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Short communication

A sensitive liquid chromatography–tandem mass spectrometry method for the quantification of mometasone furoate in human plasma

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Abstract

A robust, rapid, selective and sensitive liquid chromatography-negative atmospheric pressure chemical ionization (LC–(APCI$^-$)–MS–MS) method has been developed for the quantification of mometasone furoate (MF) in human plasma utilizing a solid-phase extraction clean-up step and ^{13}C-fluticasone propionate as internal standard. The intra- and inter-day coefficients of variation were ≤15% and the lower limit of quantification (LLOQ) was 15 pg/ml. This method is ideally suited for pharmacokinetic investigations of low MF levels following inhalation of MF.

Keywords: HPLC–MS–MS; Glucocorticoid; Drug analysis; Mometasone furoate; Liquid chromatography–tandem mass spectrometry

1. Introduction

Liquid chromatography–tandem mass spectrometry (LC–MS–MS) techniques have become popular in the determination of glucocorticoids in biological matrices because of their increased specificity and sensitivity [1–6].

Mometasone furoate (MF, Fig. 1), 9α,21-dichloro-11β, 17α-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione 17-(2-furoate), a synthetic glucocorticoid with anti-inflammatory properties is available in Europe as a dry powder inhaler and is under evaluation in the United States for the treatment of chronic mild-to-moderate persistent asthma [7,8]. Affrime et al. assessed the pharmacokinetics of MF using a LC–MS–MS method with a lower limit of quantification of 50 pg/ml [8]. Because of insufficient sensitivity of the assay, concerns were expressed that a more sensitive assay is needed to adequately describe the pharmacokinetics of this drug after inhalation [9,10]. This paper presents a specific and sensitive atmospheric pressure chemical ionization (APCI) based LC–MS–MS assay using a multiple reaction monitoring technique suitable to quantify MF with sufficient sensitivity in human plasma after oral inhalation of therapeutic doses.

2. Experimental

2.1. Chemicals and reagents

Mometasone furoate was purchased from USP (Rockville, MD). $^{13}C_3$-fluticasone propionate ($^{13}C_3$-FP) was provided by GSK R&D, Ware, Hertz, UK. HPLC grade solvents were purchased from Fisher chemicals (Springfield, NJ, USA). The solid phase LC_{18} (3 ml) cartridges for sample extraction were obtained from Supelco (Bellefonte, PA, USA). Drug-free human plasma was obtained from the Civitan regional blood system (Gainesville, FL, USA).

2.2. Preparation of calibration standards and quality control samples

The calibration standards (CC, 15–1000 pg/ml)) were prepared in plasma using working solution of MF (100 and

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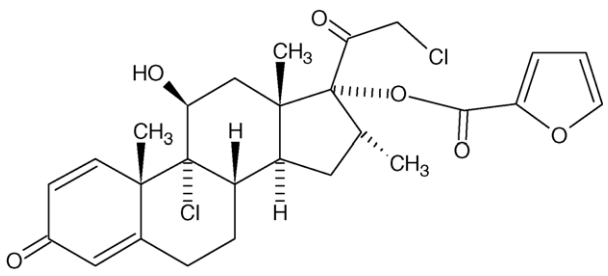

Fig. 1. Structure of mometasone furoate.

10 ng/ml of MF in a mixture of methanol–water (85:15, v/v)). Quality control (QC) samples (15, 30, 50, 80, 120 and 400 pg/ml MF in plasma) were prepared from another set of working solutions. An I.S. working solution of 20 ng/ml was prepared in methanol.

2.3. Sample processing

Plasma samples were thawed at room temperature and 50 μl of I.S. working solution was added to 1 ml of plasma. The compounds were then extracted using a previously reported method [5,11]. One milliliter of 30% ethanol was added to 1 ml of the plasma sample (to precipitate proteins) and centrifuged. Two milliliters of the supernatant was then extracted using a 6 ml end-capped C_{18} cartridge and the analytes were then eluted with 3 ml of a mixture of ethyl acetate–heptane (35:65, v/v). The residue was then evaporated under vacuum and reconstituted in 100 μl of a mixture of methanol–water (85:15, v/v). A sample volume of 80 μl was injected into the HPLC–MS–MS system.

2.4. HPLC–MS–MS conditions

Isocratic high performance liquid chromatography at ambient temperature was performed with methanol–water (85:15, v/v) as mobile phase (flow rate 1 ml/min), a Waters 5-μm Symmetry C_{18} column (50 mm × 4.6 mm i.d., Milford, MA, USA) and a Whatman 5-μm ODS C_{18} guard column cartridge (20 mm × 2.0 mm i.d., Clifton, NJ, USA) using a LDC Analytical ConstaMetric®35000 Solvent delivery system (LDC/Milton Roy, Riviera Beach, FL, USA) in conjunction with a Perkin-Elmer ISS 200 autosampler. A Micromass Quattro-LC-Z (Beverly, MA, USA) triple quadrupole mass spectrometer (Masslynx 3.1 software) equipped with an APCI ion source (negative mode) was used as detector. Multiple reaction monitoring was used for the quantification. The source temperature and the APCI probe temperature were set to 120 and 500 °C, respectively. Argon was used as the collision gas and the mass resolution was set to unit mass. The corona voltage for both MF and I.S. transitions was set to 3.5 kV. The cone voltages for the MF and I.S. transitions were set to 35 and 20 V, respectively. A dwell time of 0.7 s was used for monitoring MF transition and dwell time of 0.1 s was used for monitoring the I.S. transition. Peak area ratios of MF to I.S. were plotted against MF concentrations to obtain the calibration curves for MF in Masslynx 3.1. A weighted ($1/x$) linear regression model with 10 concentration points (including blank plasma) ranging from 15 to 1000 pg ml^{-1} in duplicates was used to plot the calibration curves.

2.5. Method validation

2.5.1. Selectivity

Drug-free plasma samples from 30 humans were assayed. Any apparent responses at the retention times of MF and the I.S. were compared to the response at the lower limits of quantification for MF and to the response at the working concentration of the I.S., respectively.

2.5.2. Recovery

The recovery of MF was determined at low (40 pg/ml), medium (80 pg/ml) and high (400 pg/ml) concentrations by comparing the responses from plasma samples spike prior to extraction with those from plasma samples extracted and spiked after extraction. The recovery of the I.S., using an identical extraction method and analytical conditions, has been reported earlier. Higher concentrations were not included during the recovery experiments, as it was unlikely to observe such high concentrations in the following pharmacokinetic studies [8].

2.5.3. Accuracy and precision

Intra-day accuracy and precision were determined by analyzing the quality control samples at concentrations of 15, 30, 50, 80, 120 and 400 pg/ml ($n = 6$ at each concentration) on the same day. Inter-day accuracy and precision were assessed by repeating the experiment on three different days. The lower limit of quantification (LLOQ) of the assay was defined as the lowest drug concentration that can be determined with an accuracy of 80–120% and a precision $\leq$20% [12]. Experiments evaluating the lower limit of detection were not performed as such determinations are of no relevance for pharmacokinetic assessments.

2.5.4. Stability

Freeze–thaw cycle stability was assessed by analyzing quality control samples at concentrations of 50, 120 and 400 pg/ml in triplicate, following three cycles of freezing at −80 °C and thawing. Short-term temperature stability was evaluated for the quality control samples at the same concentrations by thawing the samples and keeping them at room temperature for 4 h. Bench-top stability for 6 h at the same concentrations was determined after extraction and reconstitution. This was judged to be sufficient as the length of an analytical run was generally shorter. Stability was evaluated in terms of accuracy, as the percentage ratio of measured concentration to the nominal concentration.

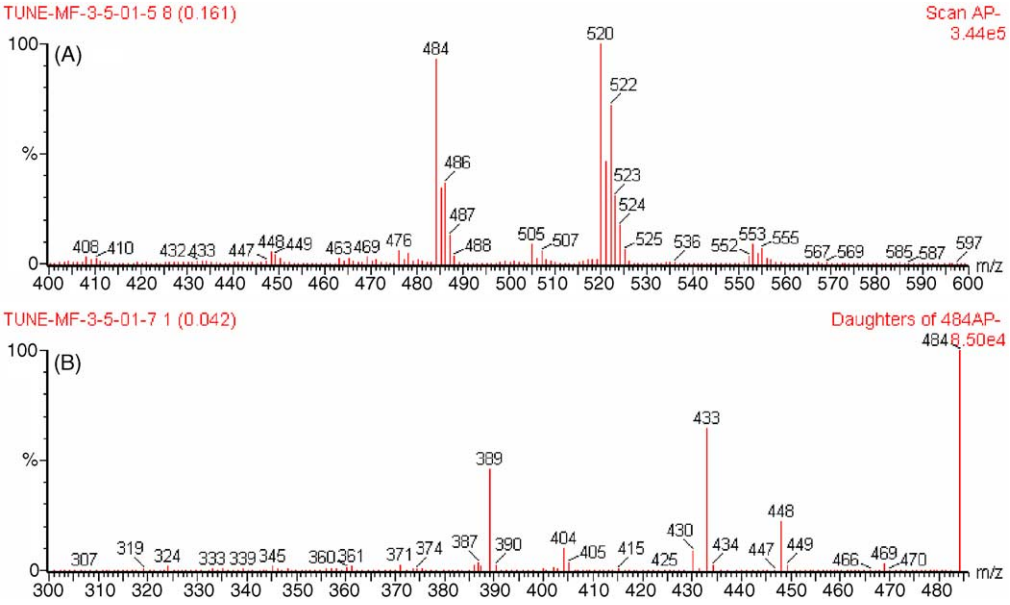

Fig. 2. Full scan (A, top panel) and daughter scan (B, bottom panel) spectra of MF.

3. Results and discussion

3.1. Mass spectrometry/chromatography

Preliminary tuning experiments were performed for MF in APCI and electrospray ionization (ESI) modes. Negative APCI ($APCI^-$) was chosen because of better sensitivity seen by the increased signal-to-noise ratio for the product ion peak. The full scan $APCI^-$ mass spectra of the parent (Fig. 2A), and the daughter ions generated by the dominant 484 peak in Fig. 2A in (Fig. 2B) are shown in the upper and lower part of Fig. 2, respectively. The best sensitivity was observed with the transition *m/z* 484.0–389.2 and was chosen as the transition channel for MF. The parent molecular ion (*m/z* 484.0) corresponds to a difference of 36 mass units from the actual mass of MF (*m/z* 520.0) and this can be attributed to the loss of an HCl molecule from the structure of MF in the APCI source. The transition for $^{13}C_3$-FP (used as internal standard, see below) was selected as *m/z* 503.3–380.0 based on a previous report [5]. Both MF and the I.S. eluted at 0.97 min and the total analysis time for each sample was 4 min. The resulting calibration curves obtained after solid phase extraction of spiked plasma calibrators were linear in the range 15–1000 pg/ml with correlation coefficients (r^2) >0.99. The solid phase extraction method was selected over a simple precipitation method (e.g. with acetonitrile) as the extraction procedure included a concentration step important for achieving optimal sensitivity.

3.2. Internal standard selection

Isotope-labeled MF was unavailable for use as an internal standard (I.S.). $^{13}C_3$-FP was chosen as the I.S. because of the similar physicochemical properties, excellent sensitivity under negative ion mode APCI [5] and, contrary to unlabelled fluticasone propionate, because of the lack of interferences in the MF transition.

3.3. Selectivity

No endogenous interferences from human plasma were observed at the retention times of MF and the I.S. (Fig. 3). Fig. 4 shows the chromatograms for extracted plasma samples spiked with 15 pg/ml MF and I.S. (1 ng/ml of $^{13}C_3$-FP).

3.4. Recovery

The recovery of MF ($n = 5$) for the low (40 pg/ml), medium (80 pg/ml) and high (400 pg/ml) concentrations was 81.5 ± 5.4, 74.5 ± 3.9 and 73.7 ± 5.8, respectively. The recovery for the I.S. using an identical extraction method has been reported to be 82% for a concentration of 100 pg/ml in an earlier report by this group [5].

3.5. Precision and accuracy

The intra- and inter-day accuracy and precision data for the six QC samples of MF used in the assay are listed in Table 1. The assay was reliable, consistent and showed good accuracy (88.0–104.4% at LLOQ and 93.8–111.1% at other concentrations) and precision (5.2–12.8 at LLOQ and 3.8–12.0 at other concentrations). A 15 pg/ml was chosen as the LLOQ for MF based on the accuracy (80–120%) and precision (≤20%) on a day-to-day basis [12]. The linear range for MF was 15–1000 pg/ml with correlation coefficients (r^2) >0.99. In

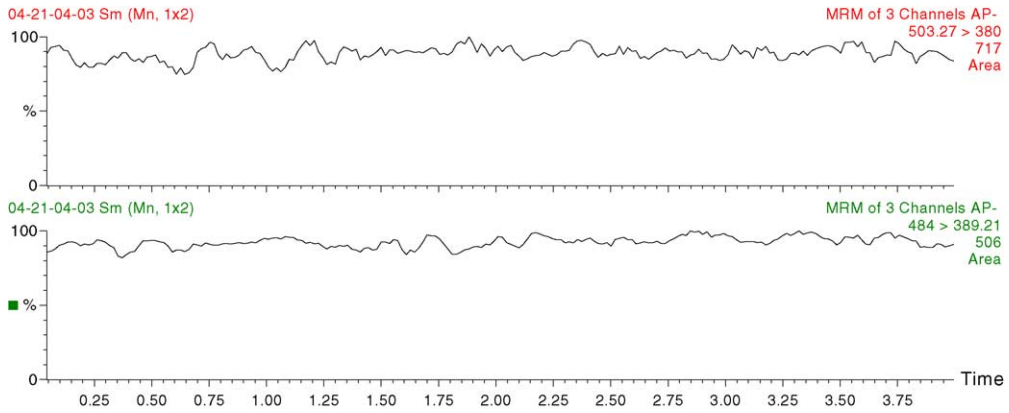

Fig. 3. Chromatograms of blank human plasma for the I.S. ($^{13}C_3$-FP, top panel) and MF (bottom panel) channels.

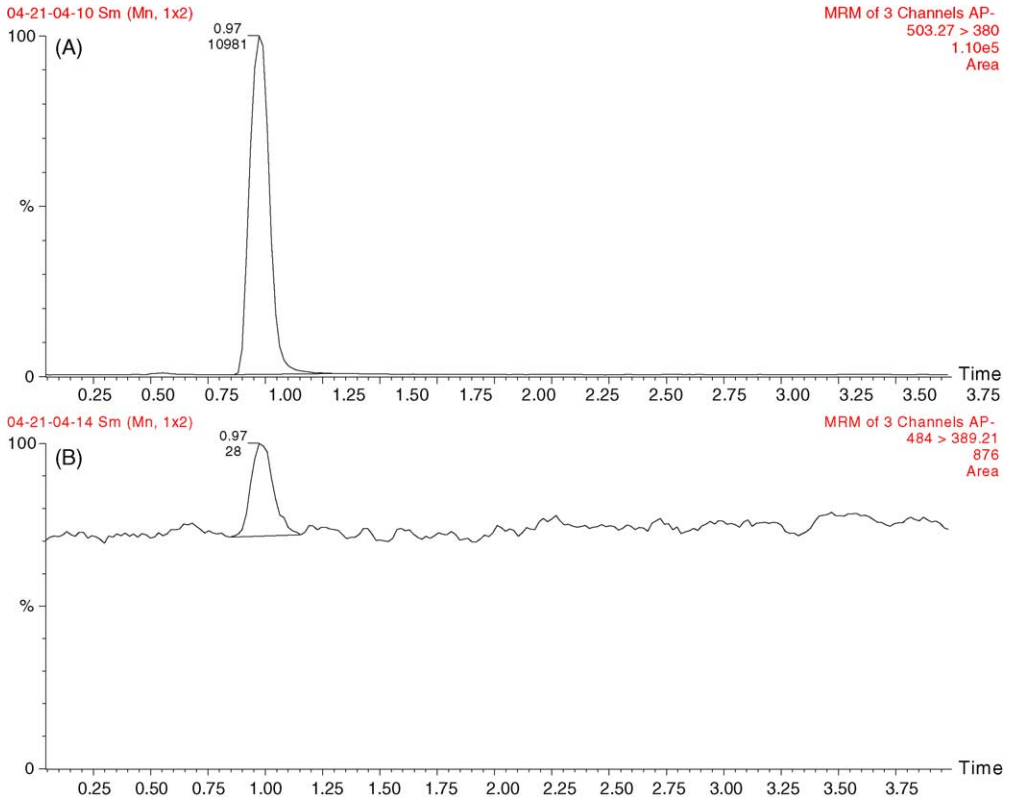

Fig. 4. Chromatograms of human plasma spiked with 1000 pg/ml I.S. ($^{13}C_3$-FP, A, top panel) and 15 pg/ml MF (B, bottom panel) channels.

addition, accuracy and precision of quality controls obtained with a different batch of the same column brand were similar and fulfilled assay acceptance criteria. Thus, accuracy and precision seems to be rather robust.

3.6. Stability

The data for the human plasma freeze-thaw cycle stability, short-term stability under room temperature and bench-top

Table 1
Intra- and inter-day accuracy/precision for MF in human plasma

	15 pg/ml	30 pg/ml	50 pg/ml	80 pg/ml	120 pg/ml	400 pg/ml
Day 1	88.0/9.9	97.3/12.0	98.0/9.5	93.8/7.0	103.8/8.1	101.0/5.8
Day 2	104.4/5.2	93.9/9.6	98.8/7.2	104.3/4.4	106.5/5.0	105.8/7.1
Day 3	91.1/12.8	100.0/8.7	104.7/2.9	104.2/3.8	110.3/5.4	111.1/4.9
Inter-day	94.9/11.8	97.1/9.8	100.8/7.0	100.5/7.0	106.9/6.6	106.0/6.9

Table 2
Stability[a] (%) of MF in human plasma

	50 pg/ml	120 pg/ml	400 pg/ml
Three cycle freeze–thaw	100.0 ± 7.2	101.9 ± 7.5	94.2 ± 5.2
Short term (4 h)	95.3 ± 8.1	89.5 ± 6.5	87.4 ± 0.8
Bench top (6 h)	89.3 ± 8.3	89.2 ± 6.5	87.6 ± 3.5

[a] Stability expressed as the % ratio of the measured concentration to the nominal concentration ($n = 3$).

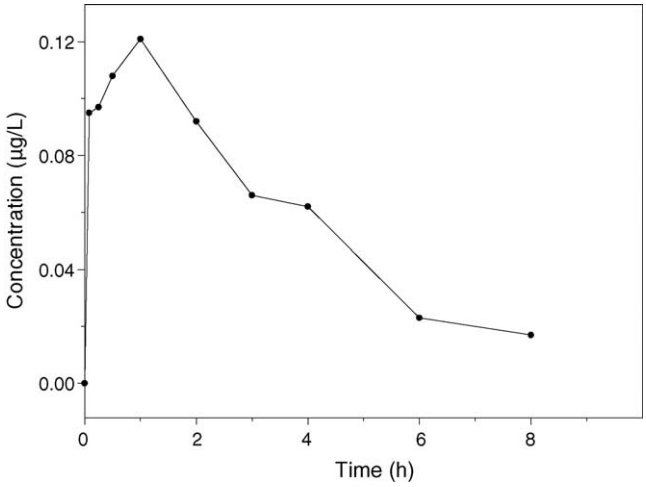

Fig. 5. Concentration-time profile of a subject dosed with 800 μg of MF via oral inhalation.

stability at the low, medium and high concentrations of MF is shown in Table 2. The results show that MF was stable under the investigated conditions as the measured concentrations were within acceptable limits (85–115% of the nominal concentrations).

3.7. Application

The developed LC–APCI–MS–MS method has been used successfully for the quantification of MF in human plasma following oral inhalation in human volunteers. The volunteers were given 800 μg dose of MF and were sampled pre-dose (0 h) and at 0.08, 0.25, 0.5, 1, 2, 3, 4, 6 and 8 h after dosing. A representative concentration-profile of MF from one of the volunteers is shown in Fig. 5.

4. Conclusions

A rapid, simple, sensitive and selective LC–(APCI$^-$)–MS–MS method was developed and validated for the quantification of MF in human plasma samples. The method is robust and linear over a wide range of concentrations. The assay has a limit of quantification of 15 pg/ml, and is more sensitive than a previously reported competitive enzyme immunoassay (LLOQ 50 pg/ml) and LC–MS–MS method (LLOQ 50 pg/ml) for the quantification of MF in human plasma [8,13] and equivalent to other HPLC–MS–MS assays for other inhaled glucocorticoids (FP: 20 pg/ml [5]; budesonide: 50 pg/ml [14,15]). Considering that no assay conditions have been published by the developer of MF [8], it is difficult to determine the reasons for the difference in sensitivity for the two MF assays. It might be related to differences in the volume of serum or plasma utilized in the two assays or differences in the equipment used. It is well established that difficulties to transfer HPLC–MS–MS assays are often related to the use of detectors from different companies [15].

In conclusion, the validated assay is a significant improvement over existing assays of MF and is suitable to assess in detail the pharmacokinetics of MF following oral inhalation of therapeutic doses of this new inhaled glucocorticoid.

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