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Research Article

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC DETERMINATION OF DOXOFYLLINE AND AMBROXOL HYDROCHLORIDE IN BULK AND COMBINED TABLET FORMULATION

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ABSTRACT

Two simple, rapid, precise and reproducible UV spectroscopic methods has been developed for simultaneous estimation of two component drug mixture of doxofylline (DOX) and ambroxol hydrochloride (AMB) in bulk and combined tablet dosage form. First method employs simultaneous equation method using 274nm (λ max of DOX) and 244.5nm (λ max of AMB) as two wavelengths for estimation. The second method involves absorbance correction method the wavelength used were 274nm (λ max of DOX) and 308nm (second λ max of AMB it is zero for DOX). For the two methods distilled water was used as solvent. Linearity was observed in the concentration range of 7 - 35 µg/ml for DOX and 1-5 µg/ml for AMB. The percentage recovery was found in the range of 99.64-100.07 for doxofylline and 98.48-100.55 for ambroxol hydrochloride. The developed method was validated statistically and by recovery studies. The % RSD value was found to be less than 2. Thus the proposed method was simple, precise, economic, rapid and accurate and can be successfully applied for simultaneous determination of doxofylline and ambroxol hydrochloride in bulk and combined tablet dosage form.

Keywords: Doxofylline, Ambroxol hydrochloride, Simultaneous equation, Absorbance correction method, ICH guidelines.

INTRODUCTION

Doxofylline (DOX) is a novel bronchodilator, chemically it is 7-(1, 3-Dioxolan-2-ylmethyl)-3, 7-dihydro-1, 3-dimethyl-1H-Purine-2, 6-Dione[1]. Various analytical methods have been reported for the assay of doxofylline alone. They include UV spectroscopy[2], high performance liquid chromatography[3], high performance thin layer chromatography[4] and LC-MS/MS[5].

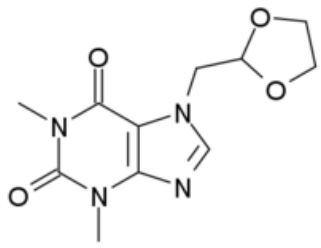

Fig. 1: Chemical structure of doxofylline

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 a mucolytic agent. Ambroxol hydrochloride is an N – desmethyl metabolite of bromohexine[6,7]. Methods such as UV spectroscopy[8-14], high performance liquid chromatography[15-20] ,high performance thin layer chromatography[21,22] and UPLC[23] are reported for estimation of ambroxol hydrochloride alone or in combination with other drugs.

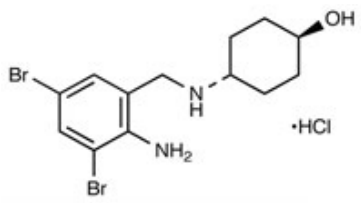

Fig. 2: Chemical Structure Of Ambroxol Hydrochloride

Both the drugs are available in combined tablet dosage form, as an antiasthmatic agent. The extensive literature survey revealed that numbers of methods are reported for the individual drugs but no method is so far reported for the simultaneous estimation of both the drugs in combined pharmaceutical dosage forms. So the present article discusses the attempts made to develop two simple, sensitive and reproducible methods for the simultaneous estimation of DOX and AMB in tablet formulation using simultaneous equation and absorbance correction method[24].

MATERIALS AND METHODS

Instrumentation

The present work was carried out on Shimadzu-1700 double beam UV-Visible spectrophotometer with pair of 10 mm matched quartz cells. Glassware's used were of 'A' grade and were soaked overnight in a mixture of chromic acid and sulphuric acid, rinsed thoroughly with double distilled water and dried in hot air oven.

Reagents and chemicals

Pharmaceutically pure sample of DOX and AMB were generously gifted by Shine Pharmaceuticals Pvt Ltd. Chennai and Apex Pharmaceuticals Pvt Ltd. Allathur. Combination product containing 400mg doxofylline and 30mg ambroxol hydrochloride. All solvents were of AR grade obtained from Qualigens India Pvt. Limited, Mumbai.

Experimental condition

According to the solubility characteristics, the common solvent for the two drugs was found to be distilled water.

Preparation of standard stock solution

Accurately weighed drug samples of both DOX and AMB (50 mg each) were transferred to a suitable standard volumetric flask separately, dissolved and diluted to mark with distilled water. Both the drug solutions were diluted so as to get 10 µg/ml. These solutions were scanned in the UV region of 200-400 nm in 1cm cell against distilled water as blank and the overlain spectra was recorded.

Method A: Simultaneous Equation Method

From the overlain spectra of DOX (10 µg/ml) and AMB (10 µg/ml) in distilled water [Fig 3] wavelengths 274nm (λ max of DOX) and 244.5nm (λ max of AMB) were selected for the formation of Simultaneous equation method. From the above stock solution, aliquots were drawn and suitably diluted so as to get the final concentration range of 7 – 35 µg/ml of DOX and 1-5 µg/ml of AMB.

Absorbances of these solutions were recorded in the respective wavelengths. Both the drugs were linear in the concentration range of 7 – 35 µg/ml of DOX and 1-5 µg/ml of AMB and Calibration curves [n=5] were plotted between concentration and absorbances of drugs with correlation coefficient value not less than 0.999. Optical and regression characteristics are found out. E (1%, 1cm) is determined for DOX at 274 and 244.5nm were 351.69 and 88.964 while respective values for AMB are 319 and 212.5. These values are the mean of six independent determinations.

The simultaneous equations formed were,

At λ_1 $A_1 = a\,x_1bc_x + ay_1bc_y$ ------ (1)

$A1 = 351.69C_X + 319.0\,C_Y$------ (2)

At λ_2 $A_2 = a\,x_2bc_x + ay_2bc_y$------- (3)

$A2 = 88.964\,C_X + 212.5C_Y$ ------ (4)

Where A_1 and A_2 are the absorbances of sample solution at 274nm and 244.5nm respectively. Cx and C_Y are the concentration of DOX and AMB respectively (µg/ml) in sample solution.

The absorbances [$A_{1\&}$ A_2] of the sample solution were recorded at 274 and 244.5nm respectively and concentration of both the drugs were calculated using above mentioned equation (2&4). Precision of the method was determined by carrying out Intra-Day

[n = 3] and Inter Day [n = 3] studies.

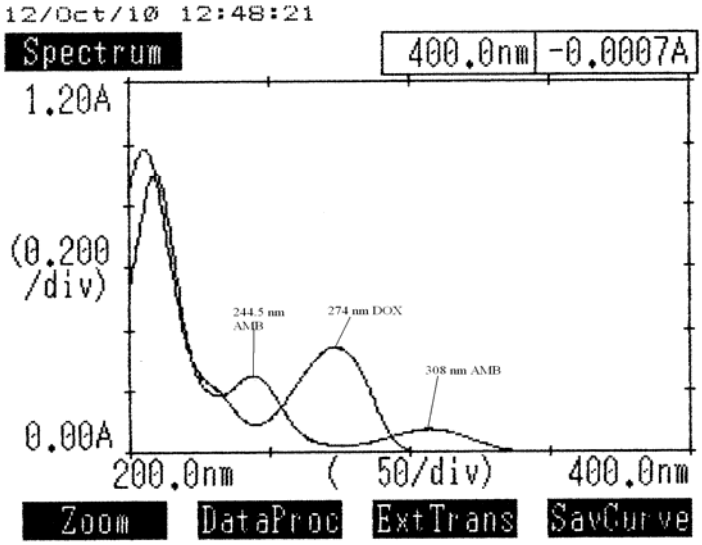

Fig. 3: Overlain zero order spectrum of DOX and AMB

Method B: Absorbance Correction Method

From the overlain spectrum of DOX and AMB in distilled water, it was observed that DOX have zero absorbance at 308 nm, where as AMB has substantial absorbance. Thus AMB was estimated directly at 308 nm without interference of DOX. For estimation of AMB, the absorbance of DOX was measured at 274nm using standard solution of AMB (10 µg/ ml). The contribution of AMB was deducted from the total absorbance of sample mixture at 274nm. The calculated absorbance was called as corrected absorbance for DOX. To estimate the amount of DOX, the absorbance of AMB were corrected for interference at 274nm by using absorptivity values. A set of two equations were framed using absorptivity coefficients at selected wavelengths.

$$cx = \frac{A1}{ax1} \qquad cy = \frac{A2 - ax2\,cx}{ay2}$$

Where,

A1 and A2 are absorbance of sample solution at 308nm and 274nm, respectively.

ax1 and ax2 absorptivity coefficients of DOX at 308nm and 274nm, respectively.

ay1 and ay2, absorptivity coefficients of AMB at 308nm and 274nm, respectively.

From the above stock solution, aliquots were drawn and suitably diluted so as to get the final concentration range of 7 – 35 µg/ml of DOX and 1-5 µg/ml of AMB. Absorbances of these solutions were recorded in the selected wavelengths.

Analysis of tablet formulation

Twenty tablets were weighed and average weight was found. The tablets were triturated to a fine powder. An accurately weighed quantity of powder equivalent to 70 mg of DOX was transferred in to 100ml volumetric flask, sufficient distilled water was added and the solution was sonicated for 15 minutes and diluted to the mark with distilled water. It was filtered through Whatmann filter paper no: 41, filtrate was suitably diluted to get final concentration of 14 µg/ml of DOX and 1 µg/ml of AMB with distilled water. The absorbance of sample solution was measured at all selected wavelengths. The content of DOX and AMB in sample solution of tablet was calculated. This procedure was repeated for six times.

Table 1: Results of analysis of tablet formulation

Parameters	**DOX**		**AMB**	
	Method A	**Method B**	**Method A**	**Method B**
Labeled claim (mg)	400 mg	400 mg	30 mg	30 mg
% Assay*	99.97	100.32	98.64	99.60
SD	0.32750	2.0275	0.13841	0.1959
%RSD	0.08189	0.5052	0.46779	0.6558

*Mean of six determinations

Validation of methods

The methods were validated with respects to linearity, LOD (Limit of detection), LOQ (Limit of quantitation), precision and accuracy and ruggedness[25].

Linearity

Linearity was checked by diluting standard stock solution at five different concentrations. DOX was linear with the concentration range of 7-35 µg/ml and AMB showed linearity in the range of 1-5 µg/ml and calibration curves [n=5] were plotted between concentration and absorbance of drugs. Optical parameters were calculated.

Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) parameters were calculated, in accordance with ICH guidelines, LOD=3.3σ/S and LOQ=10σ/S respectively, where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

Accuracy

To check the accuracy of the developed method and to study the interference of formulation excipients, analytical recovery experiments were carried out by using standard addition method in three different concentrations. From the total amount of drug found, the percentage recovery was calculated. This procedure was repeated for three times for each concentration. The % RSD was calculated.

Precision

The precision of the method was confirmed by repeatability and intermediate precision. The repeatability was performed by the analysis of formulation and it was repeated for six times with the same concentration. The amount of each drug present in the tablet formulation was calculated. The % RSD was calculated. The intermediate precision of the method was confirmed by intraday and inter day analysis i.e. the analysis of formulation was repeated three times in the same day and on three successive days. The amount of drugs was determined and % RSD also calculated.

Ruggedness

The ruggedness test of analytical assay method is defined as the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different labs, different analysis, different lots of reagents etc. Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory to laboratory and from analyst to analyst. In present study, determination of DOX and AMB were carried out by using different instruments and different analysts.

Table 2: Spectral and Linearity Characteristics Data

Parameters	DOX		AMB	
	Method A	Method B	Method A	Method B
λmax nm	274nm	274nm	244.5nm	308nm
Linearity range (µg/ml)	7-35	7-35	1 -5	1-5
Correlation coefficient (r^2)	0.9999	0.9999	0.9994	0.9993
Molar absorptivity (L mol^{-1} cm^{-1})	9330.737	9330.737	14467.40	4868.786
Sandell's sensitivity (µg/cm^2/0.001A.U)	0.028723	0.028723	0.029669	0.088475
Slope (m)	0.034833	0.034833	0.034782	0.011726
Intercept (c)	0.002097	0.002097	0.001124	0.000166
LOD(µg/ml)	0.216615	0.216615	0.129641	0.053809
LOQ(µg/ml)	0.656410	0.656410	0.392853	0.163058
Standard Error	0.000465	0.000465	0.000233	9.3797E-05

*Mean of six determinations

Table 3: Results of recovery studies

Method	Drug	Amount in µg/ml		% Recovery	S.D*	% RSD*
		Added*	Recovered*			
		11.2	11.2079	100.07	0.0113	0.1005
	DOX	14.0	13.9972	99.98	0.0121	0.0867
Simultaneous		16.8	16.8049	100.02	0.0008	0.0048
equation method		0.8	0.7874	98.42	0.0012	0.1526
	AMB	1.0	0.9998	99.86	0.0037	0.3659
		1.2	1.1935	99.46	0.0022	0.1848
		11.2	11.1975	99.97	0.0043	0.0391
	DOX	14.0	13.9496	99.64	0.0044	0.0317
Absorbance		16.8	16.8012	100.00	0.0087	0.0521
correction method		0.8	0.8044	100.55	0.0050	0.6172
	AMB	1.0	1.0005	100.06	0.0049	0.4904
		1.2	1.1968	99.73	0.0048	0.4101

*Mean of three observations

Table 4: Intermediate Precision And Ruggedness Of The Method

Parameters	%Label Claim Estimated [Mean±%RSD]			
	DOX		AMB	
	Method A	Method B	Method A	Method B
Intraday Precision [n=3]	99.88 ± 0.064	100.21 ± 0.166	98.76 ± 0.097	99.42 ± 0.584
Interday Precision [n=3]	99.95 ± 0.076	100.18 ± 0.239	98.84 ± 0.265	99.54 ±0.639
Different instruments [n=6]				
Instrument I	99.66 ± 0.099	99.89 ± 0.09	98.68 ± 0.26	99.03 ± 0.659
Instrument II	99.97 ± 0.082	100.32 ± 0.505	98.64 ± 0.467	99.60 ± 0.655
Different analyst [n=6]				
Analyst I	99.88 ± 0.096	99.95 ± 0.136	98.91 ± 0.234	99.30 ± 0.721
Analyst II	99.90 ± 0.130	99.99 ± 0.152	98.67 ± 0.260	99.31 ± 0.720

RESULTS AND DISCUSSION

The proposed methods for simultaneous estimation of DOX and AMB in combined dosage form were found to be accurate, simple and rapid. Hence it can be used for routine analysis of two drugs in combined dosage forms.

There was no interference from tablet excipients was observed in these methods. The values of % RSD and correlation of coefficient for simultaneous determination (Tablet) were found to be (% RSD 0.0048- 0.617) and correlation coefficient was 0.9999 for DOX and 0.9994 for AMB. The result of recovery studies for tablet was found to be in the range of 98.48 -100.07% for method A, 99.64-100.55 for method B. Values are reported in Table 3. It indicates that there is no interference due to excipients present in the formulation. It can be easily and conveniently adopted for routine quality control analysis. Both methods are accurate, simple, rapid, precise, reliable, sensitive, reproducible and economic and are validated as per ICH guidelines.

CONCLUSION

The results indicate that the proposed UV spectrophotometeric methods are simple, rapid, precise and accurate. The developed UV spectrophotometeric methods were found suitable for determination of DOX and AMB as bulk drug and in marketed tablet dosage formulation without any interference from the excipients. Statistical analysis proves that, these methods are repeatable and selective for the analysis of DOX and AMB.

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