Use of Sodium Adduct Combined with Improved Sample Extraction for Femtogram Level Detection of Mometasone Furoate by UPLC-MS/MS

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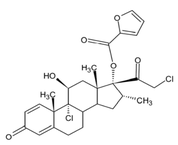
Introduction



Mometasone Furoate
MW: 521.4

In order to quantitate low LLOQ in the femtogram range, sensitivity and signal to noise ratio are crucial parameters to be optimized to obtain decent response. An exhaustive optimization of the sample extraction is mandatory in order to get rid of every co-extracts which could affect ionization. Also, UPLC chromatography allows the use of smaller sample volumes and shorter retention times, all in favor of a higher signal to noise ratio and overall response of the molecule of interest. Mometasone furoate, which do not exhibit strong enough response to be detected at such low level, easily forms a sodium adduct. This adduct, coupled with improved sample extraction and chromatography, provides accurate quantitation at an LLOQ of 250 fg/mL.

Method

Mometasone furoate was originally validated with an LLOQ of 2 pg/mL. In order to decrease the LLOQ down to 250 fg/mL, an exhaustive evaluation of the sample extraction was deemed necessary along with the improvement of the chromatographical process. The extraction procedure consisted of a liquid-liquid extraction followed by a solid phase extraction. The chromatography used was performed on regular reversed phase HPLC. However, the extraction procedure was optimized and the chromatography was improved to UPLC using the ACE Excel 2 C18 50 X 3.0, 2 µm column. The use of UPLC allowed to achieve the sensitivity needed for proper quantitation.

Extraction Procedure

- Internal Standard: Mometasone furoate-d_3
- Sample Volume: 1000 µL
- Extraction: Liquid-liquid followed by solid-phase extraction
- Matrix: human EDTA K_2 plasma
- Dynamic Range: 250-50000 fg/mL
- Concentration factor: 10

LC-MS/MS Analysis

	Old Method	Improved Method
Analytical Range	2-100 pg/mL	250-50000 fg/mL
Analytical Column	ACE 3 C18, 50x4.6mm, 3um	ACE Excel 2 C18, 50 x 3.0 mm, 2 µm
Elution Mode	HPLC Gradient	UPLC Gradient
Mobile Phase A	Water/Methanol (10/90), Na Acetate 0.2M	Milli-Q type Water, Sodium Acetate 1 mM
Mobile Phase B	Water/Methanol (30/70), Na Acetate 0.2M	Methanol 100%
Flow Rate	1 mL/min	0.65 mL/min
Injection Volume	30 µL	40 µL
Retention Time	2.64 minutes	1.74 minutes
Ion Monitored	543→ 507 amu	543→ 507 amu
Ionization Mode	Positive TurboIonSpray	Positive TurboIonSpray

Results

The final improved method used a liquid-liquid extraction (LLE) using 1-chlorobutane and a solid phase extraction using MCX extraction cartridges. The method was validated over the dynamic range of 250-50000 fg/mL **(Fig. 1)**. The new UPLC conditions decreased the overall run time of a sample from 7.0 minutes to 4.5 minutes, improving considerably the throughput of the assay **(Fig. 2 and 3)**. Optimization of extraction procedures allowed clean samples in order to improve signal to noise ratios at low concentrations. This was even more improved by careful ionization source optimization. The signal to noise ratio of a 250 fg/mL samples was 15:1. Accuracy and precision were demonstrated at this low level with %bias of 15.33% and %CV of 17.25% **(Table 1)**. The matrix effect and the matrix factor were evaluated and found to have no impact on quantitation **(Table 2)**. Freeze and thaw, short-term and long-term stability in matrix were evaluated and met acceptance criteria. Recovery of analyte and internal standard showed to be dependent on the LLE extraction time. An optimal 30 minute extraction proved to be ideal and reproducible. Recovery of analyte and internal standard was 79 and 80%, respectively **(Table 3)**.

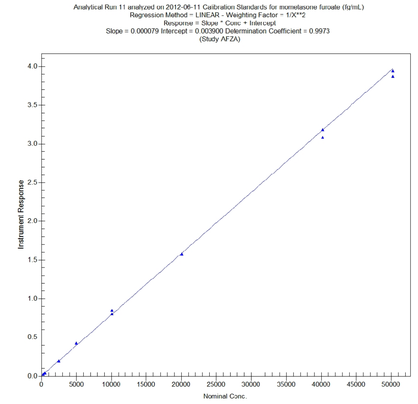


Figure 1. Representative Calibration Curve from 250 to 50000 fg/mL

Table 1. Between-Run Accuracy and Precision Summary Table

	LLQC 250 fg/mL		QC1 750 fg/mL		QC2 25000 fg/mL		QC3 37500 fg/mL	
	Conc. Found	% Bias	Conc. Found	% Bias	Conc. Found	% Bias	Conc. Found	% Bias
N	54		54		54		54	
Mean	288.3	15.3	780.0	4.00	25031.1	0.12	38349.0	2.26
SD	49.74		55.15		826.50		1848.90	
%CV	17.25		7.07		3.30		4.82	

Table 3. Recovery of Analyte and Internal Standard

Concentration (fg/mL)	Recovery (%)
750	76.99
25000	69.79
37500	79.94
Internal Standard	80.45

Table 2. Matrix Effect Evaluation

Untreated Standard (MFQC1)		Reference solution (RSQC1)		Calculated	Calculated	IS-Normalized
Analyte Responses	Internal Standard Responses	Analyte Responses	Internal Standard Responses	Matrix Factor (Analyte)	Matrix Factor (IS)	Matrix Factor
2039	36696	2231	40586	0.909172	0.904874	1.004750
1990	36051	2197	39952	0.887323	0.888969	0.998148
2368	35793	2193	40985	1.055870	0.882608	1.196307
1931	35024	2258	40752	0.861016	0.863645	0.996956
2188	36035	2283	39051	0.975610	0.888575	1.097949
2246	36276	2294	41996	1.001471	0.894518	1.119565
Mean		2242.7	40553.7			1.0689458
SD(±)						0.02238971
CV(%)						7.71

Untreated Standard (MFULOQ)		Reference solution (RSULOQ)		Calculated	Calculated	IS-Normalized
Analyte Responses	Internal Standard Responses	Analyte Responses	Internal Standard Responses	Matrix Factor (Analyte)	Matrix Factor (IS)	Matrix Factor
122810	32543	148087	38894	0.835205	0.852603	0.979594
126026	32056	149001	38302	0.857077	0.839844	1.020519
125435	33011	149640	38755	0.853057	0.864864	0.986348
108982	28039	146976	38184	0.741164	0.734601	1.008934
130080	33060	144777	37888	0.884647	0.866148	1.021358
121797	30640	143769	36991	0.828316	0.802746	1.031853
Mean		147041.7	38169.0			1.0081010
SD(±)						0.02088372
CV(%)						2.07

Chromatography

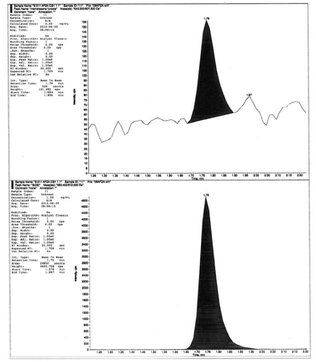


Figure 2. Chromatogram of the LLOQ (250 fg/mL) using the New UPLC Conditions

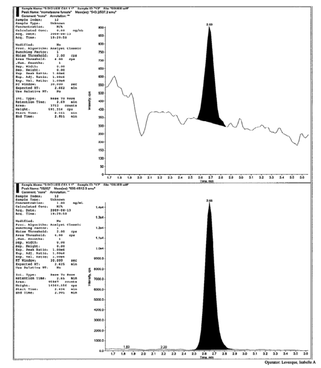


Figure 3. Chromatogram of the LLOQ (2 pg/mL) using the old Chromatographic Conditions

Conclusion

The use of UPLC chromatography helped in shortening retention times and maximizing signal response. Also, detection of mometasone furoate sodium adduct provided viable ionization and more accurate detection and quantitation. This assay was used for a recent bioanalysis study in which reliable results were produced. Moreover, the assay showed good reproducibility with more than 94% of the reassays confirming their original value in the ISR testing.

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