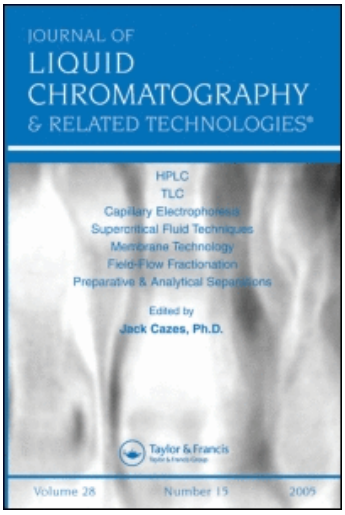

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Abstract: A reversed phase liquid chromatography (RP-LC) method was validated for the determination of fluticasone propionate (FP) in nasal sprays. The LC method was carried out on a Shim-pack CLC-ODS column (150 mm × 4.6 mm I.D.), maintained at 35°C. The mobile phase consisted of acetonitrile/methanol/phosphate buffer (0.01 M, pH 4.0) (35:35:30, v/v/v), run at a flow rate of 1.0 mL/min and using photodiode array (PDA) detection at 240 nm. The chromatographic separation was obtained with retention time of 6.1 min, and was linear in the range of 0.05–150 μg/mL ($r^2 = 0.9999$). The specificity and stability indicating capability of the method were proven through degradation studies, which also showed that there was no interference of the excipients. The accuracy was 99.36% with bias lower than 1.12%. The limits of detection and quantitation were 0.03 and 0.05 μg/mL, respectively. Moreover, method validation demonstrated acceptable results for precision and robustness. The proposed method was applied for the analysis of the nasal sprays and cream pharmaceutical formulations, contributing to improve the quality control and to assure the therapeutic efficacy.

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Keywords: Fluticasone propionate, Pharmaceutical formulations, Reversed phase liquid chromatography, Stability indicating method, Validation

INTRODUCTION

Fluticasone propionate (FP) (Figure 1) is a trifluorinated glucocorticoid specifically designed to provide enhanced anti-inflammatory effects combined with gastrointestinal absorption and fast liver metabolism, providing minimal systemic activity and low nasal bioavailability. The steroid molecule is highly lipophilic, which enhances its penetration into the cells and has a highly binding capacity for the glucocorticoid receptor.[1,2]

Glucocorticoids, administered by inhalation, remain a first line treatment of patients with seasonal and allergic perennial rhinitis, management of asthma, and advanced chronic obstructive pulmonary disease.[3–5] More recently, topical FP preparations have been formulated for use in dermatoses, including atopic dermatitis and psoriasis. The FP nasal spray therapy aims to maximize the beneficial therapeutic effects of corticosteroids while minimizing the well known side effects of systemic corticosteroids, such as suppression of the hypothalamo-pituitary-adrenal axis and inhibition of bone formation.[6,7]

The liquid chromatography tandem mass spectrometry (LC-MS/MS) methods with atmospheric pressure chemical ionization (APCI) were developed and validated for the quantification of FP in human plasma using a combination of protein precipitation and solid phase extraction (SPE).[8,9] A LC-APCI-MS/MS method coupled to

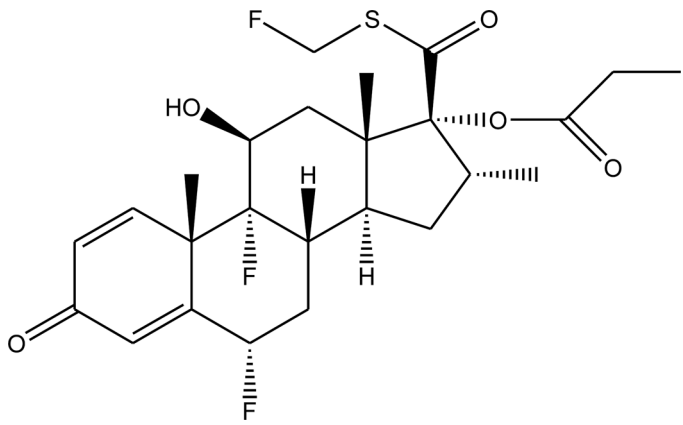

Figure 1. Chemical structure of fluticasone propionate (FP).

an automated SPE system was improved, showing it to be reliable for the analysis of samples from clinical studies.[10] Also, an LC-MS/MS method with electrospray ionization (ESI), using automated SPE, was developed to monitor systemic concentrations of inhaled FP at therapeutic doses.[11] A LC-ESI-MS/MS method was also used for the simultaneous detection and quantification of the 14 most frequently used synthetic corticosteroids, including FP, in human serum, plasma, urine, and tablets.[12]

A reversed phase liquid chromatography method (RP-LC) was developed and validated for the simultaneous determination of salmeterol and FP in combined pressured metered dose inhaler, using a RP ODS-2 base deactivated column, showing the application for the quantification of basic, acidic, and neutral compounds.[13] A RP-LC method was also performed using a RP ODS-2 column with UV detection at 250 nm for the determination of FP in nasal spray formulation,[14] but the validation was not performed and the stability indicating capability of the method not demonstrated.

The aim of the present study was to develop and validate a simple, accurate, and stability indicating RP-LC method for the quantitative analysis of FP in pharmaceutical nasal sprays, evaluating also the application for the cream formulations, contributing to improve the quality control and to assure the therapeutic efficacy of the pharmaceutical preparations.

EXPERIMENTAL

Chemicals and Reagents

Fluticasone propionate reference substance was supplied by Sigma-Aldrich (St. Louis, Missouri, USA). A total of four batches of Flixonase® (Glaxo Wellcome, Rio de Janeiro, Brazil) nasal sprays, containing 50 μg of FP per dose were identified by Arabic numbers from 1 to 4, and four batches of Flutivate® (Glaxo Wellcome, Rio de Janeiro, Brazil) cream formulations, containing 0.5 mg of FP per gram of the formulation were identified by Arabic numbers from 5 to 8. The samples were obtained from commercial sources within their shelf life period. HPLC-grade methanol and acetonitrile were obtained from Tedia (Fairfield, Ohio, USA). All chemicals used were of pharmaceutical or special analytical grade. For all the analyses, ultrapure water was purified using an Elix 3 coupled to a Milli-Q Gradient A10 system (Millipore, Bedford, MA, USA).

Apparatus and Analytical Conditions

The LC method was carried out on a Shimadzu LC system (Shimadzu, Kyoto, Japan) equipped with a SCL-10A_{VP} system controller, LC-10 AD_{VP} pump, DGU-14A degasser, SIL-10AD_{VP} autosampler, and a SPD-M10A_{VP} PDA detector. The peak areas were integrated automatically by computer using a Shimadzu Class VP V 6.14 software program. The experiments were performed on a reversed phase Shimadzu (Kyoto, Japan) Shim-pack CLC-ODS column (150 mm × 4.6 mm I.D., with a particle size of 4 μm). A security guard holder was used to protect the analytical column. The Shimadzu LC system was operated isocratically at ambient controlled temperature (35°C) using a mobile phase of acetonitrile/methanol/phosphate buffer (0.01 M, pH 4.0) (35:35:30, v/v/v) run at a flow rate of 1.0 mL/min, and using PDA detection at 240 nm. The injection volume was 20 μL of the solutions containing 50 μg/mL of reference substance and samples, respectively.

Solutions

Preparation of Reference Substance Solution

The stock solution was prepared by weighing accurately, 10 mg of FP reference substance, transferred to individual 10 mL volumetric flasks, and diluted to volume with acetonitrile, obtaining a concentration of 1 mg/mL. The stock solution was stored at 2–8°C protected from light, and daily diluted to an appropriate concentration in mobile phase.

Preparation of Sample Solutions

The sample solutions of FP nasal spray were prepared by weighing accurately 2 g of the formulation containing 50 μg of FP per dose (equivalent to 1000 μg of FP) and diluting to the final volume of 10 mL in volumetric flask with acetonitrile. Then the mixture was vortex mixed for 2 min, sonicated for 5 min, and centrifuged at 5000 × *g* for 15 min. The final concentration was stored at 2–8°C protected from light, daily filtered through a 0.45 μm membrane filter (Millipore, Bedford, USA), and diluted to an appropriate concentration with mobile phase, injected, and the amount of the drug calculated against the reference substance.

The sample solutions of FP cream were prepared by weighing accurately 1 g of the formulation containing 0.5 mg of FP per gram and adding 90% (v/v) aqueous methanol to obtain a final volume of 10 mL in volumetric flask. The mixture was shaken using a vortex mixer for 5 min,

sonicated for 20 min, and centrifuged at 5000 × *g* for 15 min. The final concentration was filtered through a 0.45 μm membrane filter, injected, and the amount of the drug calculated against the reference substance.

Validation of the Method

The method was validated using samples of pharmaceutical formulation of nasal spray by the determination of the following parameters: specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), robustness, and system suitability test following the International Conference on Harmonisation (ICH) guidelines.[15,16]

Specificity

A stability indicating method is defined as an analytical method that accurately quantifies the active ingredients without interference from degradation products, process impurities, excipients, or other potential impurities.[17] The stability indicating capability of the method was determined by subjecting a reference sample solution (100 μg/mL) to accelerated degradation by acidic, basic, neutral, oxidative, and photolytic conditions to evaluate the interference in the quantitation of FP. After the procedures, the samples were diluted in mobile phase to a final concentration of 50 μg/mL. A sample solution prepared in 1 M hydrochloric acid was used for the acidic hydrolysis, and a sample solution in 1 M sodium hydroxide for the basic hydrolysis evaluation. Both solutions were refluxed at 100°C for 4 h, cooled, and neutralized with acid or base, as necessary. For study in the neutral condition, the drug dissolved in water was heated at 80°C for 3 h. The oxidative degradation was induced by storing the samples solutions in 20% hydrogen peroxide, at ambient temperature for 24 h, protected from light. Photodegradation was induced by exposing the samples to 200 watt hours/square meter of near ultraviolet light for 24 h. Then, the stability indicating capability of the method was established by determining the peak purity of FP in the degraded samples using a PDA detector.

Linearity

Linearity was determined by constructing three analytical curves, each one with eight reference substance concentrations of FP, including the LOQ, in the range of 0.05–150 μg/mL prepared in mobile phase. Before injection of the solutions, the column was equilibrated for at least 20 min with the mobile phase flowing through the system.

Three replicates of 20 μL injections of the reference solutions were made to verify the repeatability of the detector response. The peak areas of the chromatograms were plotted against the respective concentrations of FP to obtain the analytical curve. The results were subjected to regression analysis by the least squares method to calculate calibration equation and determination coefficient.

Precision and Accuracy

The precision of the method was determined by repeatability and intermediate precision. Repeatability was examined by eight evaluations of the same concentration sample of FP, on the same day, under the same experimental conditions. The intermediate precision of the method was assessed by carrying out the analysis on three different days (inter days) and also by other analysts performing the analysis in the same laboratory (between analysts). The accuracy was evaluated applying the proposed method to the analysis of the in house mixture of the excipients with known amounts of the drug, to obtain solutions at concentrations of 40, 50, and 60 μg/mL, equivalent to 80, 100, and 120% of the nominal analytical concentration, respectively. The accuracy was calculated as the percentage of the drug recovered from the formulation and also expressed as the percentage relative error (bias %) between the measured mean concentrations and added concentrations.

Limits of Detection and Quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated, as defined by ICH,[15] using the mean values of three independent analytical curves, determined by a linear regression model, where the factors 3.3 and 10 for the detection and quantitation limits, respectively, were multiplied by the ratio from the standard deviation of the intercept and the slope. The LOQ was also evaluated in an experimental assay.

Robustness

The robustness of an analytical procedure refers to its ability to remain unaffected by small and deliberate variations in method parameters and provides an indication of its reliability for the routine analysis. The robustness was determined by analyzing the same samples (50 μg/mL) under a variety of conditions of the method parameters, such as: flow rate, column temperature, injection volume, mobile phase composition, and mobile phase pH. To assess the stability of sample

solutions of FP, the samples were tested maintained at 2–8°C for 48 h and also placed into the autosampler, at room temperature, for 24 h. The stability of these solutions was studied by performing the experiment and observing any change in the chromatographic pattern, compared with freshly prepared solutions.

System Suitability Test

The system suitability test was also carried out to evaluate the resolution and reproducibility of the system for the analysis to be performed, using five replicates injections of a reference solution containing 50 μg/mL of FP. The parameters measured were peak area, retention time, theoretical plates, and tailing factor (peak symmetry).

Analysis of FP in Nasal Sprays and Cream Formulations

For the quantitation of FP in the pharmaceutical formulations, the respective stock solutions were diluted to appropriate concentration with mobile phase, filtered, injected in triplicate, and the percentage recoveries of the drug calculated against the reference substance.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

To obtain the best chromatographic conditions, the mobile phase was optimized to provide sufficient selectivity and sensitivity in a short separation time. Phosphate buffer resulted in high sensitivity compared with ammonium acetate buffer and phosphoric acid solution. The use of acetonitrile combined with methanol as organic components resulted in better sensitivity, short analysis time, improving the peak symmetry (about 1.04). For the selection of the best wavelength detection, a PDA detector was used. The optimized conditions of the LC method were validated for the analysis of FP in nasal sprays, due to the capability and application for the quality control.

Method Validation

Specificity and Forced Degradation Studies

Forced degradations were performed to provide indications of the stability indicating properties of the analytical method, particularly

when there is no information available about the potential degradation products. Figure 2, shows that the acidic and neutral conditions resulted in significant decrease of the area without any additional peak, indicating that the degradation products were not detected by UV. Under the basic and the photolytic conditions, significant decrease of the areas were observed with one additional peak detected for each condition, at 3.5 and 8.6 min, respectively. Under the oxidative condition, FP content exhibited a non significant decrease of the area. Specificity of the method towards the drug was established through determination of purity peak of the drug in working reference solution using a PDA detector. No interference from formulation excipients was found, showing that the peak was free from any coeluting peak, with values of peak purity index higher than 0.9999, thus demonstrating that the proposed method is specific for the analysis of FP.

Linearity

The analytical curves constructed for FP were found to be linear in the 0.05–150 μg/mL range. The value of the determination coefficient calculated ($r^2 = 0.9999$, $y = (38317.42 \pm 380.18)x + (1932.05 \pm 264.19)$, where, x is concentration and y is the peak absolute area) indicated the linearity of the analytical curve for the method. Moreover, the relative standard error of slope can be used as a parameter with respect to the precision of the regression, as a general acceptance criterion for the linearity performance of the analytical procedure.[18] This parameter should be comparable to the relative standard deviation obtained in the evaluation of the precision. The result obtained for the relative standard deviation of the slope is 0.99%, which is lower than the mean value 1.11%, of the RSD of the precision.

Precision

The precision evaluated as the repeatability of the method was studied by calculating the relative standard deviation (RSD) for eight determinations of the 50 μg/mL performed on the same day and under the same experimental conditions. The RSD value obtained was 0.60%.

The intermediate precision was assessed by analyzing two samples of the nasal sprays on three different days (inter-day); the mean values obtained were 100.01 and 100.23% with RSD 0.53 and 0.68%, respectively. Between analyses, precision was determined by calculating the mean values and the RSD for the analysis of two samples of the nasal spray by three analysts; the values were found to be 100.04 and 100.86% with RSD 0.68 and 0.28%, respectively. The results are shown in Table 1.

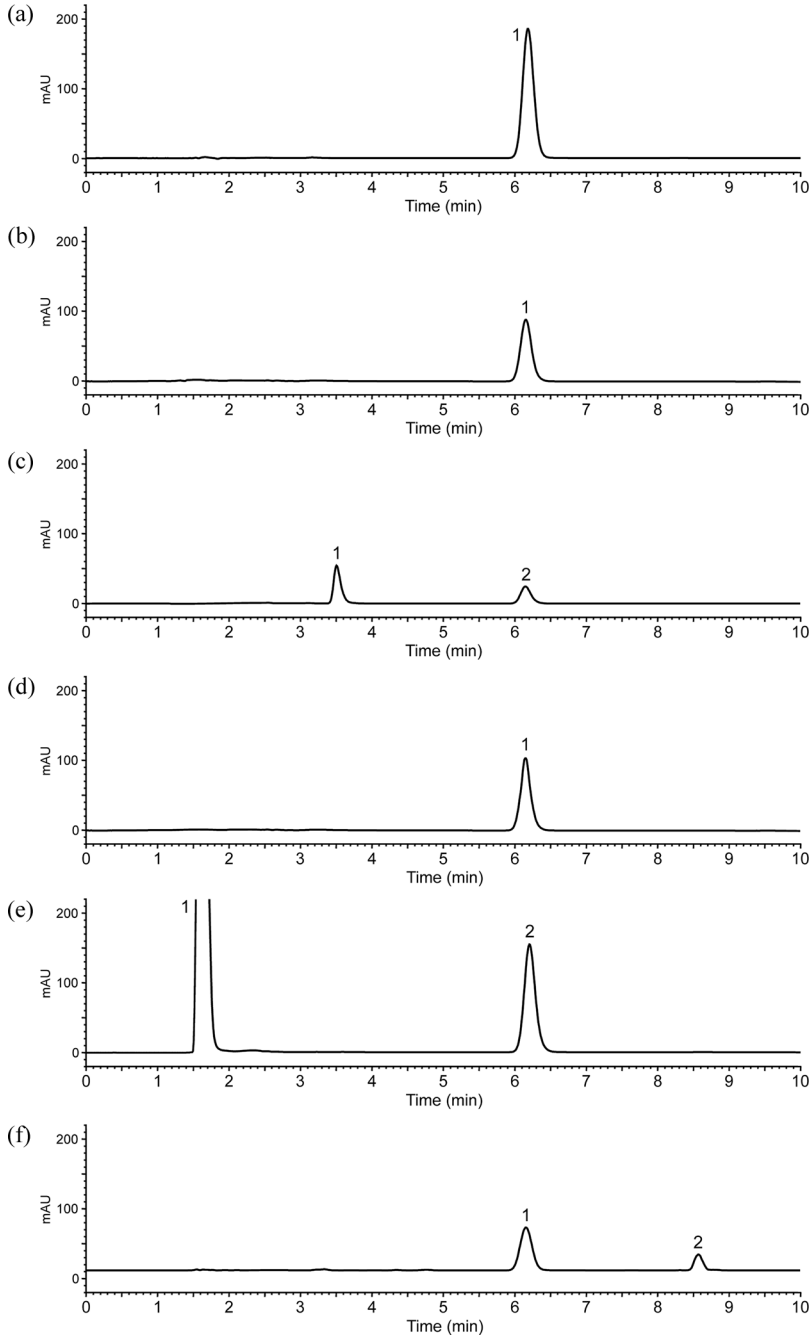

Figure 2. LC chromatograms of FP (50 μg/mL). (a) FP reference substance solution: peak 1 = FP. (b) After acidic hydrolysis: peak 1 = FP. (c) After basic hydrolysis: peaks 1 = degraded form, 2 = FP. (d) After neutral condition: peak 1 = FP. (e) After oxidation: peaks 1 = hydrogen peroxide, 2 = FP. (f) After exposition to UV light: peaks 1 = FP, 2 = photodegraded form.

Table 1. Inter-day and between-analysts precision data of RP-LC for fluticasone propionate (FP) in samples of nasal sprays

Inter-day				Between-analysts		
Sample	Day	Recovery[a] (%)	RSD[b] (%)	Analysts	Recovery[a] (%)	RSD[b] (%)
1	1	99.88		A	100.20	
	2	100.59	0.53	B	99.53	0.68
	3	99.55		C	100.40	
2	1	100.31		A	100.73	
	2	100.88	0.68	B	100.96	0.28
	3	99.51		C	100.89	

[a]Mean of three replicates.
[b]RSD = Relative standard deviation.

Accuracy

The accuracy was assessed from three replicate determinations of three different solutions containing 40, 50, and 60 μg/mL. The absolute means obtained for FP are shown in Table 2, with a mean value of 99.36% and bias lower than 1.12%, demonstrating that the method is accurate within the desired range.

Limits of Detection and Quantitation

For the calculation of the LOD and LOQ, a calibration equation, $y = 38317.42x + 1932.05$, was generated by using the mean values of the three independent analytical curves. The LOD and LOQ were obtained by using the mean of the slope, 38317.42 ± 380.18, and the standard deviation of the intercept of the independent curves, determined by a

Table 2. Accuracy of RP-LC for fluticasone propionate (FP) in samples of nasal sprays

Nominal concentration (μg/mL)	Mean concentration found[a] (μg/mL)	RSD[b] (%)	Accuracy (%)	Bias[c] (%)
40	39.86	0.79	99.65	−0.35
50	49.44	0.24	98.88	−1.12
60	59.73	0.83	99.55	−0.45

[a]Mean of three replicates.
[b]RSD = Relative standard deviation.
[c]Bias = [(Measured concentration – Nominal concentration)/Nominal concentration] × 100.

linear regression line as 264.19. The LOD and LOQ calculated were 0.03 and 0.04 μg/mL, respectively. The LOQ evaluated in an experimental assay, with the precision lower than 5% and accuracy within ±5%, was found to be 0.05 μg/mL.

Robustness

The results and the experimental range of the selected variables evaluated in the robustness assessment are given in Table 3, together with the optimized values. There were no significant changes in the chromatographic pattern when the modifications were made in the experimental conditions, thus showing the method to be robust. The stability of the sample solutions was studied and the data obtained

Table 3. Chromatographic conditions and range investigated during robustness testing

Variable	Range investigated	FP[a] (%)	RSD[b] (%)	Optimized value
Flow rate	0.8	99.69	1.12	
(mL/min)	1.0	100.17	0.52	1.0
	1.2	100.08	0.89	
Column temperature (°C)	30	101.34	0.99	
	35	99.20	0.61	35
	40	99.33	1.06	
Injection volume (μL)	10	99.99	1.32	
	20	100.03	0.64	20
	30	99.75	0.52	
Percent acetonitrile	33	99.22	0.89	
	35	98.33	0.78	35
	37	99.17	1.14	
Percent methanol	33	99.10	1.04	
	35	98.33	0.97	35
	37	99.12	0.78	
Mobile phase pH	3.7	98.82	1.55	
	4.0	99.37	1.03	4
	4.3	98.08	0.86	
Solution stability	Autosampler 24 h	102.07	1.82	–
	2–8°C 24 h	100.94	0.79	–
	2–8°C 48 h	101.21	1.03	–

[a]Mean of three replicates.
[b]RSD. = Relative standard deviation.

showed the stability during 24 h into the autosampler and during 48 h when maintained at 2–8°C.

System Suitability

The system suitability test was carried out to evaluate the resolution and reproducibility of the system for the analysis to be performed, using five replicates injections of a reference substance solution containing 50 μg/mL of FP. The RSD values calculated for the retention time, tailing factor, and peak area were 0.06, 0.14, and 0.24%, respectively. The number of theoretical plates was about 8749, with RSD of 1.15%. The experimental results show that the parameters tested were within the acceptable range (RSD <2.0%), indicating that the system is suitable for the analysis intended.

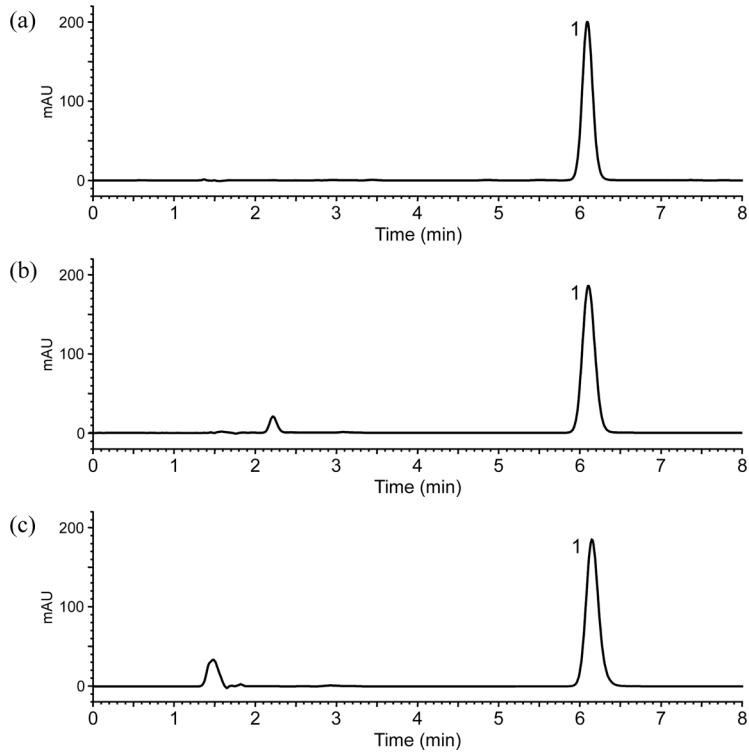

Figure 3. Representative RP-LC chromatograms of FP reference substance solution (a); FP nasal sprays formulations (b) and FP cream formulations (c). Peak 1 = FP (50 μg/mL).

Table 4. Determination of fluticasone propionate (FP) in pharmaceutical formulations by the RP-LC method

Theoretical amount			Experimental amount		
Sample		FP μg	FP[a] μg	Recovery (%)	RSD[b] (%)
Nasal spray	1	50/dose	49.45/dose	98.91	0.29
	2	50/dose	49.11/dose	98.23	0.43
	3	50/dose	47.98/dose	95.96	0.54
	4	50/dose	50.16/dose	100.33	0.31
Cream	5	500/g	497.89/g	99.58	0.24
	6	500/g	501.68/g	100.34	0.43
	7	500/g	538.50/g	106.50	0.41
	8	500/g	512.03/g	102.41	0.06

[a]Mean of three replicates.
[b]RSD = Relative standard deviation.

Method Application

The proposed method was applied for the determination of FP in nasal sprays and also in topical cream formulations, with the retention time of 6.1 min, as shown in the typical chromatograms of Figure 3. The results demonstrated the quality of the pharmaceutical samples and the applicability of the method for the quality control laboratories (Table 4).

CONCLUSION

The results of the validation studies show that the RP-LC method is specific, stability indicating, accurate, and possesses significant linearity and precision characteristics without any interference from the excipients, demonstrating also the advantages of the chromatographic technique, very well established for the quality control of most of the pharmaceuticals due to its simplicity, high resolution, satisfactory precision, and accuracy. Therefore, the proposed method was successfully applied and suggested for the quantitative analysis of fluticasone propionate in pharmaceutical formulations, contributing to improve the quality control and to assure the therapeutic efficacy.

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