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METHOD DEVELOPMENT AND VALIDATION OF BENZALKONIUM CHLORIDE IN MARKETED FORMULATION BY UV-VISIBLE SPECTROPHOTOMETRY USING SILVER NITRATE AND EOSIN SOLUTION

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ABSTRACT

A simple, accurate and precise UV-Visible Spectrophotometric (UV-VIS) method has been developed and validated for analysis of Benzalkonium chloride both as the bulk drugs and in formulations. Analysis of Benzalkonium chloride was performed at 517 nm. Regression analysis data for the calibration plots were indicative of good linear relationships between absorbance and concentration over the range of 1-5 ug/ml. The correlation coefficients, R^2, were 0.999. The values of slope and intercept of the calibration plots were 0.062 and 0.216 respectively. The method was validated for linearity, accuracy, and precision.

Key words: Benzalkonium chloride, UV-Visible spectrophotometric method, eye drops, accuracy, precision.

INTRODUCTION

The determination of low concentration preservative in pharmaceutical formulation constitutes a challenging problem in the current pharmaceutical analysis. Ophthalmic solutions of ciprofloxacin containing the pharmaceutical association between active pharmaceutical ingredient Ciprofloxacin and preservative Benzalkonium chloride (BKC). Cipla (marketed dosage form) eye drops contain ciprofloxacin, a synthetic broad-spectrum antimicrobial agent for ophthalmic solution administration. The preservative Benzalkonium

chloride and the drug ciprofloxacin are official in Indian Pharmacopoeia, British Pharmacopoeia whereas the drug Ciprofloxacin is also certified in United States Pharmacopoeia. Commercially available ciprofloxacin ophthalmic solution is a clear solution of the drug in sterile water for injection; benzalkonium chloride is added as a preservative. Benzalkonium chloride, a mixture of alkyls, whose solutions are rapidly acting biocidal agents with a moderately long duration of action.[1, 2] They are active against bacteria and some viruses, fungi and protozoa. Its use as a preservative in cosmetic such as eye and nasal drops attests to its general safety. The methods developed earlier for Benzalkonium chloride are simple High Performance Liquid Chromatography[1], Thin Layer Chromatography, Stability indicating Ultra- Performance Liquid Chromatography and HPLC[3,4], simultaneous estimation method, Spectrophotometric determination through charge transfer complexation, Gas Chromatography, Chemical Ionization Mass Spectroscopy.[5]

This paper portrays a simple, precise and accurate UV-Visible Spectrophotometric method for quantification of BKC in ciprofloxacin ophthalmic solution by using silver nitrateand eosin solution.

EXPERIMENTAL SECTION

Reagents and Materials

Benzalkonium chloride (50% aqueous solution), 0.1M Silver nitrate solution, 1% eosin solution , Double distilled water.

Instrument and apparatus: UV-Visible Spectrophotometer with UV-Probe Software 2.33 (SHIMADZU-1800), weighing digital balance (Shimadzu), volumetric flasks (1000ml, 100ml), graduated pipettes (1ml, 5ml, 10ml), beakers (sulab).

METHOD DEVELOPMENT

Preparation of Standard Solution

Standard stock solution was prepared in double distilled water by taking 0.1ml of Benzalkonium chloride in a 1000ml volumetric flask and it was diluted up to the mark (1000ppm). From the Standard stock solutions, a standard solution was prepared containing 100 µg/ml of Benzalkonium chloride.

Preparation of Calibration Curve

Linearity of the system was investigated by serially diluting the stock solutions to give concentrations in the range of 1 to 5µg/ml for Benzalkonium chloride. To each of the above working standard solutions, 5ml of 0.1 M silver nitrate solution and 0.5ml of 1% eosin solution were added. Calibration curves were obtained by plotting the Concentration vs. Absorbance.

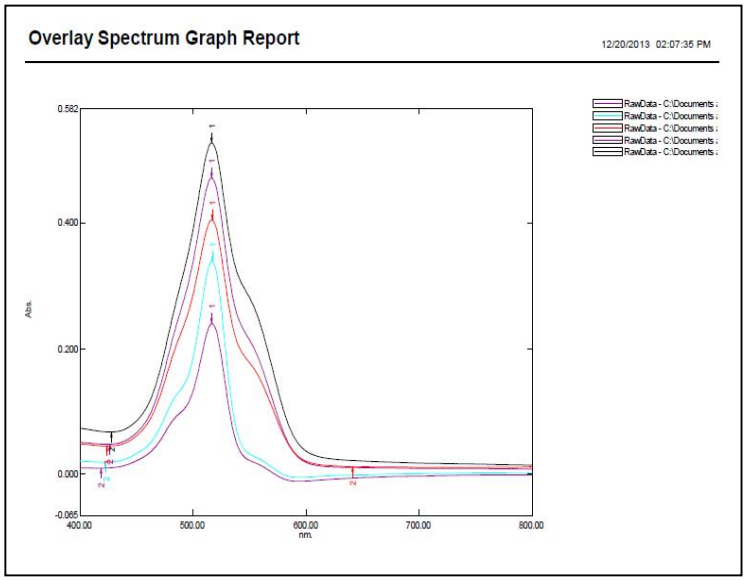

Fig 1. Calibration curve of benzalkonium chloride

Preparation of Sample Solution

Five ml of the ciplox eye drop solution was dissolved and diluted upto 100ml with distilled water. Sample solutions having concentrations 2 ug/ml were prepared from the marketed dosage form.

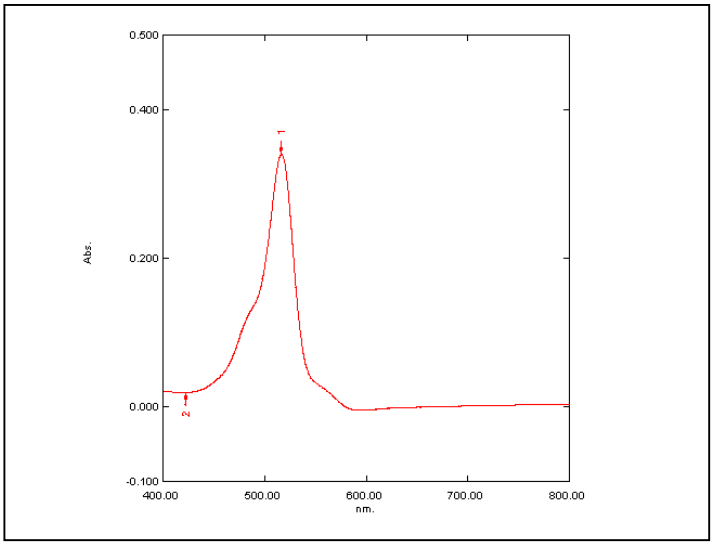

Fig 2.Spectra of sample solution (Ciplox)

Table 1. Calibration curve of benzalkonium chloride solution.

CONCENTRATION	ABSORBANCE AT λ_{max}=517 nm
1	0.280
2	0.339
3	0.405
4	0.472
5	0.523
SAMPLE(UNKNOWN)	0.342

Method Validation

As per the ICH guidelines, the method validation parameters checked were linearity, accuracy, precision, repeatability.[6]

Linearity

The linearity study verifies that the sample solutions are in a concentration range where analyte response is linearly proportional to the concentration. This study was performed by evaluating the method linearity. For the method linearity, standard solutions of Benzalkonium chloride at five concentration levels, from 1, 2, 3, 4, 5 ug/ml analyte concentration were prepared. The experimental results were plotted to obtain a calibration curve.The equations of the regression lines are for Benzalkonium chloride, $y = 0.062x+0.216$ ($R^2 = 0.999$).

Accuracy (% Recovery method)

Accuracy of a method is defined as the closeness of the measured value to the true value for the sample. The recovery method was studied at concentration levels of 50%, 100% and 150% of the claimed content. Three set for each concentration levels was prepared and the recovery was calculated with respect to the standard solutions.

Method Precision (Repeatability)

The precision is the parameter that expresses the closeness of agreement (degree of scatter) between a series of measurement obtained from multiple analysis of the same homogenous sample under the prescribed conditions. The method was evaluated using three set each having concentration of 2, 3 and 4 mcg/ml. The repeatability was expressed in terms of relative standard deviation (RSD)

Intermediate Precision (Reproducibility)

The intraday precision of the proposed method was determined by analyzing standard solution of Benzalkonium chloride at 3 different concentrations (2, 3 and 4 mcg/ml) for 3 times on the same day. The results were reported in terms of relative standard deviation. (RSD)

Validation of the Method

The linearity curves were found to be linear over the concentration range 1-5 mcg/ml for Benzalkonium chloride. A good linear relationship was observed over this range for Benzalkonium chloride (R^2= 0.999, slope = 0.062, intercept = 0.216).Repeatability of method and measurement of absorbance was expressed as RSD and was (for Benzalkonium chloride) for 3 replicate determinations. The low values of RSD indicate that the proposed method is repeatable. The RSD value obtained for intra-day variation was (for Benzalkonium chloride) respectively is low which indicates that proposed method is precise. Accuracy was performed by standard addition method. Mean recovery obtained (for Benzalkonium chloride was 98.74 ± 0.49). Validation parameters are summarized in Table 1.

Table 2. Summary of validation parameters of proposed UV-Visible Spectrophotometry method

Parameters	**Benzalkonium chloride**
Range (mcg/ml)	1-5
Regression equation y=mx+c	Y = 0.062x+0.216
Slope	0.062
Intercept	0.216
Correlation coefficient (r)	0.999
%Recovery ±SD,(n=3) Level 1: 50% Level 2: 100% Level 3: 150%	 98.03 ± 1.03 99.09 ± 0.19 99.10 ± 0.26
Precision (%RSD) (n = 3)	

Concentration (ug/ml) 2 3 4	 0.97 ± 1.56 0.157 ± 0.06 0.222 ± 0.519
Intra- Day Precision Concentration (ug/ml) 2 3 4	 0.112 ± 0.32 0.227 ± 0.41 0.278 ± 0.10

Analysis of Benzalkonium chloride in Marketed Formulation

Peaks obtained at absorbance 0.342for Benzalkonium chloride were observed in the spectra obtained from marketed eye drop solution. There was no interference from active ingredient (ciprofloxacin) present in the eye drops. The Benzalkonium chloride content was found to be 104% of the label claim. The low value of % RSD indicated the method was suitable for routine analysis of Benzalkonium chloride in pharmaceutical dosage forms.

CONCLUSION

This UV-Visible Spectrophotometric method is precise, specific, and accurate. Statistical analysis proved the method is repeatable and selective for the analysis of Benzalkonium chloride as bulk drug and in pharmaceutical formulations.

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