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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF BROMHEXINE HYDROCHLORIDE USING U.V. SPECTROPHOTOMETRIC METHOD

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ABSTRACT
Pharmaceutical analysis occupied a pivotal role in the determination of drugs in formulation and its combinations. The complexity of problems in existing methods in terms of achieving the selectivity, speed, simplicity, sensitivity, precision and accuracy has been replaced by methods of analysis. The present work attempts to minimize the time consumption and cost by simple spectrophotometric method based on use of Methanol and Formic Acid solution in which the drug Bromhexine Hydrochloride is completely soluble used a solvent system. The drug has an absorption max at 277 nm and obeys Beers Lamberts law. The absorbance was to found to increase linearly with increasing concentration of Bromhexine Hydrochloride which is corroborated by the calculated correlation coefficient value of 0.9997. The Molar Absorptivity is 2.01468±0.13778 (Mean± SD). The slope and intercept of the equation of the regression line are 0.0434, 0.0223. The mean recovery obtained for the drug was found to be in the range of 99.77-100.05%. The optimum experimental parameters for the method have been studied. The validity of the elucidated method was accessed according to the International Conference on Harmonization Guidelines. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The proposed method can be applied to the Determination of Bromhexine Hydrochloride in the Pharmaceutical dosage forms. Literature survey reveals that there is a need to develop new, simple, specific, reliable analytical methods for determination of Bromhexine Hydrochloride in pure and pharmaceutical dosage form.

KEYWORDS: Ultra Violet Spectroscopy, Active Pharmaceutical Ingredient, Limit of Detection, Limit of Quantitation, Coefficient of Variation.

INTRODUCTION[1-11]
Quality investigations play a very important role in quality specification establishments of chemical drugs. The number of the drugs introduced in the market every year very often there is a time lag from the date of introduction of a drug into a market to the date of inclusion in the pharmacopeias. Hence standards and analytical procedure for these drugs may not be included in the pharmacopeias. So this is why it becomes necessary to develop new analytical methods for such drugs. Analytical methods development and validation play important roles in the discovery, development, and manufacture of pharmaceuticals. Pharmaceutical products formulated with more than one drug, typically referred to as combination products, are intended to meet previously unmet patients need by combining the therapeutic effects of two or more drugs in one product. These combination products can present daunting

challenges to the analytical chemist responsible for the development and validation of analytical methods. There is a scope, therefore to develop newer analytical methods for such drugs. Also quality is important in every product or service but it is vital in medicines as it involves life.

Basic criteria for new method development of drug analysis:

1) Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients,
2) Analytical methods for the quantitation of the drug in biological fluids may not be available
3) Analytical methods for a drug in combination with other drugs may not be available,
4) The existing analytical procedures may require expensive reagents and solvents.
5) It may also involve cumbersome extraction and separation procedures and these may not be reliable
6) A proper analytical procedure for the drug may not be available in the literature due to patent regulations
7) The drug or drug combination may not be official in any pharmacopoeias.
8) Proper analytical method for the drug may not be available in the literature due to patent regulations.
9) Analytical method for the drug in single may not be available.
10) The existing analytical procedure may require expensive reagents and solvents.
11) Analytical method development provides the support to track the quality of the product from batch to batch

Analytical method development provides the tracking of quality of the product from batch to batch.

Method development involves considerable trails and error procedures. The most usual problem is from where to start, what type of solvent is worth trying.

Drug Profile[12-15]

Drug name: Bromhexine Hydrochloride[30]

Chemical structure

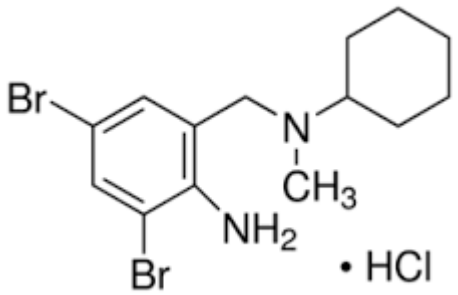

Fig 1: Chemical structure of Bromhexine HCL.

Bromhexine Hydrochloride is a mucolytic agent used in the treatment of respiratory disorders associated with viscid or excessive mucus. In addition Bromhexine has anti oxidant properties. Are used clinically for the treatment of acute exacerbations of chronic bronchitis.. It is official in B.P

AIM AND PLAN OF WORK

AIM AND OBJECTIVE

Aim: To develop a new UV Spectrophotometric method for estimation of Bromhexine Hydrochloride in pharmaceutical dosage form.

Objective:

1. The main objective is to develop and validate a method which can be successfully applicable to Bromhexine Hydrochloride.
2. To estimate Bromhexine Hydrochloride with high selectivity and sensitivity.
3. To validate for various validation parameters such as accuracy, linearity, precision, range, limit of detection and limit of quantitation, selectivity and selectivity, robustness and ruggedness.

Plan of Work

Step 1- survey on literature

The survey on literature performed for Bromhexine Hydrochloride for its physiochemical properties, solubility, pharmacology and analytical techniques, so this basic information gives notation for new method development and validation of the particular developed method.

Step 2- method development

1) Determination of solubility of the drug and its pka, pH, Dissociation.
2) Development of a rapid and accurate UV method.
3) Analysis of the marketed formulations.

Step 3- validation of the developed method

The developed method is to be validated as per ICH guidelines. The parameters used to validate the developed method are accuracy, precision, linearity, range, limit of detection and limit of quantitation, robustness and ruggedness, sensitivity and selectivity, system suitability.

MATERIALS AND METHODOLOGY[16-31]

Experimental

Apparatus or instruments used

Table 1: List of apparatus and instruments used.

S.no	Name	Model	Manufacturer or supplier
1	UV-spectrophotometer	UV-1800	SHIMADZU
2	Sonicator	C002	Life Care Equipment Pvt. Ltd.
3	Weighing balance	BL-220H	Shimadzu corporation
4	Pipettes	-	-
5	Volumetric flasks	-	-
6	Beakers	-	-

Chemicals used

Table 2: List of chemicals used.

S.no	Name	Specification	Manufacturer or supplier
1	Methanol	AR	Merck specialties, Pvt. ltd. Mumbai
2	Formic acid	AR	Sd Fine Chemical, Pvt. ltd. Mumbai
3	Acetonitrile	AR	Thermo Fischer Scientific India Pvt. Ltd
4	Water	AR	Sd Fine Chemical, Pvt. ltd. Mumbai

Bulk drugs or Active Pharmaceutical Ingredients

Table 3: Data of Bulk Drugs / Active Pharmaceutical Ingredients.

S. no	Name	Specification	Manufacturer/ Supplier
1	Bromhexine Hydrochloride	As Working standard	Gift sample from Aurobindo labs

Marketed formulations

Table 4: Data on marketed formulations.

Brand Name	Content	Mfg. Date	Expiry Date	Manufacturing Company
BISOLVON	8mg	24-10-2015	15-10-2017	Aurobindo labs.

Analytical Method Development

Selection of solvents

The solvent was selected based on the solubility of Bromhexine Hydrochloride in various solvents like Acetonitrile, Methanol, glacial acetic acid and formic acid.

Table 5: Solvents used.

Solvent	Preparation	Solubility
50% methanol	50 ml of methanol is diluted with 50 ml of water to give the concentration of 50%	**Soluble**
50% Acetonitrile	50 ml of Acetonitrile is diluted with 50 ml of water to give the concentration of 50%	Insoluble
50% formic acid	50ml of formic acid is diluted with 50 ml of water to give 50% formic acid solution	**Soluble**

The drug was completely soluble in 50% methanol and formic acid. After checking the solubility the different ratios of diluents were prepared and further solubility is checked. Different ratios prepared are as follows: Finally the combination of 50% Acetonitrile and Formic acid in the ratio of 80:20.

Table 6: Different ratios of diluents prepared.

Methanol (vol.)	Formic acid (vol.)	Solubility of the drug
50	50	Sparingly soluble
60	40	Sparingly soluble
70	30	Sparingly soluble
80	**20**	**Completely soluble**
90	10	Completely soluble

Preparation of standard stock solution

Bromhexine hydrochloride standard stock solution (100µg/ml)

A 100mg of standard Brmhexine hydrochloride was weighed and transferred to 100ml volumetric flask and dissolved in 50ml of 50% methanol and 50% formic acid in the ratio of 80:20. The flask was shaken and volume was made up to the mark with diluent.

Bromhexine hydrochloride standard solution

From this stock solution further 10ml was transferred in 100ml volumetric flask and diluent was added up to mark to give a solution containing 10µg/ml Bromhexine Hydrochloride.

Selection of Analytical Wavelength

10 µg/ml solution of Bromhexine hydrochloride was prepared in diluent and spectrum was recorded between 200-400nm. The overlain spectrum of Bromhexine Hydrochloride at different concentration was recorded and peak maxima of drug was found. The peak maximum of Bromhexine Hydrochloride was 277 nm. The spectrum of Bromhexine Hydrochloride at target concentration was recorded. (Fig no.1).

Calibration Curve for Bromhexine Hydrochloride

Appropriate volume of aliquots (0.2, 0.4, 0.6, 0.8and 1.0 ml) from standard Bromhexine Hydrochloride stock solution was transferred to different volumetric flasks. The volume was adjusted to the mark with the diluent to obtain the concentration of 2, 4, 6, 8 and 10 µg/ml.

Calibration curve of each solution against the diluent was recorded at 277 nm was measured and the plot of absorbance v/s concentration was plotted. The absorptivity of drug at wavelength 277 nm was found by straight-line equation. Linearity range of Bromhexine Hydrochloride was found with correlation co-efficient.

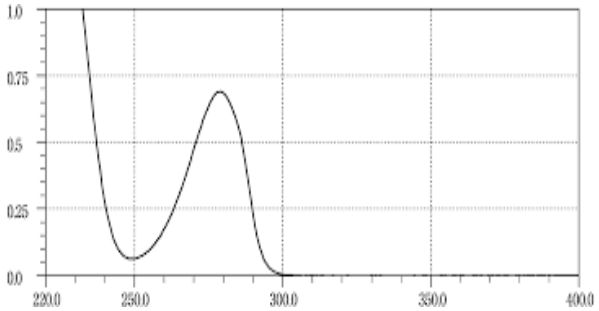

Fig 2: UV Spectrum of Bromhexine hydrochloride at 200-400 nm.

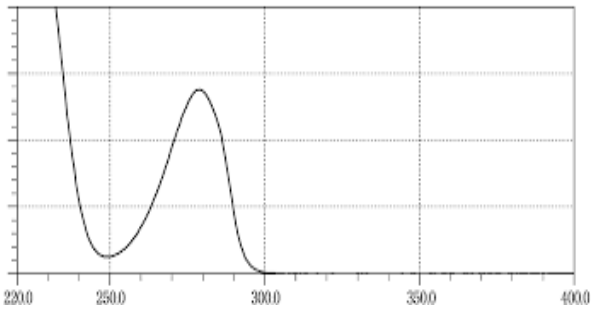

Fig 3: UV Spectrum of Bromhexine hydrochloride at 200-400 nm.

RESULTS AND DISCUSSION

Spectrophotometric method for estimation of Bromhexine Hydrochloride is simple, accurate and reproducible. Bromhexine Hydrochloride shows 277nm as λmax.

Linearity

The Zero order showed linear absorbance at 277.00 nm for Bromhexine Hydrochloride (02-10 µg/ml) with correlation Coefficient (r2) of 0.9997 (fig. 4). This method obeyed Beer's Law in the concentration range 02-10µg/ml for Bromhexine Hydrochloride.

Table 7: Result of Linearity Parameter by U.V Spectroscopy.

S.No	*Concentration (mg/ml)*	*Abs. ± SD(n=5)*	*Statistical Analysis*
1.	2	0.108±0.0083	**Slope=0.0434** **Intercept=0.0434x+0.0223** **Correlation coefficient=0.9997**
2.	4	0.196±0.0021	
3.	6	0.286±0.0021	
4	8	0.366±0.0016	
5.	10	0.457±0.018	

Linear calibration curve of Bromhexine Hydrochloride

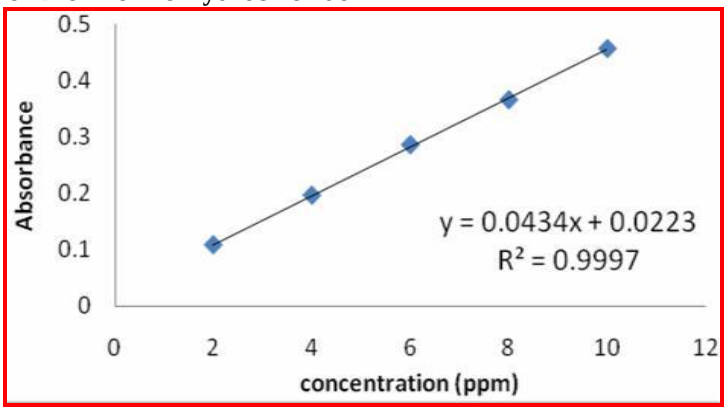

Fig 4: Linearity Calibration Curve of Bromhexine Hydrochloride at 277 nm.

Calculation of Molar Absorbtivity

Table 8: E (1%,1cm) of Bromhexine Hydrochloride at 277nm.

S.no	*Concentration[µg/ml]*	*Absorbance*	*E(1%,1cm)*	*Molar absorbtivity*
1	2	0.109	0.0545	**1.78215**
2	4	0.196	0.049	**1.6023**
3	6	0.286	0.0476	**1.55652**
4	8	0.377	0.0471	**1.54017**
5	10	0.457	0.0457	**1.49439**
Mean± SD			0.0487±0.0034	**1.5951±0.1114537**

Optical Characteristics

Table 9: Optical Characteristics of Bromhexine Hydrochloride.

Paramaters	*Results*
Absorption maximum	277 nm
Beer Lambert's Law (µg/ml)	2-10 ppm
Correlation Coefficient (r^2)	0.9997
Molar Absorptivity (mol-1 cm-1)	2.01468±0.13778
Linearity Range	4-10(µg/ml)
Regression Equation (y = mx + c)	0.0434x+0.0223
Slope (m)	0.0434
Intercept ©	0.0223
Limit of Detection (µg/ml)	0.1026
Limit of Quantitation (µg/ml)	0.3110

Selectivity and Specificity

Table 10: Result of Selectivity and Specificity by U.V Spectroscopy.

Sr.No.	*Sample*	*Absorbance*	*Conc. Found*	*% Assay*	*Specificity*
1	Standard solution	0.182	**1.970**	100%	Specific
2	Placebo set-1	0.000	**0.000**	0	Specific
3	Placebo spiked with API	0.185	**1.984**	101%	Specific

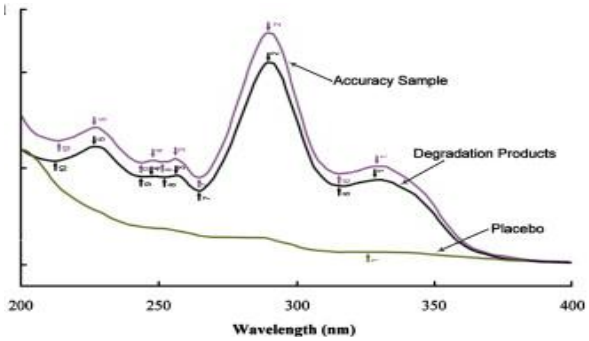

Fig 5: Specificity of the method determined by comparing the spectra of accuracy sample, placebo and degradation products.

Accuracy

Accuracy of the method was determined by recovery study at three level (80%, 100%, 120%) of standard addition. The% recovery values are tabulated in Table 11. Percentage recovery for Bromhexine HCL by this method was found in the range of 99.77-100.05%. The value of % RSD within the limit indicated that the method is accurate and percentage recovery shows that there is no interference from the excipients.

Table 11: Result of Recovery Parameter by U.V Spectroscopy (n=3).

Conc. Of Bromhexine Hcl from formulation	*Amount of Std. drug added (µg/ml)*	*Total amount of Bromhexine Hcl (µg/ml)*	*Total amount of Bromhexine Hcl found (µg/ml)*			*Total amount of Bromhexine Hcl found (µg/ml)* Mean± SD*	*% Drug Recovery (n=3)*
			n=1	**n=2**	**n=3**		
2	1.6	3.6	3.62	3.62	3.63	3.62±0.005	**100.55**
2	1.0	4.0	3.99	3.99	3.98	3.99±0.005	**99.78**
2	2.4	4.4	4.39	4.39	4.38	4.39±0.005	**99.77**

Precision

a. Repeatability Precision (Method Precision)

The %RSD values for Bromhexine Hydrochloride is found to be 0.611 %. The result of the repeatability studies are shown in the Table 12. The low %RSD values (<2%) indicates that proposed method is repeatable.

b. Intraday precision

The precision of the developed method was assessed by analyzing samples of the same batch in nine determinations with three Standard solutions containing concentrations 2, 4, 6µg/ml of Bromhexine HCl. Three replicate (n=3) each on same day. Results are presented in Table 13. These% RSD value was found to be less than ±2. 0 indicated that the method is precise.

c. Inter day precision

The precision of the developed method was assessed by analysing samples of the same batch in nine determinations with three Standard solutions containing concentrations 2, 4, 6µg/ml of Bromhexine HCl. Triplicate (n=3) per day for consecutive 3 days for inter-day precision. Interday precision data presented in Table 14. These % RSD value was found to be less than ±2.0indicated that the method is precise.

a. Repeatability Precision (Method Precision): (2 µg/ml).

Table 12: Result of Repeatability Precision Parameter by U.V. Spectroscopy.

Sr.No	*Absorbance*	*Conc. Of the Bromhexine HCL found* (µg/ml)	*% Assay*
1.	0.182	2.012	100.6
2.	0.182	2.012	100.6
3.	0.181	1.992	99.6
4.	0.179	1.975	98.75
5.	0.181	1.991	99.55
6.	0.181	1.992	99.6
Mean	**0.181**	**1.995**	**99.7**
Std.Deviation	**0.0013**	**0.014**	**0.7103**
%RSD	**0.611**	**0.71**	**0.71**

Table 13: Result of Intraday Precision Parameter by U.V. Spectroscopy.

S.no	*Conc. (µg/ml)*	*Absorbance, Conc. (µg/ml)*			*Absorbance Mean± %RSD(n=3)*	*Conc. of the Brom. HCL found Mean ±%RSD*	*% assay*
		0 hour	*4 hour*	*8 hour*			
1.	2	0.181 (1.982)	0.183 (1.993)	0.181 (1.972)	0.181±0.364	**1.989 ±0.31**	99%
2.	4	0.376 (3.993)	0.370 (4.021)	0.374 (3.954)	0.373±0.82	**3.989±0.84**	99.7%
3.	6	0.527 (5.993)	0.526 (5.861)	0.525 (5.923)	0.525±0.190	**5.92±1.11**	98.6%

b. Inter day precision

Table 14: Result of Inter day Precision Parameter by U.V. Spectroscopy.

Sr.no	*Conc. (µg/ml)*	*Absorbance, Conc. (µg/ml)*			*Absorbance Mean ±%RSD (n=3)*	*Concentration of the BROM. HCL found Mean ± %RSD* (µg/ml)	*% Assay*
		Day 1	*Day 2*	*Day 3*			
1.	2	0.182 (1.970)	0.185 (1.984)	0.183 (1.962)	0.183±0.83	**1.972±0.56**	99.13
2.	4	0.373 (4.012)	0.374 (3.991)	0.370 (4.011)	0.376±0.56	**4.004±0.30**	99.92
3.	6	0.528 (5.921)	0.521 (5.935)	0.526 (5.913)	0.525±0.69	**5.923±0.19**	98.91

Ruggedness and Robustness

The obtained Ruggedness and Robustness results are presented in table 15, 16 & 17. The % R.S.D was found to be 0.29- 0.64 % for Bromhexine HCL. These %RSD value was found to be less than ± 2.0 indicated that the method is precise from the table no. 7.7.1. No significant changes in the spectrums were observed, proving that the developed method is rugged and robust.

a. Ruggedness

1. Instrument no. 1

Table 15: Result of Ruggedness Parameter by U.V Spectroscopy (Instrument No.1).

Conc. taken (µg/ml)	*Absorbance, Conc. (µg/ml)*			*Mean Absorbance±%RSD (n=3)*	*Conc. Found Mean ±%RSD* (µg/ml)	*% Assay*
	n=1	*n=2*	*n=3*			
2	0.181 (1.959)	0.182 (1.965)	0.181 (1.962)	0.181±0.64	**1.962±0.15**	98.18
4	0.372 (3.957)	0.370 (3.956)	0.372 (3.954)	0.371±0.31	**3.955±0.04**	99.10
6	0.527 (5.963)	0.526 (5.961)	0.524 (5.967)	0.525±0.29	**5.963±0.03**	99.53

2. Instrument no. 2

Table 16: Result of Ruggedness Parameter by U.V Spectroscopy (Instrument No.2).

Conc. taken (µg/ml)	*Absorbance, Conc. (µg/ml)*			*Mean Absorbance±%RSD (n=3)*	*Conc. Found Mean ±%RSD* (µg/ml)	*% Assay*
	n=1	*n=2*	*n=3*			
2	0.182 (1.961)	0.184 (1.966)	0.182 (1.963)	0.182±0.63	**1.963±0.13**	98.19
4	0.373 (3.959)	0.371 (3.958)	0.373 (3.56)	0.372±0.31	**3.957±0.04**	99.12
6	0.528 (5.94)	0.527 (5.92)	0.525 (5.96)	0.526±0.29	**5.964±0.34**	99.52

b. Robustness

Table 17: Result of Robustness Parameter by Using U.V. Spectroscopy. (Wavelength to be changed by ±2nm (i.e, 275 and 279)).

Concentration taken (µg/ml)	*Absorbance (Mean ±%RSD) Wavelength – 275 nm*	*Mean conc. Found±%RSD*	*Absorbance (Mean ±%RSD) Wavelength -279 nm*	*Mean conc. Found±%RSD*
2	0.182±0.837	**1.961±0.14**	0.183±0.833	**1.963±0.14**
4	0.371±0.411	**3.954±0.031**	0.372±0.819	**3.955±0.04**
6	0.524±0.397	**5.962±0.04**	0.524±0.190	**5.965±0.43**

LOD AND LOQ

LOD values for Bromhexine Hydrochloride if found to be 0.1026 µ*g/ml* and LOQ value for Bromhexine Hydrochloride is found to be 0.3110 *µg/ml* , respectively. These data reveals that proposed method is sensitive for the determination of Bromhexine Hydrochloride.The obtained LOD and LOQ results are presented in Table no. 18.

LOD = 3.3*sigma/S
= 0.1026 µ*g/ml*

LOQ = 10*sigma/S
= 0.3110 *µg/ml*

Table 18: Result of LOD & LOQ Parameter by Using U.V. Spectroscopy.

Parameter	*Results(µg/ml)*
LOD	**0.1026**
LOQ	**0.3110**

The parameter LOD is determined on the basis of response and slope of the regression equation. The LOD for this method was found to be 0.1026 *µg/ml*

The parameter LOQ is determined on the basis of response and slope of the regression equation. The LOQ for this method was found to be 0.3110 *µg/ml*

Solution stability

Table 19 & 20 shows the results obtain in the solution stability study at different time intervals for test preparation. It was concluded that the test preparation solution was found stable up to 24 hrs at room temperature, as during this time the result was not decrease below the minimum percentage.

Table 19: Result of Solution Stability of Standard Solution by U.V. Spectroscopy Standard Solution.

Time(hrs)	*Absorbance*	*Conc. Found (µg/ml)*	*% Assay*
0	0.180	1.983	99.15
6	0.182	1.986	99.3
12	0.181	1.985	99.2
24	**0.193**	**1.6206**	**81.03**

Table 20: Result Of Solution Stability of Sample Solution by U.V. Spectroscopy Sample solution.

Time(hrs)	*Absorbance*	*Conc. Found (µg/ml)*	*% Assay*
0	0.178	1.980	99
6	0.181	1.992	99.6
12	0.179	1.982	99.1
24	**0.195**	**1.604**	**80.2**

System suitability

% RSD of standard reading not more than 2.0%, were full fill during all validation parameter.

Table 21: Result of system suitability.

Sample no.	*Absorbance*	*Conc. Found (µg/ml)*	*% Assay*
1	1.181	1.959	97.95
2	1.182	1.972	98.5
3	1.181	1.962	98.1
4	1.181	1.964	98.05
5	1.181	1.962	98.1
6	1.181	1.961	98.05
Average	**1.181**	**1.96**	**98.12**
SD	**0.0004**	**0.004**	**0.19**
%RSD	**0.03**	**0.23**	**0.20**

Assay of Bromhexine Hydrochloride In Pharmaceutical Dosage Form

The proposed validated method was successfully applied to determine concentration of Bromhexine Hydrochloride in its pharmaceutical dosage form. The assay results obtained for Bromhecxine Hydrochloride were comparable with the corresponding labeled amounts indicates that the method is suitable for simultaneous estimation of Bromhexine Hydrochloride without interference of excipients normally present in Table no. 22.

Table 22: Application of proposed method for analysis of tablet Dosage Form.

Sample	*Label claim*	*Amount taken*	*Amount found*	*% Assay (n=3)*	*% RSD*	*IP Limit*
Bisolvon	8mg	100mg	100.01mg	100.1	100.5%	98-101%
			100.07mg	100.7		
			100.08mg	100.8		

Table 23: Summary of Validation Parameters.

S. No	*Parameters*	*Results Obtained*	*Acceptance Criteria*
1	Linearity	Slope=0.0434 Intercept=0.0434x+0.0223 Correlation coefficient=0.9997	1.correlation coefficient should be greater than or equal to 0.999 2.y-intercept to be mentioned in the validation report 3. Slope to be mentioned in validation report
2	Selectivity and specificity:	No inference observed	There shall be no interference in absorbance from diluents and placebo due to Bromhexine Hydrochloride
3	Accuracy (% Drug recovery)	99.77-100.05%	Mean recovery of three levels shall be between 98.0 % t0 102.0%
4	Precision in % RSD Repeatability Intra day precision Interday precision	 0.611 0.190 – 0.317 0.290 – 0.546	RSD should be less than or equal to 2.0% for six assay determination for individual analyst
5	Robustness (% RSD)	0.190-0.837	%RSD value was found to be less than ± 2.0
6	Ruggedness (%RSD)	0.21-0.83	%RSD value was found to be less than ± 2.0
7	LOD & LOQ	0.1026 $\mu g/ml$ & 0.3110 $\mu g/ml$	-
8	Solution Stability	Stable for 24hrs	-
9	System suitability	% RSD -0.03	
10	% Assay	100.81	IP Limit 98.5-101.5%

CONCLUSION

The reported UV Spectrophotometric method was proved to be simple, rapid and reproducible. The validation data indicate good precision, accuracy and reliability of the method. The developed method offers several advantages in terms of simplicity of the solvent system, easy sample preparation steps and time saving which makes the method specific and reliable for its intended use in the determination of Bromhexine Hydrochloride in the pharmaceutical dosage forms. This method can be of use and value for the Quality Control Department division of pharmaceutical companies manufacturing these formulations without interference.

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