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NEW SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF AMBROXOL HYDROCHLORIDE IN PHARMACEUTICAL FORMULATIONS

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Research Article

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Three simple spetrophotometric methods (A, B and C) have been described for the assay of Ambroxol in bulk and in pharmaceutical formulations. In method A Ambroxol hydrochloride is condensed with P-dimethylaminobenzaldehyde (PDAB) to form a yellow coloured Schiff's base having an absorption maximum (λ_{max}) of 420nm. Method B is based on the oxidation / reduction reaction between the Ambroxol and Folin-Ciocalteu (FC) reagent to form a blue coloured chromogen with λ_{max} at 704 nm. Method C involves oxidation of Ambroxol hydrochloride with cerric ammonium sulphate (CAS) followed by coupling with N-methylbenzothiazolone hydrazone (MBTH) to form red coloured complex with λ_{max} at 540nm.These methods have been statistically evaluated and are found to be precise and accurate.

Key words:- Ambroxol HCL,HPLC,Validation

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INTRODUCTION

Ambroxol trans-4-[(2-amino 3, 5-dibromobenzyl) amino] cyclohexanol hydrochloride, is widely used as an expectorant and mucolytic agent in the treatment of respiratory disorders.. A survey of literature revealed that there are less number of work done on visible spectrophotometry. Therefore the need for a fast, low cost and selective method is obvious, especially for the routine quality control analysis of pharmaceutical formulations containing Ambroxol hydrochloride.

This paper describes three visible spectrophotometric methods for the determination of Ambroxol hydrochloride by making use of the reported procedures. Method A is based on the formation of coloured from Ambroxol hydrochloride and PDAB as under iodole. Among the various heteropolyacids, phosphomolybdotungstic acid the well known Folin-Ciocalteu (FC) reagent was preferred by a number of workers for the determination of drugs containing not only phenolic or amino groups but also certain other drugs which contain neither of these groups.

Method C is based on the formation of the red coloured species ($\lambda_{max:}$ 540nm) on treating Ambroxol with cerric ammonium sulphate and MBTH in the presence of dilute sulphuric acid. Reduction of heteropolyacid complexes by organic reagents was utilized as the basis for the determination of several organic compounds, particularly phenols, amines and enols.

EXPERIMENTAL

Instrument: A Systronics UV-VIS double beam spectrophotometer (model: 116) and (model: 118) and visican (model: 16) with 1 cm matched quartz cells was used for all spectral measurements.

Reagents: All the chemicals used were of analytical grade and all the solutions were prepared with double distilled water. PDAB (0.5%w/v) in methanol and conc.HCL were used in method A. Aqueous solutions of sodium carbonate (10%w/v) and commercially procured FC reagent and distilled water in 1:1 ratio were used in method B. MBTH (0.2%w/v) and Cerric ammonium sulphate (1%w/v) solution prepared by using mixture of conc.H_2SO_4 and water in 1:1 ratio were used in method C.

Standard drug solution:

Stock solution of Ambroxol hydrochloride (1 mg/ml) was prepared by dissolving 25mg of Ambroxol hydrochloride in 25 ml of distilled water. The working standards were prepared by dilution to 100 ml with distilled water (in methods A, B and C, 200µg/ml).

Sample solutions:

Tablets of one brand were used for the purpose of analysis. Twenty tablets were powdered and powder equivalent to 100mg of Ambroxol hydrochloride was weighed and the solution was prepared under standard solution preparation and filtered if insoluble portion present.

Assay procedure:

Method A:

Aliquots of standard drug solution representing 400-800 µg of Ambroxol hydrochloride, 2 ml of PDAB solution and 2 ml of conc. HCL were successively added to a series of 10 ml graduated tubes. The contents of each tube were mixed well and the volume was brought to 10 ml with distilled water. The absorbance was measured against a reagent blank at 420 nm. The amount of drug present in the sample solution was deduced from calibration curve.

Method B:

Aliquots of standard drug solution representing 300-700 µg of Ambroxol hydrochloride, 4.5ml of Na_2CO_3 and 1.25 ml of FC reagent were added simultaneously and kept aside for 10 min at room temperature. The solution was made up to 10 ml with distilled water. The absorbance was measured at 704 nm against a reagent blank. The amount of the drug in the sample was computed from Beer-Lambert's plot.

Method C:

Into a series of 10 ml graduated tubes 0.5-2.5 ml (200µg/ml) solution of Ambroxol hydrochloride was transferred and 1 ml of cerric ammonium sulphate solution was added and keep it aside for 2 minutes at room temperature and then 1 ml of MBTH solution was added and the volume was brought up to 10 ml. The absorbance was measured at 540nm against reagent blank prepared under identical conditions. The exact amount of Ambroxol hydrochloride was calculated from the calibration curve.

VALIDATION:

Accuracy: To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts of bulk samples of Ambroxol hydrochloride within the linearity range were taken and added to the preanalyzed formulation. From that percentage recovery, values were calculated. The results are shown in tables 2(a), 2(b), 2(c).

Precision: The precision of the proposed method was ascertained by actual determination of six replicate samples of fixed concentrations of the drug within the Beer's range and finding out the absorbance by the proposed method. From this absorbance, mean, standard deviation and % RSD was

calculated. The precision readings are shown in table 3(a), 3(b), 3(c).

Limit of detection: LOD calculation is based on the standard deviation of the response (σ) and the slope of the calibration curve (S) at the levels approximating the LOD according to the formula. The LOD values are shown in table 4.

LOD=3.3(σ/S)

Limit of quantitation: LOQ calculation is based on the standard of the response (σ) and the slope of the calibration curve (S) at levels approximately the LOQ according to the formula. The LOQ values are shown in table 5.

LOQ=10(σ/S)

RESULTS AND DISCUSSION

The optimum conditions for each method were established by varying one parameter at a time and keeping the other fixed and observing the effect produced on the absorbance of the coloured species and incorporated in the procedures. The optical characteristics and figures of merit are given in Table 1, together with the regression equations (obtained by linear least square treatment) for the calibration plots. The precision and accuracy were found by analysing six replicate samples containing known amount of drug and the results were summarised in Table 1.

Commercial formulations (Tablets) containing Ambroxol hydrochloride were successfully analysed by the proposed methods. The values obtained by the proposed and reference (UV method) for formulations were compared statistically by the t - and F - tests and found not to differ significantly. As an additional check of accuracy, recovery experiments were performed by adding a fixed amount of the drug to the pre analysed formulations. These results are summarized in Table 2. The ingredients usually present in the formulations of Ambroxol hydrochloride did not interfere with the proposed analytical methods.

Chemistry of coloured species:

The method A is based on the formation of colour is condensation product (Schiff's base) from Ambroxol hydrochloride and PDAB as under iodole. The colour formation by Ambroxol hydrochloride with FC reagent in method B may be explained in the following manner based on analogy. With the reports of earlier workers, the mixed acids in the FC reagent preparation involve the following chemical species.

3 H_2O. P_2O_5. 13 WO_3. 5 MoO_3. 10 H_2O and 3 H_2O. P_2O_5. 14WO_3.4MoO_3. 10H_2O

Ambroxol hydrochloride probably effects a reduction of 1, 2 or 3 oxygen atoms of tungstate and / or molybdate in FC reagent thereby producing one or more of possible reduced species which have a characteristic blue colour. The formation of the red coloured species in method A by Ambroxol hydrochloride

with cerric ammonium sulphate and MBTH is due to oxidation of Ambroxol hydrochloride and followed by the coupling with MBTH.

The proposed methods are found to be simple, sensitive, and accurate and can be used for determination of Ambroxol hydrochloride in their pharmaceutical dosage forms in a routine manner.

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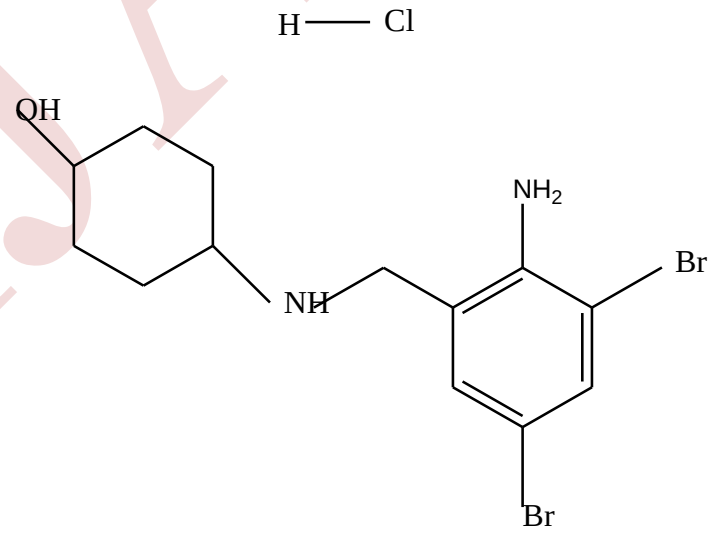
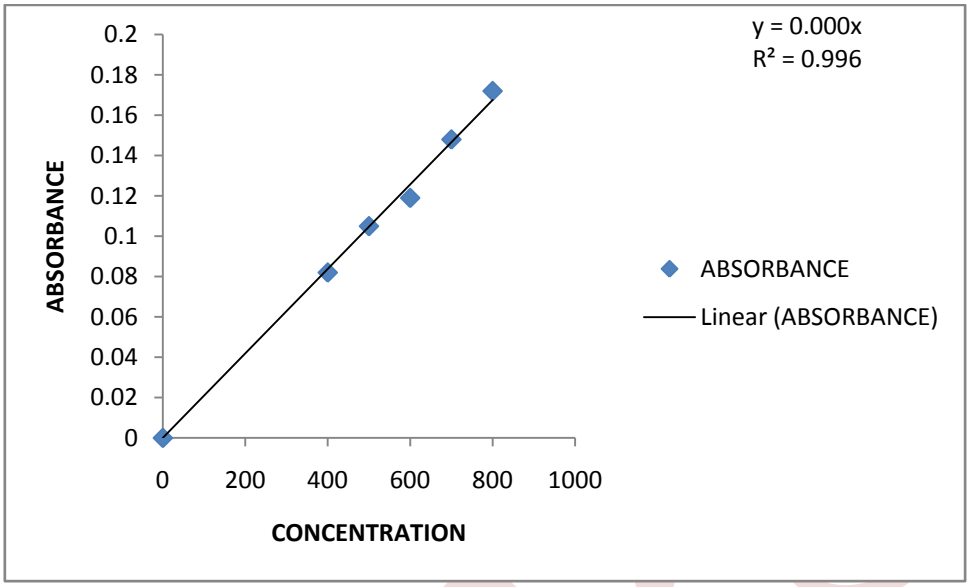
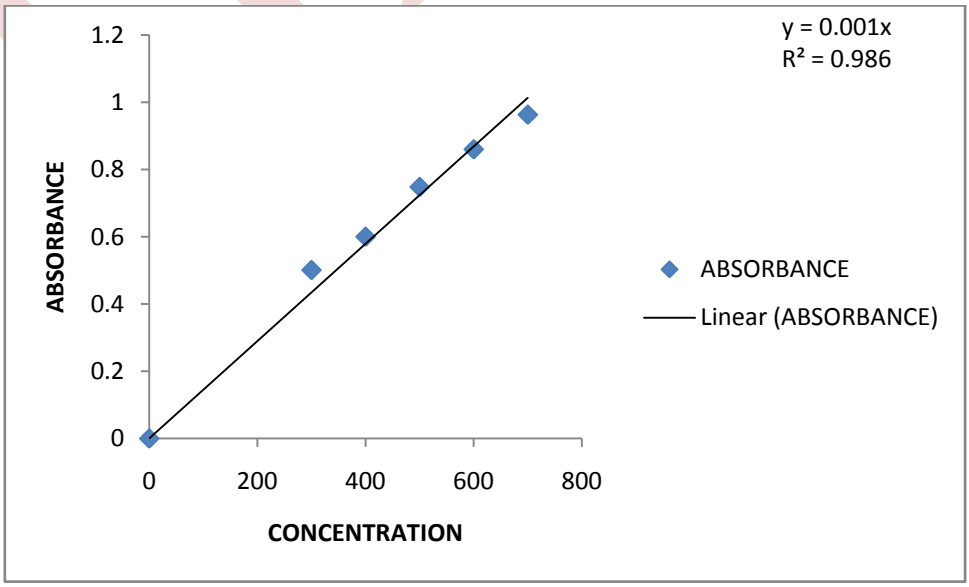
Structure of Ambroxol hydrochloride:



CALIBRATION CURVE FOR METHOD A



CALIBRATION CURVE OF METHOD B



CALIBRATION CURVE OF METHOD C

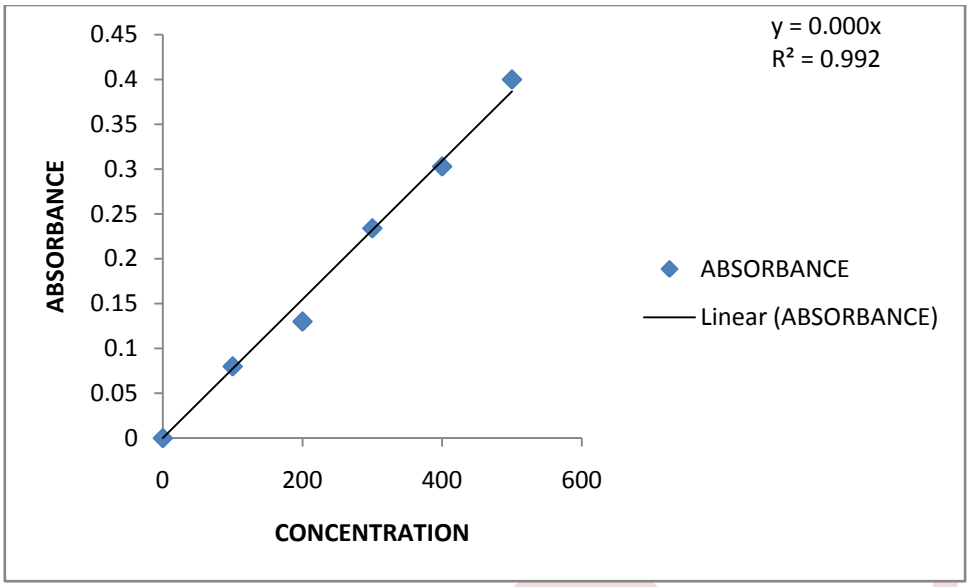


Table 1

Optical regression characteristics of Ambroxol hydrochloride using proposed methods

Optical character	Methods		
	A	B	C
λ_{max}(nm)	420	704	540
Beer's law limits (µg/ml)	40-80	30-70	10-50
Molar absorptivity (Litre.mole^{-1}.cm^{-1})	3.410×10^{3}	3.618×10^{3}	3.560×10^{3}
Sandell's sensitivity (µg/cm^2/0.01 abs. unit)	0.0565	0.0566	0.0926
Regression equation (Y)*			
Slope (b)	1.02×10^{-4}	1.04×10^{-4}	1.07×10^{-4}
Intercept (a)	5×10^{-3}	6×10^{-3}	2×10^{-3}
% RSD**	0.51	0.91	0.82
% Range of error** (0.05 level)	±0.50	±0.32	±0.45

*Y=a+bX, where X is the concentration of Ambroxol hydrochloride in µg/ml and Y is the absorbance at respective$\lambda_{max.}$

** For six replicate samples.

ASSAY OF THE TABLET:

Pharmaceutical formulation	Labelled amount (mg/tablet)	Amount found*(mg)			% Recovery***		
		Method A	Method B	Method C	Method A	Method B	Method C
MUCOLITE (Dr.REDDY'S)	30	28.8	28.5	27.99	96%	95%	93.33%

Table 2(a) Accuracy readings of method A:

S.NO.	CONCENTRATION	AMOUNT ADDED	ABSORBANCE	AMOUNT FOUNDED	% RECOVERY	STATISTICAL ANALYSIS
1	50%	400	0.084	409.7	102.4	Mean=100.36
2	50%	400	0.081	395.2	98.7	SD=1.858
3	50%	400	0.082	400	100	RSD=1.851
4	75%	500	0.118	594.9	99.15	Mean=99.71
5	75%	500	0.119	600	100	SD=0.404
6	75%	500	0.119	600	100	RSD=0.405
7	100%	600	0.172	800	100	Mean=99.6
8	100%	600	0.171	795.3	99.4	SD=0.346
9	100%	600	0.171	795.3	99.4	RSD=0.347

Table 2(b) Accuracy readings of method B:

S.NO.	CONCENTRATION	AMOUNT ADDED	ABSORBANCE	AMOUNT FOUNDED	% RECOVERY	STATISTICAL ANALYSIS
1	30%	300	0.501	300	100	Mean=99.93
2	30%	300	0.500	299.4	99.8	SD=0.212
3	30%	300	0.501	300	100	RSD=0.212
4	40%	400	0.600	400	100	Mean=98.86
5	40%	400	0.590	393.3	98.3	SD=0.98
6	40%	400	0.590	393.3	98.3	RSD=0.99
7	50%	500	0.748	500	100	Mean=99.95
8	50%	500	0.747	499.33	99.86	SD=0.08
9	50%	500	0.748	500	100	RSD=0.08

Table 2(c) Accuracy readings of method C:

S.NO.	CONCENTRATION	AMOUNT ADDED	ABSORBANCE	AMOUNT FOUNDED	% RECOVERY	STATISTICAL ANALYSIS
1	50%	100	0.068	100	100	Mean=99.5
2	50%	100	0.067	98.52	98.52	SD=0.854
3	50%	100	0.068	100	100	RSD=0.858
4	100%	200	0.130	200	100	Mean=99.73
5	100%	200	0.129	198.46	99.2	SD=0.460
6	100%	200	0.130	200	100	RSD=0.461
7	150%	300	0.234	300	100	Mean=100.1
8	150%	300	0.234	300	100	SD=0.234
9	150%	300	0.235	301.28	100.4	RSD=0.233

Table 3(a) Precision readings of method A

CONCENTRATION (µg/ml)	ABSORBANCE	STATISTICAL ANALYSIS
500	0.106	
500	0.110	MEAN=0.107
500	0.106	
500	0.106	SD=0.003521
500	0.113	
500	0.106	RSD=3.29

Table 3(b) Precision readings of method B

CONCENTRATION (µg/ml)	ABSORBANCE	STATISTICAL ANALYSIS
500	0.748	
500	0.752	MEAN=0.750
500	0.751	
500	0.754	SD=0.002449
500	0.748	
500	0.749	RSD=0.326

Table 3(c) Precision readings of method C

CONCENTRATION (µg/ml)	ABSORBANCE	STATISTICAL ANALYSIS
300	0.234	
300	0.240	MEAN=0.236
300	0.234	
300	0.236	SD=0.0024
300	0.235	
300	0.238	RSD=1.016

Table 4 LOD values of all methods

	METHOD A	METHOD B	METHOD C
LOD	0.5	0.08	0.158

Table 5 LOQ values of all methods

	METHOD A	METHOD B	METHOD C
LOQ	1.53	0.272	0.48