A Sub-Picogram LC-MS/MS Method for the Analysis of Mometasone Furoate in Human Plasma.

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INTRODUCTION

- Mometasone furoate is a potent synthetic corticosteroid with minimal bioavailability when administered topically (dermatitis) or via inhalation (allergy, asthma)
- Maximum plasma concentrations near 50 pg/mL are expected following inhalation of 100 - 400 µg, and a sensitive method is required to measure an adequate number of data points for calculating pharmacokinetic parameters
- A post-extraction derivatization procedure coupled with a dual-column LC-MS/MS set-up provided sufficient sensitivity to validate an LLOQ of 0.250 pg/mL

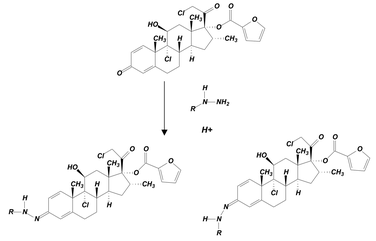
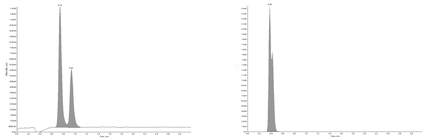


Figure 1. Mometasone furoate and hydrazone reaction products

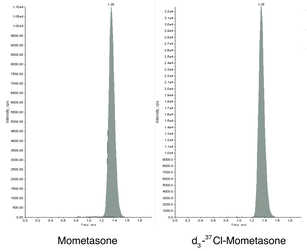
The target LLOQ of 0.25 pg/mL was challenging to achieve as the neutral steroid tends to form adducts in positive ESI and the signal is diluted by the presence of 2 chlorine atoms. Derivatization with several hydrazine compounds or dansyl chloride was investigated to increase and stabilize the response. Two isomeric products were often observed with reversed phase chromatography.



a) Dansyl Chloride derivative on ACE C8 b) Hydrazinopyridine derivative on ACE C18

Figure 2. Reversed phase chromatography of derivatised mometasone furoate.

The reaction products presented themselves as a single peak using cation exchange chromatography with moderate retention.



Mometasone d_3-^{37}Cl-Mometasone

Figure 3. Mometasone furoate derivative SCX chromatography

SAMPLE PREPARATION

Several solvents were tested across the pH range and n-butyl chloride at alkaline pH was chosen to provide the cleanest baseline and adequate recovery from 1.0 mL of EDTA plasma prior to derivatization.

1mL Sample /IS + 1mL 2% NH4OH + 4.5 mL ButylChloride

↓

Mix/Separate & evaporate organic

↓

Derivatize/Evaporate/reconstitute

Severe matrix suppression was observed for all derivatives including several highly retained species that nearly eliminated the signal from subsequent injections.

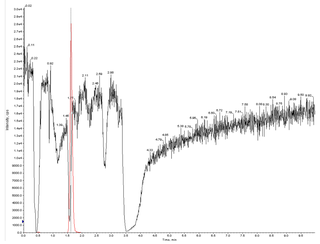


Figure 4. Post column infusion experiment, mometasone furoate retention time ca. 1.6 minutes under typical (not final) SCX conditions.

INSTRUMENTATION

A dual-column back-flush method was adopted. In this approach, 2 columns are used under isocratic conditions with one being rinsed offline in the reverse direction while the analysis column is eluting to the mass spec. Alternating columns via a valve switch eliminates downfield elution of suppressive compounds.

- Mobile Phase: Acetonitrile:Methanol:15 mM ammonium formate, pH 2.5; Analytical Flow Rate 0.9 mL/min, Back-flush Flow Rate 1.0 mL/min
- Column: SCX 3x50 mm 5µm, 50°C
- Detection: Electrospray ionization (ESI) data was acquired by multiple reaction-monitoring (MRM) in positive mode on an AB SCIEX 5500 Q-TRAP® mass spectrometer. Acquisition time 2.0 minutes

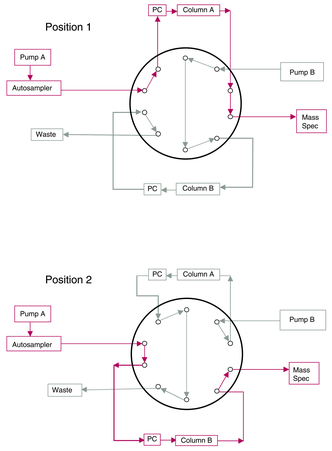
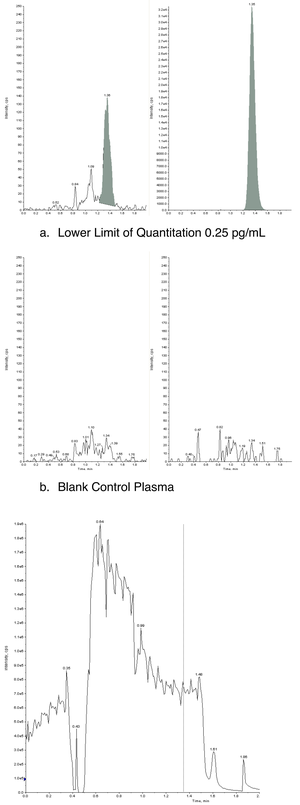


Figure 5. Dual column backflush LC-MS/MS design.

Mometasone chromatography under the final conditions, LLOQ, Blank and Post Column Infusion.



a. Lower Limit of Quantitation 0.25 pg/mL

b. Blank Control Plasma

c. Post Column Infusion

Figure 6. Final conditions for mometasone furoate derivative

RESULTS

- The validated analytical range was 0.25 to 25.0 pg/mL of mometasone furoate using 1.0 mL of human EDTA plasma
- The inter-batch precision (% C.V.) and accuracy (% Bias) of quality control samples is shown in Table 1
- The extraction recovery of mometasone furoate was 87 – 93%
- Selectivity and matrix effect data were acceptable for healthy and hemolyzed human EDTA plasma
- Mometasone furoate was stable in human EDTA plasma through 6 freeze/thaw cycles, inclusive of 53 hours at ambient temperature under white lighting and for up to 57 days when stored at -20°C
- Derivatized mometasone furoate integrity was maintained up to 78 hrs post extraction

Quality Control Samples			Precision (% CV)	Accuracy (% Bias)
Inter-Batch	LLOQ	0.25 pg/mL	16.9	4.4
	Low	0.75 pg/mL	7.6	2.8
	Medium	5.0 pg/mL	2.3	5.8
	High	20.0 pg/mL	2.3	1.5

Table 1. Validation Interday Reproducibility

A bioequivalence study of a 200 ug mometasone nasal spray generated over 3000 samples for analysis. Reanalysis of 262 samples confirmed 97% of the original results using established industry-wide criteria for incurred sample reproducibility. The average difference between all reassay results was 4.1%.

CONCLUSIONS

A robust method for analysis of mometasone furoate in human plasma has been developed and validated. Despite sub-picogram sensitivity requirements, the relatively simple method overcomes extreme matrix effects to accurately and reproducibly quantify mometasone furoate for pharmacokinetic characterization of very low dosage intranasal and topical formulations.

ACKNOWLEDGMENT

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