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Liquid Chromatograph Mass Spectrometer LCMS-8060

Application News

Rapid, Highly Sensitive and Direct Quantification of Fluticasone Propionate at Sub-pg/mL in Plasma Using LCMS-8060

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User Benefits

- Rapid, simple and sensitive method with LLOQ of 0.2 pg/mL
- Single step sample extraction method increased sample productivity

1. Introduction

Fluticasone propionate, a medium-potency synthetic corticosteroid is administered as nasal spray or drops for the treatment of allergic and non-allergic rhinitis; or by oral inhalation for the treatment of asthma. Therapeutic dose of fluticasone propionate results in very low plasma concentrations and requires a sensitive bioanalytical method for accurate quantification of the drug in plasma.
Shimadzu Application Development Centre (ADC), Navi Mumbai has developed and validated the most sensitive method with lowest limit of quantification (LLOQ) of 0.2 pg/mL. The method has used a single step sample extraction technique and direction injection approach to eliminate environmental contamination. These factors enhance productivity of the pharmacokinetic investigation involving high-throughput analysis.

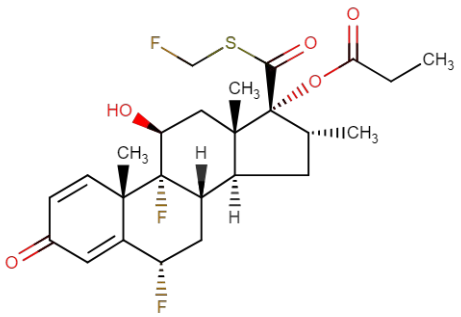

Fig. 1 Structure of Fluticasone Propionate

2. Salient Features

- A validated quantitative method was developed for the determination of fluticasone propionate in human plasma, in accordance with major US guidelines [1]. The results are presented in Table 1.
- The analytical throughput was enhanced using a rapid single-step extraction procedure and the ultra-fast technologies of the LCMS-8060 system.
- Optimization of the ionization and ion guide technologies led to improved ion production and transmission, enabling sensitive and selective quantification of fluticasone propionate down to 0.2 pg/mL.
- The plasma sample volume was carefully optimized to minimize wastage and extend the lifespan of the mass spectrometer.
- The chromatographic method was optimized to eliminate endogenous interference and reduce background noise for both the analyte and its deuterated internal standard.
- Method was partially validated as per US major guidelines for
  - ✓ Selectivity
  - ✓ Linearity
  - ✓ Inter-day and intra-day precision and accuracy (PA)
  - ✓ Recovery
  - ✓ Matrix effect
  - ✓ Stability studies (except LT stability)

Table 1 Method Validation Summary

Calibration curve range	0.20 pg/mL to 120.00 pg/mL		
Intraday precision and accuracy (For LLOQ QC)	Accuracy (% Nominal)	100.00	
	Precision (% RSD)	16.33	
Intraday precision and accuracy (For LQC, MQC and HQC)	Accuracy (% Nominal)	100.24 to 107.89	
	Precision (% RSD)	4.98 to 7.41	
Global precision and accuracy (For LLOQ QC)	Accuracy (% Nominal)	102.50	
	Precision (% RSD)	18.33	
Global precision and accuracy (For LQC, MQC and HQC)	Accuracy (% Nominal)	102.17 to 108.34	
	Precision (% RSD)	7.73 to 11.14	
Global % recovery	Recovery (%)	64.29	
	Precision (% RSD)	7.26	
Matrix effect	LQC	1.06	
	HQC	0.95	
Bench top stability in matrix (6.0 hrs.)	% Change	LQC=-4.92	HQC=0.81
	Precision (% RSD)	LQC=6.13	HQC=3.05
	Accuracy (% Nominal)	LQC=96.85	HQC=106.11
Auto sampler stability in matrix (30.0 hrs.)	% Change	LQC= -3.12	HQC=-1.63
	Precision (% RSD)	LQC=12.83	HQC=5.07
	Accuracy (% Nominal)	LQC=100.32	HQC=110.57
Freeze thaw stability in matrix (Third Cycle)	% Change	LQC=-4.8	HQC=6.12
	Precision (% RSD)	LQC=8.34	HQC=7.33
	Accuracy (% Nominal)	LQC=96.98	HQC=111.70

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

3. Experimental

3.1. Sample preparation and analytical conditions

Fluticasone propionate calibration standards and quality control samples were prepared in K2EDTA human blank plasma. Calibration curve ranged from 0.20 pg/mL to 120.00 pg/mL (refer to Fig. 4) and the quality control samples were prepared at LLOQ QC (0.22 pg/mL), LQC (1.68 pg/mL), MQC (26.28 pg/mL) and HQC (105.12 pg/mL). To a 500 µL aliquot of human plasma, 50 µL 650.00 pg/mL fluticasone propionate – D5 and 400 µL of LC-MS grade water was added followed by SPE purification. Samples were loaded on the preconditioned reversed-phase SPE cartridge. The loaded samples were washed with 20% acetonitrile in water and eluted in 0.3 mL of 50% methanol in water. The eluent was directly injected on LC-MS/MS for analysis.

3.2. Instrument parameters on LCMS-8060

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

Table 2 Analytical conditions and instrument parameters

Parameter	HPLC
Column	Shim-pack™ GIST C18, 3um, 2.10x50 (P/N: 227-30008-03)
Mobile Phase	Pump A-0.1% Ammonia in Water Pump B-Acetonitrile
Flow Rate	0.5 mL/min
Oven Temp	50 °C
Injection volume	50 µL
Parameter	**MS**
Interface	ESI
Interface Voltage and temp	5 kV and 300 ˚C
MS Mode	MRM, Positive
Heat Block Temp	400 ˚C
DL Temp	150 ˚C
CID Gas	270 kPa
Nebulizing Gas	2 L/min
Drying Gas	10 L/min
Heating Gas	10 L/min

Table 3 MRM transition and parameters of Fluticasone propionate on LC-MS/MS

Compound	Precursor (*m/z*)	Product (*m/z*)	CE (V)
Fluticasone Propionate	501.00	293.05	-20.0
Fluticasone propionate-D5	506.00	313.10	-20.0

Fig. 2 Nexera™ X2 with LCMS-8060 system

4. Result and Discussion

4.1. Method Development

The initial experiments involved optimizing fluticasone propionate in both positive and negative ion ESI modes. In the positive ion mode, the [M+H]+ ion of fluticasone propionate at *m/z* 501.0 produced prominent product ions at *m/z* 293.05 with improved signal-to-noise ratio. The negative ion mode showed low signal-to-noise ratio of the product ion peak.

The internal standard (I.S.) displayed a molecular ion at *m/z* 506.0. The precursor ions (*m/z* 501.0 and 506.0) were introduced into the collision cell to generate the product ion spectrum. Both fluticasone propionate and fluticasone propionate-D5 exhibited a fragmentation pattern, with the base peak of the product ion spectrum observed at *m/z* 293.05 and 313.1. The highest abundance of the daughter ion was achieved with a collision energy of -20 eV. The transitions selected for monitoring fluticasone propionate and fluticasone propionate-D5 were *m/z* 501.0→293.05 and 506.0→313.1, respectively.

To achieve the ultra-low detection limit of fluticasone propionate in human plasma, various HPLC columns and gradient programs were tested to optimize the response factor. Initial conditions were based on a prior LC-MS/MS method with an LLOQ of 1 pg/ml. Five different types of HPLC columns were evaluated during method development. However, Shim-pack GIST C18 column showed a higher response factor, improved sensitivity and better resolution. Analyte was eluted from the column under gradient flow.

The SPE process optimization focused on achieving maximum efficiency and recovery. Key steps included preconditioning the cartridge and washing with water. The concentration of organic solvents for washing and elution was adjusted, while testing various volumes and reconstitution solvents for optimal conditions. The ideal organic solvent percentages where 20% acetonitrile in water was used as a wash solution and 50% methanol was used as an eluent. The eluent was directly injected on LC-MS/MS for analysis.

4.2. Method Validation

In the initial phase of the study, a pre-validation evaluation of the method and instrument performance was conducted. Consistently high correlation coefficient (r2> 0.9900) were observed demonstrating reliable method performance across the calibration range. The intra-day and inter-run batch precision and accuracy data for quality control levels are summarized in Table 5 and Table 6.

The data demonstrate that the LC-MS/MS method is consistent and reliable, exhibiting good accuracy (100% ±20%) and precision (<20%) at lower limit of quantitation (LLOQ). At low (LQC), middle (MQC) and high-quality control (HQC) levels, the accuracy (100 ±15%) and precision (<15%) were within the acceptable range, meeting validation criteria. This MRM method, with a lower limit of quantitation (LLOQ) of 0.2 pg/ml and signal to noise ratio of > 15, offers substantially greater sensitivity than the previously reported methods in the literature, which had an LLOQ of 1 pg/ml [2] . MRM analysis, therefore, has not only provided additional selectivity by monitoring the fragment ion specific to fluticasone propionate, but has also provided with increased sensitivity and turnover as shown in this report. The presented method is slightly more sensitive than the one reported [2] . The presented method using ESI ionization in the positive ion mode provides an alternative for quantifying fluticasone propionate using instruments that are susceptible to sodium adduct interference. The results of partial method validation are discussed below.

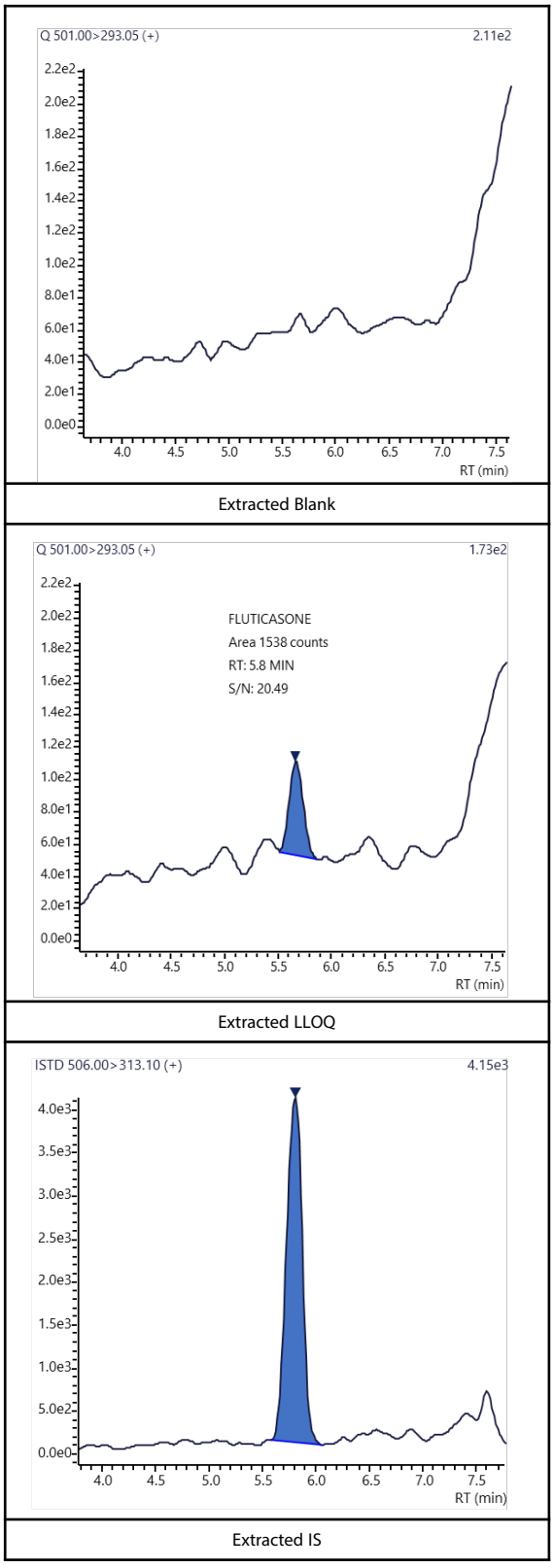

Fig. 3 Chromatograms of Fluticasone propionate
(Ext Blank, Extracted LLOQ and Extracted IS)

- **Selectivity**

Selectivity of this method was assessed in different lots of plasma. Interference from blank matrix was assessed for both fluticasone propionate and fluticasone propionate -D5 (refer to Fig. 3). Percentage interference was found to be less than 10.00 % of LLOQ area response for fluticasone propionate, as shown in Table 4 below.

Table 4 Selectivity

Plasma lot no.	Fluticasone propionate		
	Blank Plasma	LLOQ area	% Interference
P4458	46	493	9.33
P4791	0	689	0
P5515	0	627	0
P5517	68	798	8.52
P5518	0	571	0

Plasma lot no.	Fluticasone propionate-D5		
	Blank Plasma	LLOQ area	% Interference
P4458	122	66,851	0.18
P4791	220	70,195	0.31
P5515	153	51,592	0.3
P5517	143	53,529	0.27
P5518	374	59,237	0.63

- **Linearity**

The calibration curve for fluticasone propionate demonstrated linearity over a range of 0.2-120.0 pg/mL, with a regression coefficient (r2) exceeding 0.99. A representative standard curve was expressed as y=0.1641828x+0.01882287, using a weighted factor of 1/x2. Residuals showed no variation across concentrations, confirming consistent linearity, as depicted in Fig 4.

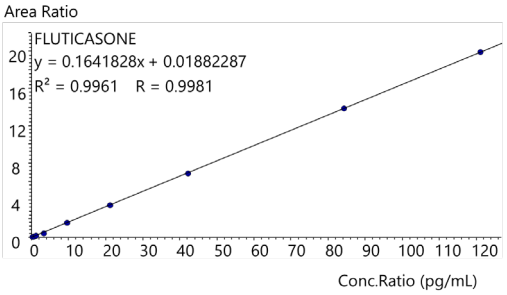

Fig. 4 Calibration curve

- **Intra-day and inter-run precision and accuracy**

Intra-day and inter-run precision and accuracy were assessed using six replicates of QC samples at four concentration levels over three days. Results, detailed in Tables 5 and 6, revealed mean RSD values below 15% for precision and accuracy values between 85% - 115% at LQC, MQC and HQC. At LLOQ QC, RSD values found below 20% for precision and accuracy values between 80% - 120%.

Table 5 Intra-day precision and accuracy

Intra-day (n=6)			
Nominal Conc (pg/mL)	Observed Conc (pg/mL)	Accuracy (%)	Precision (% RSD)
LLOQ QC (0.220 pg/mL)	0.2200	100.00	16.33
LQC (1.682 pg/mL)	1.6860	100.24	6.60
MQC (26.280 pg/mL)	28.3547	107.89	7.41
HQC (105.120 pg/mL)	107.7737	102.52	4.98

Table 6 Inter-run precision and accuracy

Inter-run (n=18)			
Nominal Conc (pg/mL)	Observed Conc (pg/mL)	Accuracy (%)	Precision (% RSD)
LLOQ QC (0.220 pg/mL)	0.2255	102.50	18.33
LQC (1.682 pg/mL)	1.7184	102.17	7.73
MQC (26.280 pg/mL)	28.4715	108.34	10.51
HQC (105.120 pg/mL)	112.3098	106.84	11.14

- **Recovery**

The recovery of fluticasone was determined by comparing the peak area of spiked extracted samples with those of spiked post-extracted samples at three QC levels (LQC, MQC and HQC). The mean recovery values for fluticasone ranged between 60.61% to 69.54%, demonstrating consistent and reproducible extraction efficiency, refer Table 7. The results indicate that the sample preparation method is efficient and the precision of the recovery values across all levels confirms the reliability of the method for routine bioanalysis.

Table 7 Statistics of Recovery

QC level	Recovery
LQC (n=6)	69.54
MQC (n=6)	60.61
HQC (n=6)	62.70
Mean	**64.29**
SD	**4.67**
% RSD	**7.26**

- **Matrix effect**

Matrix effect was studied for both fluticasone propionate and fluticasone propionate-D5 using LQC and HQC samples. Matrix factor was found to be 1.06 and 0.95 respectively at LQC and HQC level samples. Representative data of matrix effect is shown in Table 8. The results confirm the suitability of method for quantitative estimation of fluticasone propionate in human plasma.

Table 8 Matrix factor

Fluticasone Propionate	Aqueous standard	Post extracted sample	Matrix factor
LQC	4792	5550	1.16
	5320	5709	1.07
	4789	5331	1.11
	5450	5389	0.99
	5333	5522	1.04
	5292	5157	0.97
Mean			**1.06**
SD			**0.07**
% RSD			**6.77**

Fluticasone Propionate	Aqueous standard	Post extracted sample	Matrix factor
HQC	365280	348228	0.95
	352894	337526	0.96
	348430	350845	1.01
	362809	342762	0.94
	364014	326980	0.90
	357803	336132	0.94
Mean			**0.95**
SD			**0.03**
% RSD			**3.68**

- **Carry-over effect**

Carry over was evaluated by injecting extracted samples in the sequence of extracted blank, extracted highest calibrator, extracted blank and extracted lowest calibrator. No carry over was present/observed at the retention time and MRM transition of the analyte in the extracted blank sample following the highest standard calibrator.

5. Conclusion

LCMS-8060, along with special sample preparation provides best in class sensitivity for demanding bioanalytical assay of fluticasone propionate. The criticality of these assays is huge, both with respect to technical challenges and commercial impact for pharmaceutical organizations. By providing these ready to use solutions, we partner with your labs to achieve desired results in your scientific endeavors.

6. References

1. Food and Drug Administration, Bioanalytical Method Validation Guidance for Industry, 2018. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/bioanalytical-method-validation-guidance-industry (accessed Nov 27, 2024).
2. Ji AJ, Zhou D, Zhang S, Cawley MA, Fang X, Wu J. Ultrasensitive and automated 1 pg/ml fluticasone propionate assay in human plasma using LC-MS/MS. Bioanalysis. 2013 Feb;5(4):423-35. doi: 10.4155/bio.12.338. PMID: 23414376.

06-SAIP-LC-069-EN First Edition: Dec. 2024

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