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A New RP-HPLC Method Development and Validation of Ozenoxacin Cream

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ABSTRACT: This research paper is A Simple, sensitive, specific and precise RP-HPLC method for the Determination of Ozenoxacin in API and pharmaceutical dosage form. Chromatogram was run through Azilent C18 150 x 4.6 mm, 5µ. Mobile phase containing 0.1N NH_2PO_4: Acetonitrile taken in the ratio 60:40was pumped through column at a flow rate of 1.0ml/min.. Temperature was maintained at 30°C. Optimized wavelength selected was 314.0nm. Retention time of Ozenoxacin was found to be 2.429 min. %RSD of the Ozenoxacin were and found to be 067. %RSD of Method precision of Ozenoxacin was found to be 0.9. %Recovery was obtained as 100.34% for Ozenoxacin. LOD, LOQ values obtained from regression equation of Ozenoxacin were 0.03, 0.09. Regression equation of Ozenoxacin is y = 14216x + 495.45. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries

KEYWORDS: Ozenoxacin, RP-HPLC

I. INTRODUCTION

Due to the various advantages such as speed, specificity, consistency, accuracy, precision, and ease of automation in these methods, most of the drugs in multicomponent dosage form can be analysed by the HPLC system. The HPLC approach avoids repetitive processes for extraction and isolation. In HPLC, there are distinct modes of differentiation. They are Normal Phase Mode, Inverted Phase Mode, Chromatography of Reversed Phase Ion Phase, Chromatography of Affinity and Chromatography of Size Exclusion.

The quality of a drug plays an important role in ensuring the safety and efficacy of the drugs. Quality assurance and control of pharmaceutical and chemical formulations is essential for ensuring the availability of safe and effective drug formulations to consumers. Hence Analysis of pure drug substances and their pharmaceutical dosage forms occupies a pivotal role in assessing the suitability to use in patients. The quality of the analytical data depends on the quality of the methods employed in generation of the data (1) . Hence, development of rugged and robust analytical methods is very important for statutory certification of drugs and their formulations with the regulatory authorities.

The wide variety of challenges is encountered while developing the methods for different drugs depending on its nature and properties. This along with the importance of achieving the selectivity, speed, cost, simplicity, sensitivity, reproducibility and accuracy of results gives an opportunity for researchers to come out with solution to address the challenges in getting the new methods of analysis to be adopted by the pharmaceutical industry and chemical laboratories. Different physico-chemical methods (1) are used to study the physical phenomenon that occurs as a result of chemical reactions. Among the physico-chemical methods, the most important are optical (refractometry, polarimetry, emission and fluorescence methods of analysis), photometry (photocolorimetry and spectrophotometry covering UV-Visible, IR Spectroscopy and nepheloturbidimetry) and chromatographic (column, paper, thin layer, gas liquid and high performance liquid chromatography) methods. Methods such as nuclear magnetic resonance (NMR) and para magnetic resonance (PMR) are becoming more and more popular. The combination of mass spectroscopy (MS) with gas chromatography is one of the most powerful tools available. The chemical methods include the gravimetric and volumetric procedures which are based on complex formation; acid-base, precipitation and redox reactions. Titrations in non-aqueous media and complexometry have also been used in pharmaceutical analysis. The number of new drugs is constantly growing. This requires new

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methods for controlling their quality. Modern pharmaceutical analysis must need the following requirements.

1. The analysis should take a minimal time.
2. The accuracy of the analysis should meet the demands of Pharmacopoeia.
3. The analysis should be economical.
4. The selected method should be precise and selective.

1.1 CHROMATOGRAPHY

Chromatography (Chroma means 'color' and graphein means to 'write') is the collective term for a set of laboratory techniques for the separation of mixtures. It involves passing a mixture dissolved in a "mobile phase" through a stationary phase,(2-4) which separates the analyte to be measured from other molecules in the mixture based on differential partitioning between the mobile and stationary phases. Differences in compounds partition coefficient results in differential retention on the stationary phase and thus changing the separation.
Different types of chromatographic techniques were summarized in

1.1.1 Different types of chromatographic techniques

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for further use (and is thus a form of purification). Analytical chromatography is done normally with smaller amounts of material and is for measuring the relative proportion of analytes in a mixture**.**

1.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Liquid chromatography(3) is an analytical chromatographic technique that is useful for separating ions or molecules that are dissolved in a solvent. If the sample solution is in contact with a second solid or liquid phase to differing degrees due to differences in adsorption, ion exchange, partitioning or size. These differences will allow the mixture components to be separated from each other by using these differences to determine the time of the solutes through a column. During 1970's, most chemical separations were carried out using a variety of techniques including open-column chromatography, paper chromatography and thin layer chromatography (TLC). However, these chromatographic techniques were inadequate for quantification of compounds and resolution between similar compounds. During this time pressure liquid chromatography began to be used to decreased flow through time, thus reducing separation time of compounds being isolated by column chromatography. However, flow rates were inconsistent, and the question of whether it was better to have constant flow rate or constant pressure debated. High pressure liquidchromatography quickly improved with the development of column packing materials. Additional convenience of on-line detectors became rapidly a powerful separation technique and is today called as High Performance Liquid Chromatography (HPLC).

CLASSIFICATION OF HPLC

Based on modes of chromatography:

- Normal phase chromatography
- Reverse phase chromatography

Based on principle of separation:

- Adsorption chromatography
- Partition chromatography
- Ion exchange chromatography
- Size exclusion chromatography
- Affinity chromatography
- Chiral phase chromatography

Based on elution technique:

- Isocratic separation
- Gradient separation

Based on the scale of operation:

- Analytical HPLC
- Preparative HPLC

Normal Phase - High Performance Liquid Chromatography (NP-HPLC)

NP-HPLC explores the differences in the strength of the polar interactions of the analytes in the mixture with the stationary phase. The stronger the analyte-stationary phase interaction, the longer the analyte retention. Analyte molecules compete with the mobile phase molecules for the adsorption sites on the surface of the stationary phase. The stronger the mobile phase interactions with the stationary phase, the lower the difference between the stationary phase interactions and the analyte interactions, and thus the lower the analyte retention. Mobile phases in NP-HPLC are based on non-polar solvents (such as hexane, heptanes, etc.) with the small addition of polar modifier (i.e., methanol, ethanol). Packing materials traditionally used in NP-HPLC are usually porous oxides such as silica (SiO2) or alumina (Al2O3). Surface of

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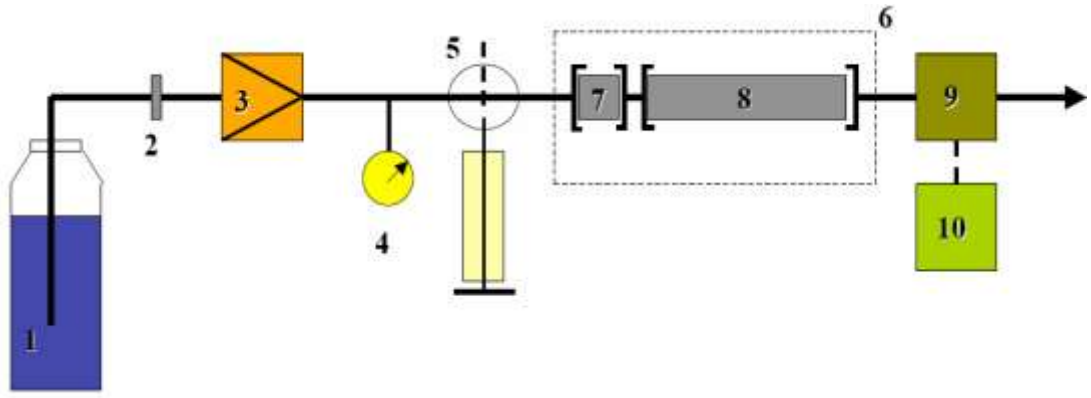
these stationary phases is covered with the dense population of OH groups, which makes these surfaces highly polar. Chemically modified stationary phases can also be used in NP-HPLC. Silica modified with trimethoxy glycidoxypropyl silanes (common name: diol-phase) is typical packing material with decreased surface polarity. Since NP-HPLC uses mainly non-polar solvents, it is the method of choice for highly hydrophobic compounds (which may show very stronger interaction with non polar mobile phases), which are insoluble in polar or aqueous solvents.

Reversed Phase - High Performance Liquid Chromatography (RP-HPLC)

As opposed to NP-HPLC, RP-HPLC employs mainly dispersive forces (hydrophobic or vanderwal's interactions). The polarities of mobile and stationary phases are reversed, such that the surface of the stationary phase in RP-HPLC is hydrophobic and mobile phase is polar, where mainly water-based solutions are employed. RP-HPLC is by far the most popular mode of chromatography. Almost 90 % of all analyses of low-molecular-weight samples are carried out using RP-HPLC. Dispersive forces employed in this separation mode are the weakest intermolecular forces, thereby making the overall background interaction energy in the chromatographic system very low compared to other separation techniques. This low background energy allows for distinguishing very small differences in molecular interactions of closely related analytes. Adsorbents employed in this mode of chromatography are porous rigid materials with hydrophobic surfaces. The majority of packing materials used in RP-HPLC are chemically modified porous silica.

II.INSTRUMENTATION OF HPLC



HPLC is a special branch of Column Chromatography in which the mobile phase is forced through the column at high speed. As a result, the analysis time is reduced by 1-2 orders of magnitude relative to classical Column Chromatography and the use of much smaller particles of the absorbent or support becomes possible increasing the column efficiency substantially. The Basic HPLC Instrumentation (5-9) was shown in the Fig.

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1 = eluent reservoir
2 = filter
3 = high pressure pump with pulse dampener
4 = pressure gauge
5 = sample injection valve with syringe
6 = column oven
7 = guard column
8 = column
9 = detector
10 = recorder (integrator, PC etc.)

III.METHOD

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

Preparation of Standard stock solutions: Accurately weighed 10mg of Ozenoxacin is transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (200µg/ml of Ozenoxacin)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (20µg/ml of Ozenoxacin).

Preparation of Sample stock solutions: 1% of Cream were weighed and the average weight of cream was calculated, then the weight equivalent to 1% Cream was transferred into a 50ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (200µg/ml of Ozenoxacin)

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (20µg/ml of Ozenoxacin)

Preparation of buffer:

0.01N NA_2HPO_4 Buffer: Accurately weighed 1.41gm of Sodium Hydrogen Phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water (4.0-pH).

Validation:

System suitability parameters:

The system suitability parameters were determined by preparing standard solution of Ozenoxacin (50ppm) and the solution were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Precision:

Preparation of Standard stock solutions: Accurately weighed 10mg of Ozenoxacin is transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (400µg/ml of Ozenoxacin)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (20µg/ml of Ozenoxacin).

Preparation of Sample stock solutions: 1% of Cream were weighed and the average weight of cream was calculated, then the weight equivalent to 1% Cream was transferred into a 50ml volumetric flask, 50ml of diluents was added and sonicated and Centrifuged for 25 min, further the volume was made up with diluent and filtered by HPLC filters 0.25µm(Nylon Filters) (200µg/ml of Ozenoxacin)

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution

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was transferred to 10ml volumetric flask and made up with diluent. (20µg/ml of Ozenoxacin)

Linearity:

Preparation of Standard stock solutions: Accurately weighed 10mg of Ozenoxacin is transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (200µg/ml of Ozenoxacin)

25% Standard solution: 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (25µg/ml of Ozenoxacin)

150% Standard solution: 1.5ml each from two up to 10ml (30µg/ml of Ozenoxacin)

Accuracy:

Preparation of Standard stock solutions: Accurately weighed 10mg of Ozenoxacin is transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (200µg/ml of Ozenoxacin)

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

up to 10ml. (5µg/ml of Ozenoxacin)

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (5µg/ml of Ozenoxacin)

75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (15µg/ml of Ozenoxacin)

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (20µg/ml of Ozenoxacin)

125% Standard solution: 1.25ml each from two standard stock solutions was pipetted out and made standard stock solutions was pipettede out and made

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Ozenoxacin, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Ozenoxacin, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Degradation studies:

Oxidation:

To 1 ml of stock solution of Ozenoxacin, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 20µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:

To 1 ml of stock solution Ozenoxacin, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c. For HPLC study, the resultant solution was diluted to obtain 20µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

To 1 ml of stock solution Ozenoxacin, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c. For HPLC study, the resultant solution was diluted to obtain 20µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the

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stability of sample.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 105°C for 6h to study dry heat degradation. For HPLC study, the resultant solution was diluted to obtain 20µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a

Photo Stability studies:

The photochemical stability of the drug was also studied by exposing the 400µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 20µg/ml solution and 10µl were injected into the temperature of 60º. For HPLC study, the resultant solution was diluted to obtain 20µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

IV.RESULTS

Parameters		**Ozenoxacin**	**LIMIT**
Linearity Range(µg/ml)		5-30µg/ml	R< 1
Regressioncoefficient		0.999	
Slope(m)		14216	
Intercept(c)		495.4	
Regression equation (Y=mx+c)		y = 14216x + 495.45	
Assay (% mean assay)		100.14%	90-110%
Specificity		Specific	No interference of any peak
System precision %RSD		0.6	NMT 2.0%
Method precision %RSD		0.9	NMT 2.0%
Accuracy%recovery		100.34%	98-102%
LOD		0.03	NMT 3
LOQ		0.09	NMT 10
Robustness	**FM**	1.0	%RSD NMT 2.0
	FP	0.6	
	MM	0.3	
	MP	0.5	
	TM	0.8	
	TP	0.5	

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V.CONCLUSION

A Simple, sensitive, specific and precise RP-HPLC method for the Determination of Ozenoxacin in API and pharmaceutical dosage form. Retention time of Ozenoxacin was found to be 2.429 min. %RSD of the Ozenoxacin were and found to be 067. %RSD of Method precision of Ozenoxacin was found to be 0.9. %Recovery was obtained as 100.34% for Ozenoxacin. LOD, LOQ values obtained from regression equation of Ozenoxacin were 0.03, 0.09. Regression equation of Ozenoxacin is y = 14216x + 495.45. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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