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Research Article

Simultaneous Estimation of Ambroxol Hydrochloride and Olopatadine in Formulated Tablet Dosage Form by UV Spectroscopic Method

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ABSTRACT

A novel, simple, rapid, precise, accurate, cost effective and reproducible spectroscopic method has been developed for simultaneous estimation of ambroxol HCl and olopatadine in formulated combined tablet dosage form. The method employs measurement of absorbance at two wavelengths, 246.nm and 298nm, of ambroxol and olopatadine respectively. Beer´s law obeyed in the concentration range of 12-72µg/ml and 2-16µg/ml for ambroxol and olopatadine respectively. The proposed method is recommended for routine analysis since it is rapid, simple, accurate, and also sensitive and specific. Results of percentage recovery studies confirmed the accuracy of the proposed method. The present uv spectroscopic method was validated following the ICH guideline.

Keywords: Ambroxol , Olopatadine, Simultaneous equation method, UV spectroscopy.

INTRODUCTION

Ambroxol hydrochloride is chemically, 1 ({[2 –Amino – 3, 5 dibromo phenyl] methyl} amino) cyclohexanol monohydrochloride which is a semi synthetic derivative of vasicine from the Indian shrub "Adhatoda vasica". It is an expectoration improver and a mucolytic agent used in the treatement of bronchial asthama and chronic bronchitis. Ambroxol hydrochloride has also been reported to have a cough suppressing effect and anti inflammatory action. Recently the inhibition of nitric oxide dependent activation of soluble guanylate cyclase was suggested one of the molecular mechanism of the therapeutic action of ambroxol hydrochloride, also used in pulmonary alveolar proteinosis in pulmonary distress and infant respiratory distress syndrome. Olopatadine hydrochloride, chemically, 11-[(Z)-3-(Dimethylamino) propylidene]-6-11-dihydrodibenz [b,e] oxepin-2-acetic acid hydrochloride is a dibenzoxipine derivative used for systemic treatment of allergic rhinitis, urticaria, and bronchial asthma. It is a selective inhibitor for the release of histamine and other pro-inflammatory mediators from the mast cell. The objective of present study was to develop simple, precise, accurate and validated, economic and analytical methods for estimation of Ambroxol Hcl and Olopatadine in tablet dosage forms and hydrolytic stress degradation studies of the mixture.

MATERIALS AND METHODS

Instrument

A Shimadzu UV/Vis double beam spectrophotometer model 1601 with spectral bandwidth of 0.1 nm, wavelength accuracy of ± 0.5 nm with automatic wavelength correction and with a pair of 1 mm quartz cells.

Chemicals

OLO and AMB (Purity 99.89%w/w and 99.92% were procured as a gift sample from Sun pharma and anantha pharmaceuticals, India). Acetonitrile AR grade (Merck India Ltd.,), dihydrogen phosphate AR grade were used in the present study.

Method Validation

Linearity

Standard stock solution of 100 µg/ml of OLO and AMB were prepared by dissolving separately in 50 ml of Acetonitrile:phosphate buffer(70:30) in 100 ml volumetric flask, the volume was made up to mark with the same. Standard solutions were prepared by dilution of the stock solution with solvent to give the final concentration range of 2-16 µg/ml and 12-70 µg/ml for OLO and AMB respectively.

Sample preparation

Twenty tablets were weighed accurately. The average weight was determined and then ground to a fine powder. A quantity equivalent to 5 mg of OLO and 30 mg of AMB were transferred into a 100 ml volumetric flask. The contents were ultrasonicated for 15 min with 50 ml acetonitrile: phosphate buffer (70:30) and made up to the mark with same. The resulting solution was allowed to settle for about an hour, and the supernatant was suitably diluted to give the desired concentration with solvent.

Precision

Intra-day and inter-day accuracy and precision of the assay samples containing (6, 12 and 18µg/ml) for AMB and (1, 2 and 3µg/ml) for AMB were analyzed six times in the same day (intra-day) and for three consecutive days (interday).

Specificity

The specificity of the method was assessed by analyzing standard drug, pharmaceutical product and placebo and

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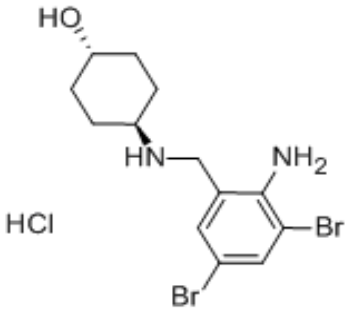
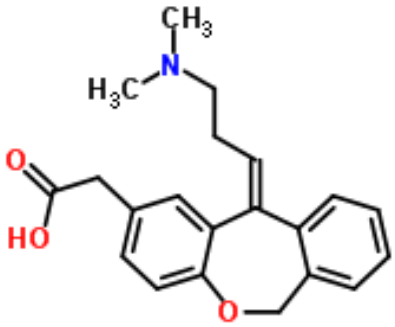


Structure of Ambroxol hydrochloride



Structure of Olopatadine

Table 1: Intermediate Precision Studies.

Actual conc.(µg/ml)	Interday precision		Intraday precision	
	SD	%RSD	SD	%RSD
OLO				
4.8	0.0106	0.0225	0.0126	0.266
6	0.0097	0.164	0.0055	0.093
7.2	0.02	0.28	0.021	0.297
AMB				
28.8	0.0049	0.017	0.0099	0.034
36	0.0255	0.07	0.038	0.105
43.2	0.0024	0.005	0.0032	0.007

Table 2: Recovery studies.

Amount added(µg/ml)	Mean recovery ± SD	% RSD
OLO		
1	98.178	0.816
2	±	
3	0.806	
AMB		
6	99.715	0.162
12	±	
18	0.162	

comparing the λ max of the standard with that of the sample to determine whether the pharmaceutical product and placebo led to interfere.

Accuracy as Recovery studies

Recovery studies were done at three different levels. The pre-analyzed sample was spiked with known concentration of the pure samples, and the mixtures were reanalyzed by the proposed method. Percentage recovery was calculated from the amount of drug found in the solution.

Robustness of the method

Small deliberate changes in the wavelength (± 5 nm) were introduced and the effects on the results were examined.

RESULTS AND DISCUSSION

The development of simultaneous determination of drugs has received considerable attention in recent years because of their importance in the quality control of drugs and drug products. Since there are no literature reported methods of OLO and AMB simultaneously for routine analysis, our primary goal is to develop a simple UV spectroscopic method is to achieve simultaneous determination of OLO and AMB in the compound formulation under common conditions that are applicable for the routine quality control of this product in ordinary laboratories. For simultaneous estimation of OLO and AMB, a series of standard solutions were prepared by diluting appropriate volumes of the standard stock solutions. The scanning of the solutions of OLO and AMB was carried out in the range of 200 to 400 nm against acetonitrile:phosphate buffer(pH 6.8)(70:30v/v) as blank for obtaining the overlain spectra. Absorbance and absorptivities of series of standard solutions were recorded at selected wavelengths λ1 and λ2. The method employed simultaneous equations Two simultaneous equations were formed using the above obtained absorptivity coefficient values. At 246.8 nm (λ_1) 13.5 (a_{x1}) and 15.3 (a_{y1}) are absorptivities were found for Ambroxol hydrochloride and Olopatadine respectively, while At 298 nm 64.0(a_{x2}) and 38.57 (a_{y2}) are absorptivities were found for Ambroxol hydrochloride and Olopatadine hydrochloride respectively.

$A_1 = 13.5\ C_x + 15.3 C_y$ (1)

$A_2 = 64.0\ C_x + 38.57\ C_y$ (2)

Where, Cx and Cy are the concentrations of AMB and OLO respectively in sample solution (µg/ml). A1 and A2 are the absorbance of the sample solution measured at 246.8 and

298 nm, respectively.

Linearity

A set of six solutions of OLO and AMB at concentrations ranging from 2 to 16µg/ml and 12 to 72µg/ml were prepared. Each sample was analyzed in triplicate; calibration curve was constructed by plotting the absorbance against concentration using linear regression analysis. The correlation coefficient was found to be 0.998 and 0.999 for OLO and AMB respectively. The results show that an excellent correlation existed between absorbance and concentration of each drug within the concentration range tested.

Precision

The intra-day precision of the developed method was determined by preparing the tablet samples of the same batch in six determinations with three concentrations. Intra-day precision study showed a RSD of 0.048% for Ambroxol hydrochloride and 0.218 % for Olopatadine hydrochloride, which is less than 2%. Thus, it can be concluded that the analytical method showed a good intra-day precision. Inter-day precision study showed a RSD of 0.03% for Ambroxol hydrochloride and 0.223% for Olopatadine, which is less than 2%. Thus, it can be

Table 3: Summary of validation parameters.

Parameters	Olopatadine	Ambroxol
Beers's law limit (µg/ml)	2-16	12-72
Molar absorptivity (l $mole^{-1}cm^{-1}$)	21.6x 10	6.34x 10
Sandell's sensitivity (mg/cm^2/.001absorbance unit)	0.005	0.027
Regression equation (y= mx+ c) slope (m) intercept (c)	0.0087x+0.014	0.09137x+0.0155
Correlation coefficient (r^2)	0.998	0.999
Mean recovery%	98.178 ± 0.806	99.715 ± 0.162
Precision %(RSD)		
Interday	0.223	0.03
Intraday	0.218	0.048
Specificity	Specific	specific
Robustness	Robust	robust

concluded that the analytical method showed a good inter-day precision.

Specificity

The developed method was found to be specific as percent interference obtained was 0.064 and 0.031 for Ambroxol hydrochloride and Olopatadine hydrochloride respectively, which is less than prescribed limit (0.5%) as per ICH guideline. Thus from the results obtained for the specificity study (n=3), it can be also concluded that the addition of excipients had a very negligible change in the concentration.

Accuracy as Recovery studies

From the results obtained for the accuracy study from three samples studies (n=3), it can be concluded that the mean of the % recovery was 99.715 ± 0.162 for AMB and 98.178± 0.806 for OLO which is within the range of 98-102% and % RSD. was 0.162 for AMB and 0.816 for OLO which is less than 2%.

CONCLUSION

The results of our study indicate that the proposed UV spectroscopic method is simple, rapid, precise and accurate. The developed UV spectroscopic method was found suitable for determination of AMB and OLO as bulk drug and in formulated solid dosage formulation without any interference from the excipients. Statistical analysis proves that, the method is repeatable and selective for the analysis of OLO and AMB. It can therefore be concluded that use of the method can save much time and money and it can be used in small laboratories with very high accuracy and a wide linear range.

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