC-01	Quality Control	3.33	702474.3	1194853.6	0.5879	612	592.2	96.77
M1QC-02	Quality Control	3.33	670865.1	1144735.0	0.586	612	590.4	96.46
M1QC-03	Quality Control	3.33	694641.2	1166183.6	0.5957	612	600	98.04
MQC-01	Quality Control	3.33	2446621.3	1137845.3	2.1502	2185.6	2165.2	99.07
MQC-02	Quality Control	3.33	2325804.2	1075608.0	2.1623	2185.8	2177.4	99.62
MQC-03	Quality Control	3.33	2475862.4	1151230.8	2.1506	2185.8	2165.6	99.08
HQC-01	Quality Control	3.33	4779896.1	1063384.0	4.495	4371.5	4526	103.53
HQC-02	Quality Control	3.33	4501349.2	1012613.1	4.4453	4371.5	4476	102.39
HQC-03	Quality Control	3.33	4809731.3	1071184.2	4.4901	4371.5	4521.1	103.42

Table 6 Continued: Results of the full accuracy and precision batch data for Efavirenz in human plasma sample measured on the SCIEX Triple Quad™ 3500 system.

Sample ID	Sample Type	Retention Time (Min)	Analyte Peak Area	IS Peak Area	Area Ratio	Analyte Con. (ng/mL)	Calculated Con. (pg/mL)	% Accuracy
LQC -01	Quality Control	3.33	31512.9	1289156.3	0.0244	25.7	24.9	96.97
LQC-02	Quality Control	3.33	30034.1	1227469.8	0.0245	25.7	24.9	97.06
LQC-03	Quality Control	3.33	29365.7	1234676.1	0.0238	25.7	24.3	94.38
LQC-04	Quality Control	3.33	29804.0	1232233.2	0.0242	25.7	24.7	95.96
LQC-05	Quality Control	3.33	29469.6	1226527.7	0.0240	25.7	24.5	95.33
LQC-06	Quality Control	3.33	30646.2	1261427.9	0.0243	25.7	24.8	96.38
LQC-07	Quality Control	3.33	28812.0	1189267.7	0.0242	25.7	24.7	96.12
LQC-08	Quality Control	3.33	29464.2	1228718.3	0.0240	25.7	24.5	95.15
LQC-09	Quality Control	3.33	29714.4	1210667.2	0.0245	25.7	25	97.36
LQC-10	Quality Control	3.33	29915.9	1207836.2	0.0248	25.7	25.2	98.24
LQC-11	Quality Control	3.33	30753.2	1261179.5	0.0244	25.7	24.9	96.73
LQC-12	Quality Control	3.33	28725.3	1195582.7	0.0240	25.7	24.5	95.33

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