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Analytical method development and validation of ambroxol hydrochloride by UV spectroscopy and forced degradation study and detection of stability.

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ABSTRACT:

Aim: The aim is to develop simple validated analytical method for analysis of Ambroxol hydrochloride by UV Spectroscopy and to study the forced degradation and stress conditions have been used to detect the stability of Ambroxol hydrochloride. **Method:** Ambroxol hydrochloride was estimated at 306nm. Linearity range was found to be2-10mcg/ml. The correlation coefficient was found to be 0.99987. The molar absorptivity was found to be 3947 L mol/cm. The proposed method Sandell's sensitivity was found to be 0.111111 µg cm−2/0.001AU. The limit of detection and limit of quantification were found to be 3.94210and 11.94577µg /ml respectively. The degradation behavior of Ambroxol hydrochloride was carried out as per the standard procedures and guidelines. Forced acid hydrolytic degradation, alkali degradation and oxidative degradation of Ambroxol hydrochloride was performed in bulk and solid oral formulation using 1N Hydrochloric acid and 0.1M Sodium hydroxide at room temperature in different time intervals such as 0mins,30mins, 60mins and 90mins. The resulting solutions were analyzed for content by UV spectrophotometry at the maximum absorption of 306 nm.. The assay value ofAmbroxol hydrochloride in bulk and formulation was calculated at different time intervals for intraday and interday experiments. **Results and conclusion:** The proposed method was successfully applied for the determination of Ambroxol hydrochloride in bulk and Pharmaceutical formulations (Tablets). The results were demonstrated, that the procedure is accurate, precise and reproducible (relative standard deviation < 2%), For acid degradation studies the assay values of Ambroxol hydrochloride at the end of the 90mins and 3rd day study for standard and sample were 58.42% and 50.53% respectively. For alkali degradation studies the assay values of Ambroxol hydrochloride at the end of the 90mins and 3rd day study for standard and sample were were 70.85% and 77.06% respectively. For oxidative degradation studies the assay values of Ambroxol hydrochloride at the end of the 90mins and 3rd day study for standard and sample were were 58.42% and 43.42% respectively. Ambroxol hydrochloride was found to degrade extensively under acid, alkali and oxidative conditions. Ambroxol hydrochloride has to be stored under such condition where the possibility of acid, alkali and oxidative hydrolysis does not arise.

KEYWORDS: Ambroxol hydrochloride, UV –Spectroscopy, Validation, ICH guidelines, Forced degradation studies.

INTRODUCTION:

Ambroxol hydrochloride[1,2] (AMH) is chemically 4-[(2-amino-3,5-dibromophenyl)methylamino]cyclohexan-1-ol;hydrochloride[3] which is a semi-synthetic derivative of vasic[illegible] a mucolytic agent. Ambroxol is indicated as "secretolytic therapy in brc[illegible]mal mucus secretion and impaired mucus transport. It promotes mucus cle[illegible]ctive cough, allowing patients to breathe freely and deeply".[4]

Fig.1:Structure of Ambroxol Hydrochloride

Only limited analytical methods were reported in the literature for Ambroxol. The aim of this work was to develop and validate a simple, fast, and reliable isocratic ultraviolet (UV) spectroscopic method for the determination of Ambroxol hydrochloride in bulk and pharmaceutical dosage forms. Forced degradation study like alkali, acid and oxidative degradation were performed. Confirmation of the applicability of the developed method validated according to the International Conference on Harmonisation (ICH) to determine the Ambroxol hydrochloride in bulk and pharmaceutical preparations. The objective of this study was to develop and validate an assay for the estimation of Ambroxol using HPLC.

Chemicals reagents:

Preparation of stock solution

10 mg of Ambroxol hydrochloride raw material was weighed and transferred in to 100 ml volumetric flask, then dissolved in methanol and made up to the volume with the same solvent. This solution contains 100µg/ ml concentration.

The standard stock solution was further diluted with water to get the concentration of 10 µg/ ml and the solution was scanned between 200 and 400 nm using the same solvent as blank. The spectrum was observed in that range and the λ max was found to be 306 nm and was selected as analytical wavelength.[7,8,9]

Preparation of linearity studies

The standard stock solution of Ambroxol hydrochloride was transferred into series of 10 ml volumetric flasks and made up to the volume with water. The absorbance of 2,4,6,8,10 g/ ml solutions were measured at 306 nm. The calibration curve was plotting between concentration vs. absorbance. Ambroxol hydrochloride was linear within the concentration range of 2,4,.....,10g/ ml at 306 nm. [9]

Validation of method development

Linearity

A calibration curve was plotted between concentration and absorbance. Ambroxol hydrochloride was linear in the concentration range of 2-10g/ ml at 306 nm. The linearity was repeated for five times and LOD and LOQ values for calculated.[5,7] The linearity is shown in fig.3 and the concentration with the absorbance value are given in table.1

Quantification of formulation:

20 tablets (mucolite containing ambroxol equivalent to 30mg)were weighed accurately. The average weight of the tablets are calculated and the tablets are made into powdered form. The powdered equivalent to 30mg of ambroxol was weighed and transferred into 100 ml volumetric flask. Added a minimum quantity of methanol to dissolve the substance and made upto the volume with the same(100µg/ml). The solution was filtered with whatmann filter paper. From the clear solution, further dilutions were made by 1ml to 10 ml volumetric flask with water to get 10µg/ml solution theoretically. The absorbance of six replicates were measured and the amount was calculated by using regression equation. This procedure is repeated for six times.[6,11]

Precision

The repeatability of the developed method was confirmed by the precision analysis. The intermediate precision of the method was confirmed by intraday and interday analysis i.e. the analysis of formulation was repeated three time in the same day and on three successive days. For this process Ambroxol hydrochloride 30mg was used. The amount of drugs present was determined and the percentage RSD also calculated.[5,7,10,12]

Accuracy

Accuracy of the method was confirmed by the recovery studies. To the preanalysed formulation a known quantity of raw material of Ambroxol hydrochloride was added in 6 concentration and recovery process are followed as per the quantification process. The amount of recovery was calculated. This procedure is repeated for 6 times and the %RSD was calculated. The results are shown below.[9,10,13]

Study of acid degradation Ambroxol Hydrochloride by UV spectroscopy method

Standard Preparation (stress) Ambroxol hydrochloride was transferred to volumetric flask and dissolved methanol to achieve a concentration of 1mg/mL. After 30mins, an aliquot solution was diluted with 1N hydrochloric acid to get a final concentration of 100mcg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 306 nm. The same procedure was repeated for 60mins, and 90mins time interval.[14]

Sample Preparation (stress) Ambroxol hydrochloride tablets were powdered and weighed and then transferred into volumetric flask and dissolved methanol to achieve a concentration of 1mg/mL. After 30mins, an aliquot solution was diluted with 1N hydrochloric acid to get a final concentration of 100mcg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 306 nm. repeated

Blank Preparation: A blank preparation of Hydrochloric acid (1 e procedure was repeated thrice. After the stipulated time, the absorption m (Table:8) against reagent blank treated in the same way. Three such determinations were made and the assay value was estimated the obtained values are concurrent.

For Inter day study Standard preparation The standard preparation was prepared in a similar manner which was mentioned in an intraday preparation Standard stress preparation Same method was followed, but the final solution was scanned and absorption was recorded at the following time intervals 1st, 2nd, and 3rd day.

Sample Preparation: Same method was followed, but the final solution was scanned and absorption was recorded at the following time intervals 1st, 2nd, and 3rd day. Blank preparation Similar to intraday preparation The procedure was repeated thrice. After the stipulated time, the absorption of the resulting solution showed maxima 306nm (Table:9) against reagent blank treated in the same way. Three such determinations were made and the assay values are estimated and concurrent values were obtained.

Study of alkali degradation Ambroxol Hydrochloride by uv spectroscopy method

Stand ard Preparation (stress) Ambroxol hydrochloride 10mg was transferred to volumetric flask and dissolved methanol to

Standard Preparation (stress) Ambroxol hydrochloride 10mg was transferred to volumetric flask and dissolved methanol to achieve a concentration of 1mg/mL. After 30mins, an aliquot solution was diluted with distilled with 0.1M Sodium hydroxide to get a final concentration of 100mcg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 306nm. The same procedure was repeated for 60mins, and 90mins time interval. [15]

Sample Preparation (stress) Ambroxol hydrochloride granules were weighed and transferred to volumetric flask and dissolved methanol to achieve a concentration of 1mg/mL. After 30mins, an aliquot solution was diluted with 0.1M Sodium hydroxide to get a final concentration of 100mcg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 306nm. The same procedure was repeated for 60mins, and 90mins time interval

Blank Preparation: A blank solution of Sodium hydroxide (0.1M) solution was prepared in a similar manner. The procedure was repeated thrice. After the stipulated time, the absorption of the resulting solution showed maxima 306nm (Table:10) against reagent blank treated in the same way. Three such determinations were made and the assay value was estimated the obtained values were concurrent.

For Inter day study Standard preparation The standard preparation was prepared in a similar manner which was mentioned in an intraday preparation Standard stress preparation Same method was followed, but the final solution was scanned and absorption was recorded at the following time intervals 1st, 2nd, and 3rd day.

Sample Preparation: Same method was followed, but the final solution was scanned and absorption was recorded at the following time intervals 1st, 2nd, and 3rd day. Blank preparation Similar to intraday preparation The procedure was repeated thrice. After the stipulated time, the absorption of the resulting solution showed maxima 306nm (Table:11) against reagent blank treated in the same way. Three such determinations were made and the assay value was estimated and concurrent values were obtained.

Study of oxidative degradation Ambroxol Hydrochloride by uv spectroscopy method

Standard Preparation (stress) Ambroxol hydrochloride 10mg was transferred to volumetric flask and dissolved methanol to achieve a concentration of 1mg/mL. After 30mins, an aliquot solution was diluted with distilled with 10% hydrogen peroxide to get a final concentration of 100mcg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 306nm. The same procedure was repeated for 60mins, and 90mins time interval.[16]

Sample Preparation (stress) Ambroxol hydrochloride granules were weighed and transferred to volumetric flask and dissolved methanol to achieve a concentration of 1mg/mL. After 30mins, an aliquot solution was diluted with 10% hydrogen peroxide to get a final concentration of 100mcg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 306nm. The same procedure was repeated for 60mins, and 90mins time interval.

Blank Preparation: A blank solution of hydrogen peroxide(10%) solution was prepared in a similar manner. The procedure was repeated thrice. After the stipulated time, the absorption of the resulting solution showed maxima 306nm (Table:12) against reagent blank treated in the same way. Three such determinations were made and the assay value was estimated the obtained values were concurrent.

For Inter day study Standard preparation The standard preparation was prepared in a similar manner which was mentioned in an intraday preparation Standard stress preparation Same method was followed, but the final solution was scanned and absorption was recorded at the following time intervals 1st, 2nd, and 3rd day.

Sample Preparation: Same method was followed, but the final solution was scanned and absorption was recorded at the following time intervals 1st, 2nd, and 3rd day. Blank preparation Similar to intraday preparation The procedure was repeated thrice. After the stipulated time, the absorption of the resulting solution showed maxima 306nm (Table:13) against reagent blank treated in the same way. Three such determinations were made and the assay value was estimated and concurrent values were obtained.

RESULT AND DISCUSSION:

The new, simple and cost effective UV-Spectrophotometric f Ambroxol hydrochloride in bulk and pharmaceutical formulations and study of acid, alkali and oxidative degradation

Ambroxol hydrochloride was estimated at 306 nm by using water. The drug was soluble in aqueous solvent but it is not produce stable λmax and absorbance. So we tried with water and 0.1N HCl.

Linearity range was found to be 2−10 µg/ml. The correlation coefficient was found to be 0.999876 and the molar absorptivity was found to be 3947 L mol^{-1} cm^{-1} in water and in 0.1M HCl.. The proposed method Sandell's sensitivity was found to be about 0.111111 µg cm^{-2}/ 0.001AU.

Fig.2:UV absorption spectrum of Ambroxol hydrochloride.

Fig.3: Calibration curve of Ambroxol hydrochloride

Table no.1: Linearty of Ambroxol hydrochloride

S. no.	Concentration (µg /ml)	Absorbance
1.	2	0.023
2.	4	0.041
3.	6	0.060
4.	8	0.077
5.	10	0.095

The limit of detection and the limit of quantification were determined by the linearity studies, theprocess was repeated for six times and the limit of detection (3.9420) and the limit of quantification (11.9477) were calculated. It has been shown in table no.2.

Table no:2. Optical Characteristics of Ambroxol Hydrochloride

PARAMETERS	VALUES*
λmax (nm)	306
Beer's law limit (µg/ ml)	2-10
Sandell's sensitivity (g/cm^2/0.001 A.U)	0.111111
Molar absorptivity ($L\ mol^{-1}\ cm^{-1}$)	3947
Correlation coefficient (r)	0.999876
Regression equation (y=mx+c)	Y = 0.009x + 0.00520
Slope(m)	0.009
Intercept(c)	0.0052
LOD (µg/ ml)	3.9420
LOQ (µg/ ml)	11.9477
Standard error	0.0032

Table no.3: Quantification of formulation.

	Sample no.	Amount added (µg/ ml)	Amount present (µg/ ml)	Percentage obtained	Average%	S.D	%RSD	S.E
	1.	6	5.86	97.66				
	2.	6	6.08	101.4				
Ambroxol	3.	6	5.97	99.61	98.6	1.25	1.00	0.0032
	4.	6	5.86	97.6				
	5.	6	5.64	94.07				

Table no.4: Recovery analysis of Ambroxol hydrochloride

Drug	Sample no.	Amount present µg/ ml	Amount added µg/ ml	Amount found µg/ ml	Amount recovered µg/ ml	% recovered	S.D	%RSD	S.E
	1.	3	3	5.86	2.86	97.77			
	2.	3	3	6.03	3.03	100.55	1.89	2.00	0.0032
Ambroxol	3.	3	3	6.08	3.08	101.4			
	4.	3	3	5.97	2.97	99.61			
	5.	3	3	6.06	3.06	101.10			

From the linearity curve, the mean concentration of 6µg/ml was selected and quantification in tablets was performed. The 30 mg tablets was selected for analysis. The amount present was determined by average of six replicate analysis and the amount present were found to be 5.86,6.08, 5.97,5.86,5.64 mg respectively. The results were shown in Table no.3

The accuracy of the developed method was carried out by standard addition method. The known amount of pure drug was added to the previously analyzed solution containing tablets and the mixture was analyzed by the proposed method and the recoveries were calculated. The percentage recovery o nd to be 97.77,100.55,101.4,99.61,101.10. The resultswere shown in Tab

Precision of the method has done by making repeated analysis c nes in a day for 3 days. The percentage standard deviation for inter day and intraday analysis of Ambroxol hydrochloride was found to be 1.4120 and 1.89 respectively and shown in table .5 and 6.

Table no.5: Intraday analysis of Ambroxol hydrochloride

Drug	Sample no.	Amount Present (µg/ml)	Amount found (µg/ml)	Percentage obtained	Average %	S.D	%RSD	S.E
	1.	6	6.08	101.4				
Ambroxol	2.	6	5.97	99.62	98.36	1.1352	1.4120	0.0032
	3.	6	5.64	94.07				

Table no.6: Interday analysis of Ambroxol hydrochloride

Drug	Sample no.	Amount present (µg/ml)	Amount found (µg/ml)	Percentage obtained	Average %	S.D	%RSD	S.E
	1.	6	5.86	97.77				
Ambroxol	2.	6	5.97	99.92	98	1.85	1.89	0.0032
	3.	6	5.75	95.62				

Table no.7: Ruggedness study by different analysts

Table no.7:Ruggedness study by different analysts

Drug	Condition	Sample No	Amount present (µg/ml	Amount found (µg/ml)	Percentage Obtained	Average (%)	S.D	%R.S.D	S.E
AMB	Analyst 1	1 2 3 4 5 6	6 6 6 6 6 6	6.088 6.2 6.077 5.97 6.033 6.066	101.46 103.35 101.26 99.62 100.55 101.10	101.22	0.95	0.94	0.0032
AMB	Analyst 2	1 2 3 4 5 6	6 6 6 6 6 6	5.86 6.08 6.06 6.05 6.2 6.08	97.77 101.46 101.1 100.97 103.35 101.46	101.01	0.87	0.86	0.0032

Ruggedness

The ruggedness of the developed method was confirmed by using different instruments and different analysts.The % RSD was calculated. The % RSD by using different analyst 0.94% and 0.86%. The results are within the limit. So it indicates the developed method was more rugged. The results were shown in Table 7.

Acid Degradation studies:

Ambroxol hydrochloride was found to be unstable under acid condition. Table: 8shows the results of intraday degradation and how much of standard and sample remaining in the solution after certain time intervals.

Table no. :8. Acid degradation study of AMB(Interday)assay stress conditions

Stress condition	Time in minutes	Standard*	Sample*	Remarks
Hydrochloric acid (1N) Acid Hydrolysis	30	63.08%	54.75%	Degradation observed
	60	61.31%	51.97%	Degradation observed
	90	59.46%	50.53%	Degradation observed

The assay values of the standard and sample was found to be 58.42% and 50.53% at the end of 90 mins.Table:9 shows the interday degradation and how much standard and sample were remaining after the degradation. The assay value of standard and sample was found to 58.42% and 50.03% at the end of first day of acid hydrolysis. At the end of 3rd day the assay value of standard and sample were found to be 52.69% and 44.24%

Table no.9: Acid degradation study of AMB(Intraday)assay stress conditions

Stress condition	Time in Hours	Standard*	Sample*	Remarks
Hydrochloric acid (0.1N) Acid Hydrolysis	24	58.42%	50.03%	Degradation observed
	48	56.97%	47.08%	Degradation observed
	72	52.69%	44.24%	Degradation observed

Alkali degradation studies:

Ambroxol hydrochloride was found to be unstable under alkali condition. Table: 10 shows the results of intraday degradation and how much of standard and sample remaining in the solution after certain time intervals.

Table no.:10. Alkali degradation study of AMB(Intraday)assay stress conditions

Stress condition	Time in minutes	Standard
Sodium Hydroxide (0.1M) Alkali Hydrolysis	30	76.08
	60	73.86
	90	70.85

The assay values of the standard and sample was found to be 70.85% and 73.75% at the end of 90 mins.Table:11 shows the interday degradation and how much standard and sample were remaining after the degradation. The assay value of standard and sample was found to 66.35% and 71.62% at the end of first day of alkali hydrolysis. At the end of 3rd day the assay value of standard and sample were 54.24% and 60.31%.

Table no.11. Alkali degradation study of AMB(Interday)assay stress conditions.

Stress condition	Time in Hours	Standard*	Sample*	Remarks
Sodium Hydroxide (0.1M) Alkali hydrolysis	24	66.35	71.62	Degradation observed
	48	60.64	66.53	Degradation observed
	72	54.24	60.31	Degradation observed

Oxidative Degradation:

Oxidative Degradation:

Ambroxol hydrochloride was found to be unstable under oxidative condition.Table.12shows the results of intraday degradation and how much of standard and sample remaining in the solution after certain time intervals.

Table no.: 12. Oxidative Intraday Degradation study of Ambroxol Hydrochloride.

Stress condition	Time in minutes	Standard*	Sample*	Remarks
Hydrogen Peroxide (10%) Oxidative Hydrolysis	30	64.86%	49.86%	Degradation observed
	60	61.62%	46.31%	Degradation observed
	90	58.42%	43.42%	Degradation observed

The assay values of the standard and sample was found to be 58.42% and 43.42% at the end of 90 mins.Table:13 shows the interday degradation and how much standard and sample were remaining after the degradation. The assay value of standard and sample was found to 57.28% and 40.09% at the end of first day of oxidative hydrolysis. At the end of 3rd day the assay value of standard and sample were 45.22% and 33.54% respectively.

Table no. 13: Oxidative Interday Degradation study of Ambroxol Hydrochloride.

Stress condition	Time in Hours	Standard*	Sample*	Remarks
Hydrogen Peroxide (10%) Oxidative Hydrolysis	24	57.28%	40.09%	Degradation observed
	48	51.31%	37.32%	Degradation observed
	72	45.22%	33.54%	Degradation observed

CONCLUSION:

In this study a simple ,precise, accurate and sensitive UV-spectroscopy methods were developed for the simultaneous estimation of Ambroxol hydrochloride in bulk and in tablet dosage form. The Correlation coefficient (γ) values of the proposed method was close to 1.0, it indicate that the concentration used for plotting calibration curve were obeying Beer's law strictly. Additives and impurities commonly present in the dosage forms but did not show any interference in the proposed method. Statistical validation was done it shows that the method was reproducible and accurate. Also the various parameters were calculated such as standard deviation and percentage relative standard deviation. The values are complies all the limit as per ICH guidelines. The forced acid, alkali and oxidative degradation study of Ambroxol hydrochloride was studied by UV spectroscopy at various time interval (30mins, 60mins, 90mins; 1st, 2nd, 3rd day ;) it is observed that the drug Ambroxol hydrochloride is degrading. Therefore the drug Ambroxol hydrochloride has to be stored under such condition where the possibility of acid, alkali and oxidative hydrolysis does not arise.

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