METHOD DEVELOPMENT AND VALIDATION OF OXYMETAZOLINE HCl AND SORBITOL IN BULK AND PHARMACEUTICAL DOSAGE FORM

Dr. M. David Raju
Department of chemistry, PB Siddhartha College of Arts and Sciences, Vijayawada, AP.

Abstract
An accurate, rapid economical and simple, reliable assay method was developed and validated for the simultaneous estimation of Oxymetazoline HCl and Sorbitol using RP-HPLC. In the proposed method efficient chromatographic separation was achieved using Waters X-Terra RP-18 column (150x4.6mm, 3.5µ) as a stationary phase and acetonitrile: 0.1%OPA (70+30), as a mobile phase with a flow rate of 1ml/min and UV detection at 230nm. Chromatography was carried out isocratically at ambient temperature and the run time was approximately 10min. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 10% to 150% levels, R^2 value was found to be as 0.999. By using above method assay of marketed formulation was carried out 100.1% was present. Degradation studies of Oxymetazoline HCl and Sorbitol were done, in all conditions purity threshold was more than purity angle and within the acceptable range.
Key Words: Oxymetazoline HCl, Sorbitol, HPLC.

INTRODUCTION

Oxymetazoline is a selective α_1 adrenergic receptor agonist [1] and α_2 adrenergic receptor partial agonist [2]. It is a topical decongestant [3], used in the form of Oxymetazoline hydrochloride. It was developed from xylometazoline [4] at E. Merck Darmstandt by Fruhstorfer in 1961. Oxymetazoline is generally available as a nasal spray. Oxymetazoline is available over the counter as a topical decongestant in the form of oxymetazoline hydrochloride in nasal spray such as Otrivin, Afrin, Operil, Dristan, Dimetapp, Oxyspray, Facimin, Nasivin, Nostrilla, Utabon, Sudafed OM, Vicks Sinex, Zicam, SinuFrin, Drixoral and Mucinex full force. Due to its vasoconstricting [5] properties, oxymetazoline is also used to treat nose [6,7] and eye redness [8] due to minor irritation (marketed as Visine L R in the form of eye drops).

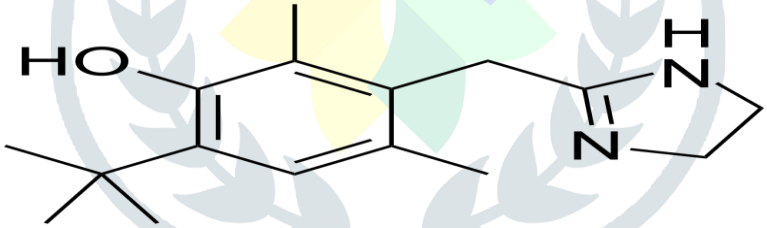

Fig. 1: Chemical Structure of Oxymetazoline

Sorbitol, less commonly known as glucitol, is a sugar alcohol [9] with a sweet taste which the human body metabolizes [10] slowly. It can be obtained by reduction of glucose, which changes the aldehydes group to a hydroxyl group. Most Sorbitol is made from corn syrup, but it is also found in nature, for example in apples, pears [11], peaches [12] and prunes [13, 14]. It is converted to fructose [15] by Sorbitol-6-phosphate 2-dehydrogenase. Sorbitol is an isomer [16] of mannitol [17], another sugar alcohol; the two differ only in the orientation of the hydroxyl group on carbon 2. While similar, the two sugar alcohols have very different sources in nature, melting points [18], and uses.

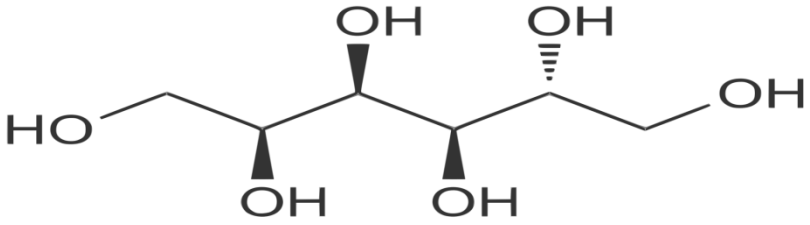

Fig. 2: Chemical structure of Sorbitol

Different chromatographic methods were studied in an attempt to optimize simple, reliable and sensitive and an accurate method for the estimation of studied compounds in bulk and pharmaceutical dosage forms. But literature search reveals that there was no HPLC method for the simultaneous estimation of these drugs has been reported so far. The purpose of the present work therefore was to development a fast, economical, sensitive and confirmation of Oxymetazoline HCl and Sorbitol in bulk and pharmaceutical dosage forms.

MATERIALS AND EQUIPMENTS

Chemicals

Acetonitrile, Ortho Phosphoric Acid and water (HPLC grade) were purchased from Merck (India) Ltd. Worli, Mumbai, India. All active pharmaceutical ingredients (APIs) of Oxymetazoline HCl and Sorbitol as reference standards were procured from Glenmark pharmaceuticals private Ltd., Andheri (E), Mumbai, India (99.7-99.9% purity).

Equipment

Waters alliance-2695 chromatographic system consisting of quaternary pump, PDA detector-2996 and chromatographic software Empower-2.0 was used.

Chromatographic conditions

Chromatographic separation was carried out in isocratic mode at room temperature using a Waters X- Terra RP-18 column (150x4.6mm, 3.5µ) column. The mixture of 0.1% OPA: acetonitrile 30+70 at a flow rate of 1ml/min was used as a mobile phase. The injection volume was 10µl and eluent was monitored at 230nm using PDA detector. The run time was 10min and each of the studied components was quantified by using total peak height.

Preparation of Buffer

Add 1ml of Ortho Phosphoric acid in 1lt of water and mix well. Filtered through 0.45µ membrane filter paper and degassed.

Selection of Mobile Phase

Prepared a mixture of Buffer and Acetonitrile (30+70 v/v). The selected mobile phase has given a sharp peaks with low tailing factor i.e. < 2.0 and also plate count will be less than 3000.

Selection of Wavelength

The absorption spectra of solution of each Oxymetazoline and Sorbitol were scanned over the range 200-400nm by using photodiode spectrophotometer and the spectra were recorded. Two drugs combined at common wavelength 230nm was selected.

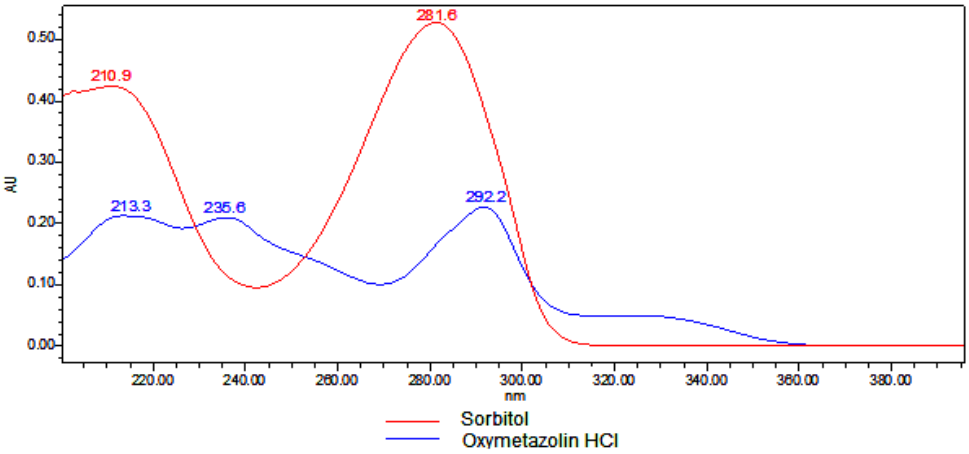

Fig. 3: PDA Spectrum of Oxymetazoline HCl and Sorbitol

Diluent

Use mobile phase as a diluent.

Preparation of standard solution

Standard stock solution: Weigh about 0.25mg of Oxymetazoline HCl and 10mg of Sorbitol and transferred into a 100ml volumetric flask. Then they were dissolved in 70ml diluent and sonicated for about 10min with intermittent shaking and diluted up to the mark with diluent.

Further transferred 5ml of above solution into 50ml volumetric flask and diluted up to the mark with diluent.

Preparation of sample solution

Accurately weigh 0.25mg of Oxymetazoline HCl and 10mg of Sorbitol equivalent weight of sample and transferred into a 100ml volumetric flask and dissolved in 70ml of diluent and sonicated to dissolve and diluted up to the mark with the diluent. Then, 5ml of above solution was diluted with the 50ml of diluent and was filtered through 0.45µ nylon syringe filter.

Procedure for Analysis

A steady baseline was recorded by the optimized chromatographic conditions. It was stabilized for about 30min and successive aliquots of the standard solution of the same concentration were injected and chromatogram was recorded until the reproducibility of the peak areas was satisfactory. This procedure was repeated using the sample solution so that duplicate injection of the sample solution was bracketed by injection of the standard solution. The response factor of the standard peak and sample peak was obtained and the amount of each drug in the sample was determined. This procedure was repeated six times. The concentration of each drug in the triple component dosage form was calculated using the formula,

$$\text{Concentration of drug} = \frac{\text{Response factor of the sample} \times \text{Concentration of standard}}{\text{Response factor of the standard}}$$

Validation Procedure

The analytical method was validated as per ICH Q_2 (R1) guidelines for the parameters like system suitability, specificity, accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantification (LOQ), forced degradation and stability.

System Suitability

System suitability parameters were measured to verify the system performance. The parameters including USP plate count, USP tailing and %RSD are calculated and found to be within the limits.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of other components (impurities, degradates or excipients), which may be expected to be present in the sample and standard solution. It was checked by examining the chromatograms of blank samples and samples spiked with Oxymetazoline HCl and Sorbitol.

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. It was assessed by the recovery studies at three different concentration levels. In each level, a minimum of three injections were given and amount of the drug present, percentage recovery and related standard deviation were calculated.

Precision

Precision of an analytical method is the degree of agreement among individual test results. It was studied by analysis of multiple sampling of homogeneous sample. The precision of the present method was assessed in terms of repeatability, intra-day and inter day variations. It was checked by analyzing the samples at different time intervals of the same day as well as on different days.

Linearity and range

Linearity of an analytical method is its ability to obtain results directly proportional to the concentration of the analyte in the sample within a definite range. The six series of standard solutions were selected for assessing linearity range. The calibration curve was plotted using peak area versus concentration of the standard solution and the regression equations were calculated. The least squares method was used to calculate the slope, intercept and correlation coefficient.

LOD and LOQ

LOD is the lowest amount of analyte in a sample that can be detected while LOQ is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy. LOD and LOQ were separately determined based on the calibration curves. The LOD and LOQ for Oxymetazoline HCl and Sorbitol were determined by injecting progressively low concentrations of standard solutions using the developed RP-HPLC method. The LOD and LOQ were calculated as 3.3s/n and 10s/n respectively as per ICH guidelines, where s/n indicates signal-to-noise ratio.

Robustness

Robustness of an analytical procedure is a measure of its ability to remain unaffected by small deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness study was performed by injecting standard solution into the HPLC system and altered chromatographic conditions such as flow rate (±0.2ml/min), Wavelength (±5nm), variation in pH (±0.2), organic content in the mobile phase (±7). The separation factor, retention time and peak asymmetry were calculated by determining the effect of the modified parameters.

Stability

Analytical solution was prepared and injecting into the HPLC system at periodic intervals of 0h to 24h at 6h intervals depending on the instrument utilization and sequence of injection.

Results and Discussion

The current study was designed to develop a simple, precise and rapid analytical RP-HPLC method, which can be used for the analysis of assay method for simultaneous estimation of Oxymetazoline HCl and Sorbitol in bulk and pharmaceutical dosage forms. The chromatographic conditions were optimized in order to provide a good performance of the assay. To optimize mobile phase, various combinations of 0.1% OPA: acetonitrile were tried for Oxymetazoline and Sorbitol and the final working mobile phase is buffer and acetonitrile in composition of 30:70 v/v. Mobile phase for each drug was selected based on its polarity. Detection was carried out in several wavelengths in order to obtain enough sensitivity for the three APIs in smaller proportion (Oxymetazoline HCl and Sorbitol). At last, the wavelength 230nm, at which the two drugs showed good absorbance, was selected as a detection wavelength. The flow rate was 1ml/min, which is critical as it affects the peak symmetry parameters. The retention time for Sorbitol Oxymetazoline HC and Oxymetazoline HCl were 3.382 and 6.611min respectively. The proposed method is validated in accordance with the

ICH guidelines with all of the results within the limits. In this method a good linearity was observed in the range of 1-15 µg/ml of Sorbitol and 0.025-0.375µg/ml of Oxymetazoline HCl respectively.

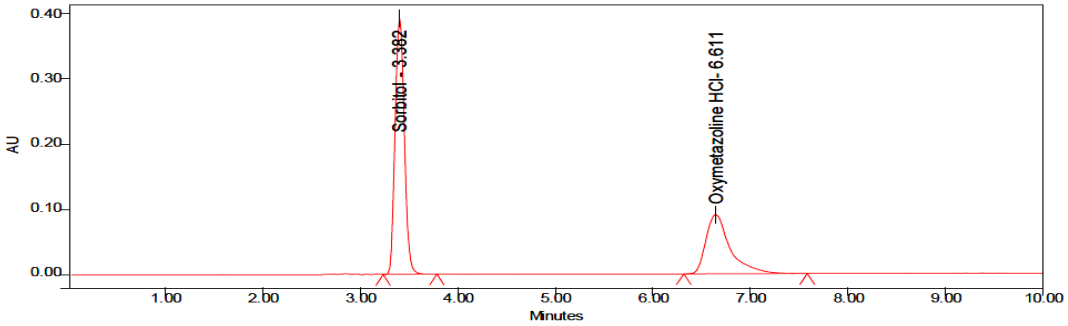

Fig. 4: Typical chromatogram of Oxymetazoline HCl and Sorbitol

Method Validation tests

System Suitability

The HPLC system was stabilized for 60min to get a stable base line. Six injections of the mixture containing 10µg/ml of Sorbitol and 0.25µg/ml of Oxymetazoline and were assessed to check the system suitability. The system suitability parameters were evaluated from six replicate injections. The study concludes that the suitability of the HPLC system being used and results were summarized below.

Table 1: System suitability data

System suitability Parameter	Acceptance criteria	Drug Name	
		Oxymetazoline HCl	Sorbitol
%RSD	NMT 2.0	1.02	1.33
USP Tailing	NMT 2.0	1.11	1.09
USP Plate count	NLT 3000	7822	5834

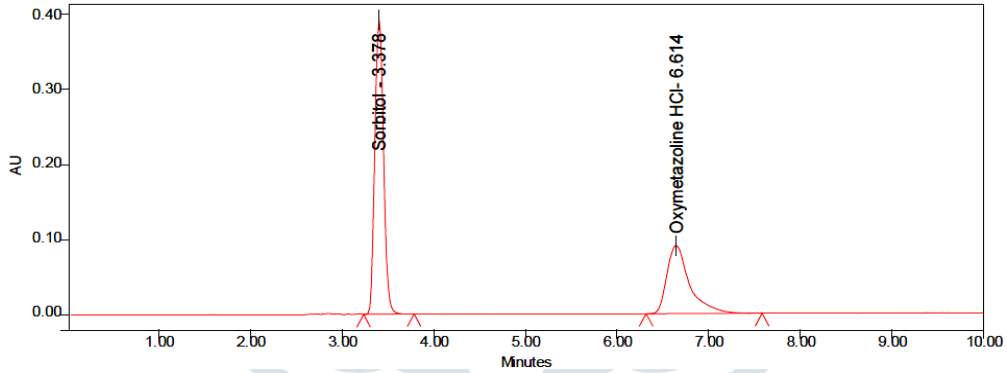

Fig. 5: Chromatogram of system suitability

Specificity

There was no interference from blank at the retention time of Oxymetazoline HCl and Sorbitol. Hence the method is specific.

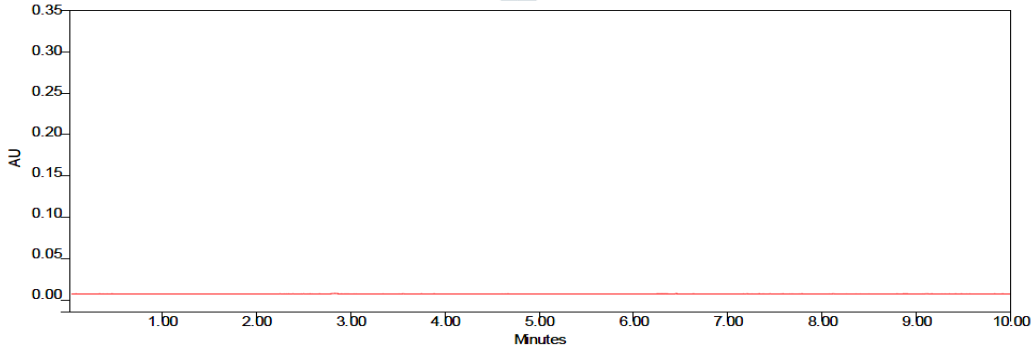

Fig. 6: Typical chromatogram of Blank

Linearity

Linearity was determined by plotting a calibration curve of peak area against their respective concentration. From this calibration curve it was found that the curve was linear in the range of 1-15µg/ml of Sorbitol and 0.025-0.375µg/ml of Oxymetazoline HCl. The regression equations for calibration curves was Y= 3842403x+x1217.3 (R^2= 0.999) for Oxymetazoline HCl and Y= 256082x+26239 (R^2= 0.999) for Sorbitol respectively.

Table 2: Results of Linearity

Linearity level	Oxymetazoline HCl µg/ml	Area counts	Sorbitol µg/ml	Area counts
Linearity-1	0.03	92635	1.00	275964
Linearity-2	0.06	256978	2.50	689536
Linearity-3	0.13	486325	5.00	1362578
Linearity-4	0.25	932635	10.00	2546315
Linearity-5	0.31	1196354	12.50	3162597
Linearity-6	0.38	1462358	15.00	3926450
Corelation coefficinet	0.9992		0.999	
Slope	3842403		256082	
Intercept	1217.3		26239	

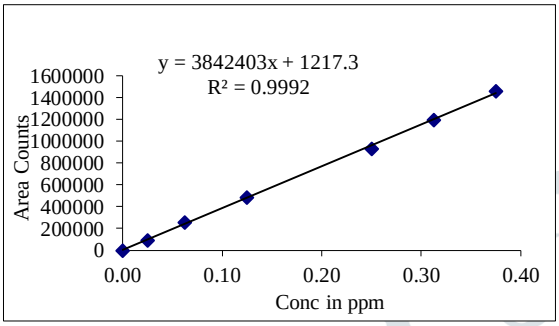

Fig.7: Linearity plot for Oxymetazoline HCl

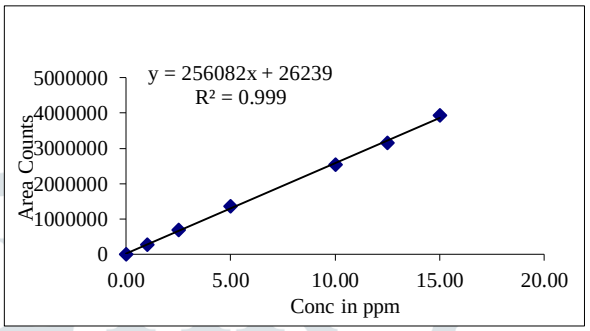

Fig.8: Linearity plot for Sorbitol

Accuracy

The accuracy of the method was performed by calculating the recovery experiments at three levels (50%, 100% and 150%). APIs with concentration 50, 100 and 150µg/ml of Oxymetazoline HCl , 0.125, 0.25 and 0.375µg/ml of Oxymetazoline HCl and 5, 10 and 15µg/ml of Sorbitol were prepared. The test solution was injected three times for each spike level and assay was performed as per the test method. The recovery results were close to 100% and also the RSD values were less than ±2%. The percentage recovery, mean and relative standard deviation were calculated. Recovery values demonstrated that the method was accurate within the desired range. The results are summarized below.

Table 3: Results of Accuracy

Accuracy	Amount of Oxymetazoline µg/ml	Recovery (area) mAU	% drug recovery	Amount of Sorbitol µg/ml	Recovery (area) mAU	% drug recovery
50%	0.125	496358	99.9	5	1284526	100.1
100%	0.25	835624	99.7	10	2563978	100.3
150%	0.375	1315264	100. 1	15	4013269	99.8

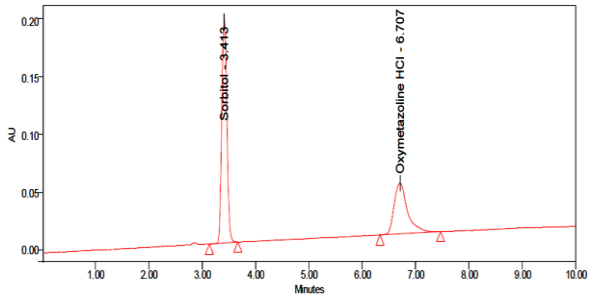

Fig. 9: Chromtogram of Accuracy 50%

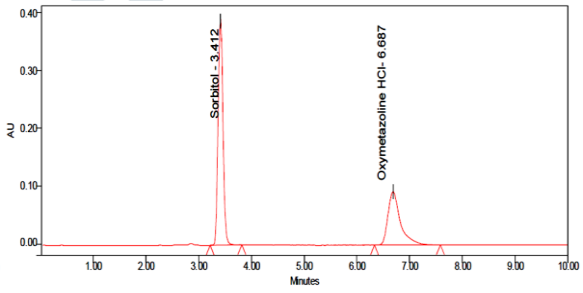

Fig.10: Chromatogram of Accuracy 100%

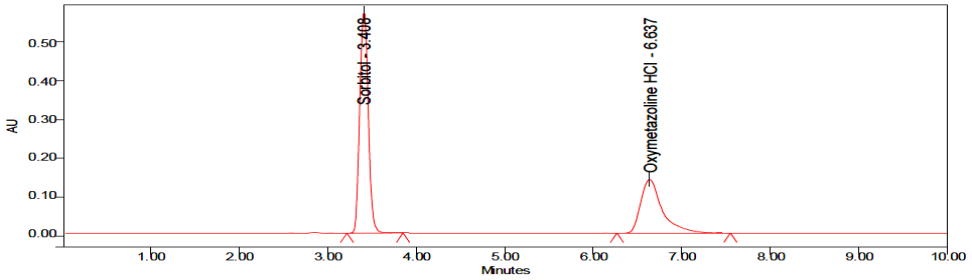

Fig. 11: Chromatogram of Accuracy 150%

Precision

Precision of this method was assesd interms of intraday (repeatability) and interday (intermediate precision) variations. The intraday studies were determined by performing six repeated analysis of the sample solution of Oxymetazoline HCl and Sorbitol on the same day under the same experimental conditions. The intermediate precision of the method was carried out in the same laboratory by studying the analysis with different analyst and different instrument. The method is highly precise as % RSD values were found to be < 2%. Good recoveries (98-102%) of the drug were obtained at each added concentration, indicating that the method was accurate. The results and chromatograms were furnished below

Table 4: Method precision results

Oxymetazoline HCl µg/ml	Area (mAU)	% RSD	Sorbitol µg/ml	Area (mAU)	%RSD
0.25	823652	0.24	10	2536975	0.68
	825468			2503677	
	825310			2540361	
	824356			2516926	
	825012			2503625	
	820157			2536365	

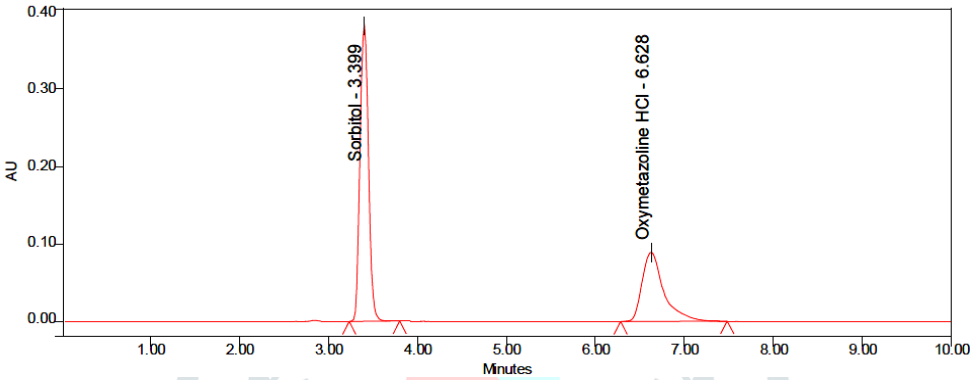

Fig. 12: Chromagram of Method Precison

Intermediate Precision

Table 5: Results of Intermediate precision

Concentration of Oxymetazoline HCl µg/ml	Area (mAU)	%RSD	Concentration of Sorbitol µg/ml	Area (mAU)	% RSD
0.25	813548	0.17	10	2563978	1.24
	812635			2502364	
	813062			2504015	
	815360			2546035	
	814502			2548657	
	816305			2579658	

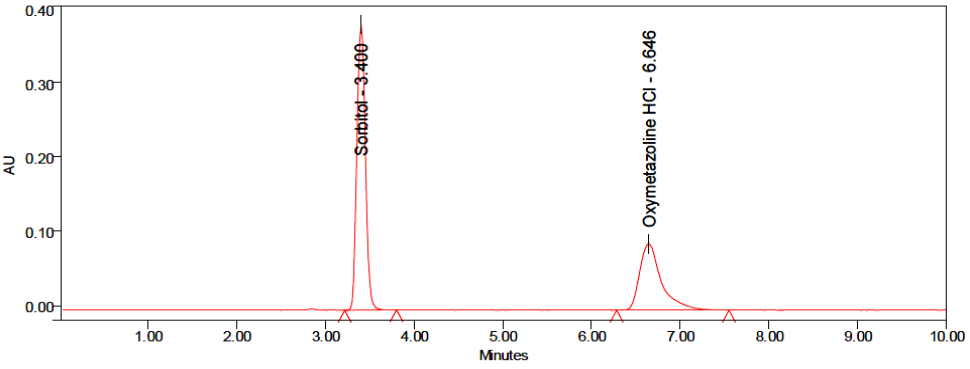

Fig. 13: Chromatogram of Intermediate Precision

LOD and LOQ

LOD and LOQ were separately determined by calibration curve method. LOD and LOQ of the compounds were determined by injecting progressively lower concentrations of standard solutions using the developed RP-HPLC method. The LOD values for Oxymetazoline HCl and Sorbitol were found 0.01µg/ml and 0.0025µg/ml respectively. LOD s/n values are 4, 8. The LOQ values were found to be 0.1µg/ml and 0.025µg/ml for Oxymetazoline HCl and Sorbitol respectively. LOQ s/n values are 23, 27.

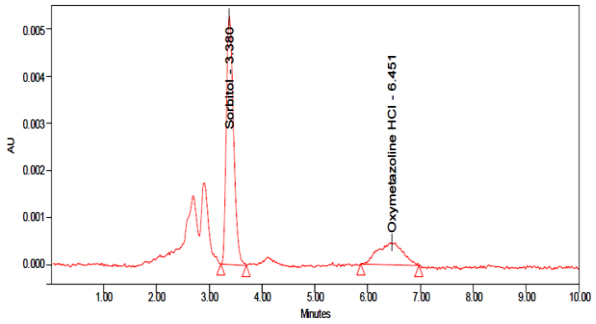

Fig. 14: Chromatogram of LOD

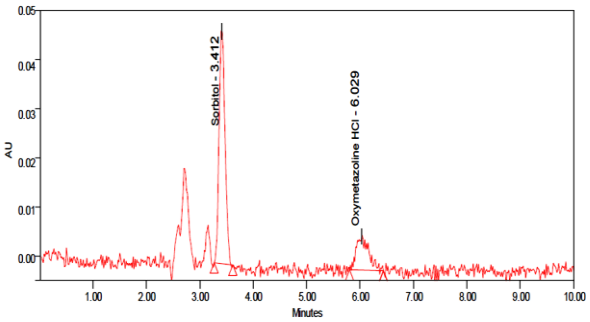

Fig. 15: Chromatogram of LOQ

Robustness

As per ICH norms, small but deliberate variations were made in the method parameters such as change in the flow rate (±0.2), organic content in the mobile phase (±10%), wavelength of detection (±5) to check the method capacity to remain unaffected. The robustness of the method was evaluated by observing the effect of the modified parameters on retention time, tailing factor, area, percentage content. The degree of reproducibility of the results which were obtained by small deliberate variations has proven that the method is robust.

Table 6: Results of Robustness studies

Change in parameter	% RSD for Oxymetazoline HCl	% RSD for Sorbitol
Flow rate (0.8ml/min)	0.25	0.75
Flow rate (1.2ml/min)	0.32	0.84
Organic phase composition (-10%)	0.22	0.65
Organic phase composition (-10%)	0.31	0.88
Wave length Minus (225nm)	0.38	0.78
Wave length Plus (235nm)	0.42	0.95

Stability

To assess the stability of sample solutions, they were analysed initially to 24h at different intervals of time at room temperature. No significant degradation was observed during this period and the % deviation was not more than 5.0%, suggesting that the solutions were stable for at least 24h, which was sufficient for the whole analytical procedure. Results are furnished below.

Table 7: Results of stability studies

Stability	% Lable claim Oxymetazoline HCl	%Deviation Oxymetazoline HCl	% Lable claim Sorbitol	% Deviation Sorbitol
Initial	99.7	0.00	100.2	0.00
6 Hr	99.6	-0.10	100	-0.20
12 Hr	99.4	-0.30	99.9	-0.30
24 Hr	99.3	**-0.40**	99.8	**-0.40**

Conclusion

In this study a novel, simple, rapid, economical, sensitive and easily available HPLC method was developed for the simultaneous determination of Oxymetazoline HCl and Sorbitol in bulk and spray dosage form. The main advantages of this method there is no HPLC methods are reported. In this method shorter run time, low price, accessibility, sensitivity, reliability and reproducibility. These properties are important when a large number of samples are to be analysed. The validation of all the parameters like linearity, accuracy, specificity, robustness, stability was done and found to be within the acceptance criteria. The RSD values for all parameters were found to be less2, which indicates the validity of method and results obtained by this method are in fair agreement. So the proposed method could be easily applied for the routine analysis and pharmaceutical formulations of Oxymetazoline HCl and Sorbitol in quality control laboratories without any preliminary separation.

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