Ultra-sensitive quantification of the low-level inhalant drugs fluticasone propionate and salmeterol xinafoate in human plasma

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ABSTRACT

Fluticasone propionate is a synthetic glucocorticoid and salemetrol xinafoate is a selective LABA, which cause bronchodilation and inhibition of the release of hypersensitivity mediators from mast cells. Fluticasone propionate and salmeterol xinafoate, which are frequently co-administered drugs, were anlyzed in human plasma on the SCIEX 7500 system after preparation using a protein precipitation method followed by solid phase extraction. The developed simultaneous quantification method was able to reach a lower limit of quantification of 0.050 pg/mL for fluticasone propionate and salmeterol xinafoate using 400 µL of human plasma .An 8min LC gradient was used in this method to ensure separation of endogenous interferences from the peaks of intertest for fluticasone propionate and salmeterol xinafoate. Sample was concentrated 4x after solid phase extraction cleanup and 10 µL of final sample was injected for LC-MS/MS analysis.

INTRODUCTION

Fluticasone propionate is a synthetic trifluorinated glucocorticoid with potent and generalized anti-inflammatory activity. It is currently one of the most effective drugs used in the treatment of asthma. Salmeterol xinafoate is a long-acting β-adrenergic receptor antagonist with very high selectivity for the β_2-receptor subtype, which produces airway smooth muscle relaxation and consequently bronchodilation.[1] For patients with persistent asthma, inhaled corticosteroids (ICS) have been the first-line treatment regardless of disease severity. Considering the guidelines, asthma sufferers whose condition is not sufficiently controlled with ICS alone are recommended to include a long-acting β2-agonist (LABA) in their treatment. Salmeterol is a selective LABA, which causes bronchodilation and inhibition of the release of hypersensitivity mediators from mast cells. The corticosteroid fluticasone propionate inhibits eosinophil activation and the subsequent release of inflammatory mediators.[2]

The lowest daily pediatric doses of fluticasone propionate and salmeterol xinafoate, for children 12 years and older are 90 micrograms and 42 micrograms, respectively, for the aerosol and 100 micrograms and 50 micrograms, respectively, as an inhalation powder.[5] This leads to very low circulating levels of plasma concentrations of these drugs in human blood. Fluticasone propionate concentrations in plasma following administration of fluticasone propionate in the form of an aqueous nasal spray are 10.8 pg/mL to 14.1 pg/mL.[5,7]

Therefore, it is very important to have an analytical method with ultra-low detection limits for both analytes in human plasma to understand pharmacokinetics. Here, using a smaller sample volume[8] than was previous possible, an LC-MS/MS method has been developed for the ultra-low-level quantification of inhalants in human plasma using the SCIEX 7500 system with fluticasone propionate and salmeterol xinafoate as model compounds.

MATERIALS AND METHODS

Sample preparation: Fluticasone propionate and salmeterol xinafoate were spiked in 400 µL of human plasma aliquots in the range of 0.050 to 50.000 pg/mL, with 5 pg of internal standard. Samples were extracted using protein precipitation with 500 µL of 0.2 M zinc sulfate, samples were vortexed and centrifuged at 6000 rpm for 5 minutes followed by SPE using supernatant liquid with phenomenex C18-SPE cartridges. Cartridges were conditioned using 1 mL of methanol followed by 1 mL of water. The sample was loaded onto the cartridge and followed by a series of washes: water, then 25% methanol in water. Analytes were eluted using acetonitrile and dried under a nitrogen stream at 40°C. Samples were reconstituted with 100µL of 60% mobile phase A and 40% mobile phase B, then analyzed using LC-MS/MS.[3]

HPLC conditions: Samples were analyzed using the ExionLC AD system at a flow rate of 0.3 mL/min using Phenomenex Kinetex 1.7 µm EVO C18 100 Å, LC column 100 x 2.1 mm with an 8-minute gradient (Table 1). 10 µL injection volume was used for analysis.

MS/MS conditions: Samples were analyzed using the SCIEX 7500 system equipped with the OptiFlow Pro ion source, and the system was controlled by SCIEX OS software. The optimized MS parameters are listed in Table 2.

Table 1. Chromatographic gradient.

Time (min)	Flow (mL/min)	%B Conc
1.00	0.300	10.0
4.00	0.300	70.0
5.50	0.300	70.0
5.60	0.300	95.0
6.90	0.300	95.0
7.00	0.300	10.0
8.00	0.300	10.0
Mobile phase A: 0.5 mM ammonium trifluoroacetate in water **Mobile phase B: acetonitrile**		

Table 2. Optimized MS parameters.

Name	Q1/Q3	Q0D	CE	CXP
Fluticasone propionate-1	501.2/293.2	40	24	10
*Fluticasone propionate-2	501.2/313.2	40	24	10
Salmeterol xinafoate	416.4/232.1	40	34	10
Fluticasone propionate d5	506.4/293.3	40	24	10
Salmeterol d3	509.4/235.1	40	34	10
Source parameters	**Value**	**Source parameters**		**Value**
Curtain gas	50	CAD gas		12
Ion source gas 1	50	Ion spray voltage		3000
Ion source gas 2	60	Source temp		500°C
***Transition used for quantification**				

Data processing: Data processing was done using SCIEX OS software using the Analytics module.

QUANTIFICATION RESULTS

Calibration curves were acquired across the concentration range of 0.050 to 50.00 pg/mL. Each concentration was analyzed in triplicate in order to assess method reproducibility. The resulting curves are shown in Figure 2 and Figure 3. Very good linearity was observed across the concentration ranges analyzed. The quantification results are summarized in Table 3. Samples analyzed in triplicate showed good reproducibility. Excellent %CV were achieved across all concentration levels with no interference in blank human plasma samples for fluticasone propionate and salmeterol xinafoate. The method provided a lower limit of quantification (LLOQ) of 0.050 pg/mL for fluticasone propionate and salmeterol xinafoate, with 0.4 mL of human plasma and a total run time of 8 minutes.

As summarized in Table 3, the assay accuracy for fluticasone propionate was between 93.16% and 106.87%, and all %CV were below 10%. As shown in Table 4 for salmeterol xinafoate, the assay accuracy was between 94.91 and 102.75% and all %CV were below 13%. Both assays provided accuracy and reproducibility well within acceptance criteria for all tested samples.

The extracted ion chromatograms (XICs) for the observed LLOQs for each compound are shown in Figure 4 and 5. The double blank and blank injections are also provided for comparison. In both cases the blank is clean and there is a very distinct peak at the LLOQ.

Current results were achieved using plasma sample volumes of 400 µL and results demonstrate that very good LLOQs and reproducibility were achieved.

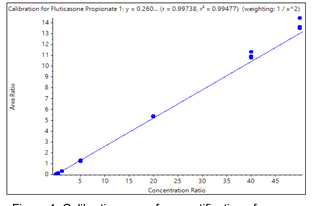

Figure 1. Calibration curve for quantification of fluticasone propionate in human plasma.

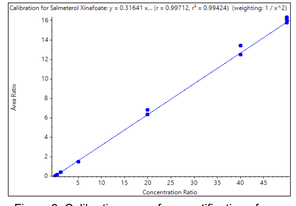

Figure 3. Calibration curve for quantification of salmeterol xinafoate in human plasma.

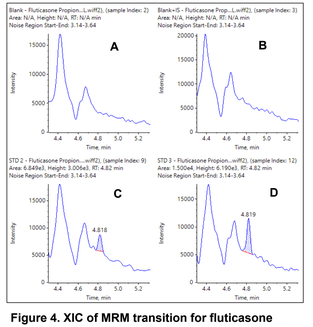

Figure 4. XIC of MRM transition for fluticasone propionate. Double blank and blank from extracted plasma (A and B) and LLOQ at 0.050 pg/mL (C) and 0.100 pg/mL (D).

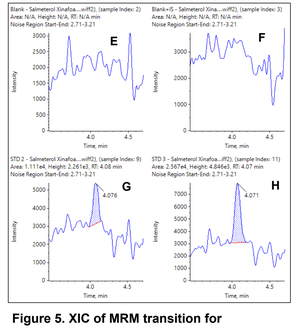

Figure 5. XIC of MRM transition for salmeterol xinafoate. Double blank and blank from extracted plasma (E and F) and LLOQ at 0.050 pg/mL (G) and 0.100 pg/mL (H).

Table 3. Quantification summary for fluticasone propionate.

Actual concentration (pg/mL)	Calculated concentration (pg/mL)	Accuracy (%)	CV (%)	N
0.050	0.051	101.17	6.14	3
0.100	0.102	101.61	4.16	3
0.200	0.191	95.66	5.19	3
0.500	0.466	93.16	8.24	3
1.250	1.187	94.96	2.63	3
5.000	4.866	97.32	4.18	3
20.000	20.670	103.34	0.46	3
40.000	42.360	105.89	2.64	3
50.000	53.440	106.87	3.64	3

Table 4. Quantification summary for salmeterol xinafoate.

Actual concentration (pg/mL)	Calculated concentration (pg/mL)	Accuracy (%)	CV (%)	N
0.050	0.049	98.66	12.81	3
0.100	0.103	102.75	11.82	3
0.200	0.202	101.07	8.28	3
0.500	0.487	97.32	5.74	3
1.250	1.250	99.98	3.77	3
5.000	4.746	94.91	0.36	3
20.000	20.530	102.66	4.32	3
40.000	40.440	101.10	4.06	3
50.000	50.770	101.53	1.82	3

CONCLUSIONS

Here, a highly sensitive and reproducible assay for the quantification of both fluticasone propionate and salmeterol xinafoate in human plasma has been developed on the SCIEX 7500 system.

- Lower limits of quantification of 0.050 pg/mL for fluticasone propionate and salmeterol xinafoate were achieved. This was done using smaller sample volumes than previously described.[7]
- The method showed very good reproducibility and %CV for triplicate injections at all concentration levels
- The method demonstrated the ability to routinely detect ultra-low levels of both analytes in a 8-minute run time, which will allow bioanalytical labs to deliver high-quality data with good throughput

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