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Development Of Reverse-Phase High-Performance Liquid Chromatographic and UV-Spectrophotometric Method with Validation For Octenidine Dihydrochloride

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ABSTRACT

The purpose of this research is to develop and validate a precise method for UV-Vis spectrophotometric and Reverse-Phase High Performance Liquid Chromatography (RP-HPLC) for determination of Octenidine dihydrochloride in bulk and pharmaceutical preparation. According to the relevant experiment the maximum wavelength was found to be 285nm and it is used for further process of development of method and its validation. The developed methods used for quantitative estimation of Octenidine dihydrochloride in pharmaceutical preparation and bulk drug which shows the satisfactory results as per ICH guidelines, so these developed and validated methods are found very simple, sensitive and rapid according to the ICH guideline and can be successfully applied to estimate the ODCL in bulk and pharmaceutical dosage form.

Keywords: - ODCL, UV-Vis spectrophotometry, RP-HPLC, Development, Validation.

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INTRODUCTION

The FDA and different types of food, medicine regulatory agencies around the world not only want a product that satisfies their specifications, but they also want a method, processes, intermediate inspection phases, and testing that are in order to provide consistent, repeatable, and desired results that fulfil the standard requirements of the manufacturing a product. These procedures are developed using the validation method.[1]

The performance in terms of numbers of an appropriate chemical reaction and determination of the quota of reagent added to absolute reaction or the total quota of reaction product, a substance's distinctive flow through a specific medium under carefully monitored circumstances, Measurement of the compound's electrical properties, as well as some of its spectroscopic qualities needs to develop a specific method.[2]

Spectroscopy is a term that deals with the interaction of various types of radiation with matter. The visible and ultraviolet spectral regions are commonly used to quantify a wide range of inorganic, chemical, and biological substances. Spectrophotometry in the ultraviolet and visible ranges is mostly utilized for quantitative analysis and structural elucidation. The ultraviolet spectrum ranges from 4000-2000A^0, while the visible spectrum ranges from 8000-4000A^0.[3]

HPLC is versatile and widely employed chromatography elution technique. It is a vital and important analytical instrument used in the modern pharmaceutical industry at all stages of drug discovery, development, and production. Depending on the type of stationary phase used, HPLC is a dominant technique for separating substances. [4-7]

Reversed phase HPLC is the most widely used type of chromatography, accounting for about 90% of all low molecular weight sample analysis. The rationale for this widespread use is that it offers various advantages over normal-phase chromatography. [8-11]

MATERIALS AND METHOD

Materials:

Octenidine Dihydrochloride purchased from Disham Pharmaceuticals and Chemicals Ltd. Ahmedabad

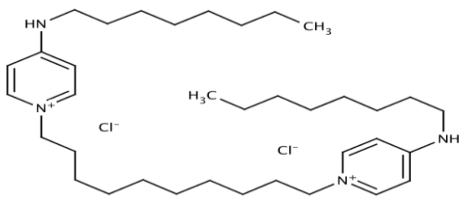

Figure 1: Octenidine Dihydrochloride

Chemical Name:

N,N- (decane – 1 ,10 - diyldipyridin – 1 – yl – 4 – ylidene) dioctan -1- amine dihydrochloride

Molecular Formula:

$C_{36}H_{64}Cl_2N_4$, Molecular Weight: 623.82616 g/mol.

Appearance:

Octenidine Dihydrochloride is white to off white powder.

Solubility:

Freely soluble in AR grade water, methanol and dichloromethane. Slightly soluble in N,N-dimethyl formamide.

Melting Point:

203°C -204°C.

Category:

Antiseptic, Disinfectant.

Instruments:

Ultra-violet spectrophotometer Agilent (Cary-60), HPLC Carry1260 Agilent Technologies

Method:

TLC carried out by using Silica gel G as a stationary phase on prepared TLC slides sample spots are applied and acetonitrile: phosphate buffer (4:6) used as mobile phase.

Octenidine Dihydrochloride UV Spectroscopic Method Development and Validation:

According to the solubility characteristics of the drug, Methanol was selected as solvent for analysis Determination of λ max 10 mg of Octenidine (100µg/ml), calibration curve of Octenidine Dihydrochloride carried out by Spectrophotometric method based on absorbance measurements between 200 and 400 nm of UV region concentration of solution is 100 micro gram per milliliter. Standard stock was used to make the dilutions like 1, 0.8, 0.6, 0.4, 0.2 ml aliquots, which were extracted and diluted with methanol up to 10 ml to obtain 10, 8, 6, 4, 2 micro gram per milliliter solutions in the same order. Absorbance measured at 285 nm.

Octenidine Dihydrochloride RP-HPLC Method Development and Validation:

In methanol, a medication's stock solution was made and UV spectrum of 10µg/ml solution of ODCL was taken, it showed maximum absorbance at 285 nm. From the various mobile phases tried, the mobile phase used to consist of 40:60% by volume of Acetonitrile: Phosphate Buffer with pH 6.4 was selected for the HPLC which obtain good resolution and sharp Peak. Standard stock solution containing ODCL was prepared of 1000µg/ml Suitable dilutions were made using diluent. Aliquots of 10µl of clear filtrate were injected into the HPLC column. The sample solution

contains 100mg/100ml of ODCL (label claim). further diluted to obtain final concentration 6µg/ml. Chromatographic condition for RP-HPLC-Stationary Phase: C18-(250 x 4.6 milimeter , 5 micro meter), variable wavelength detector with 1 milliliter per minute flow rate:, Injection Volume: 20 µl, Retention time: 3.38 min, Run time: 10 min

Validation of these methods carried out by using criteria for validation of analytical methods according to ICH guidelines

RESULTS AND DISCUSSION

As per preliminary studies and characterization of the sample of Octenidine Dihydrochloride (ODCL) was found to be white/off white amorphous substance and having specific odour with melting point 200°C -202°C compound which is freely soluble in methanol and water

TLC of standard and marketed formulation of ODCL:

Table 1: TLC of ODCL

Name of compound	Solvent system	Rf value
ODCL	Acetonitrile: Phosphate Buffer pH 6.4	0.81
Marketed formulation	(4:6)	0.78

Figure 2: TLC profile of standard ODCL

Figure 3: TLC profile of Marketed formulation

FTIR Spectroscopic studies of Octenidine Dihydrochloride:

IR spectroscopy showed dominant characteristics peaks of Octenidine Dihydrochloride. FTIR spectrum of Octenidine Dihydrochloride complies with its chemical structure. Since the characteristic bands for functions such as -NH, -CH, C-Cl, Ar C-N was observed.

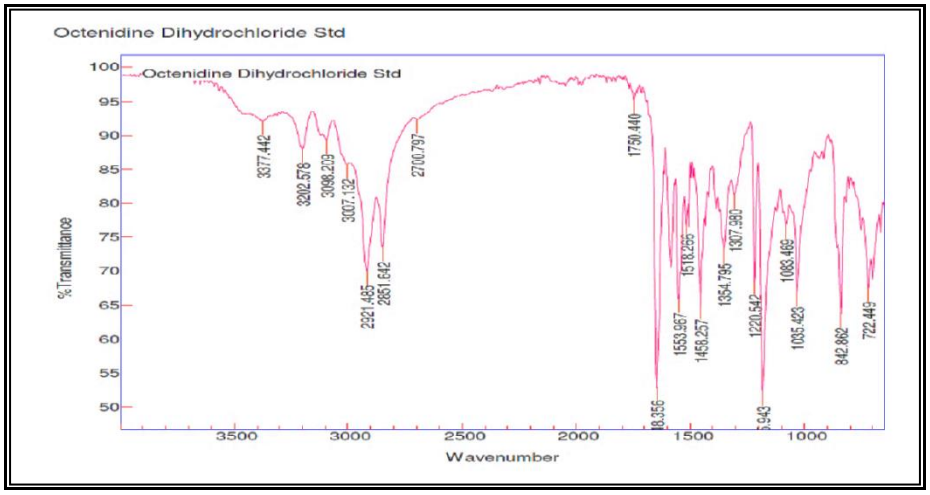

Figure 4: IR spectra of Octenidine Dihydrochloride

UV-Visible Spectrophotometry:

The λ max of Octenidine Dihydrochloride was found to be 285 nm.

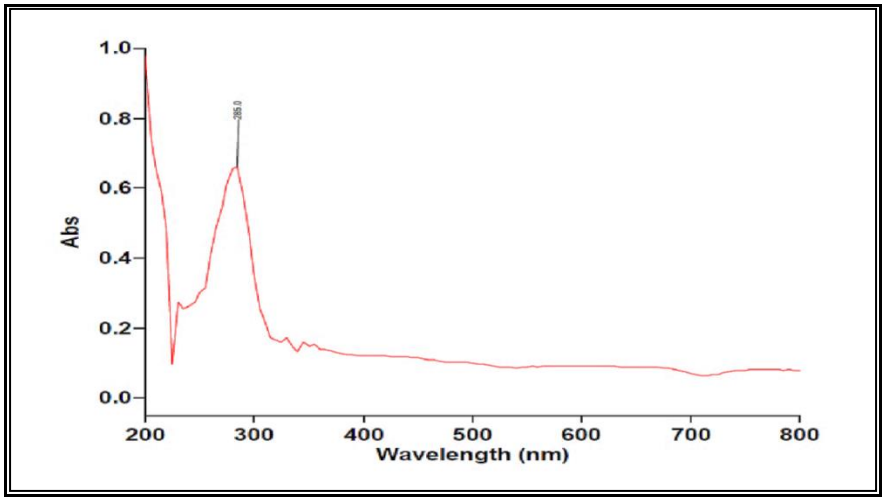

Figure 5: UV-spectra of Octenidine Dihydrochloride.

RP-HPLC method development and validation of for octenidine dihydrochloride:

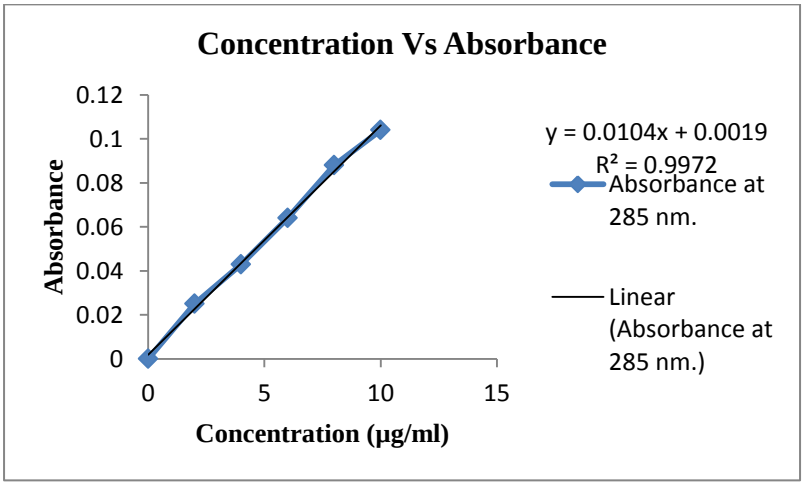

Figure 6: ODCL Calibration curve.

Linearity:

Recovery Studies (Accuracy):

Percent recovery of Octenidine Dihydrochloride was found to be was found to be 98.5-102.22 %. Which is within limit indicating method has good accuracy.

Precision Studies:

The solutions were analyzed twice for the purpose to record intra and inter-day variations in the result. The result obtained for mean are good, standard deviation is within the range and % relative standard deviation was found be below 2 percent.

Table 2: Intra and Inter-day precision Octenidine Dihydrochloride.

Conc. µg/ml	Precision (%RSD) Intra-day	Inter-day
3	0.4282	0.4287
6	1.5069	1.67211
9	1.8429	1.3617

Specificity:

This method was tested by for its Specificity. The result shows that the present amount found was in the range 98% to 101% Octenidine Dihydrochloride indicating this method is specific.

Table 3: Specificity of Octenidine Dihydrochloride.

Conc. µg/ml	Mean % Recovery	%RSD
3	99.6094	1.5794
6	100.625	1.2190
9	100.55	0.5383

Limit of detection and quantification:

The slope of the calibration curve and the standard deviation of the response were used to calculate (LOD) and (LOQ).

Table 4: Limit of detection and quantification determination

Drug	Parameters	
Octenidine Dihydrochloride	LOD (µg/ml)	LOQ (µg/ml)
	1.475	3.0303

Ruggedness:

This method was also tested for ruggedness by different analyst. The proposed method gives precise and repeatable result when analyzed by different analyst.

Table 5: Result of Analyst I and II.

Conc. µg/ml	Analyst I %RSD.	Analyst II %RSD.
3	0.4282	0.4287
6	1.5069	1.67211
9	1.8429	1.3617

The proposed UV-Spectrophotometric method is used to evaluate the pharmaceutical dosage form.

Table 6: The outcome of a UV spectroscopy analysis of a pharmaceutical formulation

Formulation	ODCL		
Octenisept (solution)	Labeled amount (mg)	Amount discovered (mg)	% Total Amount ± S.D.
	100	99.8	99.8±0.05

RP-HPLC method for octenidine dihydrochloride development and validation

Mobile phase selection:

Copious mobile phases were attempted to obtain good resolution and sharp Peak. The Standard solution containing ODCL was run in different mobile phases, the mobile phase used to consist of 40:60% by volume of Acetonitrile: Phosphate Buffer with pH 6.4 was selected for the HPLC, since it gave sharp and resolved peak with symmetry and significant retention time for both standard and dosage Form of drug.

Chromatogram of octenidine dihydrochloride:

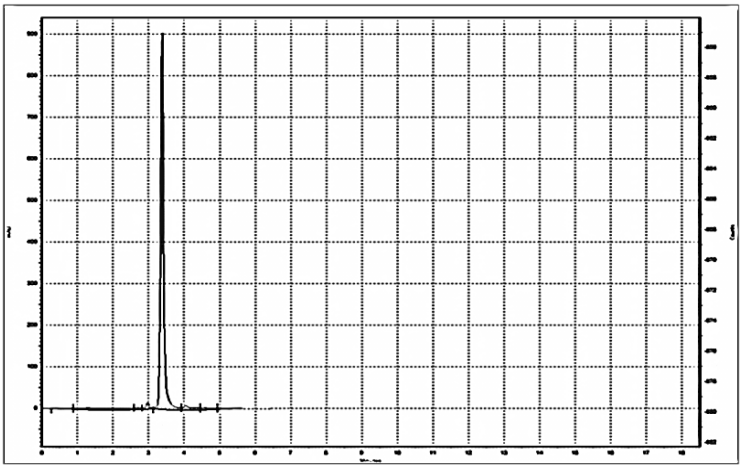

Figure 7: HPLC chromatogram of ODCL in solution at wavelength detection 285nm

Linearity:

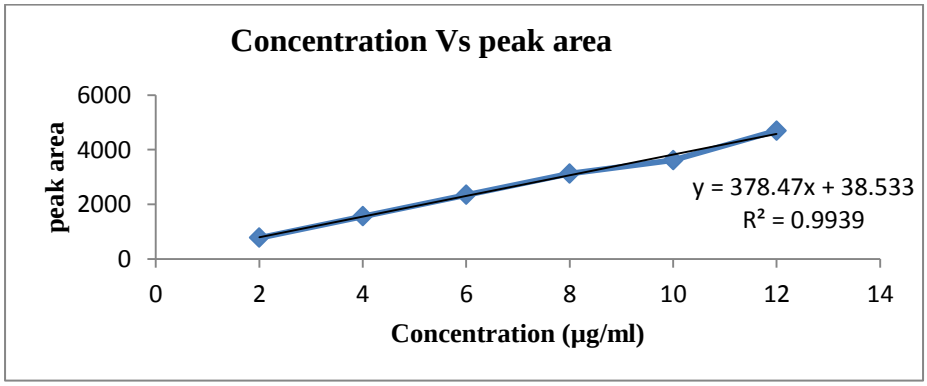

Figure 8: Linearity study for ODCL

Recovery studies (Accuracy):

Percent recovery of Octenidine Dihydrochloride was found to 99.11-101.66 %. Which is within limit indicating method has good accuracy.

LOD and LOQ:

Table 7: LOD and LOQ of ODCL

Drug	Specifications	
Octenidine Dihydrochloride	**LOD (µg/ml)**	**LOQ (µg/ml)**
	1.081	3.277

Precision

The solutions were analyzed twice, in order to keep track of intra-day and inter-day variations in the outcome. The result obtained for mean are good, standard deviation is within the range and % relative standard deviation was found be below 2 %.

Table 8: Octenidine Dihydrochloride intra-day and inter-day precision.

Level	Precision (%RSD)	
	Inter-day	**Intra-day**
50	0.1303	0.1293
100	0.0851	0.0862
150	0.0716	0.0722

Specificity:

Table 9: Specificity of ODCL

Concentration (µg/ml)	Drug	Area (mAU*)	
		Standard	**Test**
100	ODCL	39145	39147

Robustness:

Table 10: % RSD for different mobile phase ratio

Level	% RSD for MOBILE PHASE RATIO	
	70:30 % (V/V)	**75:25 % (v/v)**
100	0.0851	0.088

The proposed RP-HPLC technology is being used to determine the pharmaceutical dosage form.

Table 11: Analysis of Pharmaceutical Formulation by RP-HPLC method

Formulation	**ODCL**		
Octenisept (solution)	**Labeled amount (mg)**	**Amount discovered (mg)**	**% Total Amount ± S.D.**
	100	99.6	99.6 ± 0.05

UV-Visible Spectrophotometry and RP- HPLC method were effectively employed for Octenidine Dihydrochloride to examine dosage forms which gets satisfactory findings.

CONCLUSION

According to the ICH guidelines, a simple, sensitive, and quick UV-Vis spectrophotometric and RP-HPLC technique was successfully designed and validated. The developed methods for routine quantitative determination of Octenidine Dihydrochloride pharmaceutical dosage form include UV Visible spectrophotometry and RP-HPLC. The validation procedures confirm that these are an appropriate method for their quantification in the formulation and bulk drug compound.

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