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Application News

Liquid Chromatograph Mass Spectrometer LCMS-8060NX

Sensitive and Robust Method for Estimation of Mometasone at Sub-pg/mL in Human Plasma Using Shimadzu LCMS-8060NX

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User Benefits

- Simple, Selective and most sensitive method with LLOQ of 0.5 pg/mL
- Linear dynamic range suitable for PK studies and range between 0.5 pg/mL to 20 pg/mL
- Single step sample extraction method increased sample productivity

1. Introduction

Mometasone furoate is a long-acting beta2-agonist used in the management of asthma and/or chronic obstructive pulmonary disease (COPD). Inhaled Mometasone works like other beta2-agonists, causing bronchodilatation by relaxing the smooth muscle in the airway so as to treat the exacerbation of asthma. Mometasone furoate is a corticosteroid used as an anti-inflammatory[1] and chemical name is (11β, 16α)-9, 21-dichloro-11-hydroxy-16- methyl-3, 20-dioxopregna-1, 4-dien-17-yl 2-furoate. Mometasone have very less bioavailability (less than 1 %) and very low circulating plasma concentrations (50 pg/mL) following a 100–400 µg inhaled dose, accurate quantification of mometasone from plasma can be challenging[2]. In this work, a robust, sensitive, and selective method was developed for the accurate quantification of mometasone furoate.

Such method should address many problems such as low Bioavailability, carry-over and low extraction recovery.

We have therefore developed a method with high chromatographic resolution and ample sensitivity giving lowest limit of quantification (LLOQ) of 0.50 pg/mL for Mometasone in human plasma using LCMS-8060NX. Method was developed keeping some key criteria in focus- namely simpler extraction procedure, highly optimized chromatography and enhanced sensitivity. These factors enable selective and high-throughput analysis of Mometasone for the pharmacokinetic investigation.

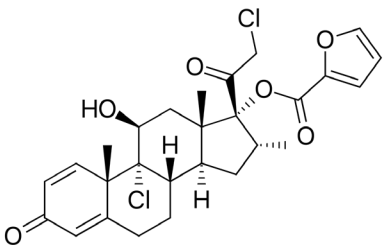

Fig. 1 Structure of Mometasone

2. Salient Features

- Quantitative method for estimation of Mometasone in human plasma was developed and validated as per US major Guidelines (results are presented in Table 1)
- Effective throughput for quantitative assessment is increased by use of a quick single step extraction procedure.
- Heated ESI along with New UF-Qarray™ ion guide technology contributes by increasing ion production and enhancing transmission, respectively. This ensures sensitive and selective quantification of Mometasone at 0.50 pg/mL.
- Isocratic method enhances the chromatographic resolution of Mometasone with consistent and reproducible peak area and retention time
- Method was validated as per US major guidelines for
  - ✓ Linearity
  - ✓ Inter-day and intra-day precision and accuracy (PA)
  - ✓ Recovery
  - ✓ Matrix effect

Table 1 Method Validation Summary

Calibration curve range		0.50 pg/mL to 20 pg/mL
Intraday precision and accuracy (For LLOQ-QC)	Accuracy (% Nominal)	105.68
	Precision (% RSD)	15.03
Intraday precision and accuracy (For LQC, MQC and HQC)	Accuracy (% Nominal)	90.31 to 100.72
	Precision (% RSD)	6.83 to 14.80
Global precision and accuracy (For LLOQ-QC)	Accuracy (% Nominal)	109.59
	Precision (% RSD)	16.88
Global precision and accuracy (For LQC, MQC and HQC)	Accuracy (% Nominal)	91.19 to 103.63
	Precision (% RSD)	7.95 to 12.20
Global % recovery	Recovery (%)	50.02
	Precision (% RSD)	14.59
Matrix effect	Mean Matrix Factor	0.92

Note: LLOQ QC- Lower Limit of Quantification Quality Control, LQC- Lower Quality Control, MQC- Middle Quality Control and HQC- Higher Quality Control

3. Experimental

3.1. Sample preparation and analytical conditions

- Four hundred microliters of Extraction Buffer was added to plasma samples and vortexed to mix for 30 seconds.
- After Vortexing the samples were processed by using solid phase extraction technique. The sample extraction protocol is mentioned below:

Extraction protocol

- Conditioning and equilibration- 1mL methanol followed by 1 mL water
- Sample loading
- Wash 1 - 1.000 mL wash solution 1
- Wash 2 - 1.000 mL wash solution 2
- Dry the cartridges for few seconds.
- Elution - 0.500 mL of eluting solution.
- SPE eluent was collected into prelabelled RIA vials and evaporate for 15 minutes under 40 ℃ at 15 psi Pressure (103.4 kPa)
- Reconstitute the samples with 0.100 mL of reconstitution solution and vortexed to mix
- Transfer the solution into the prelabelled HPLC vials for analysis.

3.2. Instrument parameters on LCMS-8060NX

Refer to Table 2 for analytical conditions and instrument parameters. Refer to Table 3 for MRM transition.

Table 2 Analytical conditions and instrument parameters

Parameter	**HPLC**
Column	Shim-pack Velox™ C18 100 × 2.1 mm, 2.7 µm (P/N: 227-32015-03)
Mobile Phase	Methanol : 0.01% formic acid in 5 mM Ammonium Acetate (90 :10)
Flow Rate	0.5 mL/min
Oven Temp	50 ℃
Injection	50 µL
Parameter	**MS**
Interface	ESI
Interface temp and Voltage	5 kV and 400 ℃
MS Mode	MRM, Positive
Heat Block Temp	400 ℃
DL Temp	300 ℃
CID Gas	270 kPa
Nebulizing Gas	3 L/min
Drying Gas	10 L/min
Heating Gas	10 L/min

Table 3 MRM transition and parameters of Mometasone on LCMS

Compound	**MRM (*m/z*)**	**CE (V)**
Mometasone	520.90-355.15	-10.0

Fig. 2 Nexera™ X2 with LCMS-8060NX system

4. Result and Discussion

4.1. Method Development

In this study, mass spectrometric (MS) analyses, focusing on both precursor ion and product ion spectra, were conducted for mometasone in positive ion mode. The precursor ion spectra for mometasone revealed prominent signal corresponding to the protonated molecule ($[M+H]^+$) at *m/z* 520.9. Subsequently, product ion spectra for mometasone exhibited fragment ions at *m/z* 355.15, 373.2 and 263.2. To optimize sensitivity and selectivity, multiple reaction monitoring (MRM) transitions were established at *m/z* 520.9 > 355.15. This detailed mass spectrometric analysis provides valuable insights into the identification and quantification of mometasone, laying the foundation for precise analytical methodologies. The established MRM transitions contribute to the development of a sensitive and selective assay for the determination of mometasone in complex biological matrices.

Optimization of chromatographic conditions, including column type, mobile phase composition, and nature, was conducted through multiple trials to enhance the retention and signal of mometasone. Various combinations of methanol and acetonitrile with buffers such as ammonium acetate, ammonium formate, and acid additives like formic acid and acetic acid were explored. The optimal mobile phase, identified as methanol : 0.01% formic acid ammonium acetate (90 : 10), was selected for its superior retention, sensitivity as well as peak shape.

A simple sample preparation technique was employed to isolate mometasone from other components in the sample matrix. Evaluation of different extraction methods revealed that SPE yielded the best overall results, demonstrating acceptable precision and accuracy (within 20%) at the lower limit of quantitation (LLOQ) without significant interference in human plasma blank samples (n = 6 for each species).

Representative chromatograms of an extracted human blank plasma, along with an extracted LLOQ standard in human plasma, are depicted in Fig. 3.

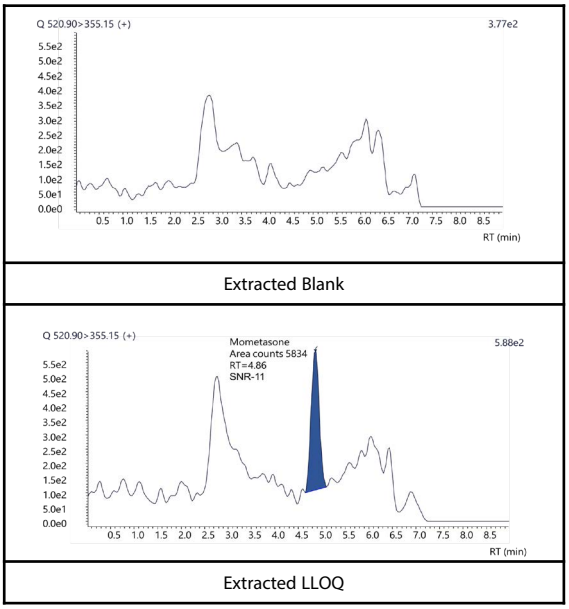

Fig. 3 Chromatograms of Mometasone (Extracted Blank and LLOQ)

4.2. Method Validation

- **Selectivity**

  Six blank plasma samples from different donors were analyzed. Fig. 3 presents representative chromatograms of blank plasma and plasma spiked with mometasone furoate at LLOQ. The method exhibited high selectivity for mometasone furoate and the IS, with no noteworthy interference from endogenous compounds during the retention times of the analyte and IS in blank plasma samples. Results are presented in Table 4.

Table 4 Selectivity

Plasma lot no.	Mometasone		
	Blank Plasma	LLOQ area	% Interference
V1102	0	5,146	0.00
V8245	0	6,589	0.00
V6132	0	5,412	0.00
V11886	0	5,834	0.00
V11782	0	7,456	0.00
V11911	0	6,718	0.00

- **Sensitivity and Calibration Curve:**

  Mometasone furoate calibration curves displayed linearity within the range of 0.50 to 20 pg/mL, with a regression coefficient exceeding 0.99 (refer to Fig. 4). A typical standard curve was described by y=7658.820x + 652.1041. Residuals demonstrated consistent behaviour across concentrations. The achieved Lower Limit of Quantification (LLOQ) was 0.50 pg/mL, exhibiting precision of 15.03% and accuracy of 105.68% in terms of %RSD and accuracy, respectively.

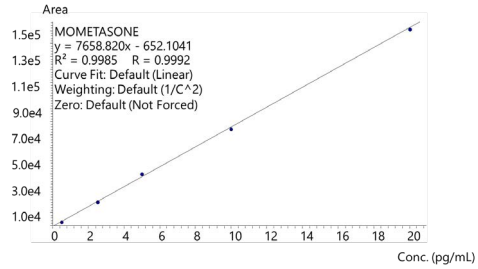

Fig. 4 Calibration curve of mometasone

- **Accuracy and precision**

  Accuracy and precision were assessed by analyzing six replicates of Quality Controls (QCs) at four levels on three separate days. Table 5 and Table 6 displays the results. Intra and Inter-day biases for mometasone furoate were within ± 20% at LLOQ level and ± 15% at LQC, MQC and HQC each level respectively. %RSD for intra and Inter-day samples were below 20% at LLOQ level and below 15% at LQC, MQC and HQC each level for mometasone furoate, indicating consistent outcomes and confirming the accuracy and reliability of the assay

Table 5 Intra-day precision and accuracy

Intra-day (n=6)			
Nominal Conc (pg/mL)	Observed Conc (pg/mL)	Accuracy (%)	Precision (% RSD)
LLOQ QC (0.50 pg/mL)	0.53	105.68	15.03
LQC (1.00 pg/mL)	1.01	100.72	6.83
MQC (5.00 pg/mL)	4.87	97.48	9.67
HQC (10.00 pg/mL)	9.03	90.31	14.8

Table 6 Global precision and accuracy

Inter-day (n=18)			
Nominal Conc (pg/mL)	Observed Conc (pg/mL)	Accuracy (%)	Precision (% RSD)
LLOQ QC (0.50 pg/mL)	0.55	109.59	16.88
LQC (1.00 pg/mL)	1.04	103.63	12.2
MQC (5.00 pg/mL)	4.87	97.47	7.95
HQC (10.00 pg/mL)	9.12	91.19	11.23

- **Recovery and Matrix Effect**

  Extraction recovery was assessed across three QC levels, with mometasone furoate recovery ranging from 44.82% to 58.36%. The %RSD remained under 15%. Results in Table 7 indicates the global recovery 50.02% for mometasone. Recovery was found precise, consistent and reproducible at all QC levels.

  Matrix effect was evaluated by comparing peak area ratios with and without the matrix at two QC levels. Results in Table 8 indicated mean matrix factors of 0.87 at LQC and 0.97 at HQC for mometasone furoate, with %RSD values below 8.42% at LQC and 3.86% at HQC. Hence, extraction recovery and matrix effect were satisfactory across all mometasone furoate QC levels.

Table 7 Recovery

QC level	Recovery
LQC (n=6)	58.36
MQC (n=6)	46.87
HQC (n=6)	44.82
Mean	**50.02**
SD	**7.30**
% RSD	**14.59**

Table 8 Matrix effect

Mometasone	Aqueous sample	Post extracted sample	Matrix factor
LQC	9,515	9,214	0.97
	11,147	9,011	0.81
	10,674	8,479	0.79
	10,469	8,419	0.80
	11,976	10,880	0.91
	9,814	8,942	0.91
Mean			**0.87**
SD			**0.07**
% RSD			**8.42**

Mometasone	Aqueous sample	Post extracted sample	Matrix factor
HQC	1,01,662	98,021	0.96
	97,610	93,510	0.96
	1,01,568	99,440	0.98
	98,620	1,01,982	1.03
	1,00,257	93,501	0.93
	1,04,280	97,457	0.93
Mean			**0.97**
SD			**0.04**
% RSD			**3.86**

- **Carry-over**

  Carryover was evaluated by injecting extracted samples in the sequence of extracted blank, extracted highest calibrator, extracted blank and extracted lowest calibrator. No carryover was observed at the retention time and MRM transition of mometasone in the extracted blank sample following the highest standard calibrator.

5. Conclusion

We have successfully developed an analytical approach to quantitate mometasone furoate in human plasma, demonstrating significant peak resolution enhancement. To our knowledge, this represents the initial LC-MS/MS technique for quantifying mometasone furoate in human plasma, achieving an LLOQ of 0.25 pg/mL. Sample preparation utilized SPE, effectively eliminating matrix interferences and enhancing extraction recovery. The method underwent partial validation following regulatory guidelines, confirming its robustness and compliance. This sensitive and selective approach can be applied to a bioequivalence study involving mometasone furoate aqueous nasal sprays in healthy volunteers.

6. References

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2. Daley-Yates, PT (2015) Inhaled Corticosteroids: Potency, Dose Equivalence and Therapeutic Index. Br J Clin Pharmacol, 80: 372–380. doi: 10.1111/bcp.12637.

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