Assessing Ease of Method Transfer from the API 4000™ System to the SCIEX Triple Quad™ 4500 System for the Quantification of Loratadine in Human Plasma

SCIEX Triple Quad 4500 System

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Key Features of the SCIEX Triple Quad 4500 System

- The SCIEX Triple Quad 4500 system takes the best features of the API 4000 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

- Cross validation – 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.

Figure 1. The SCIEX Triple Quad 4500 System.

INTRODUCTION

The transfer of bioanalytical LC-MS/MS methods from lab to lab or between instruments within the same lab is essential for any drug development program and is increasingly important in a globalized world. Delays in the transfer process, especially post transfer failure can be extremely costly, delaying projects and costing time and money to remedy the issues.

Many factors are involved in the failure of method transfer; however some important considerations are instrument manufacturer, model, age, location, servicing standards and its historical use.

The SCIEX 4500 Series of mass spectrometers takes the legendary API 4000 platform and intelligently re-engineers it to set a new benchmark for ease of method transfer and reliable quantitation.

In a bioanalytical lab, as in any other industry, customers expect maximum uptime and a system that can easily handle the most difficult samples and matrices. The 4500 Series fulfills these expectations – with new levels of dependability and consistency built into the system.

The Turbo V™ source and Curtain Gas™ interface set the benchmark for reliability and the proven QJet® ion guide, eQ™ electronics, curved LINAC® collision cell, and AcQuRate™ detector set new standards for system robustness.

The aim of the present study was to transfer a liquid chromatography coupled with mass spectrometry (LC-MS/MS) method from the API 4000™ system to the SCIEX Triple Quad™ 4500 system for quantification of Loratadine (LOR) in human plasma using Loratadine-D_5 (LOR-D_5) as internal standard.

This study also evaluated the signal stability in plasma samples overnight after a direct method transfer from the API 4000 system to the SCIEX Triple Quad 4500 system.

MATERIALS AND METHODS

Sample Preparation

Human plasma (300 µL) spiked with LOR was prepared by adding internal standard (50 µL, 10000 pg/mL of LOR-D_5), then further diluting with 100 µL of buffer solution. Liquid-liquid extraction was performed by adding 1.5 mL of extraction solvent and vortexing. The organic layer was then removed, concentrated to dryness and reconstituted in mobile phase.

Table 1. Calibration Standard and QC sample concentrations

Sample Name	LOR Concentration (pg/ml)
Std A	50
Std B	100
Std C	1879
Std D	3758
Std E	7516
Std F	9774
Std G	12780
Std H	15000

Sample Name	LOR Concentration (pg/ml)
LOQQC	50
LQC	135
M1QC	1502
MQC	5007
HQC	12218

HPLC Conditions

Table 2. Isocratic conditions for sample analysis.

System	Shimadzu SIL HTC
Column	Zorbax SB C18 (75 × 4.6mm, 3.5µ)
Mobile Phase A	10mM Ammonium formate + 0.1% Formic acid
Mobile Phase B	Acetonitrile : Methanol (95:5 v/v)
Isocratic Conditions	40:60, A:B
Flow rate	1.5 mL/min (50% to waste)
Column temperature	40°C
Injection volume	20 µL
Run Time	4 minutes
Rinsing Solution	50:50 Methanol:Water

MS/MS Conditions:

MS experiments on both systems were performed using the Turbo V™ source and electrospray ionization (ESI) probe operating in positive mode. The compound dependent parameters: declustering potential (DP), entrance potential (EP), collision energy (CE) and collision cell exit potential (CXP) of LOR and its internal standard LOR-D_5 was optimized on each system via infusion. The optimized parameters were as follows

Table 3A. Optimized MS parameters for MRM transitions of LOR and LOR-D_5 on the API 4000 System.

Analyte	MRM	DP (V)	EP (V)	CE (V)	CXP (V)
LOR	383.2/337.2	70	10	35	22
LOR-D_5	388.2/337.2	70	10	35	22

Table 3B. Optimized MS parameters for MRM transitions of LOR and LOR-D5 on the SCIEX Triple Quad 4500 System.

Analyte	MRM	DP (V)	EP (V)	CE (V)	CXP (V)
LOR	383.2/337.2	70	10	33	14
LOR-D_5	388.2/337.2	70	10	33	14

The API 4000™ System mass spectrometer was operated with electrospray voltage +2800 V and source temperature of 600°C. Nitrogen was used as nebulizing gas (GS1), drying gas (GS2) and curtain gas at 45, 55 and 40 units respectively.

The SCIEX Triple Quad™ 4500 System mass spectrometer was operated with electrospray voltage +5500 V and source temperature of 450°C. Nitrogen was used as nebulizing gas (GS1), drying gas (GS2) and curtain gas at 55, 50 and 40 units respectively.

RESULTS AND DISCUSSIONS

Loratadine is a long acting tricyclic antihistamine with selective peripheral histamine H1-receptor antagonist activity.

A sensitive method for the quantification of LOR in human plasma was previously developed and validated on the API 4000 System at Jubilant Generics. To test method transfer to the SCIEX Triple Quad 4500 System, processed samples from an accepted precision and accuracy batch which was run on the API 4000 System machine was reinjected multiple times on the SCIEX Triple Quad 4500 System at SCIEX. Chromatographic conditions used for analysis on the SCIEX Triple Quad 4500 System were the same as those for analysis on the API 4000 System.

Analyst® software version 1.6.2 was used for data processing. The calibration curve for LOR from the SCIEX Triple Quad 4500 System is shown in figure 4. Linearity was established in the range of 50 to 15000 pg/ml for LOR in human plasma with correlation coefficient r = 0.99. A $1/x^2$ weighted linear regression was used to calculate the concentrations.

Table.4 shows the global %CV and accuracy data of all reinjected QC samples at different concentration levels of LOR. As with the original injection on the API 4000 System All are within the acceptance criteria of accuracy and %CV ±20% at LLOQ level and ±15% at other levels as defined by regulatory guidelines.

The stability of peak area response in low concentration samples was evaluated over 12 hours by re-injecting analyte LLOQ and LQC samples. Acceptable stability in response was observed with the %CV less than 11.4 for LOR. (Refer Table 5).

CONCLUSIONS

- A sensitive bioanalytical method for the quantitation of Loratadine was successfully transferred from the API 4000 System to the SCIEX Triple Quad 4500 system.
- Stability in signal was observed throughout the overnight analysis. %CV for peak area response was less than 12% for LOR.

REFERENCES

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- Verma S, et al. LC-ESI-MS/MS estimation of loratadine-loaded self-nanoemulsifying drug delivery systems in rat plasma: Pharmacokinetic evaluation and computer simulations by GastroPlus™. J Pharm Biomed Anal. 2016 May 30;124:10-21

Table 4: Global precision and Accuracy statistics

S.NO	LORATADINE (pg/ml)				
	LOQQC	LQC	M1QC	MQC	HQC
1	54.66	141.02	1544.22	5135.31	12263.16
2	57.20	134.57	1538.08	5114.08	12203.47
3	50.41	131.76	1533.37	5122.63	12292.76
4	50.66	132.27	1540.20	5245.07	12469.00
5	50.43	138.82	1541.53	5112.26	12341.64
6	52.50	126.19	1541.82	5100.24	12432.92
7	56.00	130.76	1527.79	5201.61	12386.15
8	54.47	134.01	1559.17	5183.36	12671.91
9	51.21	131.23	1565.00	5193.71	12487.99
10	50.66	130.64	1558.53	5058.78	12589.96
11	50.57	134.88	1566.89	5112.45	12526.15
12	56.07	136.54	1599.38	5213.42	12685.43
13	54.75	137.00	1571.75	5077.01	12412.99
14	58.13	125.69	1556.78	5215.82	12710.22
15	55.43	130.82	1554.87	4998.97	12454.39
16	54.35	137.68	1567.78	5149.25	12494.72
17	56.10	135.58	1511.99	5056.43	12195.58
18	51.02	125.79	1529.21	5088.42	12312.18
19	50.20	137.60	1555.69	5142.52	12317.58
20	55.94	138.54	1556.55	5242.81	12554.24
21	53.51	132.91	1577.47	5143.17	12218.44
22	53.31	125.92	1539.20	5086.85	12421.61
23	52.74	127.37	1527.86	5182.75	12291.66
24	45.25	133.29	1542.08	5003.23	12294.63
Mean	**53.14**	**132.95**	**1550.30**	**5132.50**	**12417.86**
S.D (+/-)	**2.961**	**4.512**	**19.245**	**68.405**	**151.868**
C.V (%)	**5.57**	**3.39**	**1.24**	**1.33**	**1.22**
Nominal	**50**	**135**	**1502**	**5007**	**12218**
Accuracy (%)	**105.85**	**98.34**	**103.21**	**102.50**	**101.63**

Table 5: Peak area response of LLOQ and LQC samples

S.NO	(LOQQC Area response)	(LQC Area response)
	LOR	LOR
1	9665	22526
2	10226	25387
3	8934	24879
4	9556	22964
5	8584	21432
6	9251	22270
7	9682	22134
8	10030	25567
9	8463	20268
10	8875	22185
11	8867	23531
12	9542	23751
13	8040	16820
14	8426	19208
15	8139	20239
16	7349	21121
17	7636	16738
18	7284	18961
19	7477	20881
20	7549	19325
21	7444	16267
22	7666	19256
23	8030	20032
24	6992	20612
Mean	**8487.792**	**21098.083**
S.D (+/-)	**965.9796**	**2579.6380**
C.V (%)	**11.38**	**12.23**

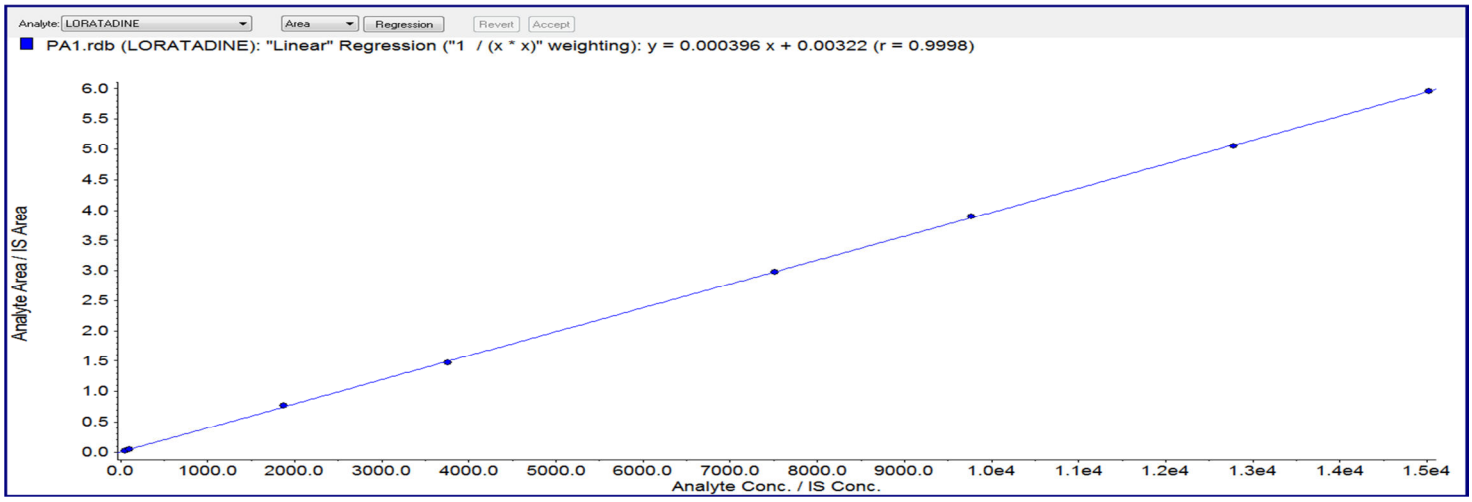

Figure 2: Calibration curve of LOR (50 to 15000 pg/ml) analysed on the SCIEX Triple Quad™ 4500 System.

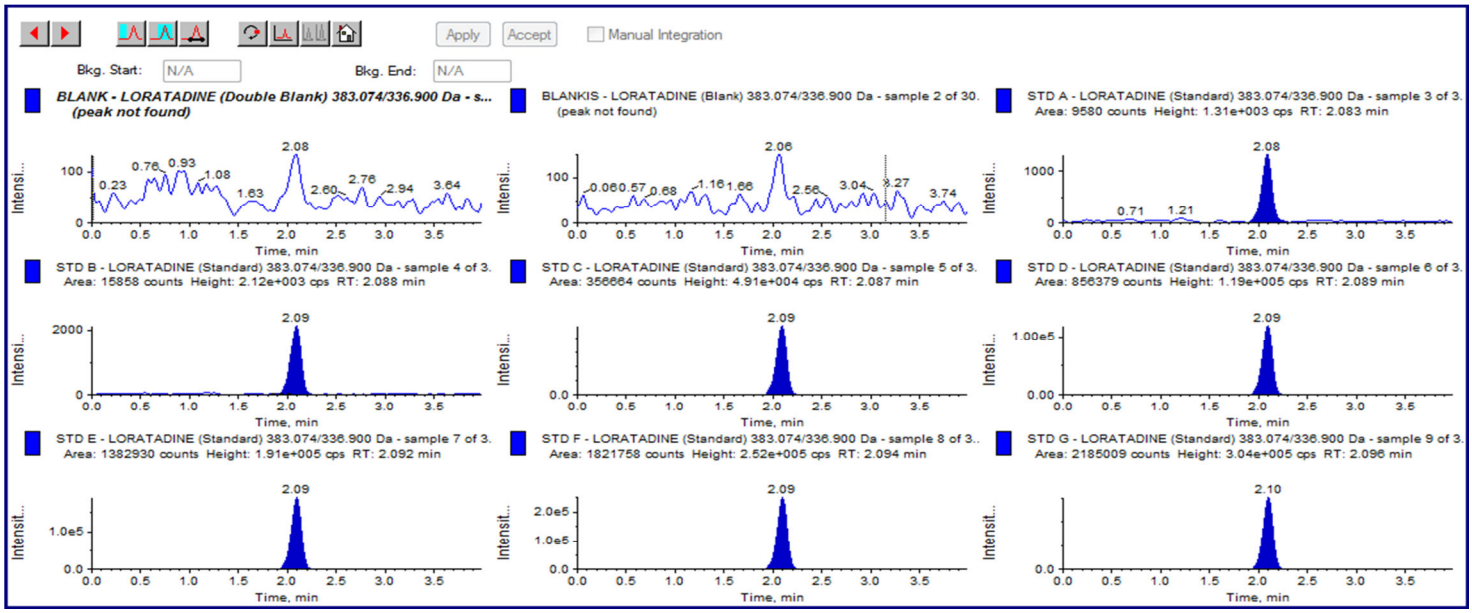

Figure 3: Representative chromatograms of LOR analysed on the SCIEX Triple Quad 4500 System.

Document number: RUO-MKT-02-6689-A

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