

SCIEX Solution for Highly Sensitive LC-MS/MS Method for the Quantification of mometasone furoate in Human Plasma using SCIEX QTRAP® 6500 System



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INTRODUCTION

Mometasone Furoate (MF) is a topical corticosteroid and is a potent anti-inflammatory drugs for the treatment of allergic diseases such as asthma, allergic rhinitis, eczema and psoriasis. Mometasone acts by reducing the number and the activation process of inflammatory cells in the airway. Mometasone is a synthetic 17-heterocyclic glucocorticoid currently available in nasal and oral inhaled preparations.

Recently, it has been reported that inhaled mometasone furoate has a systemic bioavailability of less than 1%, which is much lower than other corticosteroids. Plasma concentrations of mometasone furoate in therapeutic inhaled dose ranges are extremely low and require sensitive assays to determine the pharmacokinetic parameters which necessitate the use of a sensitive analytical method that can quantify at sub-pico gram per mL levels.

A few analytical methods have been developed for pharmacokinetic studies or clinical trials of mometasone furoate, however, to achieve the necessary sensitivity, most of the methods use a large plasma sample aliquot and a low reconstitution volume which limits the feasibility of performing reinjection reproducibility or repeat analysis in a GLP regulated bioanalytical laboratory.

To provide a strong support for its pharmacokinetic studies, a robust, rapid, selective and sensitive LCMS-ESI method has been developed for the quantification of mometasone furoate and mometasone Furoate D3 internal standard in human plasma using a solid-phase extraction Clean-up step. The main objective of this work is to develop a sub-pico gram level (LLOQ 250 fg/mL) LC-MS/MS method using SCIEX QTRAP® 6500 LC-MS/MS system

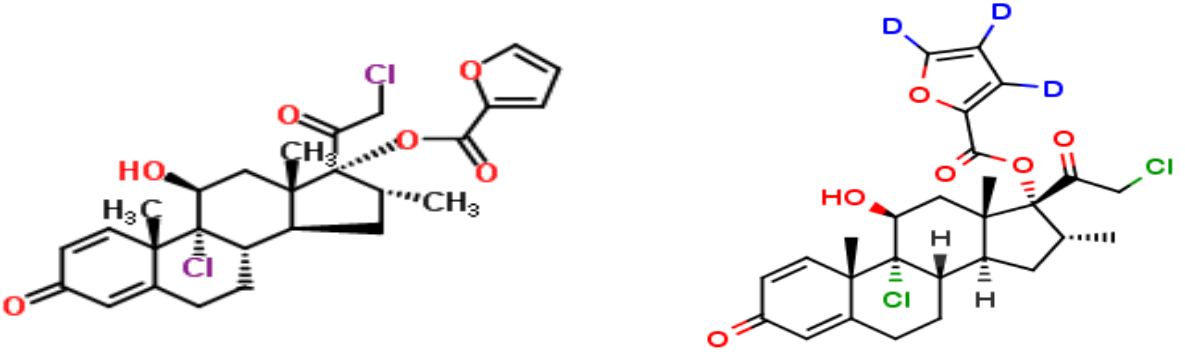


Figure 1: Structure of Momeatsone Furoate and Momeatsone Furoate –D3



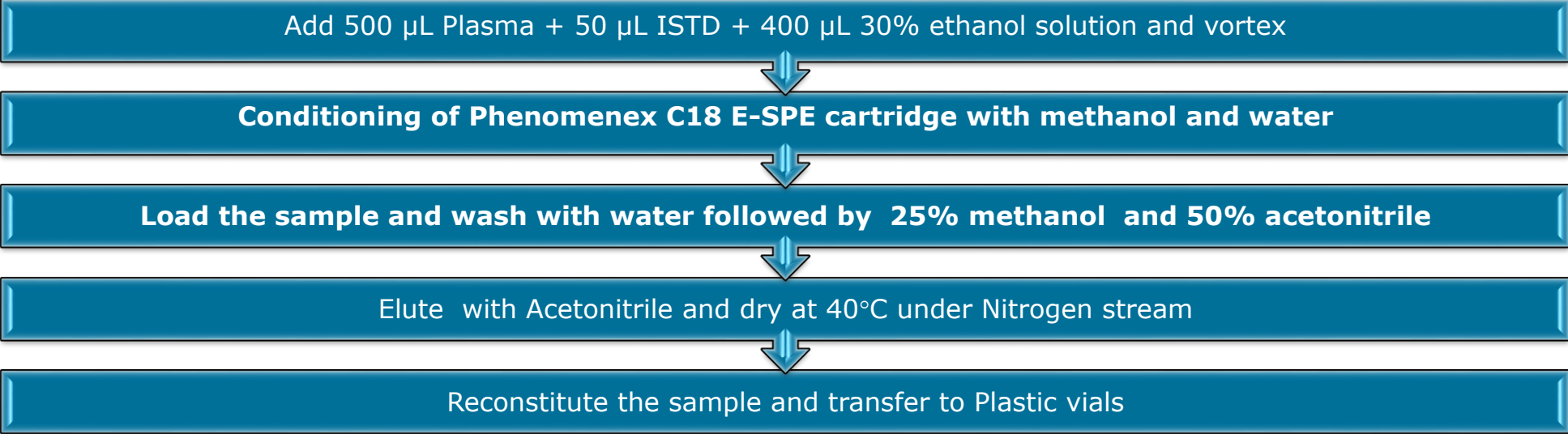
Figure 2: SCIEX QTRAP® 6500 LC-MS/MS system



Figure 3: Unique features of QTRAP® 6500 System (IonDrive™ Turbo V Source, IonDrive™ QJet Ion Guide, IonDrive™ High Energy Detector)

MATERIALS AND METHODS

Sample Preparation:



Chromatographic Conditions:

LC System : Exion LC AD
Column : Kinetex XB C18 (100mm × 2.1mm, 2.6µ)
Column Temp : 45 °C
Flow Rate: 0.600 ml/min
Flow type : Gradient
Mobile phase : 0.5mm Ammonium Trifluoroacetate buffer and Acetonitrile



MultiQuant™ Software for streamlining data processing and increasing MS *quantitation* productivity

Mass Spectrometry Conditions:

The SCIEX QTRAP® 6500 LC-MS/MS system was operated in positive electrospray ionization mode. The MS conditions were as follows: scan type positive MRM, Q1 resolution at unit and Q3 at unit; curtain gas set at 30; ion source temperature 450°C, ion source gas (GS1) at 50 and drying gas (GS2) at 50; ion spray voltage at 5500 V; and dwell time 200 ms for all transitions. The compound dependent parameters for analyte and internal standard were as follows:

COMPOUND	Q1	Q3	DP	EP	CE	CXP
Mometasone Furoate	521.1	355.1	60	10	22	35
Mometasone Furoate-D3	524.1	355.1	60	10	22	35

RESULTS AND DISCUSSIONS

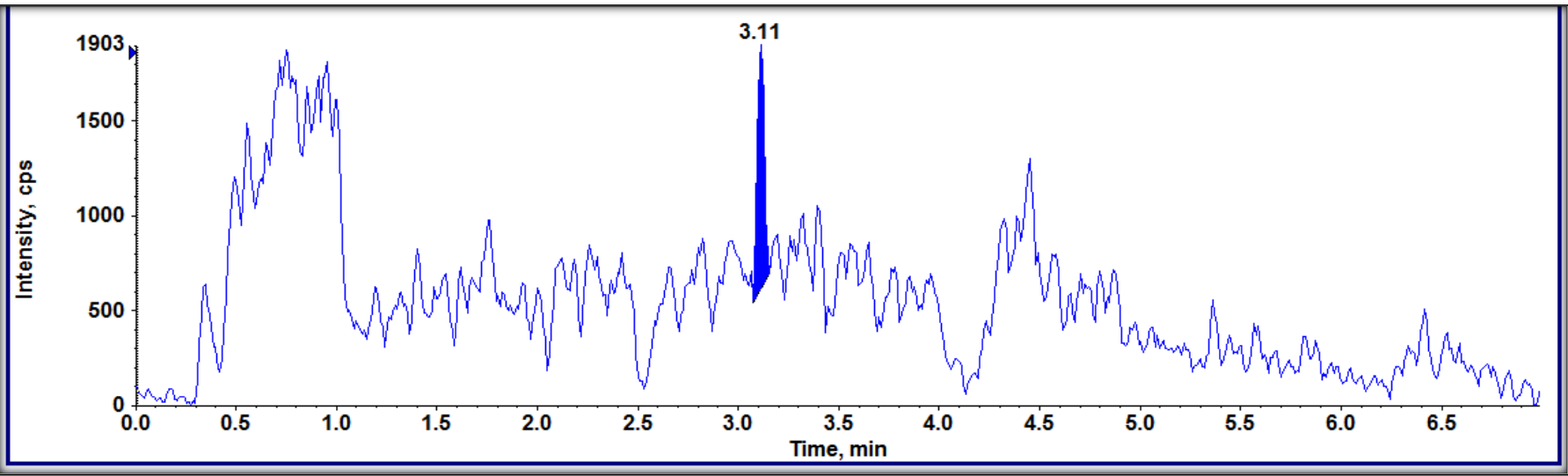


Figure 4: S/N ratio peak at LLOQ 0.250pg/mL in plasma

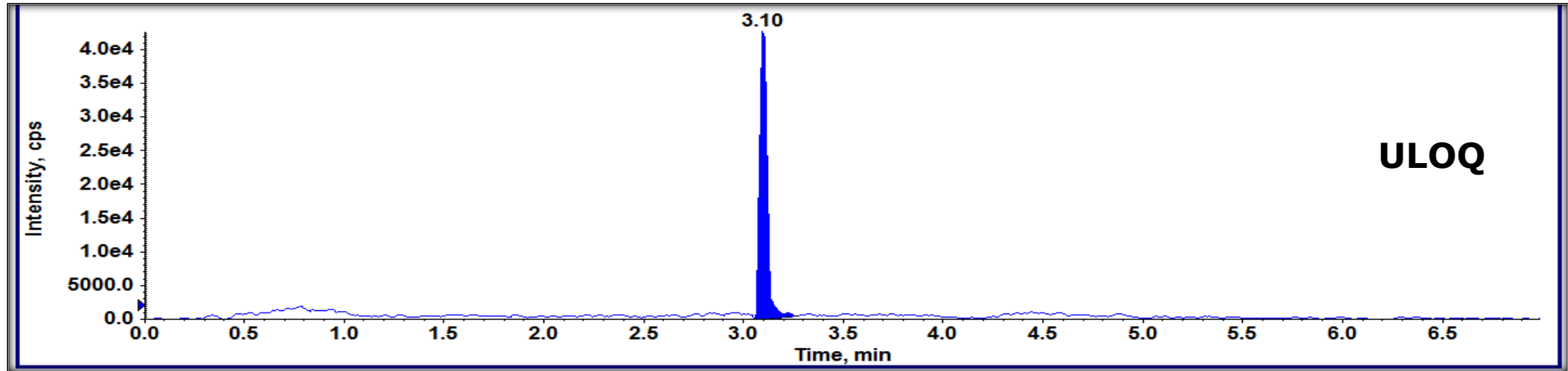
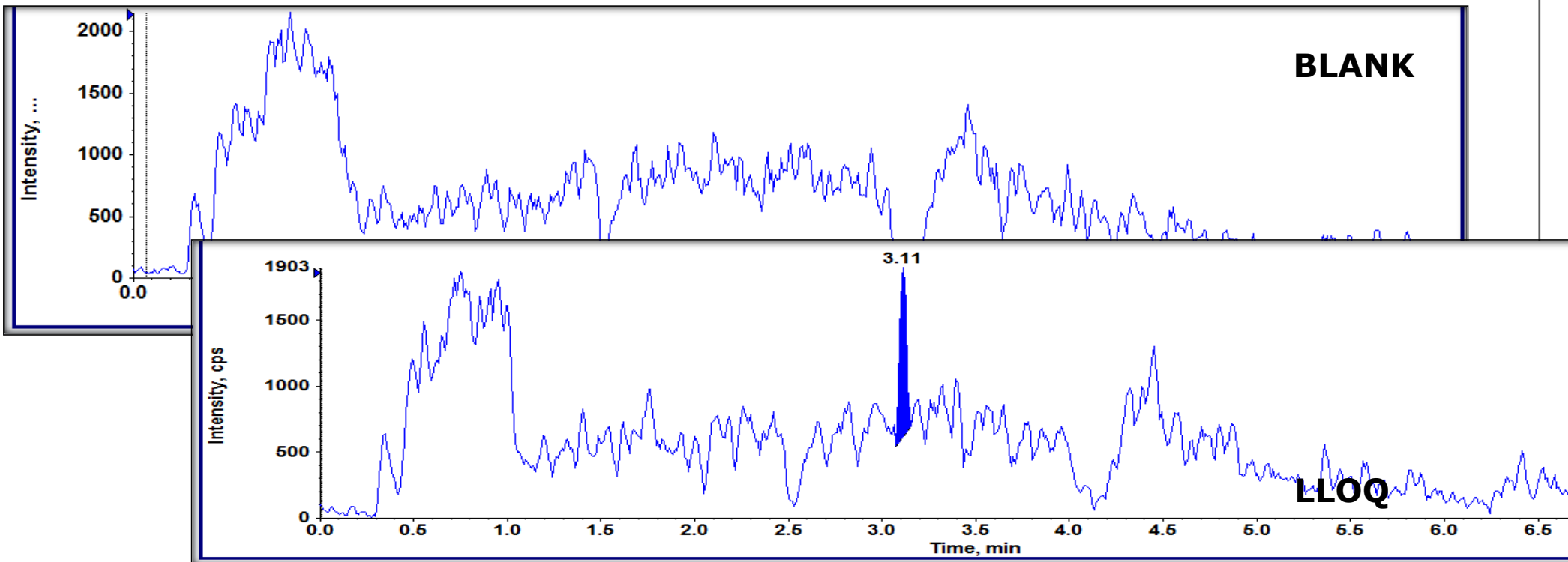


Figure 5: Chromatograms of blank plasma, LLOQ and ULOQ samples from the mometasone furoate method

Mometasone fuorate and mometasone fuorate D3 were produced two intense product ions at *m/z* 355.1 and 263.1 but finally 355.1 was selected and used for the quantitation in plasma. The mass spectrometric parameters for both analyte and internal standard were given in Table 1.

Various sample extraction techniques like liquid-liquid extraction, protein precipitation and SPE were tried to separate the analyte of interest from the matrix components. The SPE technique outlined above produced promising results over the published sample preparation methods because it removed a significant number of matrix components and given good recovery for both analyte and internal standard.

Extensive SPE clean up and optimised chromatographic condition were the key parameters to achieve the lowest LOQ (250fg/ml) and removal of interferences at the retention time of the analyte.

In the present method, linearity was established in the range of 0.250 to 30 pg/mL in human plasma. The calibration curve is shown in figure 6 with correlation coefficient *r* = 0.99.

Table 2 shows the accuracy and precision data at different QC levels of mometasone fuorate. All are within the acceptance criteria of %CV ±20% at LLOQ level and ±15% at other levels. Example chromatograms of the blank, LLOQ and ULOQ calibration standards are shown in figure 5.

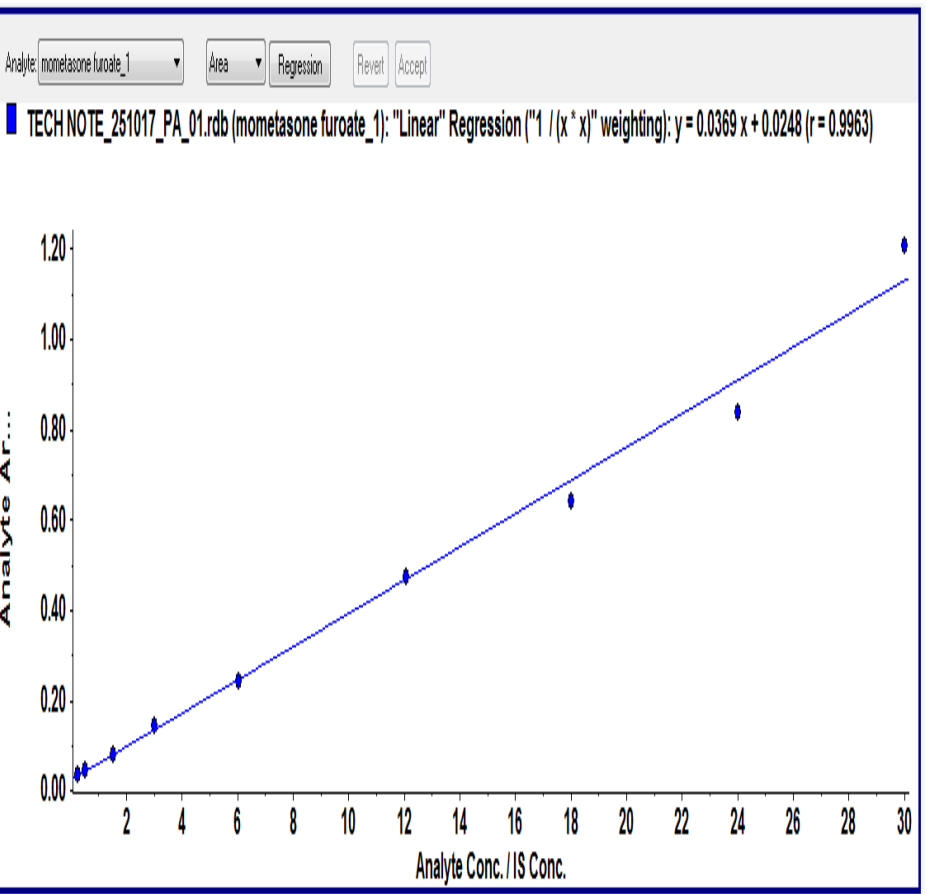


Figure 6:- Linearity Plot for mometasone range 0.25 to 30 pg/mL

Sample Number	LOQ QC (pg/ml)	LQC (pg/ml)	MQC (pg/ml)	HQC (pg/ml)
1	0.255	1.380	10.600	22.100
2	0.240	1.420	11.000	22.600
3	0.241	1.480	11.200	21.600
4	0.244	1.330	11.500	23.400
5	0.236	1.610	12.100	22.700
6	0.258	1.360	11.500	22.200
7	0.231	1.600	11.100	22.000
8	0.245	1.560	11.300	22.500
9	0.226	1.470	11.500	24.300
10	0.266	1.530	11.500	23.000
11	0.295	1.700	11.100	21.400
12	0.230	1.600	12.200	22.000
Mean	0.247	1.503	11.383	22.483
S.D (+/-)	0.0192	0.1156	0.4469	0.8043
C.V (%)	7.76	7.69	3.93	3.58
% Nominal	0.250	1.510	12.100	24.000
Accuracy (%)	98.90	99.56	94.08	93.68

Table 2: Precision and Accuracy Statistics

CONCLUSIONS

- A highly selective, sensitive and reproducible bioanalytical method was developed for the detection as low as 250 fg/mL of mometasone furoate, in Human plasma on the SCIEX QTRAP® 6500 LC-MS/MS system.
- Lower plasma volume of 500µl and final reconstitution volume of 200µl made this method possible for reinjection of samples or repeat analysis in a regulated bioanalytical laboratory

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- Sahasranaman S, Tang Y, Biniasz D, Hochhaus G A sensitive liquid chromatography-tandem mass spectrometry method for the quantification of mometasone furoate in human plasma.

TRADEMARKS/LICENSING

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