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Validation of ambroxal hydrochloride in bulk drug and pharmaceutical dosage form by third order derivative method in UV spectrophotometry

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ABSTRACT

A simple and precise UV- spectrophotometric, third order derivative method has been developed and validated for the estimation of ambroxal hydrochloride in bulk drug and its pharmaceutical formulation. Ambroxal hydrochloride was estimated by third order derivative method at 215 nm. Beer's law was obeyed in the concentration range of 1 to 10 µg / ml with coefficient of correlation value 0.9987. This method was tested and validated for various parameters according to ICH guidelines. The precision expressed as relative standard deviation, which was 2.7023 % for the above method. The proposed method was successfully applied for the determination of ambroxal hydrochloride in pharmaceutical formulation. Results of the analysis were validated statistically and were found to be satisfactory. The proposed method is simple, easy to apply, low-cost and require relatively inexpensive instruments.

Keywords: Ambroxal hydrochloride, UV third order derivative method.

INTRODUCTION

Ambroxal Hydrochloride is trans-4-[(2Amino-3,5-dibromobenzyl)amino] cyclohexanol. It shows molecular formula as $C_{13}H_{18}Br_2N_2O.HCl$ with molecular weight 414.57. It is official in BP[1] and IP[2]. Ambroxal is a metabolite of bromhexine. It is an expectoration improver and mucolytic agent used in the treatment of acute and chronic disorders characterized by the production of excess or thick mucus. A literature survey reveals a spectrophotometric[3-7], HPLC[8-13] and miscellaneous[14-20] methods.

MATERIALS AND METHODS

Instrument and reagents

Spectral scan was made on a Shimadzu UV-spectrophotometer, model 1800 (Shimadzu, Japan) with spectral band width of 0.5 nm with automatic wavelength corrections by using a pair of 10 mm quartz cells. All spectral measurements were done by using UV-Probe 2.42 software. Reference standard of ambroxal hydrochloride was obtained from reputed firm with certificate analysis.

Preparation of standard drug solution

100 mg standard ambroxal hydrochloride was weighed accurately and transferred to a 100 ml volumetric flask and sonicated with 30 ml of distilled water for 15 minutes. The volume was made up to the mark with distilled water to give a stock solution of concentration 1000 µg /ml. From this solution, 10 ml of solution was pipetted out and

transferred into 100 ml volumetric flask. The volume was made up to mark with distilled water to give a working standard solution of concentration 100 µg/ml.

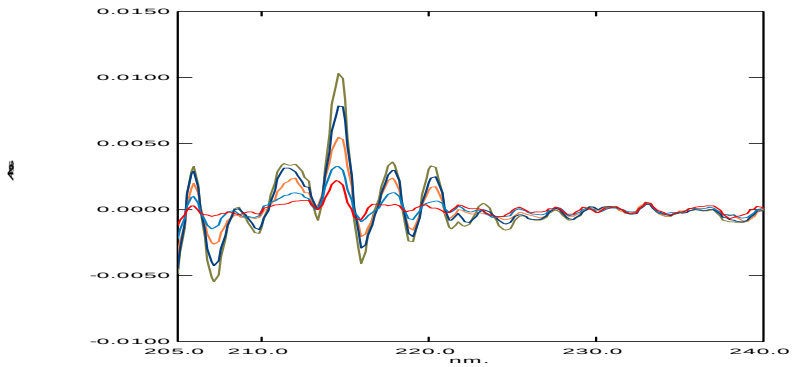
Estimation from tablets

Twenty tablets were weighed accurately and average weight of each tablet was determined. Powder equivalent to 10 mg of ambroxal hydrochloride was weighed and transferred in 100 ml of volumetric flask. A 30 ml of distilled water was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with distilled water to give concentration as 100 µg /ml. Such solution was used for analysis.

Method

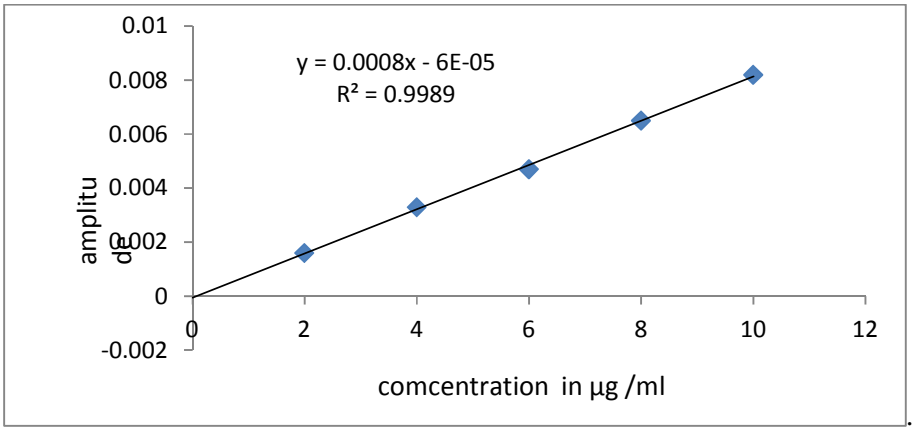
For the selection of analytical wavelength, 10 µg /ml solution of ambroxal hydrochloride was scanned in the spectrum mode from 300 nm to 200 nm by using distilled water as blank. The third order derivative spectrum was obtained by using derivative mode by UV probe 2.42 software. From the spectrum, the amplitude of the derivative spectrum was measured between. 215nm. (Fig.1).

Fig. 1: Overlay spectra of third order derivative of ambroxal hydrochloride at 215nm in the concentration range of 2 – 10 µg/ ml



Into series of 10 ml graduated flask, varying amount of standard solutions of ambroxal hydrochloride was pipette out and volume was adjusted with distilled water as solvent. Solutions were scanned between 300 nm to 200 nm in spectrum mode. The third order derivative spectra were obtained by using derivative mode. Amplitudes of the resulting solutions were measured at 215nm by using distilled water as blank. The calibration curve was prepared in the concentration range of 1 to 10 µg/ml. (Fig. 2)

Fig. 2. Calibration curve for ambroxal hydrochloride at 215nm by third order derivative Spectroscopy



Results of analysis are given in table 1.

Table 1: Values of results of optical and regression of drug

Parameter	values
Detection Wavelength (nm)	215
Beer Law Limits (µg/ml)	1-10
Correlation coefficient(r^2)	0.9989
Regression equation (y=b+ac)	
Slope (a)	0.0008
Intercept (b)	0.00006

Validation

Accuracy

Accuracy of the proposed method was carried as on the basis of recovery studies. It is performed by the standard addition method. Recovery studies were performed by adding standard drug at different levels to the pre-analyzed tablets powder solution and the proposed method was followed. From the amount of the drug estimated, the percentage recovery was calculated. The results of the analysis are shown in table 2.

Table 2: Results of recovery of ambroxal hydrochloride for third order derivative method

Amount of Sample Added in (µg/ml)	Amount of Standard Added in (µg/ml)	Total amount recovered in (µg/ml)	Percentage recovery (%)	Standard deviation	Percentage of relative standard deviation (C.O.V.)
2	0	1.9821	99.10714	0.112467	5.674007
2	2	3.9642	99.10714	0.094491	2.38356
2	4	6.0357	100.5952	0.094491	1.565533
2	6	7.9642	99.55357	0.094491	1.186436
				Mean = 0.9898	Mean =2.7023

Precision

The method precision was established by carrying out the analysis of homogenous powder blend of tablets. The assay was carried out of drug by using proposed analytical method in six replicates. The values of relative standard deviation lie well within the limits indicated the sample repeatability of the method. The results obtained are tabulated in table 3.

Table 3: Precision- method precision

Experiment no.	Weight of ambroxal hydrochloride taken in mg	Weight of ambroxal hydrochloride Found in mg
1	10	10.125
2	10	10.000
3	10	9.875
4	10	100.125
5	10	10.000
6	10	10.125
	Standard deviation	0.102062
	%RSD	1.0158

Inter-day and intra-day precision

An accurately weighed quantity of tablets powder equivalent to 10 mg of ambroxal hydrochloride was transferred to 100 ml of volumetric flask. A 30 ml of distilled water was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with distilled water to give concentration as 100 µg /ml. Such solution was used for analysis.

Solution was scanned between 300 nm to 200 nm in spectrum mode. The third order derivative spectrum was obtained by using derivative mode. Amplitude of the resulting solution was measured at between 215nm by using distilled water as blank. The amplitude of final solution was read after 0 hr., 3 hrs. and 6 hrs. in 10 mm cell 220.5 nm for third order derivative. Similarly the amplitude of the same solution was read on 1^{st}, 2^{nd} and 5^{th} day. The amount of distilled water was estimated by comparison with standard at 215nm for third order derivative, table 4.

Table 4: Summary of validation parameter for intra-day and inter-day

Sr. no.	Parameters	values
(A)	Intra-day precision (n=3) Amount found ± % RSD	100.592 % 0.09438
(B)	Inter-day precision (n=3) Amount found ± % RSD	98.164 % 0.0366
(C)	Ruggedness Analyst to analyst(n= 3) %RSD	0.92266

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations-

$LOD = 3.3\ \sigma/S$ and $LOQ = 10\ \sigma/S$

Where σ is the standard deviation of the signal to noise ratio of the sample and S is the slope of the related calibrations graphs.

The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability .The values of LOD and LOQ are given in table 6.

Table 6: Values of results of LOD and LOQ

parameters	values
Limit of Detection (µg/ml)	3.3
Limit of Quantification (µg/ml)	10

Ruggedness

The ruggedness of the method is defined as degree of reproducibility of results obtained by analysis of ambroxal hydrochloride sample under variety of normal test conditions such as different laboratories, different analysts and different lots of reagents. Quantitative determination of ambroxal hydrochloride was conducted spectrophotometrically on one laboratory. It was again tested in another laboratory using different instrument by different analyst. The assays obtained in two different laboratories were well in agreement. It proved ruggedness of the proposed methods.

RESULTS AND DISCUSSION

The third order derivative method is useful for routine analysis of ambroxal hydrochloride in bulk drug and formulation. The derivative spectroscopy method applied has the advantage that it locates hidden peak in the normal spectrum. It eliminates the interference caused by the excipients and the degradation products present, if any, in the formulation. The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures. The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 1 to 10 µg/ml and given in table1. Recovery studies were carried out by adding the pure drug to the previously analyzed tablet powder sample and shown in table 2, 3. The percentage recovery value indicates non interference from excipients used in formulation. The reproducibility and accuracy of the method were found to be good, which was evidenced by low standard deviation.

CONCLUSION

The most striking feature of method is its simplicity and rapidity, not requiring tedious sample solutions preparations which are needed for other instrumental methods. From the results obtained it can be concluded that the proposed method is fully validated and found to be simple, sensitive, accurate, precise, reproducible, rugged and robust and relatively inexpensive. So, the developed methods can be easily applied for the routine quality control analysis of rupatadine fumarate in pharmaceutical formulation.

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