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ORIGINAL RESEARCH PAPER

A sensitive, heart-cutting, two-dimensional liquid chromatography–tandem mass spectrometry method for the determination of mometasone furoate in human plasma: Application for a bioequivalence study in nasal spray formulations

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ABSTRACT

We developed and validated a sensitive, heart-cutting, two-dimensional liquid chromatography–tandem mass spectrometry (2D-LC–MS/MS) method to determine the concentration of mometasone furoate in human plasma after nasal spray administration. Isotopically labeled mometasone furoate-$^{13}C,d_6$ was used as an internal standard (IS). Plasma samples were prepared using a solid-phase extraction (SPE) method. With this 2D-LC strategy, the analytes were trapped in the first dimension (1D) column, and only judiciously selected portions of the 1D effluent were transferred to the second dimension (2D) column for further separation to obtain high-resolution information. MS/MS quantification was performed in positive ionization mode via multiple-reaction monitoring (MRM). This analytical method was fully validated according to related regulatory guidance, and the results showed that the method is robust and sensitive enough for pharmacokinetic investigation of mometasone furoate with satisfactory linearity from 0.25 to 30 pg mL^{-1}. This method was successfully applied to a bioequivalence (BE) study of mometasone furoate aqueous nasal sprays in healthy volunteers.

KEYWORDS

heart-cutting bidimensional liquid chromatography, LC–MS/MS, mometasone furoate, nasal spray, bioequivalence

1. INTRODUCTION

Allergic rhinitis (AR) is considered one of the most common chronic inflammatory disorders. Its prevalence ranges from 17% to 28.5% in Europe and from 6.9% to 36% in Asia and continues to increase every year [1]. AR is reported to affect approximately 25% and 40% of children and adults globally, respectively [2]. Common symptoms of AR are nasal congestion, rhinorrhea, sneezing, nasal itching, and postnasal drainage. This disorder can significantly impair the quality of life and poses a massive economic burden not only due to the costs of medical treatment and healthcare utilization but also due to absenteeism and reduced productivity at work [3]. Conventional treatments for AR include allergen avoidance and

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environmental control, immunotherapy, nasal turbinate surgery, and various pharmacotherapies, such as intranasal corticosteroids, antihistamines and/or oral and nasal decongestants [1, 3, 4]. Inhaled corticosteroids are recognized as the most effective agents for treating AR.

Mometasone furoate is a potent 17-heterocyclic corticosteroid formulated in an aqueous suspension for intranasal use with a metered-dose, manual pump nasal spray. Mometasone furoate nasal spray has been proven effective for the prophylaxis and treatment of seasonal allergic rhinitis, the treatment of perennial allergic rhinitis and nasal polyposis and as an adjunct to antibacterials for the treatment of acute rhinosinusitis [5]. Nasally administered drugs not only produce local effects but also potentially result in systemic exposure either by absorption into the systemic circulation through the nasal mucosa or absorption gastrointestinally after oral administration [6, 7].

To receive approval of an abbreviated new drug application (ANDA), a generic drug must be verified to be bioequivalent to the reference listed drug (RLD). As an increasing number of pharmaceutical companies conduct research on generic inhalation preparations, it is imperative to develop accurate, precise, selective, sensitive, and reproducible bioanalytical methods for bioequivalence (BE) studies. Unfortunately, mometasone furoate in nasal spray is poorly absorbed into the systemic circulation with minimal bioavailability (<1%) because of its lipophilicity and extensive first-pass metabolism in the liver [8, 9]. In addition, mometasone furoate is a glucocorticoid. Structurally speaking, it has no easily charged groups and is easily affected by other charged substances. To improve the response, the injection volume (50 µL) must be increased. The presence of interfering substances with similar structures, such as endogenous molecules, also affects the quantification of mometasone furoate. Therefore, the following problems must be addressed to develop a sensitive and rapid analytical method to quantify the amount of mometasone furoate in plasma. First, sufficient separation is required to reduce the interference from matrix components; second, the chromatographic peak must have a sufficient signal-to-noise ratio and good peak shape during bulk injection. The current reported lower limit of quantitation (LLOQ) is 0.5 pg mL^{-1} from 600 µL of plasma, with a method using UPLC separation, tandem quadrupole MS with UniSpray ionization, and selective solid-phase extraction (SPE) sample preparation [9]. However, we found that it was not sufficient to quantify the plasma concentration of the drug 48 h after nasal spray administration with a dose of 200 µg.

Two-dimensional liquid chromatography (2D-LC) enhances the separation power compared to conventional LC, particularly in complex samples, and has been widely recognized among researchers in the field of bioanalysis. This technology uses two solid phases with different polarities and selectivities to achieve significantly higher separation power and peak capacity [10, 11]. 2D-LC can be divided into two major types, and heart-cutting chromatography is one of them. Different from the continuous transfer of the effluent from the first dimension (1D) column to the second dimension (2D) column, only judiciously selected portions of the 1D effluent are transferred to the 2D column in heart-cutting chromatography. It is especially useful when high-resolution information is needed on a relatively small number of peaks in the 1D chromatogram and is mostly applied in the case of pronounced matrix interferences that could be substantial for the determination of selected analytes [12].

Therefore, the present study aimed to develop and validate a sensitive heart-cutting 2D-LC coupled to tandem mass spectrometer (2D-LC–MS/MS) method and optimization of sample preparation procedures for the quantification of mometasone furoate in human plasma and apply it to a BE study in Chinese health volunteers who received mometasone furoate through nasal spray administration.

2. EXPERIMENTAL

2.1. Chemicals and reagents

Mometasone furoate (99.9% purity) reference standard was purchased from the National Institutes for Food and Drug Control (Beijing, China), and its isotope labeled internal standard (IS) mometasone furoate-$^{13}C,d_6$ (IS, 99.7% purity) was purchased from BDG Synthesis (Lower Hutt, New Zealand). Ultrapure water was produced by a Millipore Milli-Q system (Billerica, MA, USA). Acetonitrile (HPLC grade) and methanol (HPLC grade) were purchased from Chron Chemicals (Chengdu, Sichuan, China), while sodium acetate trihydrate (mass spectrometry grade) was obtained from J.T. Baker (Phillipsburg, NJ, USA).

2.2. Instrumentation and analytical conditions

Heart-cutting 2D-LC–MS/MS analyses were carried out on a Shimadzu LC-30AD system (Shimadzu, Kyoto, Japan) composed of a quaternary pump, an autosampler, a column oven, and an API 6500 + triple quadrupole mass spectrometer (SCIEX, Framingham, MA, USA) equipped with an electrospray ionization source. Analyst v.1.7.2 software was used for data acquisition and analysis. Liquid chromatography separation was performed on a 2D-LC column.

The scheme of the heart-cutting 2D-LC system is demonstrated in Fig. 1. The system consisted of an autosampler, a switching valve (two-position microelectric actuator, Valco, Houston, TX, USA), and a quaternary pump. The 1D and 2D analytical columns were thermostated in the column manager of the UHPLC system. A volume of 50 µL of the extracting solution was injected into the developed system. At valve position 1, 75% solvent C and 25% solvent D served as the loading and washing solution and were delivered to the 1D column by a Shimadzu LC-30AD quaternary pump system at a flow rate of 0.4 mL min^{-1}. After 2.2 min, the switching valve was switched to position 2, and the sample was flushed with a mixture of 30% solvent C and 70% solvent D at a flow rate of 0.4 mL min^{-1}. The trapped target portion was directed to a 40 °C preheated reversed-phase (RP) analytical

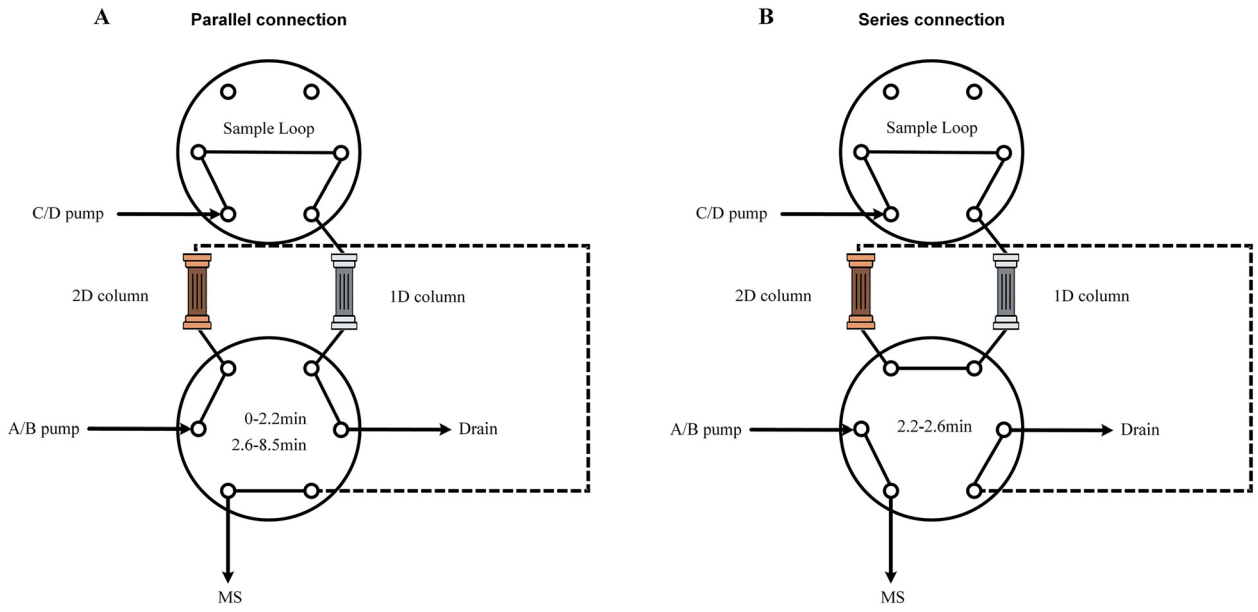

Fig. 1. Schematic of the heart-cutting 2D-LC system. At valve position 1 (A), the extracting solution was delivered to the 1D column, and the analytes were trapped. After 2.2 min, the switching valve was switched to position 2 (B), and the trapped target portion was directed to the 2D column for further separation. Then, the switching valve was switched back to position 1 (A) at 2.6 min to flush analytes into the MS

2D column. After that, the switching valve was switched back to position 1 at 2.6 min to flush analytes into MS with a gradient from 80% solvent A and 20% solvent B to 10% solvent A and 90% solvent B from 3.0 to 6.5 min, which was held for 1.5 min and then returned to the conditional composition (80% solvent A and 20% solvent B) before the next sample injection (Table 1). Because the two columns have completely different packings, the mometasone furoate and the interfering compounds will undergo different separation modes, resulting in more efficient separation. In addition, by using different separation modes, 2D-LC significantly reduces the analysis time and improves peak shape compared with the classical LC-MS/MS method, which requires a sufficient number of plates and a long analysis time (more than 20 min) to obtain a beautiful peak shape, acceptable signal-to-noise ratio and sufficient resolution for large injection volumes (50 µL).

Mass spectrometric detection was performed using an atmospheric pressure chemical ionization ion source in positive ionization mode. The source temperature (TEM) was 450 °C, with an ion spray voltage (IS) of 5,500 V, declustering potential (DP) of 75 V, ion source gas (GS1) setting of 60 psi, drying gas (GS2) setting of 70 psi, collision gas (CAD) setting of 8 psi and curtain gas (CUR) setting of 30 psi. The collision energy (CE) was optimized at 22 V for both mometasone furoate and the IS. Multiple-reaction monitoring (MRM) was employed for quantification with ion pairs including m/z 543.1→m/z 507.1 and

Table 1. The gradient programs of the first and second dimensions with two six-port valve positions and analysis mode

Gradient program of 1st dimension			Gradient program of 2nd dimension			Timetable of valves		Mode
Time (min)	C%	D%	Time (min)	A%	B%	Time (min)	Valve	
0.00	75	25	0.00	80	20	0.00	Position 1	trapping
0.10	75	25						
1.00	30	70						
						2.20	Position 2	
						2.60	Position 1	analysis
			3.00	80	20			
3.50	30	70						
3.51	10	90						
6.00	10	90						
6.01	75	25						
			6.50	10	90			
			8.00	10	90			
			8.01	80	20			
8.50	System controller stop		8.50	System controller stop				

m/z 550.1→m/z 514.0 for mometasone furoate and IS, respectively. A dwell time of 0.2 s was used for both compound transitions.

2.3. Preparation of standard solutions

Stock solutions of mometasone furoate and the IS at 200.0 μg mL^{-1} concentration were prepared by dissolving the accurately weighed reference standards in methanol. Working solutions of mometasone furoate and the IS and quality control (QC) solutions were prepared by serially diluting the stock solutions with methanol and water (50:50) and stored at −20 °C. The final concentrations of the working solutions were 5, 10, 30, 100, 300, 480, and 600 pg mL^{-1} for mometasone furoate. The serial concentrations of QCs were 15 pg mL^{-1}, 60 pg mL^{-1}, 200 pg mL^{-1} and 450 pg mL^{-1}. The final concentration of the IS was prepared at 300 pg mL^{-1} for sample extraction.

2.4. Preparation of calibration and quality control samples

All working solutions were thawed at room temperature before use. Seven calibration standards were prepared by spiking appropriate amounts of the working solution of mometasone furoate in blank human EDTA-K_2 plasma obtained from healthy volunteers. The concentrations of the mometasone furoate in plasma were 0.25, 0.5, 1.5, 5, 15, 24 and 30 pg mL^{-1}. The LLOQ was 0.25 pg mL^{-1}, and the upper limit of quantification (ULOQ) was 30 pg mL^{-1}. QC samples were prepared in a similar manner, and the very low (LLOQ), low (LQC), middle (MQC) and high (HQC) concentrations were 0.25, 0.75, 10 and 22.5 pg mL^{-1}, respectively.

2.5. Sample preparation

An SPE procedure was used for sample pretreatment. Plasma samples (500 μL) were spiked with 40 μL of IS working solution (300 pg mL^{-1}) and diluted with 400 μL of 30% methanol followed by reversed-phase SPE purification using 30 mg Cleanert PEP-SPE cartridges that were conditioned and equilibrated with methanol and water. After loading, the samples were washed with water followed by 50% methanol twice and then eluted using acetonitrile. The eluent was dried under a stream of nitrogen at 40 °C. Dried samples were then reconstituted with 150 μL of 30% methanol for heart-cutting 2D-LC–MS/MS analysis.

2.6. Method validation

Method validation was carried out according to the related guidelines for bioanalytical validation issued by the US Food and Drug Administration (FDA) and National Medical Products Administration (NMPA) of China [13, 14].

2.6.1. Selectivity. The selectivity of the method was evaluated by analyzing human blank plasma samples from twelve different donors, including lipemic and hemolyzed plasma samples. The samples were compared to those containing mometasone furoate at the LLOQ at 0.25 ng mL^{-1}. The detected responses attributable to interfering components should not represent more than 20% of the analyte response at the LLOQ and not more than 5% of the IS response in the LLOQ sample for each matrix.

2.6.2. Linearity and sensitivity. The linearity of the method was assessed by constructing seven-point calibration curves using standard plasma samples in duplicate for each batch. The evaluated concentration ranged from 0.25 to 30 pg mL^{-1}. A least square linear regression model weighted by $1/x^2$ was used to fit the peak area ratio of each analyte to IS versus the nominal concentration of calibration standards. Acceptance criteria were that nonzero calibrators should fall within ±15% of nominal concentrations (±20% for LLOQ) and 75% as well as a minimum of six nonzero calibrator standards should meet the above criteria in each validation run. There should be at least one parallel sample acceptable for each nominal concentration. The correlation coefficient (r^2) was not less than 0.98. The LLOQ was considered the lowest concentration of the calibration curve, whose precision was 20% and accuracy was within 80~120%, as determined by six replicated analyses. The peak signal-to-noise ratio of the LLOQ samples was required to be greater than 5.

2.6.3. Accuracy and precision. The intrabatch accuracy and precision of the method were evaluated by assaying six replicates of QCs at four levels (LLOQ~HQC) within a batch, whereas interbatch accuracy and precision were evaluated on three separate days. The accuracy should be acceptable when the mean values were within ±15% of the nominal values, except ±20% at the LLOQ. The precision should be acceptable when the percentage of the relative standard deviation (%RSD) at each concentration level did not exceed 15%, except 20% at the LLOQ.

2.6.4. Extraction recovery and matrix effect. The extraction recovery was evaluated by analyzing six replicates of samples at the LQC, MQC, and HQC levels. After blank matrix extraction, the determined amounts of analytes and IS were spiked, followed by measuring the mean peak area response of unextracted QC samples. The samples prepared by adding analytes and IS to blank plasma prior to extraction were used to obtain the mean peak area response of the extracted QC samples. Extraction recovery at each QC concentration was calculated as the mean peak area ratio of extracted to unextracted QC samples. Acceptance criteria were that the %RSD of the recovery for each QC level did not exceed 15%. For matrix effect evaluation, plasma samples from six different donors containing LQC and HQC concentrations of mometasone furoate and a working concentration of the IS and analyte dissolved in mobile phase at the same QC levels without biological matrix were prepared. The matrix effect was evaluated by calculating the IS normalized peak area ratio with and without biological matrix samples. The %RSD should be less than 15%.

2.6.5. Carryover. Carryover was evaluated by injecting a blank sample after a ULOQ sample of the calibration standards. Acceptance criteria were that peak areas of analytes in

the double blank samples were less than 20% of LLOQ and less than 5% for the IS.

2.6.6. Dilution integrity. Dilution QC samples were prepared in six replicates with a concentration of mometasone furoate at two times the ULOQ at 60 pg mL^{-1} and were diluted 5-fold with blank human plasma. The acceptance criteria were that the test values fall within ±15% of nominal concentrations and that the %RSD between six parallel samples at the same concentration was less than 15%.

2.6.7. Stability. Short-term, long-term, extracted sample, and freeze/thaw stability were evaluated with five replicates of plasma samples at LQC and HQC levels in each batch. Specifically, short-term stability was performed by putting LQC and HQC plasma samples aside under white light at room temperature (23 °C) for 21 h. For long-term stability, LQC and HQC plasma samples were prepared and stored at −30 °C ∼ −10 °C for 67 days or −80 °C ∼ −60 °C for 158 days. Extracted sample stability was evaluated by storing processed samples in the autosampler at 10 °C for 194 h. For freeze/thaw stability, prepared plasma samples were stored at −30 °C ∼ −10 °C or −80 °C ∼ −60 °C for 24 h and then thawed at room temperature. Four freeze/thaw cycles were carried out. The analytes were considered stable when 85∼115% of the initial concentrations could be identified, and the %RSD between parallel samples was not more than 15%.

2.7. Incurred sample reanalysis (ISR)

Since this analytical method was applied to a BE study, it must demonstrate assay reproducibility by using incurred samples, commonly referred to as ISR [15]. Reanalysis of incurred samples could provide information on the robustness and reliability of the method. Samples at peak levels (around C_{max}) and from the elimination phases were selected. ISR is considered acceptable if the differences in concentrations between the original and reanalysis values from 67% of the repeated samples are less than 20% of their means.

2.8. Application to a BE study

The validated method was used to determine the concentration of mometasone furoate in human plasma and to investigate the BE of two generic mometasone furoate aqueous nasal sprays (50 μg/spray) produced by Sichuan Purity Pharmaceutical Co., Ltd. and NASONEX® (50 μg/spray) produced by Merck & Co., Inc., test and reference, respectively. A randomized, single-dose, open-label, three-sequence, three-period, crossover BE study was designed on healthy adult Chinese volunteers under fasting conditions. The washout period between dosing was not less than one week.

In this study, twelve healthy volunteers were enrolled and randomly divided into three groups. All the subjects were informed of the aim and risk involved in the study, and written consent was obtained. The inclusion and exclusion criteria for subject screening were based on age, body mass index, general physical examination, electrocardiography, and laboratory testing, including hematologic, biochemical and urinalysis. The study protocol was reviewed and approved by the Ethics Committee of Beijing Unicare Eye ENT Hospital.

The administration of the drug was performed in a room isolated from external contamination, and subjects had adequately cleaned their nostrils before administration. All subjects received two sprays in each nostril alternately, corresponding to 200 μg of mometasone furoate. In each period of the study, twenty-one blood samples (4 mL) were collected into EDTA-K_2 tubes from each subject according to the following time schedule: predose (−1 h) and at 5, 10, 15, 20, 30, 40, and 50 min, as well as at 1, 1.25, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 48, and 72 h after drug administration. The whole blood was immediately centrifuged at 1,700×*g* for 10 min at 4 °C, and the upper plasma was transferred to clean tubes. All plasma samples were frozen and stored at temperatures below −60 °C before further analysis.

The plasma concentration-time profiles and pharmacokinetic parameters (observed maximum plasma concentration, C_{max}; sampling time of the maximum plasma concentration, T_{max}; terminal elimination half-life time, $T_{1/2}$; area under plasma concentration/time curve until the last quantifiable value, AUC_{last}; area under plasma concentration/time curve extrapolated to infinity, AUC_{inf}) obtained from the individual subjects were derived by non-compartmental analysis using Phoenix WinNonlin Version 8.1 (Certara, Princeton, USA).

3. RESULTS AND DISCUSSION

3.1. Development of the heart-cutting 2D-LC–MS/MS method

Nasally administered drugs, due to less systemic absorption than other conventional formulations, provide a considerable technical difficulty in measuring plasma concentrations. A heart-cutting 2D-LC–MS/MS analytical method for mometasone furoate in human plasma was developed to meet extremely low quantitative requirements. The CAPCELL PAK C_{18} MG II column (50 × 2.0 mm I.D.) was used as a 1D column to trap the target compounds with solvent C (ultrapure water) and solvent D (methanol) as the 1D mobile phase. Only a single fraction of 1D effluent captured from a particular 1D peak was transferred to the CAPCELL CORE C_{18} column (150 × 2.1 mm I.D.) as the 2D column with solvent A (ultrapure water with 1 mM sodium acetate) and solvent B (methanol) as the 2D mobile phase for further separation to obtain high-resolution information. The analyte and IS were eluted at approximately 8 min.

During the optimization of chromatographic separation and selectivity, several parameters were investigated, including the mobile phase composition, flow rate, column switching times, gradient steepness, and the stationary phase of analytical columns for both dimensions. Methanol was selected as the organic mobile phase instead of acetonitrile

for the final method because of the higher signal intensity for the compounds. The interaction of the parent molecule with different atoms or molecules to form adduct ions, such as $[M + H]^+$, $[M + NH_4]^+$, $[M + Li]^+$ and $[M + Na]^+$, was compared, and the addition of sodium acetate in the 2D mobile phase was found to be important in improving the signal intensities in positive ion mode. Finally, the sodium adducts $[M + Na]^+$, with *m/z* 543.1 and *m/z* 550.1 for mometasone furoate and the IS, respectively, were selected as precursor ions. The selected product ions were *m/z* 507.1 and *m/z* 514.0 for mometasone furoate and the IS, respectively.

3.2. Sample extraction

Samples for analysis were prepared according to a previously published method with slight modification [9]. Due to the large plasma sample volume and the high plasma protein binding of mometasone furoate, it is necessary to disrupt protein binding prior to extraction to ensure maximum compound recovery. Typically, this process is carried out using pure methanol. However, as the sample is retained on the sorbent by a hydrophobic mechanism, it is important not to load the sample under conditions with too much organic solvent, as this will result in compound breakthrough. Therefore, plasma samples were pretreated with 30% methanol to precipitate proteins. After centrifugation, the supernatant was loaded onto the cartridge to be purified. During method development, up to 50% methanol could be applied to the SPE cartridge without causing the compounds to be eluted. Thus, the wash solution used to remove matrix interferences was 50% methanol. Pure acetonitrile was applied to elute the compounds of interest to ensure maximum recovery. The recovery of this extraction method was over 80%, and most of the matrix interferences were effectively removed.

3.3. Method validation

3.3.1. Selectivity. Drug-free plasma samples from twelve donors were assayed. Representative chromatograms of blank plasma and plasma spiked with mometasone furoate and the IS are shown in Fig. 2. The results showed that the method developed is highly selective for mometasone furoate and the IS. There were no significant interferences from endogenous compounds at the retention times for the analyte and IS in blank plasma samples.

3.3.2. Sensitivity and calibration curve. The mometasone furoate calibration curves proved to be linear, ranging from 0.25 to 30 pg mL^{-1}, with a regression coefficient higher than 0.99. A typical standard curve was $y = 0.08153x + 0.0005884$. The residuals showed no tendency of variation with concentration. The obtained LLOQ was 0.25 pg mL^{-1}, with a precision of 5.7% and 0.6% in terms of %RSD and accuracy, respectively.

3.3.3. Accuracy and precision. The intra- and interday accuracy and precision were calculated by analyzing six replicates of QCs at four levels on three separate days. The results are shown in Table 2. The intra- and interday biases for mometasone furoate were less than 4.8% and 3.3%, respectively. The %RSD of intra- and interday samples are

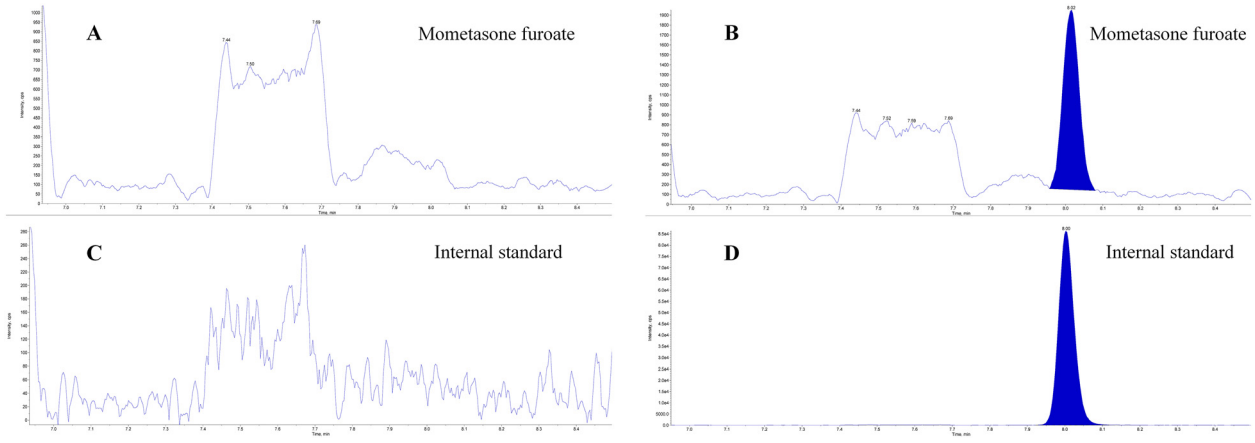

Fig. 2. Representative chromatograms of mometasone furoate and internal standard in blank plasma (A/C) and LLOQ samples (B/D)

Table 2. Intra- and interday precision and accuracy ($n = 6$)

Analyte	Concentration	Intraday			Interday		
		Mean ± SD	Bias (%)	CV (%)	Mean ± SD	Bias (%)	CV (%)
Mometasone furoate	LLOQ (0.25 pg mL^{-1})	0.26 ± 0.01	4.1	5.6	0.25 ± 0.02	0.4	7.9
	LQC (0.75 pg mL^{-1})	0.73 ± 0.03	3.2	4.4	0.73 ± 0.04	3.3	6.0
	MQC (10 pg mL^{-1})	9.52 ± 0.66	4.8	6.9	9.74 ± 0.55	2.6	5.7
	HQC (22.5 pg mL^{-1})	23.5 ± 1.43	4.4	6.1	22.6 ± 1.31	0.5	5.8

no more than 9.9% and 7.9% for mometasone furoate. These data indicated reproducible results and that the assay was accurate and reliable.

3.3.4. Extraction recovery and matrix effect. The extraction recovery was evaluated at three QC levels. The extraction recovery of mometasone furoate ranged from 80.9% to 83.6%. The %RSD did not exceed 8.8%. The matrix effect was evaluated by the peak area ratio with the matrix/peak area ratio without a matrix at two QC levels. The results are shown in Table 3. The mean matrix factors were 0.99 and 0.98 for mometasone furoate. The %RSD values are within 7.6%. Therefore, the extraction recovery and matrix effect were acceptable at each QC level for mometasone furoate.

3.3.5. Carryover. The peak areas of mometasone furoate in blank samples injected following ULOQ were compared with its LLOQ samples. In ten tested batches, there was no significant carryover. Similarly, there was no carryover for the IS.

3.3.6. Dilution integrity. Dilution integrity was determined by six replicates with twice the concentration of ULOQ diluted with blank plasma. The mean accuracy bias was 2.3% for mometasone furoate. The %RSD was 5.3%. Therefore, the developed method can accurately quantify the analyte in human plasma samples subjected to a maximum of 5-fold dilution.

3.3.7. Stability. The results of the stability experiments are shown in Table 4. Mometasone furoate plasma samples were stable for up to 21 h at 23 °C, for 67 days at −30 °C and for 158 days at −80 °C for 194 h after extraction in the autosampler and after four complete freeze/thaw cycles on consecutive days. The accuracy biases at the LQC and HQC levels were within 7.1%, and the %RSD was within 6.6% for each batch. All human plasma samples obtained from clinical trials were regularly processed at room temperature under white light based on these data. The clinical samples will be analyzed within two weeks after collection. The processed samples were injected for analysis within 24 h after transfer to the autosampler.

3.4. Application to a clinical BE study

The validated method was applied to determine the presence of mometasone furoate in plasma samples and calculate the pharmacokinetic parameters in a BE study among two generic mometasone furoate aqueous nasal sprays (50 μg/spray) produced by Sichuan Purity Pharmaceutical Co., Ltd. and NASONEX® (50 μg/spray) produced by Merck & Co., Inc., which were the test and reference samples, respectively, in twelve healthy volunteers. After that, 82 (10.8%) of the 756 samples were selected for reanalysis, of which 68 samples (82.9%) passed the ISR criteria.

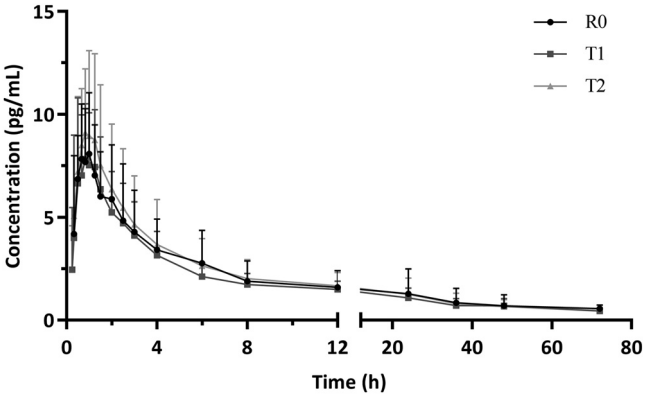

Fig. 3. Geometric mean (standard deviation) plasma concentration-time curves of mometasone furoate for the reference and two test formulations after the administration of nasal spray in healthy volunteers (n = 12). R0, reference formulation; T1 and T2, two test formulations

Table 3. Extraction recovery and matrix effect (n = 6)

Analyte	Concentration	Extraction recovery		Matrix effect	
		Mean ± SD (%)	%RSD	Mean ± SD	%RSD
Mometasone furoate	LQC (0.75 pg mL^{-1})	83.3 ± 6.0	7.2	0.99 ± 0.08	7.6
	MQC (10 pg mL^{-1})	83.6 ± 2.9	3.5	NA	NA
	HQC (22.5 pg mL^{-1})	80.9 ± 7.1	8.8	0.98 ± 0.02	1.7

Table 4. Short-term stability, long-term stability, processed sample stability, and freeze/thaw stability (n = 5)

Analyte	Validation	Condition	LQC (0.75 pg mL^{-1})		HQC (22.5 pg mL^{-1})	
			Mean ± SD	%RSD	Mean ± SD	%RSD
Mometasone furoate	short term	21 h under white light at 23 °C	0.71 ± 0.03	4.3	21.7 ± 0.3	1.6
	long term	67 days at −30 ~ −10 °C	0.80 ± 0.03	3.6	22.4 ± 0.9	4.1
		158 days at −80 ~ −60 °C	0.73 ± 0.03	4.3	21.9 ± 0.4	1.7
	processed sample	194 h at 10 °C	0.70 ± 0.03	4.0	22.2 ± 0.5	2.3
	freeze/thaw	4 cycles at −30 ~ −10 °C	0.74 ± 0.05	6.6	22.4 ± 0.6	2.6
		4 cycles at −80 ~ −60 °C	0.70 ± 0.03	3.8	22.0 ± 0.4	1.8

Table 5. Statistics of pharmacokinetic parameters of mometasone furoate for reference and test formulations after nasal administration in healthy volunteers (n = 12)

Formulation	Statistic	C_{max} (pg mL^{-1})	T_{max} (h)	$T_{1/2}$ (h)	AUC_{last} (h*pg mL^{-1})	AUC_{inf} (h*pg mL^{-1})
Reference (R0)	Mean ± SD	9.76 ± 2.57	0.97 ± 0.37	19.04 ± 11.44	89.14 ± 44.09	103.18 ± 49.89
	%RSD	26.3	38.1	60.1	49.5	48.4
Test (T1)	Mean ± SD	9.30 ± 2.72	0.79 ± 0.31	24.78 ± 16.82	76.96 ± 23.79	95.33 ± 30.98
	%RSD	29.2	39.2	67.9	30.9	32.5
Test (T2)	Mean ± SD	11.15 ± 4.43	0.90 ± 0.28	22.00 ± 11.46	92.01 ± 32.32	106.80 ± 36.09
	%RSD	39.7	31.1	52.1	35.1	33.8

C_{max}, observed maximum plasma concentration; T_{max}, sampling time of the maximum plasma concentration; $T_{1/2}$, terminal elimination half-life time; AUC_{last}, area under plasma concentration/time curve until the last quantifiable value; AUC_{inf}, area under plasma concentration/time curve extrapolated to infinity.

Table 6. The statistical results of the bioequivalence study of mometasone furoate for reference and test formulations

Formulation	Parameters	%Ratio to reference	Lower 90% CI	Upper 90% CI
T1	C_{max} (pg mL^{-1})	94.84	80.51	111.71
	AUC_{last} (h*pg mL^{-1})	92.28	71.41	119.26
	AUC_{inf} (h*pg mL^{-1})	97.85	77.14	124.12
T2	C_{max} (pg mL^{-1})	110.91	94.16	130.64
	AUC_{last} (h*pg mL^{-1})	107.77	83.39	139.28
	AUC_{inf} (h*pg mL^{-1})	108.15	85.26	137.19

C_{max}, observed maximum plasma concentration; AUC_{last}, area under the plasma concentration/time curve until the last quantifiable value; AUC_{inf}, area under the plasma concentration/time curve extrapolated to infinity.

The mean concentration profiles obtained for the reference (R0) and two test (T1, T2) formulations are shown in Fig. 3. The extrapolated area of plasma concentration versus time was less than 20% of AUC_{inf}, demonstrating the suitability of the method and experimental design. Mometasone furoate was detected in plasma 10 min after administration in most of the subjects, demonstrating rapid nasal absorption. The main pharmacokinetic parameters for the reference and test formulations are shown in Table 5. For both test formulations, the ratios of the geometric means for the logarithmic transformations of C_{max}, AUC_{last} and AUC_{inf} were within the BE limit of 80%–125%, as shown in Table 6. However, the 90% CI of the C_{max}, AUC_{last} and AUC_{inf} were out of the boundary, which indicates that the two test formulations did not pass the acceptance criteria for the BE study. This may be because the intrasubject variability of the two test formulations was large (>30%), and the number of samples enrolled was small (this is only a prestudy). A clinical trial with a larger sample size is required to verify the BE of the two generic mometasone furoate aqueous nasal sprays with the reference formulation.

4. CONCLUSION

We successfully developed an analytical method for the quantification of mometasone furoate with greatly enhanced peak resolution in human plasma. To the best of our knowledge, this is the first 2D-LC–MS/MS method concerning the quantification of mometasone furoate in human plasma that has reached an LLOQ of 0.25 pg mL^{-1}. SPE was used for sample preparation. This method could effectively remove the matrix interferences and improve the extraction recovery. The method was fully validated according to related regulatory guidance, and the results showed that the method is robust and meets the requirements of the pharmacokinetic investigation after the nasal spray administration of therapeutic doses. This sensitive and selective method was successfully applied to a BE study of mometasone furoate aqueous nasal sprays in healthy volunteers.

Declaration of competing interests: Leting Yang, Huiru Xie and Hui Liu are employees of Chengdu Finelyse Pharmaceutical Technology Co., Ltd. Gangmin He and Wenjing Zhong are employees of Sichuan Purity Pharmaceutical Co., Ltd. The other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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