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Low level quantitation of Fluticasone and Salmeterol from plasma using LC/MS/MS

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Introduction

The combination drug of fluticasone/ salmeterol contains fluticasone propionate and salmeterol. Fluticasone, a corticosteroid, is the anti-inflammatory component of the combination, while salmeterol treats constriction of the airways. Together, they relieve the symptoms of coughing, wheezing and shortness of breath related to asthma and Chronic Obstructive Pulmonary Disorder (COPD)[1].

LC/MS/MS has been increasingly employed in pharmacokinetic studies due to its specificity and sensitivity. This also allows the development of assays with minimal sample preparation.

LC/MS/MS method has been developed for ultra-trace level quantitation of these molecules from plasma using LCMS-8050, a triple quadrupole mass spectrometer from Shimadzu Corporation, Japan. Ultra high sensitivity of LCMS-8050 with heated ESI source enabled development of a low level quantitation method for both these molecules with good repeatability even in presence of complex matrix like plasma.

Fluticasone

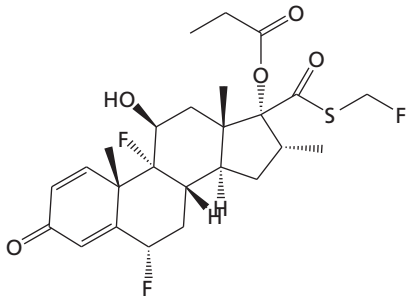

Figure 1. Structure of fluticasone

Fluticasone propionate is a corticosteroid having the chemical name S-(fluoromethyl) 6α,9-difluoro-11 β,17-dihydroxy-16α-methyl-3oxoandrosta-1,4-diene-17 β-carbothioate, 17-propionate and has a molecular formula as $C_{25}H_{31}F_3O_5S$. It's structure is shown in Figure 1.

Salmeterol

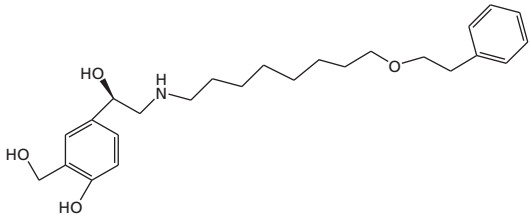

Figure 2. Structure of salmeterol

Salmeterol is a long-acting β2-adrenergic receptor agonist having the chemical name 2-(Hydroxymethyl)-4-[(1R)-1-hydroxy-2-{[6-(4-phenylbutoxy)hexyl]amino}ethyl]phenol and has a molecular formula as $C_{25}H_{37}NO_4$. It's structure is shown in Figure 2.

Method of analysis

Sample preparation

- **Preparation of aqueous calibration levels**

Fluticasone and salmeterol mix standards at concentration levels of 0.5 pg/mL, 1 pg/mL, 2 pg/mL, 5 pg/mL, 20 pg/mL, 50 pg/mL, 100 pg/mL and 200 pg/mL were prepared in water : acetonitrile (1:1 v/v).

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- **Preparation of matrix matched calibration levels**

1000 µL of acetonitrile was added to 1000 µL of plasma. It was then centrifuged at 7000 rpm for 20 minutes. Supernatant was passed through preconditioned C18 SPE cartridge (Orochem 200 mg, 3 cc). Supernatant was eluted with 3 mL of mixture of ethyl acetate : heptane (35:65 v/v)[2]. The residue was evaporated using nitrogen evaporator and reconstituted in 1000 µL water : acetonitrile (1:1 v/v). This solution was then used as a diluent to prepare matrix matched calibration levels from 0.5 pg/mL to 200 pg/mL.

Figure 3. LCMS-8050 triple quadrupole mass spectrometer by Shimadzu

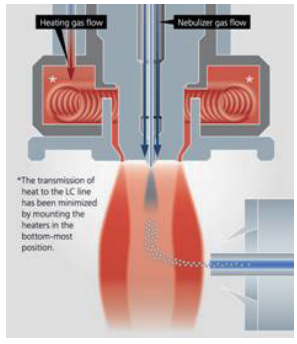

Figure 4. Heated ESI probe

LCMS-8050 triple quadrupole mass spectrometer by Shimadzu (shown in Figure 3), sets a new benchmark in triple quadrupole technology with an unsurpassed sensitivity (UFsensitivity), ultra fast scanning speed of 30,000 u/sec (UFscanning) and polarity switching speed of 5 msec (UFswitching). This system ensures highest quality of data, with very high degree of reliability.

In order to improve ionization efficiency, the newly developed heated ESI probe (shown in Figure 4) combines high-temperature gas with the nebulizer spray, assisting in the desolvation of large droplets and enhancing ionization. This development allows high-sensitivity analysis of a wide range of target compounds with considerable reduction in background.

LC/MS/MS analysis

Fluticasone and salmeterol were simultaneously analyzed using Ultra High Performance Liquid Chromatography (UHPLC) Nexera coupled with LCMS-8050 triple quadrupole system (Shimadzu Corporation, Japan). The details of analytical conditions are given in Table 1.

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Table 1. LC/MS/MS conditions for fluticasone and salmeterol

Column	: Shim-pack XR-ODS (50 mm L x 3 mm I.D.; 2.2 µm)
Guard column	: Phenomenex SecurityGuard ULTRA cartridge
Mobile phase	: A: 10 mM ammonium formate in water B: acetonitrile
Gradient program (B %)	: 0–1 min → 50 (%); 1–3.5 min → 50-90 (%); 3.5–5.0 min → 90(%); 5.0–5.5 min → 90-50 (%); 5.5–7.5 min → 50 (%)
Flow rate	: 0.3 mL/min
Oven temperature	: 40 °C
Injection volume	: 50 µL
MS interface	: Electro Spray Ionization (ESI)
Nitrogen gas flow	: Nebulizing gas 3 L/min; Drying gas 15 L/min
Zero air flow	: Heating gas 18 L/min
MS temperature	: Desolvation line 200 °C; Heating block 500 °C Interface 300 °C

Results

LC/MS/MS analysis results of fluticasone and salmeterol

LC/MS/MS method was developed for simultaneous quantitation of fluticasone and salmeterol. Analysis was performed using aqueous as well as matrix matched standards. MRM transitions used for these compounds are given in Table 2. Linearity studies were carried out using external standard calibration method and results of linearity studies are tabulated in Table 2 for both aqueous and matrix matched standards.

Overlay of MRM chromatograms of diluent, 2 pg/mL and 200 pg/mL level for fluticasone aqueous standards is shown in Figure 5. Overlay of MRM chromatograms of blank plasma, 5 pg/mL and 200 pg/mL level for fluticasone matrix matched standards is shown in Figure 6. Similarly, overlay of MRM chromatograms of diluent, 0.5 pg/mL and 200 pg/mL for salmeterol aqueous standards is shown in Figure 7. Overlay of MRM chromatograms of blank plasma, 1 pg/mL and 200 pg/mL for salmeterol matrix matched standards is shown in Figure 8. No interfering peaks were seen in diluent or in blank plasma at the retention time of these compounds, confirms the absence of any interference.

LOQ was determined for these compounds based on the following criteria – **(1)** % RSD for area < 20 %, **(2)** % accuracy between 80-120 % and **(3)** Signal to noise ratio (S/N) > 10. LOQ of 2 pg/mL and 5 pg/mL was achieved for fluticasone aqueous and matrix matched standards respectively. Similarly, LOQ of 0.5 pg/mL and 1 pg/mL was achieved for salmeterol aqueous and matrix matched standards respectively. Accuracy and repeatability results for fluticasone and salmeterol are given in Tables 3 and 4 respectively.

Table 2. Details of MRM transitions and linearity results

Name of the compound	MRM transitions	Retention time (min)	Linearity (r^2)	
			Aqueous	Matrix matched
Fluticasone	TIC (501.00 > 293.05 + 501.00 > 313.10)	3.64	0.9964	0.9992
Salmeterol	416.00 > 232.20	2.44	0.9997	0.9988

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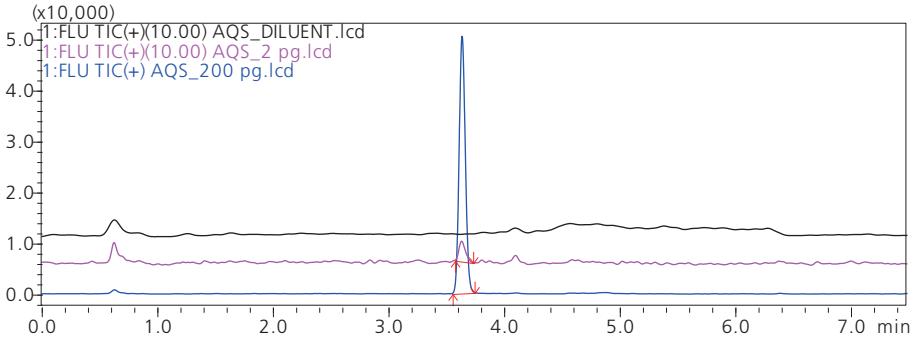

Figure 5. Overlay of MRM chromatograms of diluent, 2 pg/mL and 200 pg/mL for fluticasone aqueous standard

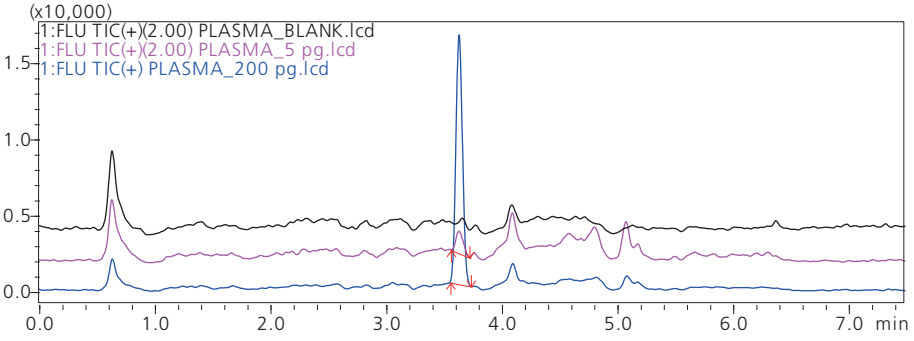

Figure 6. Overlay of MRM chromatograms of blank plasma, 5 pg/mL and 200 pg/mL for fluticasone matrix matched standard

Table 3. Results of accuracy and repeatability for fluticasone

Name of compound	Standard concentration (pg/mL)	Calculated average concentration from calibration graph (pg/mL) (n=3)		Average % accuracy (n=3)		Average % RSD for area counts (n=3)	
		Aqueous	Matrix matched	Aqueous	Matrix matched	Aqueous	Matrix matched
Fluticasone	2	2.34	NA	117.23	NA	13.42	NA
	5	4.83	5.44	96.63	108.73	1.70	12.74
	20	17.55	19.57	87.73	97.83	6.43	7.44
	50	47.08	48.72	94.17	97.43	2.56	0.56
	100	96.70	97.56	96.70	97.53	3.84	7.35
	200	209.69	203.91	104.83	101.97	1.36	0.53

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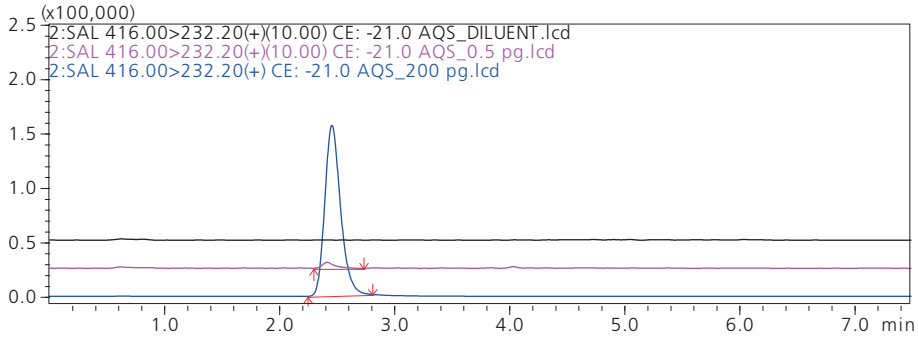

Figure 7. Overlay of MRM chromatograms of diluent, 0.5 pg/mL and 200 pg/mL for salmeterol aqueous standard

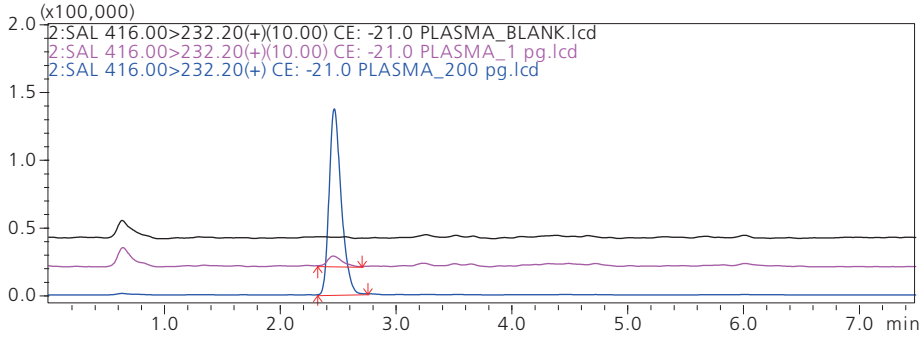

Figure 8. Overlay of MRM chromatograms of blank plasma, 1 pg/mL and 200 pg/mL for salmeterol matrix matched standard

Table 4. Results of accuracy and repeatability for salmeterol

Name of compound	Standard concentration (pg/mL)	Calculated average concentration from calibration graph (pg/mL) (n=3)		Average % accuracy (n=3)		Average % RSD for area counts (n=3)	
		Aqueous	Matrix matched	Aqueous	Matrix matched	Aqueous	Matrix matched
Salmeterol	0.5	0.55	NA	109.47	NA	10.15	NA
	1	0.94	1.01	94.33	100.67	6.99	8.99
	2	1.94	2.00	96.80	100.13	7.54	3.42
	5	5.10	5.11	101.90	102.27	1.25	1.43
	20	19.59	19.95	97.97	99.80	0.79	0.81
	50	51.17	50.02	102.37	100.03	0.71	0.28
	100	97.79	95.00	97.80	95.00	0.82	7.92
	200	201.53	205.31	100.77	102.63	0.75	0.40

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Conclusion

- LOQ of 2 pg/mL and 5 pg/mL was achieved for fluticasone aqueous and matrix matched standards respectively whereas it was 0.5 pg/mL and 1 pg/mL for salmeterol aqueous and matrix matched standards respectively.
- Heated ESI probe of LCMS-8050 system enables drastic augment in sensitivity with considerable reduction in background. Hence, LCMS-8050 system from Shimadzu gives a complete solution for bioanalysis.

References

[1] R. R. Shah et al., International Journal of PharmTech Research, Volume 3, Number 3, (2011), 1801-1806.
[2] Sriram Krishnaswami et al., Journal of Pharmaceutical and Biomedical Analysis, Volume 22, (2000), 123-129.

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