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Driving more sensitive and selective quantitation of highly potent inhaled corticosteroids in human plasma using accurate mass spectrometry

Rahul Baghla and Eshani Nandita
SCIEX, USA

ABSTRACT

Here, a selective and sensitive approach is presented for quantifying 3 potent inhaled corticosteroids—fluticasone furoate, fluticasone propionate and mometasone furoate—in human plasma using accurate mass spectrometry. This method successfully achieved a lower limit of quantitation (LLOQ) of 1 pg/mL for fluticasone propionate and fluticasone furoate and 2 pg/mL for mometasone furoate in human plasma (Figure 1).

INTRODUCTION

For long-term asthma control, inhaled corticosteroids are highly effective anti-inflammatory medications. Fluticasone furoate, fluticasone propionate and mometasone furoate are inhaled corticosteroids commonly used to treat allergic conditions such as asthma and allergic rhinitis.[1,2] Since the daily dose of inhaled corticosteroids is low, drug circulation in the blood occurs at low concentration levels. As a result, pharmacokinetic studies require highly sensitive and selective assays to quantify inhaled corticosteroids at pg/mL levels in biological matrices.[2,3]

Here, a method is presented for quantifying fluticasone furoate, fluticasone propionate and mometasone furoate using the ZenoTOF 7600 system. This accurate mass spectrometer provides exceptional selectivity for accurate and precise drug quantitation in complex biological matrices.

MATERIALS AND METHODS

Sample preparation:
All 3 corticosteroids were spiked into 300 µL of human plasma at concentrations ranging from 1 to 1000 pg/mL. A 700 µL aliquot of 30% (v/v) methanol in water was added to the sample and vortexed. Samples were centrifuged at 9400 rcf for 5 minutes. The supernatant was extracted using Strata-X 33 µm Polymeric Reversed Phase, 30 mg/well, 96-well plates from Phenomenex. The plates were conditioned with 1 mL methanol and 1 mL water. Following sample loading, the plates were washed with 1 mL water, 2 mL of 50% (v/v) methanol in water and then eluted with 1 mL acetonitrile. The eluent was dried under a nitrogen stream at 40˚ C. The dried samples were reconstituted in water with 100 µL of 50% (v/v) methanol in water. A 25 µL sample injection was used for analysis.[4,5]

Chromatography:
An ExionLC system with a Phenomenex Kinetex EVO-C18 column (2.1 x 50 mm, 2.6 µm, 100 Å) was used for chromatographic separation. The LC column was operated at 50˚ C. Mobile phase A was 1mM ammonium trifluoroacetate in water and mobile phase B was methanol. Table 1 summarizes the LC gradient conditions used.

Table 1. LC gradient.

Time (min)	Flow (mL/min)	Mobile phase A (%)	Mobile phase B (%)
0.0	0.3	70	30
0.2	0.3	70	30
6	0.3	20	80
6.1	1.00	2	98
9.0	1.00	2	98
9.1	0.30	70	30
10.0	0.30	70	30

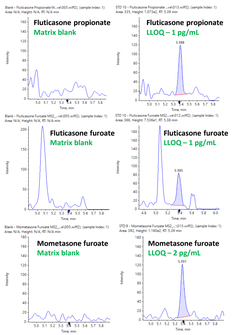

Figure 1. Representative extracted ion chromatograms (XICs) for fluticasone propionate, fluticasone furoate and mometasone furoate in human plasma. The left panels show results for the matrix blank and the right panels show the XICs at the LLOQ.

Mass spectrometry:

- 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

Data processing:

- Data processing was performed using SCIEX OS software 3.1.6
- Peaks were automatically integrated using the MQ4 algorithm with a weighting of $1/x^2$ using an extraction window of 0.02 Da

Table 2. MS and source parameters.

Parameter	MS	MS/MS
Scan mode	TOF MS	MRM^{HR}
Polarity	Positive	
Gas 1	60 psi	
Gas 2	65 psi	
Curtain gas	40 psi	
Source temperature	600°C	
Ion spray voltage	5500 V	
CAD gas	9	
Declustering potential	80 V	
Precursor ion	N/A	501.2 m/z (fluticasone propionate)
	N/A	539.2 m/z (fluticasone furoate)
	N/A	521.2 m/z (mometasone furoate)
Start mass	100 m/z	100 m/z
Stop mass	600 m/z	600 m/z
Q1 resolution	N/A	Unit
Accumulation time	0.05 s	0.03 s
Collision energy	10 V	24 V
CE spread	0 V	0 V
Zeno trap	N/A	ON
ZOD threshold (CID)	N/A	20,000 cps
Time bins to sum	8	8

RESULTS

- Quantitation was performed using Zeno MRM^{HR} mode on the ZenoTOF 7600 system. Here, the selected precursor ions are fragmented in Q2 and sent to the Zeno trap. The Zeno trap provides control of the ion beam from the collision cell into the TOF accelerator.
- All ions then arrive in the TOF accelerator at the same time and location, improving the overall MS/MS sampling efficiency. The overall MS/MS intensity is enhanced 4–25x compared to traditional MRM^{HR} workflows.[6]
- Figure 2 illustrates the sensitivity gains achieved using Zeno MRM^{HR} for all 3 corticosteroids analyzed. Compared with conventional MRM^{HR}, Zeno MRM^{HR} enabled a 7x improvement in signal-to-noise ratio (S/N) ratio for mometasone furoate and fluticasone furoate and a 5.5x improvement in S/N ratio for fluticasone propionate.

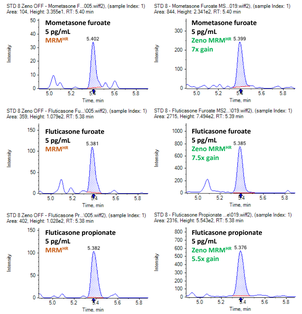

Figure 2. Chromatograms showing sensitivity gains based on S/N ratio using Zeno MRM^{HR} for all 3 analyzed corticosteroids. A 5.5–7.5x improvement in S/N ratio was achieved using Zeno MRM^{HR} compared to MRM^{HR}.

- The Zeno MRM^{HR} workflow is designed to gather all MS/MS information for each sample analyzed (Figure 3). The accessibility of the entire MS/MS spectrum can be advantageous since post-acquisition data decisions can be made on which measured fragments can be utilized for Zeno MRM^{HR}.
- For Zeno MRM^{HR}, quantitation can be performed using a single fragment ion or by summing multiple dominant fragment ions. When multiple highly abundant fragment ions are generated from the target analyte, summed XICs can further enhance assay sensitivity. Lower LLOQs were achieved using the summation approach compared to a single fragment ion for quantitation, as shown in Figure 4.
- Using summation of 2 highly abundant fragment ions, a 2x improvement in LLOQ was observed for fluticasone propionate and fluticasone furoate, while a 2.5x improvement in LLOQ was achieved for mometasone furoate (Figure 4).

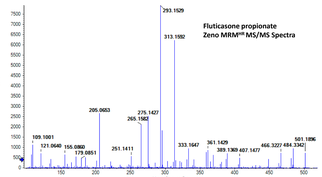

Figure 3. MS/MS spectra for fluticasone propionate using Zeno MRM^{HR}.

Table 3. Summary of the quantitative performance. Reproducibility and accuracy results were determined from the calibration curve across 3 replicates.

	Mometasone furoate		Fluticasone furoate		Fluticasone propionate	
Concentration (pg/mL)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)
1	N/A	N/A	3.43	98.3	6.20	96.7
2	14.3	99.8	9.18	105	8.25	108
5	6.26	104	8.77	98.8	3.52	98.9
20	6.69	86	5.37	85.5	4.32	86.8
40	1.81	100	4.23	107	3.22	102
100	1.75	96.9	1.66	95.2	2.54	95.0
250	1.40	105	2.50	103	0.43	103
500	4.24	107	2.67	105	1.38	104
1000	0.55	100	1.95	102	3.08	105

- Linearity was achieved across a range of concentrations, from 1 pg/mL to 1000 pg/mL, with correlations of determination (r^2) of 0.992, 0.992 and 0.993 for fluticasone propionate, fluticasone furoate and mometasone furoate, respectively (Figure 5).
- The assay accuracy was within ±15% of the nominal concentration and the %CV was <15% (Table 3). The calculated percent accuracy and %CV values were within the acceptance criteria at each concentration level.

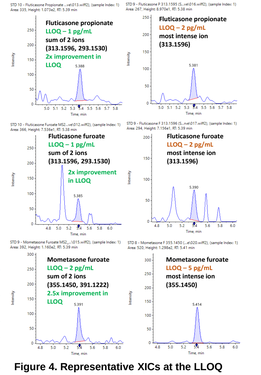

Figure 4. Representative XICs at the LLOQ using the sum of multiple ions compared to a single most intense fragment ion for all 3 corticosteroids.

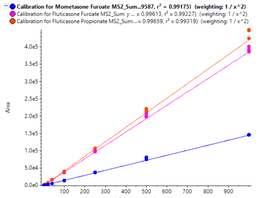

Figure 5. Calibration curves for the quantitation of fluticasone furoate, fluticasone propionate and mometasone furoate in human plasma.

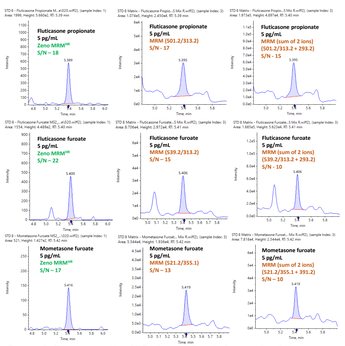

Figure 6. Representative XICs from a 5 pg/mL sample of the corticosteroid analytes analyzed using Zeno MRM^{HR} and MRM.

- Higher selectivity and S/N ratio were achieved using Zeno MRM^{HR} on the ZenoTOF 7600 system compared to both MRM experiments on a SCIEX nominal mass spectrometer (Figure 6).
- Higher isobaric background interference was observed using the sum of 2 transitions on a SCIEX nominal mass spectrometer, as shown in Figure 6. As a result, a lower S/N ratio was observed when compared to using a single transition on a SCIEX nominal mass spectrometer.
- Given the higher selectivity, the sum of multiple ions approach is more effective on the ZenoTOF 7600 system. Additionally, method development is more streamlined, as less ion path tuning is needed and the user can access the full product ion profile.

CONCLUSIONS

- LLOQs of 1 pg/mL, 1 pg/mL and 2 pg/mL were achieved in human plasma for fluticasone furoate, fluticasone propionate and mometasone furoate, respectively
- High mass accuracy and resolution from an accurate mass spectrometer yielded significant gains in selectivity and S/N ratio and reduced background noise compared to a nominal mass spectrometer
- Linearity was achieved for the concentration range of 1 pg/mL to 1000 pg/mL with r^2 values of 0.992, 0.992 and 0.993 for fluticasone propionate, fluticasone furoate and mometasone furoate, respectively
- Method development time was reduced with less ion path tuning using MRM^{HR}-based quantitation and increased data processing flexibility was achieved with access to the entire product ion profile
- The method demonstrated accurate and highly reproducible (%CV <15%) quantitative performance at all concentration levels

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