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Original Article

A STUDY OF METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF FLUNISOLIDE IN NASAL SPRAY FORMULATIONS BY RP-HPLC METHOD

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ABSTRACT

Objective: The objective of the present research work was to develop and validate reversed-phase high-performance liquid chromatography (RP-HPLC) method for quantification of flunisolide in nasal spray formulations.

Methods: The developed method was validated according to International Conference on Harmonisation (ICH) guideline with respect to system suitability, accuracy, precision, specificity, linearity, and robustness. An isocratic condition of mobile phase comprising phosphate buffer (pH 5.5): acetonitrile: tetrahydrofuran in a ratio of 73:15:12, v/v at a flow rate of 1.0 ml/minute over RP C18 (octadecylsilane (ODS), 150 × 4.6 mm, 5 µm, Phenomenex Inc.) column at ambient temperature was maintained.

Results: The method showed excellent linear response with correlation coefficient (R^2) values of 0.999, which was within the limit of correlation coefficient ($R^2 \geq 0.995$). Intra and inter-day precision studies of the new method were less than the maximum allowable limit percentage of relative standard deviation %RSD ≤ 2.0.

Conclusion: A simple reversed-phase HPLC method for the analysis of flunisolide in nasal spray formulations was developed and validated.

Keywords: Flunisolide, ICH, Nasal Spray, RP-HPLC, Validation

INTRODUCTION

Inflammation often occurs as a result of an allergic reaction, and people who suffer from nasal allergies, such as hayfever, dust, mite and p*et al.* lergies, tend to experience a variety of symptoms due to this inflammation [1]. Inflammation of the nasal passage forces fluid out of the nasal tissues, resulting in a runny and blocked nose. Other symptoms include sneezing, watery and itchy eyes.

The three main types of drugs available for anti-inflammatory and anti-allergic effect are corticosteroids [2], antihistamines [3] and decongestants. Corticosteroid drugs include beclomethasone dipropionate [4], budesonide [5], flunisolide [5], ciclesonide [6], fluticasone furoate [7], fluticasone propionate [8], mometasone furoate [9] and triamcinolone acetonide [10]. Antihistamine drugs include azelastine [11] and olopatadine [12]. Two common decongestants available in the nasal sprays or drops form are oxymetazoline and phenylephrine.

Flunisolide is an anti-inflammatory glucocorticosteroid with the chemical name: 6(alpha)-fluoro-11(beta), 16(alpha), 17, 21 tetrahydroxypregna-1, 4-diene-3, 20-dione cyclic 16,17-acetal with acetone, hemihydrate [13].

The chemical structure of flunisolide is shown in fig. 1.

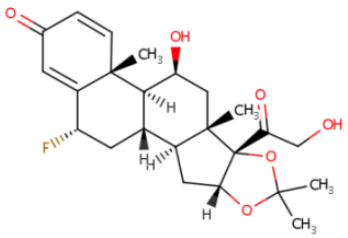


Fig. 1: Structure of flunisolide

Flunisolide is a white to creamy white crystalline powder with a molecular weight of 443.51 g/mol and a molecular formula of $C_{24}H_{31}FO_6$. It is soluble in acetone, sparingly soluble in chloroform, slightly soluble in methanol, and practically insoluble in water. It has a melting point of about 245 °C. The octanol: water partition coefficient is 2.17 at neutral pH [14].

Flunisolide is a glucocorticoid receptor agonist with anti-inflammatory action. The effects of topical corticosteroids are not immediate and require regular use and, at least, a few days to start experiencing noticeable symptom relief. As-needed use has been shown to be not as effective as regularly recommended use. Flunisolide (marketed as AeroBid, Nasalide, Nasarel) is a corticosteroid commonly prescribed as a treatment for allergic rhinitis [14].

Our work was involved with design and development of flunisolide nasal solution. Therefore, development of a suitable rapid HPLC method was required for analysis and characterization of flunisolide in developed nasal spray formulations.

MATERIALS AND METHODS

Materials

Flunisolide and flunisolide working standard were a kind gift of Healthcare Pharmaceuticals Limited, India. HPLC grade acetonitrile, Tetrahydrofuran, Potassium dihydrogen phosphate and Disodium hydrogen phosphate were purchased from Ranbaxy Fine Chemicals Ltd., India.

HPLC system

High-performance liquid chromatographic system (Shimadzu-Prominence, Japan), equipped with an autosampler (Model—SIL-20AC HT) and UV–visible detector (Model—SPD 20A), was used for the analysis. The data were recorded using LC-solution software. Analytical RP C18 column [octadecylsilane (ODS), 150 × 4.6 mm, 5 µ, Phenomenex Inc., Japan] was used for method development and its validation.

Preparation of mobile phase

A total of 13.61 g of KH_2PO_4 was dissolved into 1000 ml water (solution A). A total of 35.81 g of Na_2HPO_4 was dissolved into 1000 ml water (solution B). A phosphate buffer of pH 5.5 was prepared by mixing 96.4 ml of solution A and 3.6 ml of solution B. Then this buffer, HPLC grade acetonitrile and tetrahydrofuran were mixed together at a ratio of 73:15:12 v/v then filtered through a 0.22-µm Millipore filter and finally sonicated to degas.

Preparation of standard solution

10 mg of flunisolide was taken in a 100-ml volumetric flask, and about 10 ml diluting solution (acetonitrile: water 70:30) was added and sonicated for five minutes to dissolve properly. Then volume was made up to the mark with the same diluent. This was the stock solution.

Sample preparation

Samples of nasal spray formulations equivalent to 0.5 mg and 1.0 mg respectively, of flunisolide were taken and suitably diluted with diluting solution (acetonitrile: water 70:30) to get a 50 µg/ml concentration, and the samples were analyzed using the proposed analytical methods.

Chromatographic conditions

The analysis was carried out at ambient temperature under isocratic condition. The mobile phase was run at a flow rate of 1.0 ml/minute for 35 min. The injection volume was 10 µL for standard and samples. Before analysis, every standard and sample were filtered through 0.2 µm filter tips. The column eluent was monitored with UV detection at 240 nm.

Method validation

Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical applications. Typical parameters verified invalidation of analytical method are listed in table 1 [13, 15]. ICH Q2 (R1) is considered the primary reference for recommendations and definitions on validation characteristics for analytical procedures [16].

Table 1: Typical parameters verified in method validation

S. No.	Validation parameter
1	Accuracy
2	Precision
3	Specificity
4	Detection Limit
5	Quantitation Limit
6	Linearity
7	Range
8	Robustness

System suitability

System suitability test as an integral part of method development was used to ensure adequate performance of the chromatographic system. Retention time (RT), a number of theoretical plates (N) and tailing factor (T) were evaluated for three replicate injections of the sample solution. To determine precision system flunisolide standard solution was prepared and injected for six times into HPLC system. The mean, SD and % RSD for peak areas of flunisolide was calculated.

Accuracy

The accuracy of an analytical procedure is the closeness of test results obtained by that procedure to the true value. The accuracy of an analytical procedure should be established across its range [15]. In the present study, successive analysis (n=3) for three different concentrations of standard mixtures (50, 100 and 150 %) was carried out to determine the accuracy of proposed method.

Precision

The precision of an analytical procedure is the degree of agreement among individual test results when the procedure is repeatedly applied to multiple samplings of a homogeneous sample. The precision of an analytical procedure is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements. Precision may be a measure of either the degree of reproducibility or of repeatability of the analytical procedure under normal operating conditions. In this context, reproducibility refers to the use of the analytical procedure in different laboratories, as in a collaborative study. Intermediate precision (also known as ruggedness) expresses within-laboratory variation, as on different days, or with different analysts or equipment within the same laboratory. Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time using the same analyst with the same equipment [15]. The precision of the assay method was assessed with respect to repeatability and reproducibility. The precision of the proposed method was checked by intra-and inter-day repeatability of responses after replicate injections and expressed as %RSD among responses using the formula.

$$\%\text{RSD} = \frac{\text{standard deviation}}{\text{mean}} \text{x } 100\%$$

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components [16]. The placebo solutions containing excipients without flunisolide were prepared. To evaluate the specificity of the method blank, placebo and test solution were injected.

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. On the other hand, LOQ is the lowest amount of analyte in a sample that may be determined with acceptable accuracy and precision. LOD and LOQ values were determined from the regression curve. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines were used as the standard deviation. Based on the standard deviation of the response and the slope The LOQ may be expressed as:

$$\text{LOQ} = \frac{\sigma}{S} \text{x } 10$$

Where σ = the standard deviation of the response,

S = the slope of the calibration curve.

Linearity

Linearity was checked on seven different concentrations within 20–150% of the nominal standard concentration. The linearity of the proposed method was evaluated by using calibration curve to calculate the coefficient of correlation, slope, and intercept values.

Robustness

Robustness is an indication of the reliability of the analytical method during normal usage. The effect of the following deliberate changes in chromatographic conditions was monitored: flow rate±10%, pH of buffer solution±1, temperature±5 °C, and detector wavelength±2.

RESULTS

System suitability

The results of system suitability were within acceptable limits as shown in table 2.

The results of system precision are tabulated in table 3. The % RSD for the peak areas count of flunisolide peak from six replicate injections of standard solution was less than 2.0.

Table 2: System suitability data

Parameter	Flunisolide	Acceptable limits
Retention time (Rt)	11.538±0.06	RSD ≤ 1.0 %
Theoretical plates (N)	9270±114	N>2000
Tailing factor (T)	1.02±0.15	T ≤ 2

Values are expressed as mean±standard deviation of three replicate (n=3)

Table 3: Results from the determination of system precision for determination of flunisolide standard

N	Peak areas of flunisolide
1	617415
2	616106
3	615916
4	614856
5	615646
6	616590
Mean	616088
SD	866.721
% RSD	0.14

Values are expressed for six replicate (n=6)

Accuracy

Known amount of flunisolide was spiked in placebo at about 50, 100 and 150 % concentration of flunisolide nasal solution. The amount of flunisolide was quantified as per developed method. The % recovery was calculated from the amount found and actual amount added. The results are tabulated in table 4. The overall recovery of flunisolide in the samples was more than 95% (RSD<5%) which is sufficient for quantification of flunisolide in nasal spray formulations.

Precision

Sample solution of flunisolide nasal solution was prepared and injected for six times into HPLC system. The mean, SD and % RSD for assay of flunisolide was calculated. The % RSD for assay of flunisolide peak from six replicate injections of standard solution was less than 2.0. Results for intraday precision for quantification of flunisolide in flunisolide nasal solution are shown in table 5.

Interday precision (Ruggedness) of the method has been verified by performing an assay on six samples of flunisolide nasal solution of the same batch which was used for intraday precision on different days. Calculated the % RSD of % assay for above six preparations and calculated the overall % RSD of % assay for above six results and precision method results. Results for interday precision for quantification of flunisolide in flunisolide nasal solution are shown in table 6.

Specificity

The specificity test demonstrated that the used excipients did not interfere with the peak of the main compound. Thus, the HPLC method is useful to quantify flunisolide in the developed formulations. There was no interference between the peaks of flunisolide as shown in fig. 2. No peak was eluted at the retention time of Flunisolide in blank and placebo solution. The results showed that the developed method was selective for determination of flunisolide in nasal formulations.

Table 4: Evaluation of accuracy of the proposed method for quantification of flunisolide

Level no./spike level in %	Actual amount of flunisolide added in mcg	Amount of flunisolide found in mcg	% Recovery	Mean	SD	% RSD
Level-1	125.46	126.04	100.5	100.5	0.058	0.06
(50 %)	125.49	126.15	100.5			
	125.25	125.76	100.4			
Level-2	250.09	251.76	100.7	101.1	0.608	0.60
(100 %)	250.73	252.79	100.8			
	250.25	254.87	101.8			
Level-3	375.28	375.80	100.1	100.6	0.500	0.50
(150 %)	375.26	379.51	101.1			
	375.33	377.40	100.6			

Values are expressed as mean±standard deviation of three replicate (n=3)

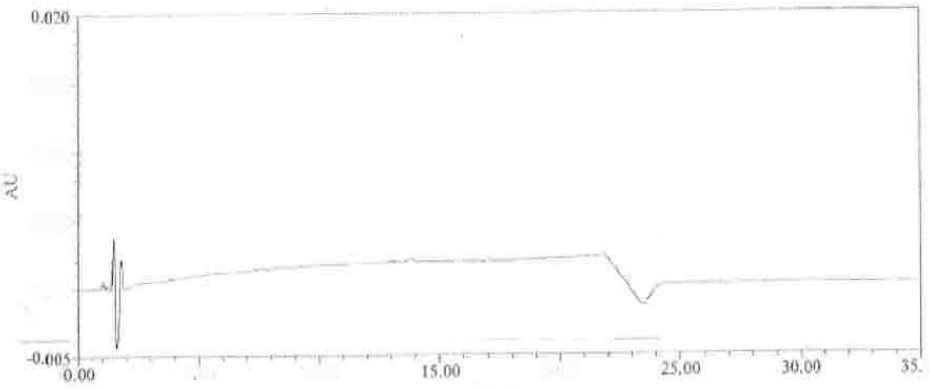
Table 5: Results of intraday precision

N	% Assay of flunisolide
1	98.6
2	98.7
3	98.6
4	98.7
5	98.6
6	98.8
Mean	98.7
SD	0.082
% RSD	0.08

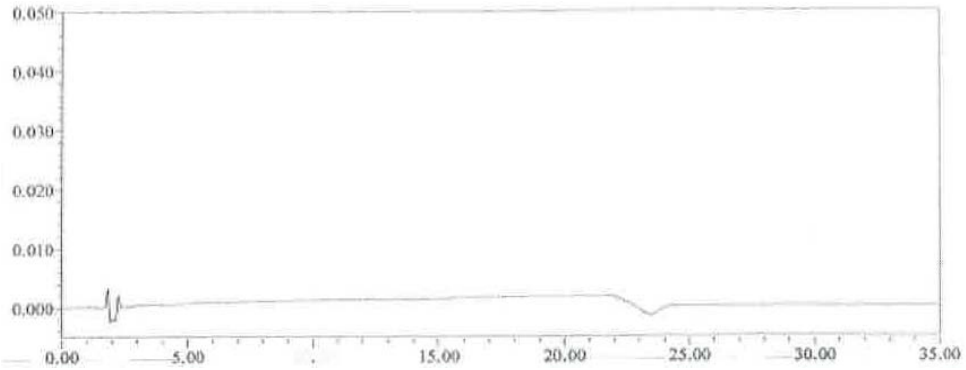
Table 6: Results of interday precision

N	% Assay of flunisolide	
	Day 1	Day 2
1	98.6	99.6
2	98.7	99.6
3	98.6	99.8
4	98.7	99.6
5	98.6	99.9
6	98.8	99.5
Mean	98.7	99.7
SD	0.082	0.151
% RSD	0.08	0.15

a) Blank



b) Placebo



c) Sample solution

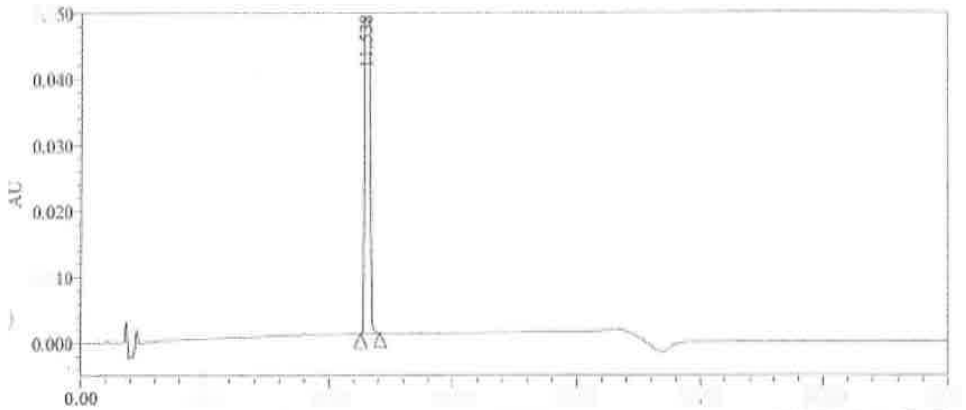


Fig. 2: HPLC chromatogram of a) Blank b) Placebo c) sample solution

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ for flunisolide were 0.05 and 0.5 µg/ml, respectively. The LOQ value shows that the method could be applied for lower concentrations of analytes. LOQ were good enough for the determination of the drug in the nasal formulations containing 3 mg of flunisolide which is the usual dose of flunisolide in currently marketed dosage forms [14].

Linearity

A graph was plotted with concentration (in µg/ml) of flunisolide on X-axis and peak areas of flunisolide on Y-axis. The results are tabulated in table 7 and graphically represented in fig. 3. The method showed excellent linear response with correlation coefficient (R^2) values of 0.999, which was within the limit of the correlation coefficient ($R^2 \geq 0.995$).

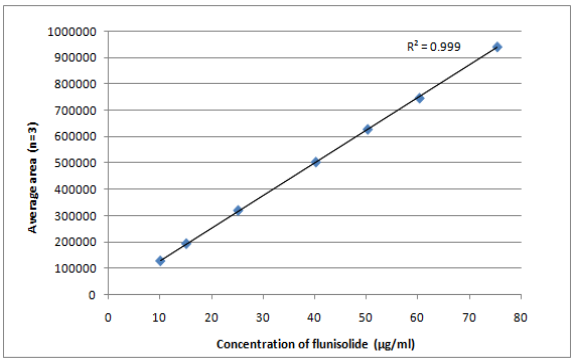


Fig. 3: Linearity plot for flunisolide

Table 7: Results of linearity

Spike level in %	Concentration of flunisolide (µg/ml)	Average area (n=3)
20	10.04	128584
30	15.06	194254
50	25.11	320374
80	40.17	504221
100	50.21	628643
120	60.25	747153
150	75.32	942140
	Slope	12380.28
	Y-intercept	6633.04
	Correlation coefficient	0.999

Robustness

Robustness of the method was verified by deliberately varying the following chromatographic conditions as shown in table 8 i.e.

- By changing the flow rate by±10%.
- By changing the column oven temperature by±5 °C.
- By changing the wavelength by±2 nm.
- By changing the pH of buffer used for mobile phase by to±1 unit

Each sample was analyzed in triplicate. The results were compared with the intraday precision data and tabulated in table 9. Data showed that the minor changes in operating conditions did not result in a huge difference in resolution and suitability of the separation parameters. Based on the robustness studies, in all studied conditions, all units were within the specification limit and % difference in the mean value between assay result of robustness and method precision using the same batch was less than 2.0%.

Table 8: Robustness experiments

S. No.	I	II	III	IV	V	VI	VII	VIII	IX
Experiment	Method precision	Plus flow	Minus flow	+Temp	-Temp	+Wavelength	-Wavelength	+pH	-pH

Table 9: Robustness results

S. No.	II	III	IV	V	VI	VII	VIII	IX
1	97.9	98.0	97.3	98.0	98.5	98.6	98.2	98.6
2	98.0	98.1	97.6	98.36	98.4	98.8	97.0	98.2
3	98.9	99.0	98.2	99.0	98.6	99.0	97.3	98.9
Mean	98.5	98.6	98.3	98.6	98.6	98.7	98.3	98.6
SD	0.346	0.320	0.539	0.289	0.117	0.1396	0.665	0.194
% RSD	0.35	0.32	0.55	0.29	0.12	0.14	0.68	0.20
% Difference with Mean	0.20	0.10	0.40	0.10	0.10	0.00	0.40	0.10

Estimation of formulations

The assay values of flunisolide in nasal spray formulations ranged from 99.80 % to 101.60 %, with a standard deviation of not more than 0.64 %. The assay values for the formulations were same as mentioned in the label claim, indicating the suitability of the proposed analytical method. The estimated drug content with low values of standard deviation established the precision of the proposed method.

DISCUSSION

Development of an analytical method for assessment of drugs in the pharmaceutical dosage form is of utmost necessity to confirm the quality of nasal formulations with respect to assay and spray content uniformity. Development and validation of liquid chromatography-tandem mass spectrometry (LC-MS/MS) method capable of quantifying flunisolide in the tissue culture matrix is reported in the literature [17]. Also, compendial methods for quantification of flunisolide drug substance and flunisolide in nasal formulation official in United States Pharmacopeia (USP) [18]. But we found some drawbacks in those methods, which are listed below:

- Almost all of those methods have used organic solvents more than 30%, which is not cost effective for routine analysis in pharmaceutical industries.
- Mobile phase containing more than 30% organic phase may be detrimental to HPLC column as at that concentration; buffer salts may precipitate.
- Interference caused by different excipients used in newly developed formulation with flunisolide peak

Our developed analytical method for estimation of flunisolide in nasal spray formulations has used very less amount of organic solvents. Also, the method was found to be simple and accurate and was able to resolve the drug from excipients in a short analytical run time.

CONCLUSION

A simple reversed-phase HPLC method for the analysis of flunisolide in nasal spray formulations was developed and validated. The proposed method is simple, accurate, precise, specific and linear over the analysis ranges and was able to resolve the drug from excipients in a short analytical run time.

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CONFLICT OF INTERESTS

Declared none

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