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Original Article

A MASS COMPATIBLE UPLC METHOD FOR THE QUANTIFICATION OF IMPURITIES IN FLUTICASONE PROPIONATE NASAL SPRAY

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ABSTRACT

Objective: The objectives of the present study were to develop and validate a mass compatible ultra-performance liquid chromatography (UPLC) method to quantify the impurities in fluticasone nasal spray, and to establish a suitable container-closure system for the formulation.

Methods: A gradient method was optimized with a flow rate of 0.5 ml/min, detector wavelength-240 nm, run time-25 min and 0.1% Trifluoroacetic acid (TFA) in water as solvent A and Methanol as solvent B.

Results: The developed method was linear over the range of 0.07-1.10 µg/ml for impurity-I, 0.16-2.47 µg/ml for impurity-II, 0.67-10.0 µg/ml for impurity-III, and 1.29-19.3 µg/ml for impurity-IV. The limit of quantification (LOQ) and limit of detection (LOD) were established as 0.07 and 0.02 µg/ml, 0.14 and 0.05 µg/ml, 0.59 and 0.19 µg/ml, 1.06 and 0.35 µg/ml for impurities I-IV respectively. The percent relative standard deviation (%RSD) of the replicate analysis for impurities I-IV, was within the acceptance criteria (0.4, 0.2, 0.3, and 0.1% respectively) that proved the precision of the method. The accuracy of the method was studied from 50%-150% of test concentration and the results ranged from 100.3% to 109.4%. The container-closure compatibility study revealed that the solution stored in the glass container system did not generate any additional peaks in the chromatogram.

Conclusion: Hence, the developed method can be employed by quality testing laboratories to quantify impurities in fluticasone propionate nasal spray. The study also suggests that glass containers could serve as a compatible system for the storage of fluticasone propionate nasal solution.

Keywords: Fluticasone propionate, UPLC, Nasal spray, Impurities, Method validation

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INTRODUCTION

Asthma and allergic rhinitis are common respiratory conditions associated with airway inflammation. Asthma is characterized by airway obstruction, which is at least partially reversible, and airway hyper-responsiveness to stimuli such as environmental allergens, viral respiratory tract infections, irritants, drugs, exercise, and cold air. Common symptoms of allergic rhinitis are nasal congestion, rhinorrhea, sneezing, nasal itch, and postnasal drainage [1]. As asthma and allergic rhinitis are inflammatory diseases, treatment with corticoids is recommended [2].

Inhaled corticosteroids have, over time, almost entirely replaced treatments based on oral corticosteroids, which commonly produced major adverse effects. These drugs, because of topical application, present a substantially better therapeutic index than oral steroids [3]. Also, the treatment of allergic rhinitis has been revolutionized by the introduction of topical nasal steroid sprays, which are the most common medications prescribed by otolaryngology professionals, given that the regular use of these sprays reduces nasal obstruction, rhinorrhea, and overall nasal symptom scores [4, 5].

Fluticasone is a potent, locally active glucocorticoid that has no demonstrable systemic side-effects when given by the oral or intranasal routes. Glucocorticoids produce several beneficial effects on the events involved in allergic inflammation. These include inhibition of production of interleukins, inhibition of cellular and protein extravasation, inhibition of arachidonic acid generation from phospholipids, and a reduction in the release of proteases and other enzymes from several cell types [6-8].

Several investigations have taken place in the quantification of fluticasone propionate in bulk drugs as well as drug products using liquid chromatography. Buscher *et al.* developed and validated liquid chromatography with tandem mass spectrometry (LC-MS-MS) method to quantify budesonide and fluticasone in human sputum samples [9]. Paczkowska *et al.* developed a high-performance liquid chromatography (HPLC) method for the simultaneous determination of salmeterol xinafoate and fluticasone propionate for the quality control of dry powder inhalation products [10]. Byrro and his co-workers presented a rapid and sensitive LC-MS-MS method to determine fluticasone in human plasma [4]. Akmese *et al.* [11] Angela *et al.* [12], studied the quantitative assessment of fluticasone using liquid chromatography. However, to the best of our knowledge, there is no reported mass spectroscopy compatible ultra-performance liquid chromatography (UPLC) method available to quantify the impurities in a fluticasone propionate nasal spray formulation. Although fluticasone is stable when exposed to various stress conditions like thermal and acid hydrolysis, it forms several unknown impurities in photolytic, oxidative, and alkaline stress conditions [13]. Hence, the proposed mass compatible liquid chromatography (LC) method will help to quantify the impurities in a fluticasone propionate nasal spray.

MATERIALS AND METHODS

Chemicals and reagents

Ultra-pure water obtained from the Milli-Q® water purification system (Millipore, USA) was used. Methanol (HPLC grade) and Trifluoroacetic acid (TFA) (HPLC grade) used for the studies, were procured from Merck, Mumbai, India. Commercially available micronized fluticasone propionate nasal sprays were used for studies. The Chromatographic column used, Ascentis® Express C18, 100 × 4.6 mm with 2.7 µm particle size (cat no. # 53827-U), was

procured from Sigma Aldrich, Bangalore, India. The centrifuge model, Heraeus Megafuge 40, procured from Thermo Fisher Scientific, Bangalore, India, was used. Unless stated otherwise, all other reagents were of HPLC grade or equivalent, at the minimum.

Liquid chromatography conditions

The analyses were performed on the ACQUITY UPLC H-Class (Waters, USA) system controlled by Waters EMPOWER 3 software and equipped with an autosampler injection system (Waters ACQ-BSM: 10 µl sample loop), a quaternary solvent manager (Separation module), a Photo Diode Array Detector (PDA), and an Ascentis® Express C18 column (150 × 4.6 mm, 2.7 µm spherical particles). The mobile phase was degassed by filtration through a 0.45 µm Millipore membrane filter followed by sonication for 10 min. The injection volume was 10 µl and detection was done at 240 nm. The UPLC system was operated at 40 °C with the gradient program specified in table 1. A mixture of water and methanol (20:80 v/v) was used as a diluent.

Table 1: LC gradient separation

Time (min)	Mobile phase (%)		Flow rate (ml/min)
	A	B	
0.0	40	60	0.5
15.0	20	80	0.5
17.0	40	60	0.5
25.0	40	60	0.5

Mobile phase A: water containing 0.1% TFA, Mobile phase B: Methanol

UPLC-MS-MS conditions

A Waters Acquity H Glass (Waters, USA) LC system was coupled to a Waters Xevo tandem quadrupole mass detector (Waters, USA). MassLynx Analyst v.8.0 software was used to control the UPLC-MS-MS system and collect the mass data. High purity Nitrogen was supplied to the mass by a PEAK Scientific generator (PEAK Scientific, Scotland). The LC system consisted of an FTN autosampler, a quartnery solvent manager, a column oven, and a controller. The autosampler was operated at ambient conditions and programmed to inject 10 µl of each sample. Gradient separation (table 1) was achieved, at a temperature of 40 °C, on an Ascentis® Express column (150 × 4.6 mm, 2.7 µm spherical particles). Mobile phase A comprised 0.1% TFA in the water, and Mobile phase B comprised of Methanol. The autosampler wash contained water-methanol at the ratio of 20:80 v/v and was programmed to wash the injection needle before and after injection. The liquid chromatogram was recorded at 240 nm.

The mass analysis was carried out with an electrospray source and the operating conditions were set as follows-scan range: mass 150-650, desolvation temperature: 350 °C, capillary voltage (kV): 3.50, cone voltage (V): 50, and gas flow: 650 L/Hr. Mass parameters were optimized by injecting fluticasone propionate standard solution into the UPLC-MS-MS system.

Preparation of solutions

Preparation of sample solution

The sample solution containing 55 µg/ml of fluticasone propionate was prepared in diluent and analyzed for its impurities as per optimized conditions.

Container-closure compatibility study

The fluticasone propionate nasal spray solution was filled in 4 ml glass vials and high-density polyethylene (HDPE) containers. Both samples were subjected to thermal stress of 60 °C for 10 d. They were then analyzed for their impurities by UPLC, followed by the identification of major peaks by MS-MS.

Degradation of fluticasone propionate nasal solution

The fluticasone propionate drug substance was subjected to various stress conditions viz., acid hydrolysis, base hydrolysis, water hydrolysis, and thermal degradation. These stressed samples were analyzed by injecting them into the LC-MS-MS system.

Preparation of impurities stock solution

500 µg/ml stock solution of each impurity-fluticasone carboxylic acid, fluticasone carbothioic acid, fluticasone carboxylic acid propionate, and fluticasone cyclic thioester was prepared in the diluent and used to validate the method.

Validation of fluticasone propionate related substances (RS) method by UPLC

The RS method of fluticasone propionate nasal spray by UPLC was validated in terms of specificity, precision, accuracy, linearity, the limit of detection (LOD), and limit of quantification (LOQ) according to the International Council for Harmonization (ICH) of Technical Requirements for Pharmaceuticals for Human Use [14].

Specificity

The specificity of the method was studied to ensure that there was no interference from the diluent at the retention time of analytes.

Method precision

The method precision was assessed by six replicate analyses of impurities, spiked in the sample solution. The method is considered to be precise if the percent relative standard deviation (% RSD) is not more than (NMT) 15.0% for each impurity.

Accuracy

The accuracy of an analytical method expresses the nearness between the expected value and the value found. It is obtained by calculating the % recovery of the analyte recovered. In this case, to evaluate the accuracy of the developed method, successive analysis (n=3) for three different levels i.e. 50, 100, and 150% of the test concentration were performed. The impurities stock solution was spiked in the sample solution at 50%, 100%, and 150% of the test concentrations. The data obtained from the experiment were statistically analyzed using the formula [$\%\ \text{Recovery} = \frac{\text{Recovered amount}}{\text{Spiked amount}} \times 100$] to study the recovery and validity of the developed method. The mean recovery should be within 85-115% to be accepted.

Linearity

Linearity is the ability to obtain test results that are directly proportional to the concentration of the analyte. Linearity was determined by plotting peak area against the concentration of the impurity. Here, a 5-point calibration curve is plotted covering from LOQ to 150% of the target concentration to calculate the coefficient of correlation, slope, intercept, and % bias. The method is considered linear if the coefficient of correlation is not less than (NLT) 0.997 and % bias at 100% response is NMT 5.0%. The % bias was calculated using the formula: [$\%\ \text{Bias} = \frac{\text{Y intercept}}{\text{Response for 100\%}} \times 100$].

Limit of detection and Limit of quantification

LOD is the lowest concentration in the sample that can be detected but not necessarily quantified, under the stated experimental conditions. LOQ is the lowest concentration of analyte that can be determined with acceptable precision and accuracy. These two

parameters were calculated using the formula LOD = $\frac{3.3 \times SD}{S}$ and LOQ = $\frac{10 \times SD}{S}$ where SD-standard deviation of response and S-slope of the calibration curve.

RESULTS

Container closure compatibility study

The container-closure compatibility study revealed that the chromatogram of the fluticasone propionate nasal solution stored in a glass container was in concurrence with the chromatogram of freshly prepared solution (fig. 1A, fig. 1B). Conversely, the solution stored in the HDPE containers developed an additional impurity at the retention time of 5.499 min (fig. 2A). These solutions were subjected to UPLC-MS-MS to get the mass of the impurity and it was found to be 396.42 (fig. 2B).

Degradation of fluticasone propionate nasal solution

No significant degradation was observed in samples subjected to acidic, water, and thermal degradation. However, around 22% of degradation was observed in the sample subjected to basic degradation (fig. 3). This observation is in concurrence with results published by Akmese *et al.* [11]. LC-MS-MS was used to identify the degradants in these samples based on their fragmentation pattern and mass. The results are presented in table 2 and fig. 3A-3E.

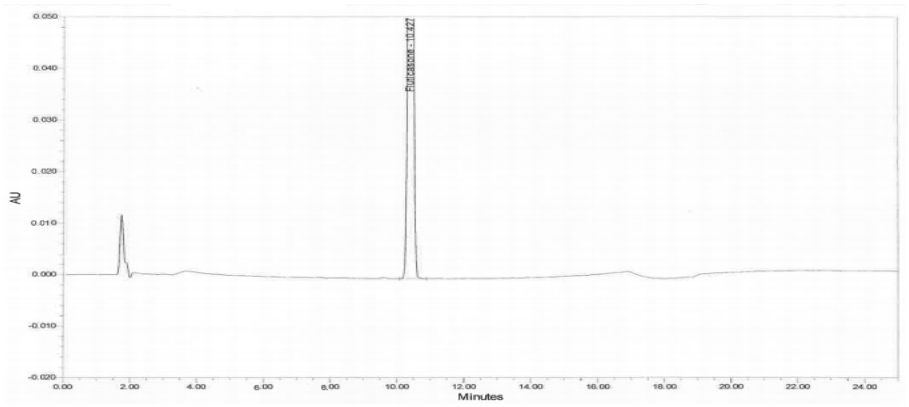

Fig. 1A: Chromatogram of freshly prepared fluticasone propionate nasal solution

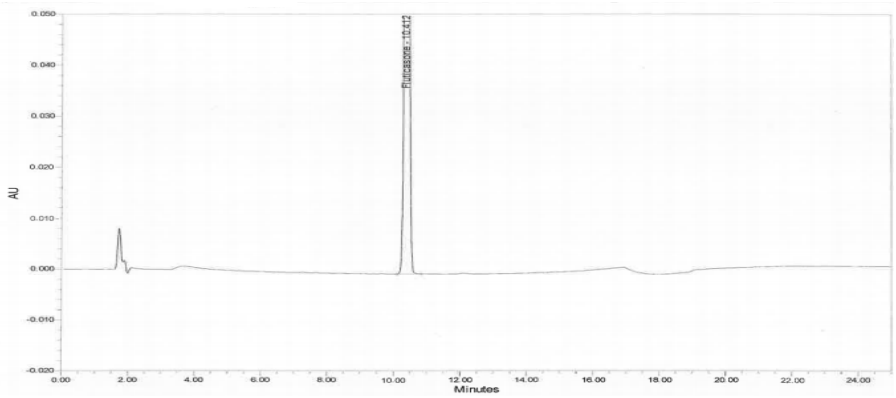

Fig. 1B: Chromatogram of fluticasone propionate nasal solution stored in a glass container

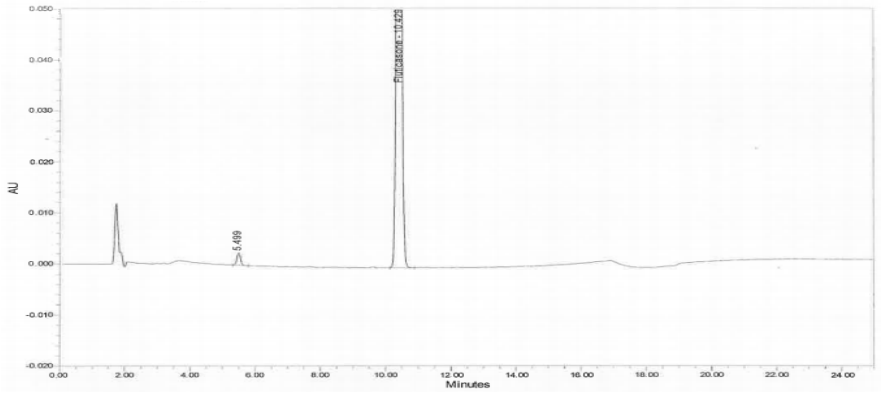

Fig. 2A: Chromatogram of fluticasone propionate nasal solution stored in HDPE container

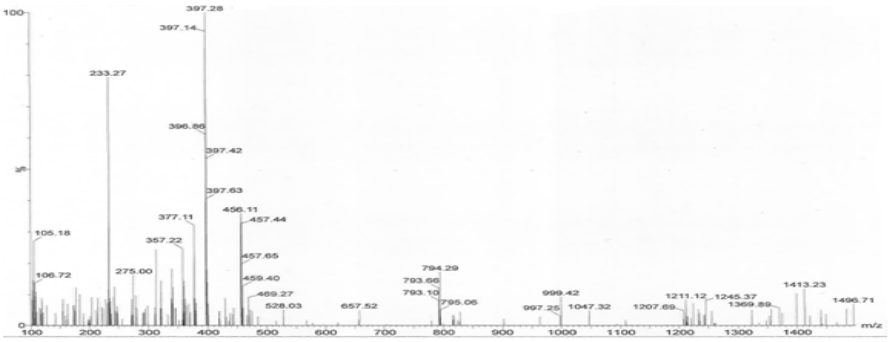

Fig. 2B: Mass spectrum of impurity eluting at RT 5.499 min

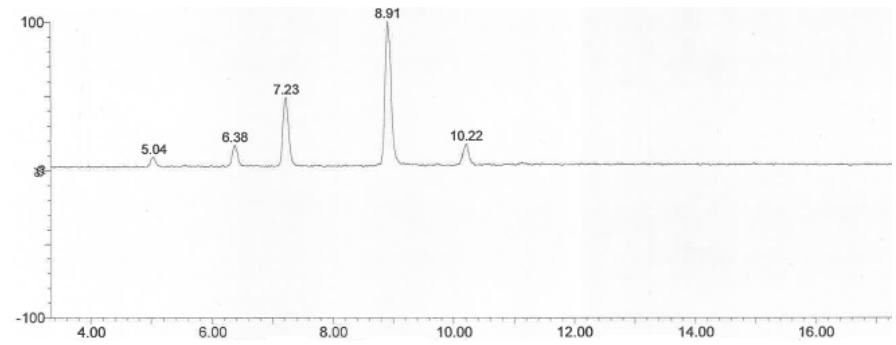

Fig. 3: Chromatogram of basic degradation sample

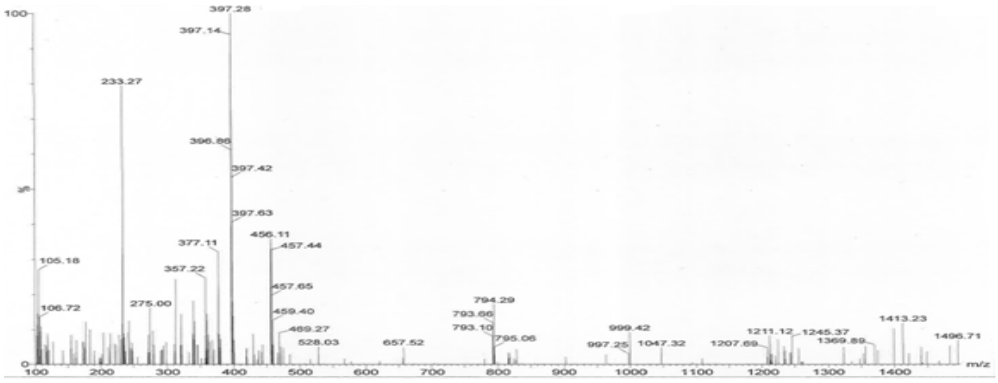

Fig. 3A: Mass spectrum of impurity at RT 5.043 min

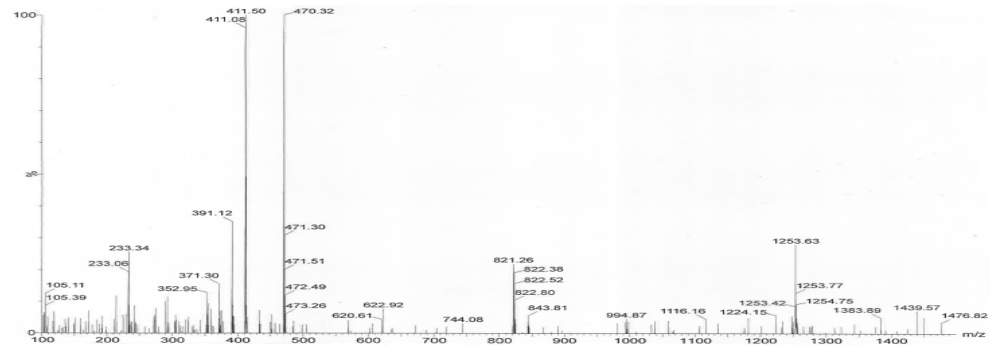

Fig. 3B: Mass spectrum of impurity at RT 6.379 min

Table 2: Impurities of fluticasone propionate nasal solution stored in HDPE container

RT (min)	Impurity	$[M+H]^+$	Mass No.
5.043	I. Fluticasone carboxylic acid	397.28	396.42
6.379	II. Fluticasone carbothioic acid	470.32	468.55
7.230	III. Fluticasone carboxylic acid propionate	453.37	452.49
8.948	IV. Fluticasone impurity (cyclic thioester)	467.31	466.55
10.198	Fluticasone propionate	501.21	500.57

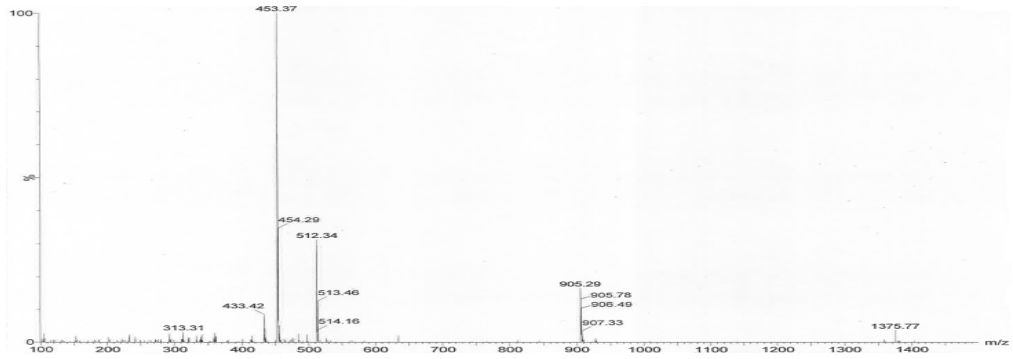

Fig. 3C: Mass spectrum of impurity at RT 7.230 min

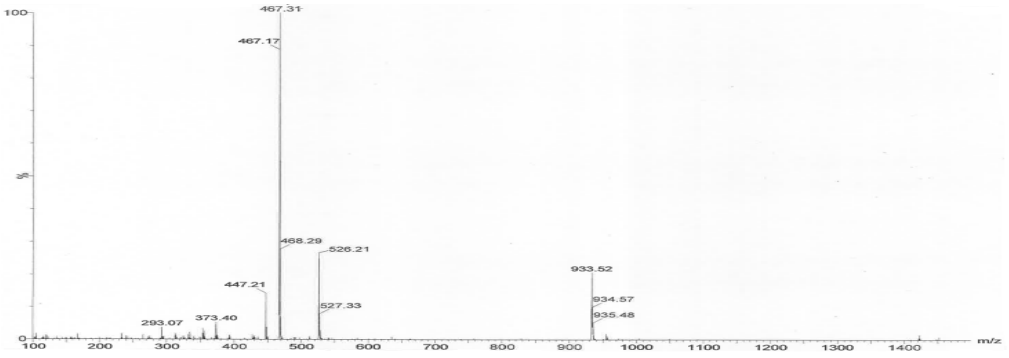

Fig. 3D: Mass spectrum of impurity at RT 8.948 min

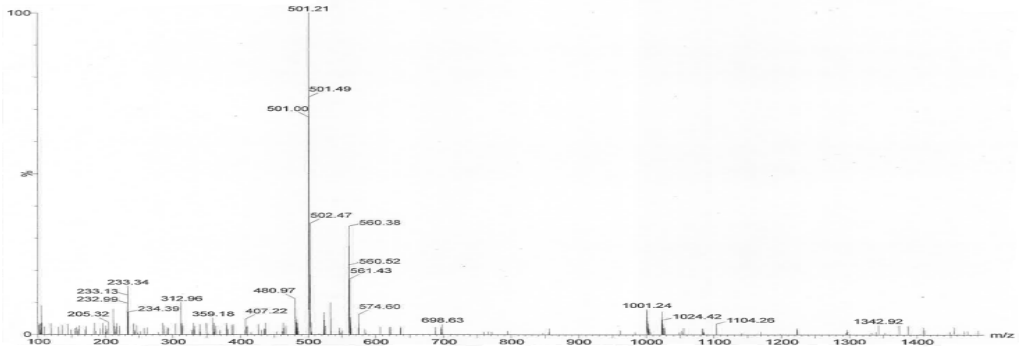

Fig. 3E: Mass spectrum of impurity at RT 10.198 min

Validation of fluticasone propionate RS method by UPLC

Specificity

The specificity of the method was studied by analyzing the diluent and sample spiked with an impurity mixture solution. No peak was detected at the retention time of fluticasone and impurities which proved the high degree of specificity of the method (fig. 4).

Method precision

The system suitability was assessed by six replicate analyses of sample solution spiked with an impurity mixture solution. The acceptance criterion was±15% for the %RSD of the peak area (table 3 and fig. 5).

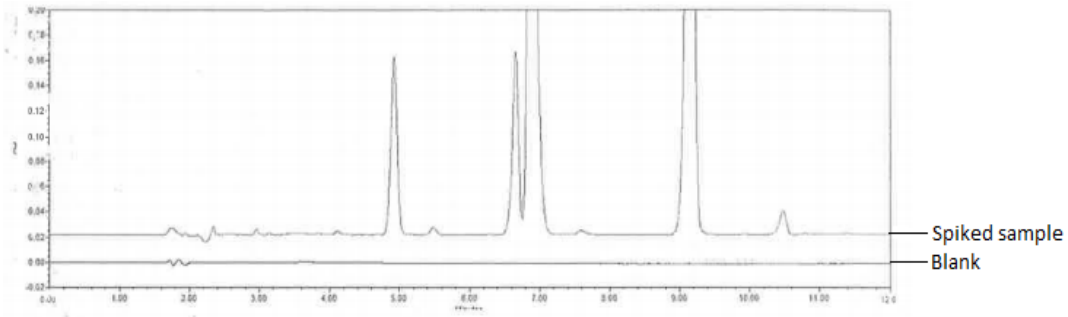

Fig. 4: Overlay chromatogram of blank and fluticasone propionate nasal solution

Table 3: Method precision

Test solution	Impurity-I	Impurity-II	Impurity-III	Impurity–IV
1	0.97	2.20	10.60	19.60
2	0.97	2.21	10.63	19.59
3	0.97	2.21	10.63	19.60
4	0.97	2.21	10.66	19.61
5	0.97	2.21	10.68	19.61
6	0.98	2.21	10.69	19.62
Average	0.97	2.21	10.65	19.61
%RSD	0.4	0.2	0.3	0.1

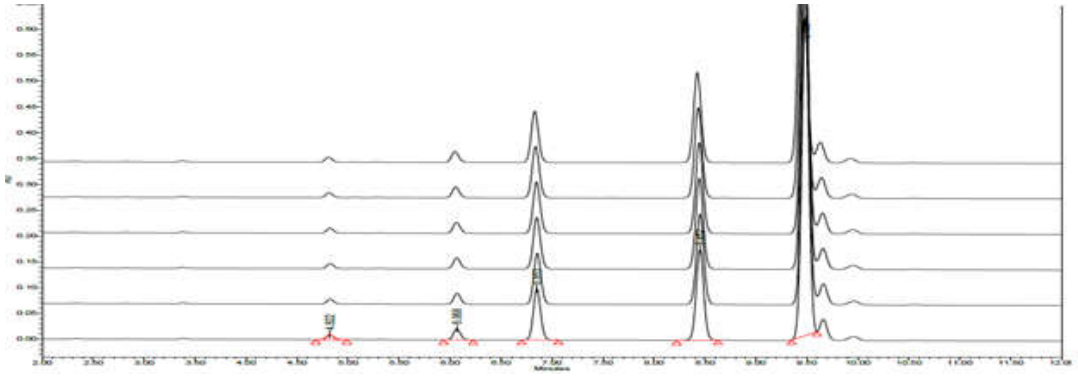

Fig. 5: Overlay chromatograms of method precision

Accuracy

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out in triplicate at 50%, 100%, and 150% of the target concentration, and the % recovery was calculated. The method was considered to be accurate if the individual recovery at each level is between 85.0% and 115.0%. The data revealed that the method is accurate (table 4).

Table 4: Accuracy

Level sample		Impurity-I	Impurity-II	Impurity-III	Impurity-IV
50%	1	109.4	105.8	109.0	104.6
	2	109.0	105.3	108.7	104.1
	3	109.9	105.2	108.9	103.9
100%	1	106.0	102.4	105.6	100.7
	2	106.0	102.4	105.7	100.5
	3	106.2	102.7	105.9	100.7
150%	1	107.6	102.4	105.8	100.4
	2	108.0	102.6	106.2	100.6
	3	108.3	102.2	106.0	100.3

Linearity

A linear relationship was observed between the area of the peak and the corresponding concentration over LOQ to 150% of the test concentration. The coefficient of correlation was found to be ≥ 0.9998 for all the impurities. And the % bias at 100% was found to be-0.5,-0.1,-0.4, and-0.1 for impurity I, II, III, and IV respectively (fig. 6A-6D). From the data obtained, it was concluded that the method is linear from LOQ to 150% of the test concentration.

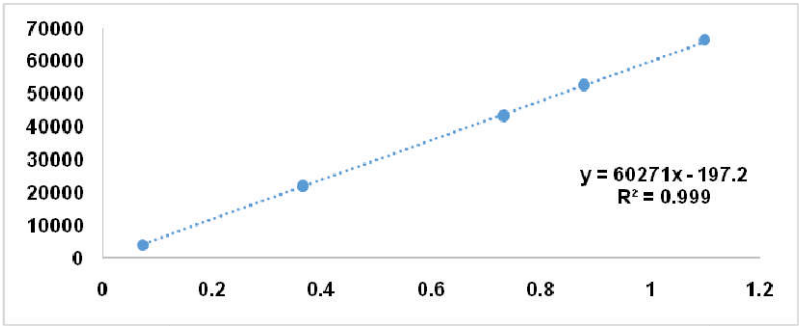

Fig. 6A: Linearity chart of impurity-I

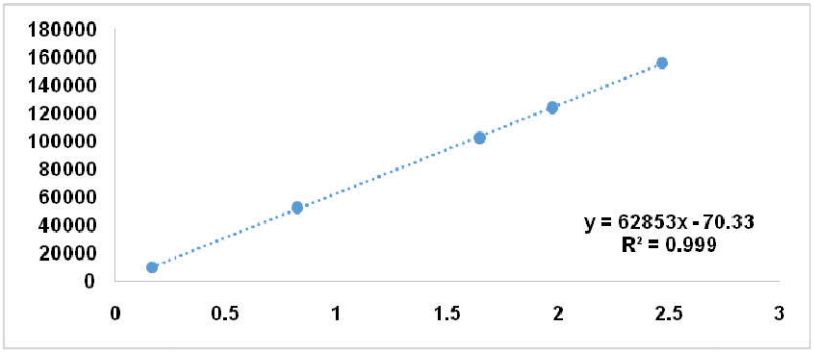

Fig. 6B: Linearity chart of impurity-II

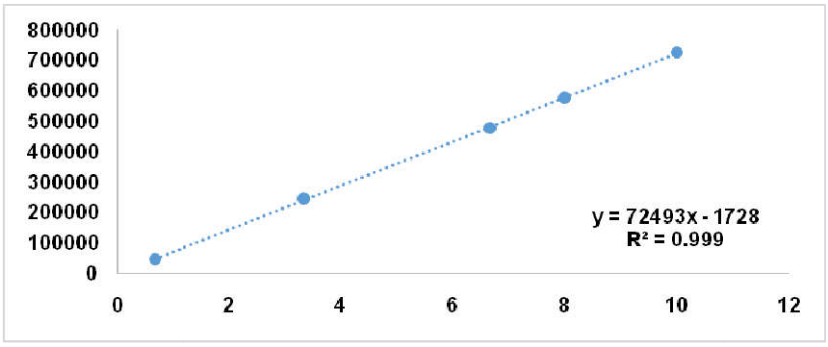

Fig. 6C: Linearity chart of impurity-III

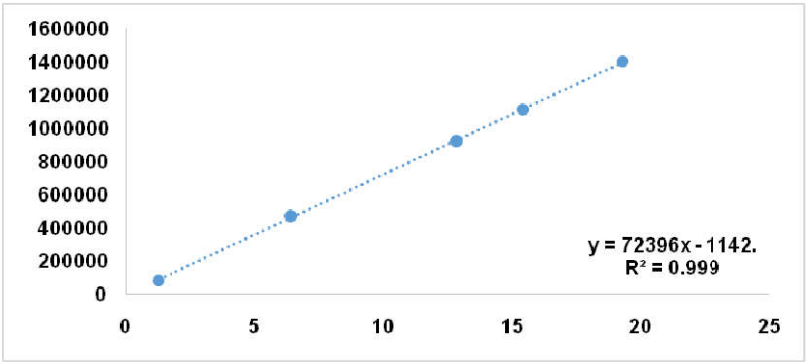

Fig. 6D: Linearity chart of impurity-IV

Limit of detection and limit of quantification

The LOD of impurity I, II, III, and IV were found to be 0.067, 0.141, 0.587, and 1.058 µg/ml respectively, and the LOQ was calculated as 0.022, 0.046, 0.194, and 0.349 µg/ml respectively.

DISCUSSION

Validation of an analytical method is imperative to assure that the developed method will consistently produce the desired result meeting its predetermined specifications and quality characteristics [15-17]. Although the λmax of fluticasone propionate was found to be 246 nm, considering the absorption values of other impurities, 240 nm was set as the detection wavelength and column temperature was set as 40 °C where all the impurities were well separated from each other with the resolution of greater than 2.0. The container closure compatibility study demonstrated that the glass container system would be a suitable one to store the fluticasone propionate nasal solution.

The sample was found to be stable in acid, water, and thermal degradation conditions. Whereas in basic conditions around 22% degradation observed. Similar degradation patterns were observed in the study conducted by Akmese *et al.* The developed method was found to be specific as no interference was observed at the retention time of analytes. The results of precision and accuracy were found to be within the limit prescribed in ICH Q2 (R1) guidelines [18]. All four impurities are shown a linear relationship between the concentration of analyte and areas of the corresponding peaks with the co-efficient of correlation value of 0.9998. The LOD and LOQ limits were established in this method are by the limit set for reporting threshold in ICH guidelines. The developed mass compatible UPLC method was successfully adopted to identify and quantify the impurities present in the fluticasone propionate nasal solution.

CONCLUSION

In this study, we have developed and validated a novel, sensitive, reproducible, and accurate mass compatible UPLC method, for the quantification of impurities in the fluticasone propionate nasal spray. Forced degradation study revealed that the solution is susceptible to alkaline hydrolysis. The container-closure compatibility study demonstrated that the fluticasone propionate nasal solution possibly undergoes degradation when the solution is stored in the HDPE container and exposed to heat. However, further investigation is warranted to better understand the interaction between the container-closure system and formulation. Although several analytical methods exist for the quantification of fluticasone, to the best of our knowledge, this is the first time that a mass compatible UPLC method was developed and validated to study the impurities in fluticasone nasal solution. Thus, the present study might aid in selecting an appropriate container-closure system, and to quantify the impurities in fluticasone propionate nasal spray.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally to this study.

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

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