Journal of Chromatography B, 879 (2011) 236–242

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Budesonide quantification by HPLC coupled to atmospheric pressure photoionization (APPI) tandem mass spectrometry. Application to a comparative systemic bioavailability of two budesonide formulations in healthy volunteers

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ARTICLE INFO

Article history:
Received 2 June 2010
Accepted 5 December 2010
Available online 13 December 2010

Keywords:
Budesonide
Nasal spray
Allergy
LC–MS/MS
Bioavailability
Pharmacokinetics

ABSTRACT

In the present study, a novel, fast, sensitive and robust method to quantify budesonide in human plasma using 3-keto-desogestrel as the internal standard (IS) is described. The analyte and the IS were extracted from human plasma by liquid–liquid extraction (LLE) using ether. Extracted samples were analyzed by high performance liquid chromatography coupled to Atmospheric pressure photoionization tandem mass spectrometry (HPLC–APPI-MS/MS). Chromatography was performed isocratically on a C18, 5 μm analytical column. The temperature of the autosampler was kept at 6 °C and the run time was 4.00 min. A linear calibration curve over the range 7.5–1000 pg ml^{-1} was obtained and the lowest concentration quantified was 7.5 pg ml^{-1}, demonstrating acceptable accuracy and precision. This analytical method was applied in a relative bioavailability study in order to compare a test budesonide 64 μg/dose nasal spray formulation vs. a reference 64 μg/dose nasal spray formulation (Budecort Aqua) in 48 volunteers of both sexes. The study was conducted in an open randomized two-period crossover design and with a one-week washout period. Plasma samples were obtained over a 14 h interval. Since the 90% CI for both C_{max}, AUC_{last} and $AUC_{0\text{-}inf}$ were within the 80–125% interval proposed by the Food and Drug Administration and ANVISA, it was concluded that budesonide 64 μg/dose nasal spray was bioequivalent to Budecort Acqua® 64 μg/dose nasal spray, according to both the rate and extent of absorption.

1. Introduction

Allergic rhinitis is usually treated with oral antihistamines or nasal steroids [1]. Topical administration of corticosteroids is highly effective, can reduce the total dose of corticosteroid required to treat the patient and minimize side effects [2,3]. Inhaled (ICS) and intranasal corticosteroids (INCS) play a pivotal role in the treatment of asthma and allergic rhinitis. INCS represents the single most effective class of medicines for allergic rhinitis and improves all nasal symptoms, including nasal congestion, rhinorrhea, itching, and sneezing [3].

Currently there is the second generation of these compounds available for rhinitis treatment: beclomethasone dipropionate, budesonide, flunisolide, fluticasone propionate, mometasone furoate, and triamcinolone acetonide [4]. The efficacy of INCS depends on the topical activity of the drug that reaches the lungs or nasal mucosa respectively, while the adverse effects mainly depend on oral deposition and on systemic activity. The drug's systemic activity depends on the amount absorbed by both the gastrointestinal tract and the lungs. The amount of ICS delivered to the lungs depends on inhalation technique, the type of inhaler used, the solvent, the propellant, the size of delivered particle, and on whether or not spacers are used [5]. The goal of topic steroids design is to achieve a high ratio of topical to systemic activity [6].

Budesonide is a non-halogenated glucocorticosteroid second generation intranasal corticosteroids, designated chemically as [[2]11-beta, 16-alpha, 17,21-tetrahydroxypregna-1,4-diene-3,20-

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doi:10.1016/j.jchromb.2010.12.003

dione cyclic 16,17-acetal with butyraldehyde], and is provided as the mixture of two epimers (22R and 22S). Its empirical formula and its molecular weight are $C_{25}H_{34}O_6$ and 430.5, respectively. Budesonide suffer extensive first-pass metabolism in the liver originating metabolites of minimal activity. The systemic bioavailability of budesonide is considered low after oral (6–13%) and topical (15–17%) administration and its half-life in human plasma after nasal inhalation is 2.9 ± 0.4 h [7–9].

Recently liquid chromatography coupled to electrospray/atmospheric pressure chemical ionization (ESI/APCI) mass spectrometry (LC/MS/MS) has been applied to the quantification of budesonide in biological fluids [10–15]. This technique provides good sensitivity and selectivity and eliminates laborious sample preparation procedures that are necessary for methodologies like RIA [16–18] and GC/MS [18]. However, most of these methods are not sufficiently sensitive to quantify the low-pg ml^{-1} levels of corticosteroids in bodily fluids that may be achieved in various therapeutic strategies.

The main objective of this study was to develop a fast, sensitive and robust method to quantify budesonide in human plasma using 3-keto-desogestrel as the internal standard (IS). This method was applied to assess the bioequivalence of two Brazilian budesonide 64 μg nasal spray formulations in healthy volunteers after dosing four sprays together of test and reference.

2. Experimental

2.1. Chemicals and reagents

Budesonide was provided by USP, USA, lot number FOE302. 3-Keto-desogestrel, the internal standard, was obtained from Synfine, USA, lot number A-1196-199. Acetonitrile, methanol (HPLC grade) and toluene were purchased from Carlo Erba, Italy. Ethyl ether and hexane were obtained from Mallinckrodt (USA). Blank human blood was collected from healthy, drug-free volunteers. Plasma was obtained by centrifugation of blood treated with the anticoagulant EDTA-Lithium. Pooled plasma was prepared and stored at −70 °C until needed.

2.2. Calibration standards and quality control

Stock solutions of budesonide and 3-keto-desogestrel were prepared in 2.5 mM ammonia diluted in methanol/water (80/20, v/v) at concentrations ranging from 0.49 to 0.58 mg ml^{-1}. Work solutions were prepared by serial dilutions of the stock solutions in methanol–water (50:50, v/v). Calibration curves for budesonide were prepared by spiking blank plasma with work solutions to obtain the final concentrations of 7.5, 25, 50, 200, 400, 800 and 1000 pg ml^{-1}. The analyses were carried out in duplicate for each concentration.

Quality control samples were prepared in blank plasma at concentrations of 15 (quality control at low level, QCL), 375 (quality control at medium level, QCM) and 750 pg ml^{-1} (quality control at high level, QCH). The spiked plasma samples (standards and quality controls) were extracted in each batch of sample analysis.

2.3. Sample preparation

All frozen human plasma samples were initially thawed at room temperature. A 500 μl volume of sample human plasma was introduced into a glass tube containing 100 μl of ammonia at 8.8 M followed by 25 μl of the IS solution (10 ng ml^{-1} of 3-keto-desogestrel in methanol–water (50:50, v/v)). After vortex mixing for 5 s, ether was added (2 ml) to all the tubes and extraction was performed by vortex mixing for 3 min. Samples were frozen for 4 min and the organic solvent was withdrawn; then samples were dried under nitrogen flux at 40 °C for 10 min. Finally, the extract was resuspended in 100 μl of methanol/water (80/20, v/v) + 2.5 mM of ammonium hydroxide.

2.4. Chromatographic conditions

Extracted samples were injected (20 μl) into a Phenomenex Gemini® C18, 5 μm analytical column, (50 × 4.6 mm i.d.) preceded by a Phenomenex Gemini® C18, 5 μm (4 × 30 mm i.d.) guard-column operating at room temperature. The compounds were eluted by pumping the mobile phase (methanol/water (70/30, v/v) at a flow-rate of 0.8 ml min^{-1} and toluene as dopant at a flow-rate of 0.15 ml min^{-1}. Under these conditions a back-pressure values of 150 bar were observed. The temperature of the autosampler was kept at 6 °C and the run time was 4.00 min.

2.5. Mass spectrometer conditions

MS detection was performed in the positive APPI mode on an Applied Biosystems Sciex API 5000 tandem mass spectrometer (Concord, Ontario, Canada) equipped with a Sciex PhotoSpray source. Interface parameters and the dopant flow rate were optimized during infusion of the budesonide and 3-keto-desogestrel through the interface connected with the LC system and were as follows: declustering potential −180 V, heater temperature 380 °C, ion transfer voltage 900 V, curtain gas 20.0, collision gas 10.0 and dwell time of 0.15 s for each transition. Tandem mass spectrometric analysis was performed using nitrogen as collision gas and collision energy at −22 eV. Toluene was pumped into the MS detector at a flow rate of 0.16 ml min^{-1}. Selected reaction monitoring (SRM) was used for the detection of both budesonide and 3-keto-desogestrel. The m/z 431.1 > 323.2 transition was monitored for budesonide (Fig. 1a) and the m/z 325.3 > 257.2 transition for 3-keto-desogestrel (Fig. 1b). Data acquisition and analysis were performed using the software Analyst (version 1.4.2).

2.6. Validation

All sample analysis was carried out in a GLP-compliant manner and in accordance to the current Brazilian regulatory agency requirements, National Health Surveillance Agency (ANVISA), and the US Food and Drug Administration Bioanalytical method validation guidance.

2.6.1. Linearity

The standard calibration curves were constructed using the peak area ratios of budesonide and IS vs. budesonide nominal concentrations of the eight plasma standards (7.5, 25, 50, 200, 400, 600, 800, 1000 and pg ml^{-1}) in duplicate. Linear least-square regression analysis, with weighting factor of $1/x$, was performed to assess the linearity. In addition, a blank (non-spiked sample) and a zero plasma sample (only spiked with IS) were run to demonstrate the absence of interferences.

2.6.2. Recovery

The budesonide recovery was evaluated by calculating the mean of the response of five replicates of each QCL (15 pg ml^{-1}), QCM (375 pg ml^{-1}), QCH (750 pg ml^{-1}) and LLOQ (7.5 pg ml^{-1}) concentration and dividing the extracted sample mean response by the unextracted (spiked blank plasma extract) sample mean of the corresponding concentration. To perform the comparison with the unextracted samples, budesonide spiked plasma residues were obtained after performing the full extraction process in blank plasma samples, was done in order to eliminate matrix effects from calculations, giving a true recovery. Since the extraction method includes an online extraction step, the unextracted samples were

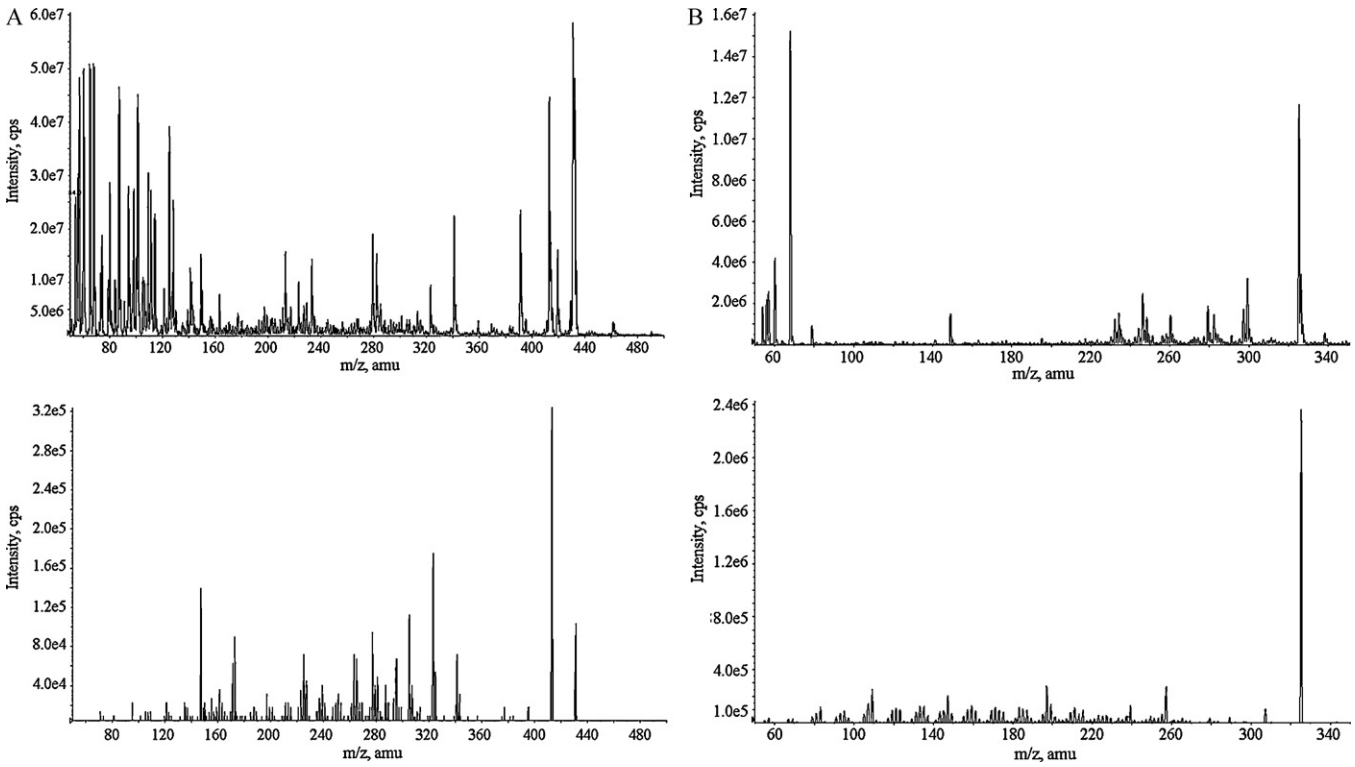

Fig. 1. Full-scan mass spectra (upper traces) and product ion spectra (lower traces) of (A) budesonide and (B) 3-keto-desogestrel.

injected directly in the mass spectrometer, bypassing the online extraction cartridge.

2.6.3. Precision and accuracy

Precision and accuracy of the method were evaluated using three different batches of quality control samples at concentrations of 15, 375 and 750 pg ml^{-1} of budesonide, also including the lowest limit of quantification, LLOQ, 7.5 pg ml^{-1}. Each batch was quantified with a specific calibration curve. For intra-batch assay precision and accuracy, six replicates of quality control samples at the three concentration levels were assayed all at once within a day to obtain CV (%) and accuracy values. The inter-batch assay precision and accuracy were determined by analyzing mean values of quality control samples from three plasma batches, yielding the corresponding inter-batches CV (%) and accuracy values.

2.6.4. Sensitivity

The lower limit of quantification (LLOQ) was determined for budesonide, based on two criteria: (a) the analyte response at LLOQ had to be at least five times baseline noise; (b) the analyte response at LLOQ being determined with sufficient precision and accuracy, i.e., precision of 20% and accuracy of 80–120%. Calculations were based on eight replicates of three blank plasma batches.

2.7. Ion suppression

Ion suppression is the effect of matrix constituents on MS signal, mainly because the presence of a non volatile compounds presenting generally a decrease of ionization ratio. The mechanism involves the decrease of small droplets production during desolvation step, impairing the formation of ions in gas phase. It is commonly observed when electrospray ion source is used, but appears less extensively in APCI or APPI [19].

A procedure to assess the effect of ion suppression on MS/MS was performed. The experimental set-up consisted of an infusion pump connected to the system by a "zero volume tee" before the spliter and the HPLC system pumping the mobile phase, which was the same as that used in the routine analysis of budesonide. The infusion pump was set to transfer a mixture of analyte and IS prepared in mobile phase to HPLC flow, before the introduction in mass spectrometer, without a significant interference in flow rate or solvent composition of mobile phase. The reconstituted extract was injected into the HPLC system while the standard mixture was being infused. The ion suppression was evaluated in three different matrices: normal, hyperlipemic and hemolyzed plasma samples. The samples of human pooled blank plasma were extracted following the full extraction procedure. In this system, any ion suppression would be observed as a depression of the MS signal.

2.8. Stability

All stability tests of budesonide were performed in human plasma or in the stock and working solutions using five replicates of plasma spiked with budesonide at 15 and 750 pg ml^{-1} (low and high QCs).

2.8.1. Freeze–thaw stability

Plasma samples were subjected to three freeze–thaw cycles of −20 °C during 24 h. In each cycle, frozen samples were allowed to thaw at controlled ambient temperature (22 °C) and were subsequently refrozen for 24 h. Aliquots of all samples were quantified at the end of the third freeze–thaw cycle. Analysis of budesonide concentrations were compared to fresh samples not subjected to the freeze–thaw cycles and expressed in percentage of degradation.

2.8.2. Short term storage stability

All samples were thawed at room temperature (22 °C) and remained on the bench top for a time exceeding the maximum period of time expected for routine sample preparation (assessed

for 17 h). Samples were extracted and further compared to fresh prepared ones at equivalent concentration.

2.8.3. Post processing stability

The post processing stability was assessed for a 50 h period. Plasma samples spiked with QCs concentration were subjected to processing and stored after liquid–liquid extraction at room temperature prior to analyzed by HPLC–MS/MS. After thawing, spiked plasma samples were processed and left 50 h sitting in the autosampler, stability assessment of samples showed a reliable stability behavior under such conditions.

2.8.4. Long term storage stability

Samples were subjected to freeze storage (−20 °C) during the entire period covered by the bioequivalence study, i.e., from the first day of volunteer sample collection up to the last day of sample analysis. In this work the time of storage was 302 days. Storage stability was defined, comparing sample concentration to the mean values obtained at first-day analysis.

2.8.5. Stock solution stability

Budesonide stock and working solutions were prepared and stored at 4 ± 2 °C and the analyte levels were evaluated after 18 and 274 days. Results were compared to fresh prepared solutions at corresponding concentrations.

2.9. Pharmacokinetics and statistical analysis

The analytical method developed here was applied to evaluate comparatively the budesonide plasma concentration from two nasal spray formulations of budesonide (256 mcg from four administrations of 64 mcg each one) in healthy volunteers.

Forty eight healthy volunteers (24 male and 24 female) of both genders aged between 18 and 50 years and index of corporal mass within 18.5 and 29.9 kg/m^2 were selected for the study after assessment of their health status by clinical evaluation (physical examination, ECG) and routine laboratory tests.

The study was a single-dose, two-way randomized crossover design with one week of washout period between the doses. At each period, the volunteers were hospitalized at 08:00 p.m., had a normal evening meal, and after an overnight fast of 8 h, they received (at approximately 7:00 a.m.) a four sprays-dose of budesonide in alternate nostrils. After first sampling, each volunteer received a single dose of budesonide (256 μg) with 200 ml of water. During administration, all flasks were weighed five times before administration and one more after it; due to national regulations, the weight of each flask was an exclusion criteria: the final weight of each flask had to be within the value of two times the value of the standard deviation values considering all flasks.

Blood samples were collected by indwelling catheter into EDTA containing tubes before dosing and 5, 10, 15, 20, 30, 40, 50 min and also 1, 1.25, 1.5, 1.75, 2, 2.33, 2.67, 3, 4, 6, 8, 10, 12 and 14 h post-dosing for budesonide. The blood samples were centrifuged at 2000 × *g* for 10 min at 4 °C and the plasma stored at −20 °C until analyzed for budesonide content.

Bioequivalence between the two formulations was assessed by calculating individual test/reference ratios for the peak of concentration (C_{max}), area under the curve (AUC) of plasma concentration until the last concentration observed (AUC_{last}) and the area under the curve between the first sample (pre-dosage) and infinite (AUC_{0-inf}). C_{max} and the time taken to achieve this concentration (T_{max}) were obtained directly from the curves. The areas under the budesonide plasma concentration vs. time curves from 0 to the last detectable concentration (AUC_{last}) were calculated by applying the linear trapezoid rule. Statistical calculations were defined at the level of $P \leq 0.10$ and bioequivalence for the two budesonide formulations was concluded as the 90.0% confidence interval for C_{max}, AUC_{last} and AUC_{0-inf} felt within the range of 80.0–125.0% defined by both the Food and Drug Administration (FDA) and the National Sanitary Surveillance Agency (ANVISA). The software used included Equivtest® 2.0, MS Excel® 97, Tinn-R1.1, Win-Edit® 2.0 and Scientific Work Place® 5.0.

3. Results

3.1. Linearity and specificity

The simplest regression method for the calibration curves of the budesonide was $Y = 4.64e^{-03} + 2.94e^{-03}x$ from 7.5 to 1000 pg ml^{-1}. Correlation coefficient ranged from 0.9993 to 0.9998. The lower limit of quantification (LLOQ) was 7.5 pg ml^{-1}.

The chromatograms obtained from the LLOQ and extracted blank plasma are presented in Fig. 2. The budesonide and IS retention times were 2.77 ± 0.41 min and 2.89 ± 0.43, respectively. The signal-to-noise ratio was higher than 7.

In the case of budesonide and its IS there was no significant ion suppression in the region where the analyte and internal standard are eluted. There was no suppression when the analysis was performed using blank normal plasma, and the two other batches of hemolyzed and hyperlipemic plasma.

Furthermore, blank plasma samples from all volunteers were run before unknown concentration sample quantification, showing a clear chromatogram in all cases.

3.2. Recovery of budesonide

Absolute recoveries for both budesonide and IS were evaluated. Results of sample extraction procedure showed the recoveries (values ± CV (%), $n = 5$) for QCL, QCM and QCH as follows: 77.5 ± 10.5, 72.1 ± 3.7, 97.8 ± 8.8, respectively. The recoveries of the IS were 89.5 ± 3.8%.

3.3. Accuracy and precision

Intra-batch precision and accuracy of the assay was measured for budesonide at each QC level (15, 375 and 750 pg ml^{-1}). Calculated inter-batch precision and accuracy (% CV) of the method ranged from 4.6% to 8.6%, and 98.7% to 105.3%, respectively, as presented in Table 1.

These results were within the acceptance criteria for precision and accuracy, i.e., deviation values were within ±15% of the nominal values, except for LLOQ, which could show a ±20% deviation.

The lower limit of quantification (LLOQ) for budesonide was validated as 7.5 pg ml^{-1}, showing intra-batch precision and accuracy (% CV) of the method of 10.5%, with an accuracy of 96.7%.

3.4. Stability of budesonide

The stability tests of budesonide in human plasma showed no significant degradation after 19 h at room temperature, three freeze and thaw cycles, 165 h post processing or 385 days at −70 °C. The variation for the low and high QC samples were: −0.1 and 6.4 after the three freeze and thaw cycles, −4.5 and 3.8 for short term, 3.2 and 11.5 for post processing and −3.7 and −7.4 for long term stability, respectively.

Stability tests also indicated no significant degradation of the stock solution at 4 ± 2 °C. After 20 days, the variation between fresh and stored samples was 1.7% and 1.6% for low and high QC samples, respectively. In addition, the variation between fresh and stored

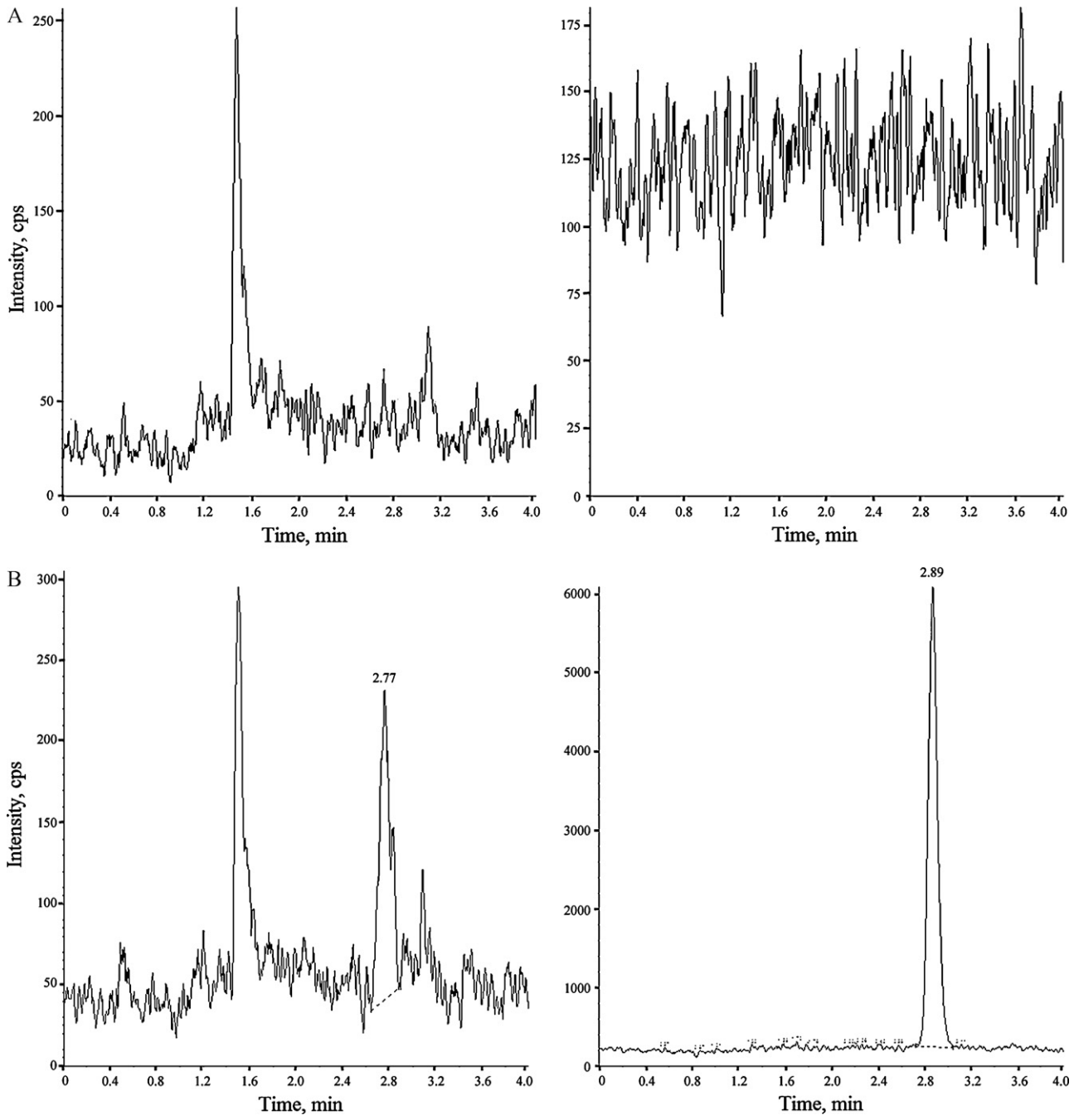

Fig. 2. MRM chromatograms of: (A) blank normal human plasma, (B) budesonide at LOQ concentration (7.5 pg ml^{-1}) and internal standard in normal plasma. The *m/z* 431.1 > 323.2 transition was monitored for budesonide (left panel) and the m/z 325.3 > 257.2 transition for 3-keto-desogestrel (right panel).

Table 1
Accuracy and precision data for budesonide quantification in human plasma. Results were obtained during the validation of QC samples, including the LLQ in human plasma.

QC samples	Nominal concentration (pg ml^{-1})	Intra-run accuracy[a]	Inter-run accuracy[b]	Intra-run precision[c] (% CV)	Inter-run precision[b] (% CV)
QC-LLOQ	7.5	102. 0	96.7	9.0	10.5
QCL	15.0	105.3	98.7	8.3	8.6
QCM	375.0	102.2	105.3	2.6	4.6
QCH	750.0	98.7	99.6	4.1	5.3

[a] (n = 6), expressed as (found concentration/nominal concentration) × 100.
[b] Values obtained from all three runs (n = 18).
[c] n = 6.

Table 2
Arithmetic mean pharmacokinetic parameters obtained from 42 volunteers after administration of each 0.256 mg budesonide tablet formulation.

	Budesonide test formulation		Diane® reference formulation	
	Mean	SD	Mean	SD
C_{max} (ng ml^{-1})	753.69	265.08	781.02	302.74
T_{max} (h)	0.30	0.16	0.28	0.13
$T_{1/2}$ (h)	3.52	0.72	3.66	0.73
AUC_{last} ([ng h] ml^{-1})	1755.14	455.26	1717.76	474.79
AUC_{inf} ([ng h] ml^{-1})	1867.56	464.60	1833.00	493.78

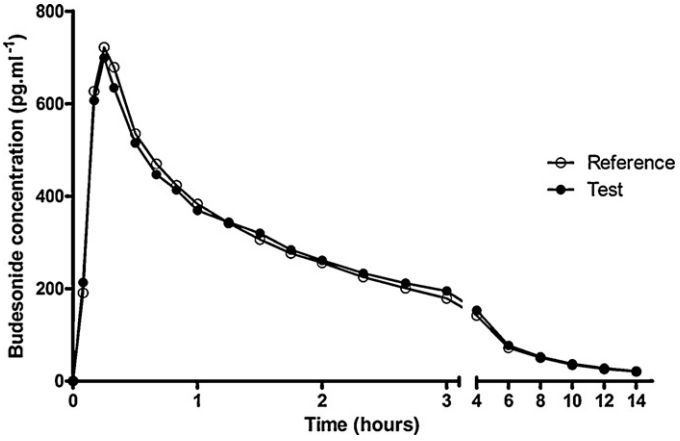

Fig. 3. Budesonide plasma mean concentration vs. time profiles obtained after the oral administration of the 256 μg dose of budesonide nasal spray formulations.

samples after 42 days was 8.4% and −4.9% for low and high QC samples, respectively.

3.5. Comparative pharmacokinetics study

Budesonide was well tolerated at the administered doses and no significant adverse reactions were observed or reported. No clinically relevant change was observed in any measured biochemical parameter. A total of 41 volunteers finished the study. The mean budesonide plasma concentration vs. time curves obtained after a inhaled dose of each formulation are shown in Fig. 3. The plasma concentration of budesonide did not differ significantly after administration of both formulations (test formulation and the reference one).

Table 2 shows the values of the pharmacokinetic parameters and Table 3 summarizes the bioequivalence analysis for budesonide formulations. Briefly, the geometric mean and respective 90% CI of budesonide test/reference percent ratios were 97.03% (88.69–106.15%) for C_{max} and 102.49% (97.11–108.19%) for AUC_{last}.

4. Discussion

The LC–MS/MS method described here for drug quantification is in accordance with both Food and Drug Administration (FDA) and the National Sanitary Surveillance Agency (ANVISA) requirements for pharmacokinetic studies.

Table 3
Geometric mean of the individual AUC_{last}, AUC_{0-inf} and C_{max} ratios (test/reference formulation) and the respective 90% CIs.

Parameters	Parametric (n = 42)			
	Geometric mean (%)	90% CI	Power (%)	CV (%)
AUC_{last} % ratio	102.74	97.22–108.58	99	14.72
AUC_{inf} % ratio	102.50	97.11–108.19	99	14.38
C_{max} % ratio	97.03	88.70–106.15	99	24.13

The sample preparation method described in this work includes a simple liquid–liquid extraction. Blank plasma samples from all 48 volunteers showed a clear chromatogram in all cases.

This is the first method developed to assess the budesonide quantification in human plasma applied to a pharmacokinetics study using LC–MS/MS with a photoionization source. This method offers the advantage over those previously reported using LC–MS/MS ([12,15,20–22]), showing a low validated LLOQ (7.5 pg ml^{-1}) associated with a faster chromatographic run time (4.0 min). Two other methods use LC–MS/MS to quantify budesonide showed a slight lower LLOQ of 6.5 pg ml^{-1} using a APCI ionization source [11] and 5 pg ml^{-1} using a Turboionspray ionization source [14].

The method described by Kronkvist et al. [11] use a very expensive equipment set, it is time consuming and used a large amount of human plasma to perform the drug extraction. Its method was built using three separate automated analytical steps with manual transfer of samples between them, including a pipetting robot to transfer 1 ml of centrifuged plasma samples and standard solutions, a second step consisting of a solid-phase extraction with 100 mg Isolute C18 columns and in the third step, the samples were chromatographed in a gradient LC system and detected using a tandem MS system (Finnigan TSQ 7000), with an atmospheric pressure chemical ionization interface. The same 1 ml of human plasma was used by Qu et al. [14] but instead of using a conventional plasma sample preparation methods such as a generic SPE, they used a selective SPE strategy that selectively concentrated the target compound.

Our method, in comparison, used the half volume (500 μl) of human plasma and a very simple liquid–liquid extraction procedure but enough to obtain a clean extract before the chromatographic separation and APPI-MS–MS analysis. The absence of an efficient extraction method seriously compromises the sensitivity and the reproducibility of the quantification method in plasma samples.

The method provides excellent analytical performance for budesonide extraction and proved to be appropriate for analyzing human plasma samples. The reported analytical method has been successfully applied to human pharmacokinetic investigations. The bioequivalence between tested and reference formulations was confirmed by the 90% Confidence Interval for the ratios of the C_{max} and AUC_{last} values being within the acceptance range of 80–125%.

5. Conclusion

This work describes a fast, sensitive and robust method to quantify budesonide in human plasma using 3-keto-desogestrel as the internal standard. Extracted samples were analyzed by high performance liquid chromatography coupled to Atmospheric pressure photoionization tandem mass spectrometry. This method agrees with the requirements proposed by the US Food and Drug Administration of high sensitivity, specificity and high sample throughput in comparative pharmacokinetic assays such as bioequivalence studies. The lowest concentration quantified was 7.5 pg ml^{-1} with suitable accuracy and precision. The intra-assay precisions ranged

from 2.6% to 9.0%, while inter-assay precisions ranged from 4.6% to 10.5%. The intra-assay accuracies ranged from 98.7% to 105.3%, while the inter-assay accuracies ranged from 96.7% to 105.3%. The described method for budesonide quantification in human plasma was successfully applied in a bioequivalence study of two budesonide 64 μg/dose formulations using an open, randomized, two-period crossover design. Since the 90% CI for C_{max} and AUC ratios were all inside the 80–125% interval, it was concluded that the test formulation of budesonideis bioequivalent to the reference formulation with respect to both the rate and the extent of absorption.

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