A sensitive method for the quantitation of mometasone furoate in human plasma

Sensitive, quantitative performance on the SCIEX 7500 system with improved front-end technology

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This technical note demonstrates a sensitive method for quantifying a highly potent orally inhaled drug, mometasone furoate, in human plasma. The method employs a solid phase extraction sample preparation for the extraction of mometasone furoate from human plasma. As a result, a lower limit of quantitation (LLOQ) of 0.25 pg/mL was achieved with %CV <1% (Figure 1).

Mometasone furoate is a synthetic 17-heterocyclic glucocorticoid and an effective anti-inflammatory medication for treating allergic illnesses such as asthma and allergic rhinitis. To exert its effects, mometasone furoate inhibits the growth and activation of inflammatory cells in the airway. Mometasone furoate is currently offered in nasal and oral inhalation forms.

According to a recent report, mometasone furoate has a systemic bioavailability of <1% compared to other corticosteroids.[3] Mometasone furoate/formoterol fumarate (400 mcg/10 mcg) administered as a single dosage corresponds to a C_{max} of 20 pg/mL.[4] Therefore, the pharmacokinetic properties of mometasone furoate in therapeutic inhalation dose ranges need sensitive assays for quantitation at sub-pg/mL levels in biological matrices.

Current methods developed for pharmacokinetic and clinical studies rely heavily on large plasma sample aliquots and small reconstitution volumes to achieve the desired sensitivity. As a result, challenges arise when performing repeat analyses or reinjection reproducibility in a GLP-regulated bioanalytical lab.

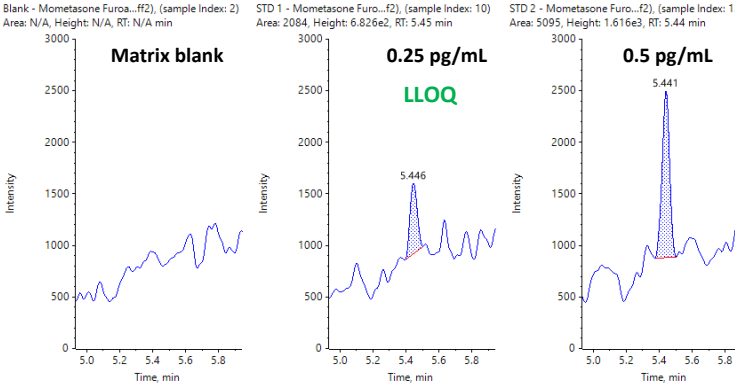

Figure 1. Representative extracted ion chromatograms (XICs) for mometasone furoate in human plasma. The left panel shows results for the matrix blank. The middle and right panels show results for mometasone furoate spiked into human plasma at 0.25 pg/mL (middle) and 0.5 pg/mL (right).

The bioanalytical method described in this technical note uses 300 µL of plasma and a solid phase extraction method to detect ultra-low levels of mometasone furoate.

Key features for the analysis of mometasone furoate using the SCIEX 7500 system

- **Sub-pg/mL level quantitation of a highly potent orally inhaled drug:** Achieve a 0.25 pg/mL LLOQ for mometasone furoate in human plasma with %CV <1%
- **Low plasma consumption:** Leverage the high sensitivity of the SCIEX 7500 system to achieve a 0.25 pg/mL LLOQ for mometasone furoate with 300 µL of human plasma
- **Enhanced sensitivity unlocked:** Improved front-end technology, including the D Jet ion guide, OptiFlow Pro ion source and E Lens probe for enhanced ion generation, capture and transmission, enables users to reach desired quantitative sensitivity
- **Streamlined data management:** Data acquisition and processing are integrated into SCIEX OS software, a 21 CFR Part 11 compliance-ready platform

Methods

Spiked sample preparation: Mometasone furoate was spiked into 300 µL of human plasma at concentrations ranging from 0.25 to 100 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 96-well plates. The plates were conditioned with 1 mL of methanol and 1 mL of water. Following sample loading, the plates were washed with 1 mL of water and 2 mL of 50% (v/v) methanol in water and then eluted with 1 mL of acetonitrile. The eluent was dried under a nitrogen stream at 40°C. The dried samples were reconstituted in 100 µL of 50% (v/v) methanol in water. A 25 µL sample injection was used for analysis.[8]

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 sodium acetate in water and mobile phase B was methanol. Table 1 summarizes the LC gradient conditions used.

Table 1. LC gradient.

Time (min)	Flow rate (mL/min)	Mobile phase A (%)	Mobile phase B (%)
0.0	*0.30*	*70*	*30*
0.2	*0.30*	*70*	*30*
6.0	*0.30*	*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*

Mass spectrometry: Samples were analyzed using the SCIEX 7500 system equipped with the OptiFlow Pro ion source. The system was controlled by SCIEX OS software. The sodium ion adduct of mometasone furoate was used for this analysis. The optimized MS parameters are listed in Table 2.

Data processing: Data processing was performed using SCIEX OS software, version 3.1.5. Peaks were automatically integrated using the MQ4 algorithm with a weighting of $1/x^2$.

Table 2. Optimized MS parameters.

Name	Q1/Q3 (*m/z*)	Q0D (V)	CE (V)	CXP (V)
Mometasone furoate	*543.1/507.1*	*50*	*22*	*10*
Source parameters	**Value**	**Source parameters**		**Value**
Curtain gas	*45 psi*	*CAD gas*		*9*
Ion source gas 1	*55 psi*	*Ion spray voltage*		*2750 V*
Ion source gas 2	*65 psi*	*Temperature*		*350°C*

Quantitative performance

A calibration curve was analyzed for concentrations ranging from 0.25 to 100 pg/mL. To evaluate reproducibility, each concentration of mometasone furoate was analyzed in triplicate.

Mometasone furoate in human plasma was quantified at an LLOQ of 0.25 pg/mL. No interferences were observed in the blank matrix sample (Figure 1). Linearity was achieved across concentrations ranging from 0.25 to 100 pg/mL with a correlation of determination (r^2) of 0.995 (Figure 2).

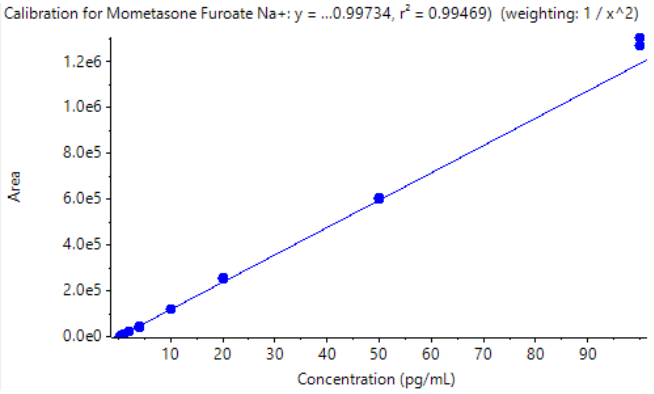

Figure 2. Calibration curve for the quantitation of mometasone furoate in human plasma.

Analytical performance was evaluated based on the requirement that the accuracy of the calculated mean should be between 80% and 120% at the LLOQ and between 85% and 115% at higher concentrations. The %CV of the calculated mean concentration should be below 20% at the LLOQ and below 15% at all higher concentrations.[5]

The assay accuracy was within ±10% of the nominal concentration and the %CV was <8% (Table 3). The calculated percent accuracy and %CV values were within the acceptance criteria at each concentration level (Table 3).

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

Concentration (pg/mL)	CV (%)	Accuracy (%)
0.25	*0.78*	*103*
0.5	*7.68*	*99.5*
1	*3.62*	*94.4*
2	*1.59*	*90.8*
4	*4.90*	*95.6*
10	*1.22*	*100*
20	*1.05*	*107*
50	*0.23*	*101*
100	*1.51*	*107*

Mometasone furoate has a ~90% plasma protein binding, which can cause challenges during extraction.[9] However, mometasone furoate was successfully extracted from human plasma using the solid phase extraction method described in this technical note, leading to a recovery of 80% (Table 4).

Carryover was evaluated by injecting a blank sample before and after the upper limit of quantitation (ULOQ) at 100 pg/mL. Figure 3 shows that no carryover was observed in the XICs run before and after the ULOQ.

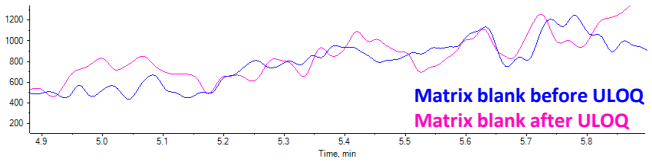

Figure 3. XICs of mometasone furoate from matrix blank injection before (blue) and after (pink) the ULOQ (100 pg/mL).

Compliance-ready SCIEX OS software

SCIEX OS software is a closed system and requires records and signatures to be stored electronically, meeting the regulations outlined by 21 CFR Part 11. SCIEX OS software can open raw data files from any visible storage location within a closed network by using designated processing workstations. Figure 4 illustrates the features of SCIEX OS software used for monitoring the audit trail, acquiring and processing data and configuring user access.

The audit trail feature enables users to audit critical user actions and locks in data integrity. The Central Administrator Console (CAC) feature allows users to centralize acquisition and processing using a single platform to maximize efficiency for multi-instrument laboratories, independent of compliance standards. The configuration module allows users to assign roles and access as the administrator, method developer, analyst and reviewer.

Table 4. Recovery based on 3 replicates at 50 pg/mL.

	Concentration (pg/mL)	Mean area response in post-spiked samples (mean ± SD)	Mean area response in pre-spiked samples (mean ± SD)	% Recovery
Mometasone furoate	*50*	*202496 ± 5629*	*161448 ± 3517*	*79.7*

Figure 4. Features of the SCIEX OS software for monitoring user access and evaluating the audit trail. The audit trail view allows users to filter for high-risk events easily and enables data integrity features to meet compliance requirements. The software features a Central Administrator Console (CAC) to manage users and groups, role definitions, workstations and projects across all systems. The CAC feature supports both regulated and non-regulated compliance standards. The configuration module enables users to quickly set up roles and levels of access for the administrator, method developer, analyst and reviewer levels.

Conclusions

- An LLOQ of 0.25 pg/mL was achieved for the quantitation of mometasone furoate in human plasma
- Linearity was achieved for the concentration range of 0.25 pg/mL to 100 pg/mL with an r^2 of 0.995
- The desired LLOQ (0.25 pg/mL) for mometasone furoate was achieved with 300 µL of human plasma
- The method demonstrated accurate and highly reproducible (%CV <8%) quantitative performance for all concentration levels
- Sensitivity was achieved on the SCIEX 7500 system with an improved front-end technology for better ion generation, capture and transmission
- SCIEX OS software is compliance-ready to support 21 CFR Part 11 and integrates with an accurate mass spectrometer to support data acquisition, processing and management on a single platform

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