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Analytical Method Development and Validation for Anti Asthamatic Drug Oxymetazoline Hydrochloride in Nasal Spray Formulations by RP-HPLC

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ABSTRACT

A new, simple, accurate and economic reverse-phase HPLC method has been developed for quantification of Oxymetazoline Hydrochloride in nasal spray formulations. This developed method has been validated according to International Conference on Harmonization (ICH) guideline with respect to system suitability, specificity, precision, linearity, accuracy, and robustness. An isocratic condition of mobile phase Phosphate buffer (pH 3.0): Acetonitrile in a ratio of 60:40, v/v at a flow rate of 1.0 mL/minute over RP C18 (octadecylsilane (ODS), 250 × 4.6 mm, 5 µm, ECLIPSE X DB C-18) column at ambient temperature was maintained. This method is specific and showed excellent linear response with correlation coefficient (R^2) values of 0.999, which was within the limit of correlation coefficient (R^2 0.995). A simple and accurate reversed-phase HPLC method for the analysis of Oxymetazoline Hydrochloride in nasal spray formulations was developed and validated successfully.

Keywords: Oxymetazoline hydrochloride, ICH, RP-HPLC, Validatation and Nasal Spray.

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1. INTRODUCTION

Asthma is a chronic disease that inflames the airways (i.e. the small tubes, called bronchi) which carry air in and out of the lungs. In asthmatic patient, the bronchi will be inflamed and more sensitive than normal and produce extra mucus. This can make breathing difficult due to reversible airflow obstruction, or bronchospasm, and trigger coughing, wheezing and shortness of breath.

The cause of asthma is probably due to a combination of the following factors:

Environmental: allergens (e.g., house dust mites, animal fur and pollen), occupational irritants (e.g. chemical fumes, gases, dust or other substances on the job), tobacco smoke, respiratory (viral) infections, strong emotional expressions and drugs (e.g. aspirin and beta blockers).

Genetic (inherited): usually occurs in children. The chances of developing asthma are increased if the patients' family members or relatives have asthma and other allergic conditions such as atopic dermatitis and hay fever[1]. Hay fever also known as Allergic rhinitis, is a type of inflammation in the nose which occurs when the immune system overreacts to allergens in the air[2].

Antiasthmatic drugs are used for the treatment of asthma. They may be useful either in the treatment or prevention of asthma attacks[3]. Asthma and allergic rhinitis are related health conditions. Effective treatment for allergic rhinitis may reduce the chance of severe asthma attacks, and make the lungs work better[4]. People those having both asthma and allergic rhinitis should use both a preventer nasal spray and an asthma preventer inhaler regularly. The addition of Oxymetazoline adds to the effectiveness of Fluticasone furoate in the treatment of perennial allergic rhinitis[5].

Oxymetazoline is available over-the-counter as a topical decongestant in the form of Oxymetazoline hydrochloride in nasal sprays such as Otrivin, Afrin, Operil, Dristan, Dimetapp, Oxyspray, Facimin, Nasivin, Nostrilla.It is used to relieve nasal discomfort caused by colds, allergies, and hay fever[6].

Its molecular formula is $C_{16}H_{24}N_2O \cdot HCl$ with the following chemical structure:

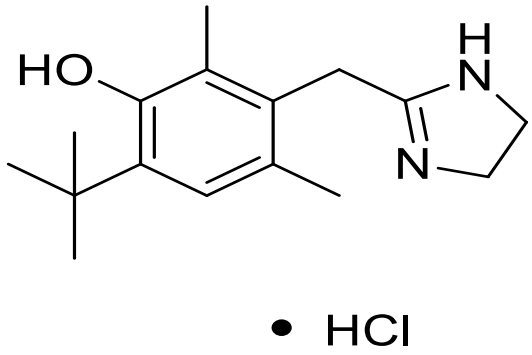


Figure 1: Chemical structure of Oxymetazoline hydrochloride

Oxymetazoline hydrochloride occurs as a white, almost odorless, crystalline powder. It has a molecular weight of 260.38 g/mol. It has a melting point of about 182 °C. It is freely soluble in water and methanol[7].

In present, official method for quantification of Oxymetazoline hydrochloride is available and that is of titrimetric method [8], which is time consuming and highly sensitive, but simple, accurate and precise reverse phase HPLC method is not available. Our interest of work was to develop suitable HPLC method required for analysis and characterization of Oxymetazoline hydrochloride from nasal spray formulation.

2. MATERIALS AND METHODS

2.1 Materials and Reagents

Oxymetazoline hydrochloride working standard and Placebo were a kind gift of Shalini Chemicals, Aurangabad, Maharashtra. Test samples purchased from market store. HPLC grade Acetonitrile, Potassium dihydrogen phosphate and HPLC Water were purchased from Ranbaxy Fine Chemicals Ltd., India.

2.2 HPLC system

High-performance liquid chromatographic system (Agilent (1100) Gradient System) equipped with UV–visible detector was used for the analysis. The data were recorded using Chemstation 10.02 software.

2.3 Preparation of mobile phase

Dissolved 6.8 gm of KH_2PO_4 in to 1000 ml water and sonicated to dissolved (pH observed 4.41), adjusted to pH 3.00 with diluted Orthophosphoric acid solution. Buffer filtered through a 0.45-µm PVDF membrane filter and sonicated to degas. Prepared a mixture of Buffer (pH 3.0) : Acetonitrile (60:40), v/v, sonicated to degas.

2.4 Preparation of standard solution (40 PPM)

Accurately weighed and transferred 40 mg of Oxymetazoline hydrochloride working standard in to 100 mL volumetric flask, added about 30 mL of Acetonitrile and sonicated to dissolve, wait to cool and diluted up to mark with diluent. Transferred 5 mL of this solution in to 50 mL volumetric and diluted up to mark with Mobile Phase.

2.5 Preparation of sample solution (40 PPM)

Brand Name:- Naselin Nasal Spray (CIPLA LTD)

Transferred 2.0 mL of Sample solution in to 25 mL volumetric flask, added about 15 mL of mobile phase and sonicated to mixed properly, diluted up to mark with mobile phase, and the samples were analyzed using the proposed analytical methods.

2.6 Preparation of Placebo solution (8 PPM)

Accurately weighed and transferred 20 mg of Placebo powder in to 200 mL volumetric flask, added about 100 mL of mobile phase and sonicated to dissolve, wait to cool and diluted up to mark with mobile phase. Transferred 2 mL of this solution in to 25 mL volumetric and diluted up to mark with Mobile Phase.

2.7 Chromatographic conditions

The analysis was carried out at temperature 27° C under isocratic condition. The mobile phase was run at a flow rate of 1.0 mL/minute for 10 min. The injection volume was 20 µL for blank, placebo, standard and sample solution . Before analysis, every standard and sample were filtered through 0.45 µm Nylon syringe filter. The analysis was monitored with UV detection at 203 nm.

2.8 Method validation

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the consistency, quality and reliability of analytical results; it is an integral part of any good analytical practice. Typical parameters verified in validation of analytical method are listed in table 1. ICH Q2(R1)[9] is considered the primary reference for recommendations and definitions on validation characteristics for analytical procedures.

Table 1: Typical parameters verified in method validation

Sr No	Validation parameter
1	System suitability
2	Specificity
3	Precision
4	Accuracy
5	Linearity
6	Robustness

2.8.1 System suitability

System suitability is an essential parameter of any analytical method development. The tests are based upon the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test ensure adequate performance of the chromatographic system and quality of the method for accurate results. It is required to done before every sample analysis. To determine system suitability Oxymetazoline Hydrochloride standard solution was prepared and injected for six times into HPLC system. The mean, SD and % RSD for peak areas of Oxymetazoline was calculated.

2.8.2 Specificity

Assuring specificity is the first step in developing and validating a good analytical method[10]. Specificity ensures the identity of the analyte of interest. The ICH documents define specificity as the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to be present in the sample matrix such as impurities and degradation products. The placebo solution containing excipients without

Oxymetazoline were injected. To evaluate the specificity of the method blank, placebo and sample solution were injected.

2.8.3 Precision

Precision is the degree of agreement among individual test results when an analytical method is used repeatedly 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. The precision of the assay method was assessed with respect to repeatability and reproducibility. Repeatability is also termed intra-assay precision. Sample of a single batch were prepared six times and analyzed as per test method, % assay of Oxymetazoline for six samples calculated for method precision.

2.8.4 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. Accuracy should be reported as percent recovery by the assay of known added amount of analyte in test solution. In this study, successive analysis (n=3) for three different concentrations of standard mixtures (80, 100 and 120%) was carried out to determine the accuracy of proposed method.

2.8.5 Linearity

The linearity of an analytical procedure is its ability within a given range to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity has been performed on different concentrations within 25–150% of the nominal standard concentration. The linearity of this proposed method was evaluated by using calibration curve to calculate the coefficient of correlation, slope, and intercept values.

2.8.6 Robustness

Robustness is a capacity of the method to remain unaffected by small deliberate, variations in method parameters. 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: Detector wavelength±2 nm, Flow rate±10%, Temperature±2 °C, and pH of Buffer solution±0.1.

3. RESULTS AND DISCUSSION

3.1 System suitability

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

Table 2: Results from the determination of system precision

Standard No	Peak area of Oxymetazoline
1	6954
2	6920
3	6998
4	6941
5	6932
6	6943
Mean	**6948**
SD	**27.02**
%RSD	**0.39**

Values are expressed for six replicate (n=6)

Table 3: Data from Method precision

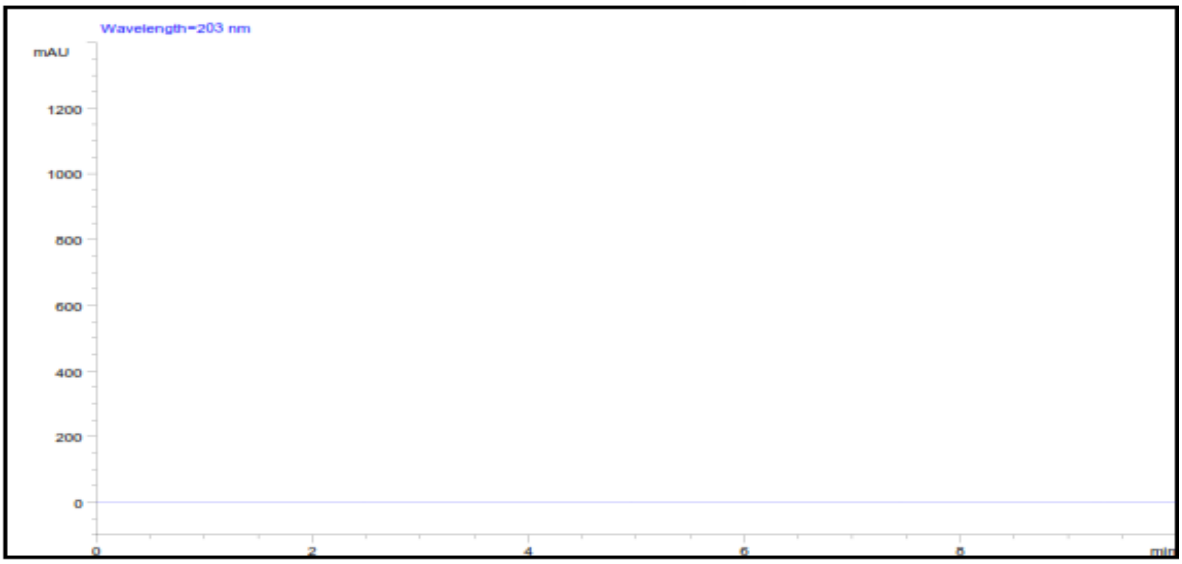
Sample No	% Assay of Oxymetazoline
1	101.3
2	100.7
3	100.4
4	101.5
5	101.1
6	101.2
Mean	**101.0**
SD	**0.42**
% RSD	**0.42**

Values are expressed as mean± standard deviation of six samples (n=6)

3.2 Specificity

Accuracy should be reported as percent recovery by the assay of known added amount of analyte in the sample

a)Blank



b)Placebo

c)Sample solution

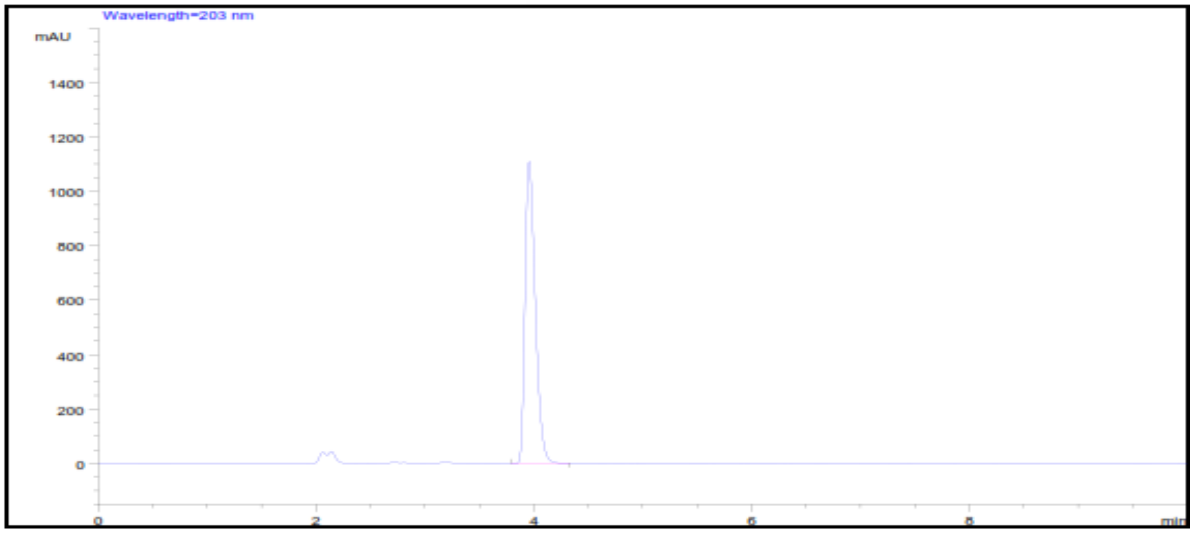


Figure 2: HPLC Chromatogram a)Blank, b)Placebo and c)Sample solution

3.3 Accuracy

Known amount of Oxymetazoline hydrochloride was spiked in placebo at about 80,100 and 120% of test concentration. The amount of Oxymetazoline hydrochloride recovered 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 Oxymetazoline hydrochloride in the samples was in between 98.0 to 102.0% (RSD<2%) which is satisfactory for quantification of Oxymetazoline hydrochloride in nasal spray formulations.

Table 4: Accuracy evaluation of the proposed method for quantification of Oxymetazoline

Spiked level (%) / Sample No	Actual amount of API added(mg)	Amount of Oxymetazoline found (mg)	% Recovery	Mean	SD	%RSD
80% Sample-1	0.8015	0.7907	98.6	98.8	0.16	0.16
80% Sample-2	0.8015	0.7926	98.9			
80% Sample-3	0.8015	0.7931	98.9			
100% Sample-1	1.0019	0.9974	99.6	99.5	0.07	0.07
100% Sample-2	1.0019	0.9966	99.5			
100% Sample-3	1.0019	0.9962	99.4			
120% Sample-1	1.2023	1.2092	100.6	100.5	0.06	0.06
120% Sample-2	1.2023	1.2086	100.5			
120% Sample-3	1.2023	1.2079	100.5			

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

3.4 Linearity

A graph was plotted with concentration (in µg/ml) of Oxymetazoline hydrochloride on X-axis and peak areas of Oxymetazoline on Y-axis. The results are tabulated in table 5 and graphically represented in figure 3.Excellent linear response has been observed with correlation coefficient (R^2) values of 0.999, which was within the limit of the correlation coefficient (R^2=0.995).

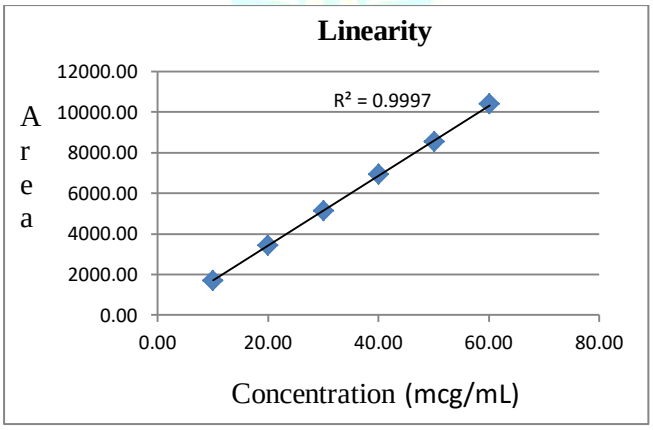


Fig.3: Linearity plot for Oxymetazoline

Table 5: Results of linearity

Spike level in %	Concentration (mcg/mL)	Average Area (N=2)
25	10.03	1695
50	20.06	3408
75	30.09	5112
100	40.12	6927
125	50.15	8539
150	60.18	10399
Slope		173
Y-Intercept		-59.47
Correlation Coefficient ®		0.99989

3.5 Robustness results

Robustness of the method was verified by deliberately applying the following chromatographic conditions as shown in table 6 i.e.

I By changing the wavelength by ±2 nm
II By changing the flow rate by ±10%
III By changing the column oven temperature by ±2 °C
IV By changing the pH of buffer used for mobile phase by ±0.1 unit

Table 6: Robustness experiment

Sr. No.	Robustness Parameter	Retention Time (min)	Tailing factor	Theoretical plates	% RSD of Standard solution
1	Wavelength 201 nm	3.77	0.96	7911	0.06
2	Wavelength 205 nm	3.78	0.96	7904	0.08
3	Flow rate (0.90 mL/min)	4.18	0.86	9321	0.12
4	Flow rate (1.10 mL/min)	3.59	0.88	8831	0.07
5	Column Temp 25° C	4.00	0.89	7869	0.04
6	Column Temp 29° C	4.00	0.89	7854	0.09
7	Buffer pH 2.9	4.01	0.91	7452	0.05
8	Buffer pH 3.1	4.03	0.89	7519	0.11

3.6 Estimation of formulations

The observation for assay of Oxymetazoline hydrochloride in nasal spray formulations ranged from 100.4 % to 101.5 %, with a standard deviation of not more than 0.42%. The assays for the formulations were observed same as mentioned in the label claim, indicating that the suitability of the proposed analytical method. The estimated drug content with low values of standard deviation established the precision of the proposed method.

4.0 DISCUSSION

Development of an analytical method for assessment of drugs in the pharmaceutical dosage form is of most necessity to confirm the quality of nasal formulations with respect to assay and spray content uniformity.

Development and validation of Oxymetazoline hydrochloride by high performance liquid chromatography is reported in literature[11,12] but we have observed below drawbacks in hose analytical methods which are as follows:

- Almost all methods are time consuming which affects on productivity in routine sample analysis.
- Usage of organic solvent more than 45% is not at all cost effective and also affects on chromatographic conditions; buffer salts may precipitate
- Complex methodology effects on accuracy of analytical method and man power utilization in pharmaceutical industry.

Our developed HPLC analytical method for estimation of Oxymetazoline in nasal spray formulations has used minimum amount of organic solvents which is cost effective, economic and environment friendly. The wavelength used in proposed analysis method also efficient to trace unexpected solvent peaks qualitatively.

5.0 CONCLUSIONS

A very simple reversed-phase HPLC method for the routine and stability analysis of Oxymetazoline hydrochloride in nasal spray formulations was developed and validated. The proposed method is new, simple, accurate, precise, robust, specific and linear over the analysis ranges and also 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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