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Development & Validation of UV Spectrophotometric Methods for Simultaneous Determination of Doxofylline & Ambroxol Hydrochloride in Bulk & their Combined Dosage Form

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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 HCL) in bulk and combined tablet dosage form. First method employs simultaneous equation method using 274nm (λ max of DOX) and 247nm (λ max of AMB) as two wavelengths for estimation. The second method involves Q-Absorption ratio method the wavelength used were 274nm (λ max of DOX) and 255nm (isoabsoptive point). For the two methods methanol was used as solvent. Linearity was observed in the concentration range of 5-30 µg/ml for DOX and 2-6 µg/ml for AMB HCL. The percentage recovery was found in the range of 98.92-101.25 for doxofylline and 98.75-100.83 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; UV Spectrophotometric; Simultaneous Equation Method; Q-Absorption Ratio Method; Validation.

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. Various analytical methods have been reported for the assay of doxofylline alone. They include UV spectroscopy, high performance liquid chromatography, high performance thin layer chromatography and LC-MS/MS. 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. Methods such as UV spectroscopy, high performance liquid chromatography ,high performance thin layer chromatography and UPLC are reported for estimation of ambroxol hydrochloride alone or in combination with other drugs. 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 HCL in tablet formulation using simultaneous equation and Q-Absorption ratio method.

MATERIALS & METHODS

Reagents and chemicals

Analytically pure DOXO and AMB HCL were kindly gifted by Ami Life sciences Pvt. Ltd., Karkhadi and Cadila Pharmaceutical Ltd, Ahmedabad. AR Grade methanol was used. Combination product containing 400mg Doxoffylline and 30mg Ambroxol Hydrochloride.

Instrumentation and Spectrophotometric condition

Shimadzu UV-1800; UV spectrophotometer with spectral bandwidth of 1.8 nm, wavelength accuracy of 2 nm and matched quartz cells of 10 mm optical path length. Calibrated analytical balance was used for weighing purpose. The common solvent for two drugs was found to be methanol.

Preparation of standard solution for calibration Plots

Weigh accurately about 100 mg of DOX and 100 mg of AMB HCL and transfer it to a 100 ml volumetric flask. Add 50 ml of methanol, sonicate it for 5 min to dissolve the content and make up the volume with methanol to give a concentration of 1000 μgmL^{-1} of DOX and 1000 μgmL^{-1} of AMB HCL. Stock solution was further diluted to give concentration of 100 μgmL^{-1} of DOX and 100 μgmL^{-1} of AMB HCL. Stock solution was diluted with methanol to give working standard solution containing 10 μgmL^{-1} of DOX and 10 μgmL^{-1} AMB HCL respectively. These solutions were scanned in the UV region of 200-400nm in 1cm cell against methanol as a blank and the overlain spectra was recorded.

Method A : Simultaneous Equation Method

From the overlain spectra of DOX (10 µg/ml) and AMB HCL (10 µg/ml) in methanol [Fig 3] wavelengths 274nm (λ max of DOX) and 247nm (λ max of AMB HCL) 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 5-30 µg/ml of DOX and 2-12 µg/ml of AMB HCL. Absorbances of these solutions were recorded in the respective wavelengths. Both the drugs were linear in the concentration range of 5 – 30 µg/ml of DOX and 2-12 µg/ml of AMB HCL and Calibration curves [n=6] 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 and for AMB HCL at 274 and 247nm. These values are the mean of six independent determinations.

The simultaneous Equation formed were,

$$C_x = (A1ax2 – A2ax1) / (ax2ay1 – ax1ay2)$$

$$C_y = (A2ay1 – A1ay2) / (ax2ay1 – ax1ay2)$$

Where A1 and A2 are the absorbances of sample solution at 274nm and 247nm respectively. ax1 and ax2 are absorptivity coefficients of DOX at 274nm and 247 nm respectively. ay1 and ay2 are absorptivity coefficients of AMB HCL at 274nm and 247 nm respectively. Cx and CY are the concentration of DOX and AMB HCL respectively (µg/ml) in sample solution.

The absorbances [A1& A2] of the sample solution were recorded at 274 and 247nm respectively and concentration of both the drugs were calculated using above mentioned equation. Precision of the method was determined by carrying out Intra-Day [n = 3] and Inter Day [n = 3] studies.

Method B : Q- Absorption Ratio Method

From the overlain spectrum of DOX and AMB HCL, one wavelength was selected for the estimation of both drugs, which is known as isoabsorptive point (at 255.0nm). The dilutions of standard and sample solutions were prepared. The absorptivity values were determined at 255.0nm. The method employs Q values and the concentrations of drugs in sample solution were determined by using the following formula,

$$C_x = (QM – QY) \text{ X } A1 / (QX – QY) \text{ X } ax1,$$

$$C_y = A1/ax1 - Cx,$$

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Where, Cx = concentration of DOX ,C_y = concentration AMB HCL respectively, A1 = absorbance of sample at 255.0 nm,

ax1 = The absorptivity of DOX 255.0nm

QX, QY & QM was obtained using the following equation

QX = (absorptivity of DOX at 255.0 nm) / (absorptivity of DOX at 274.0 nm)

QY = (absorptivity of AMB HCL at 255.0 nm) / (absorptivity of AMB HCL at 274.0 nm) and

QM = (absorbance of sample at 255.0 nm) / (absorbance of sample at 274.0 nm).

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 400mg DOX and 30mg AMB HCL were transferred into 100ml volumetric flask, sufficient methanol was added and the solution was sonicated for 15min and diluted to mark with methanol. It was filtered through Whatmann filte paper No : 41, filtrate was suitably diluted to get final concentration of 40 µg/ml of DOX and 3µg/ml of AMB HCL with methanol. Absorbance of this solution was measured at 274.0 nm (λ max of DOX), 247.0nm((λ max of AMB HCL), and 255.0 nm (Isobestic Point), values were substituted in the respective formulae of (Method A & B) to obtain concentration.Results are shown in the following table.

Validation of methods

The methods were validated with respects to specificity, precision, linearity, LOD(Limit of Detection), LOQ(Limit of Quantitation), accuracy, Robustness.

Specificity

Results of tablet solution showed that there is no interference of the excipients when compared with the working standard solution. Thus, the method was said to be specific.

Precision

The precision of an analytical method was confirmed by repeatability and intermediate precision. The repeatability was performed by the analysis of formulation and it was repeated six times with the same concentration. The amount of each drug present in the tablet formulaion was calculated. The % Relative Standard Deviation (%RSD) was calculated. Intermediate precision of method was confirmed by intraday and interday analysis i.e. the analysis of formulation was repeated three times in the same day and on three successive days. The amount of the drug was determined and %RSD also calculated. %RSD was found to be less than 2%.

Linearity

The linearity of the method is its ability to elicit test results that are directly proportional to the concentration of the analyte in samples. The calibration curve was taken in the range of 5-30µg/ml for DOX and 2-6µg/mL for AMB HCL at the respective λmax. The correlation coefficient of the linearity was found to be ≥0.999 at each wavelength for both drugs as shown in Table 3.

Sensitivity

The LOD(Limit of Detection) and LOQ(Limit of Quantitation) parameters were calculated, in accordance with ICH guidelines, LOD = 3.3σand LOQ = 10σ/ S respectively. where, σ is the standarad deviation of the response(y-intercept) and S is the slope of the calibration plot.

Accuracy

The accuracy of the method was determined by recovery study carried out using standard addition method at three different levels. The resulting spiked sample solutions were assayed in triplicate and the results obtained were compared with the expected results and expressed as percentage. The recovery by proposed methods was satisfactory as % RSD is less than ±2% .The results of the recovery study are summerised in Table 2.

Robustness

Robustness of the method was determined by analyzing standard solutions at normal operating conditions and also by changing some operating conditions such as different labs, different lots of reagents, different analysts. The deliberate aforementioned changes in parameters not have the significant change. The robustness of the method is established as the % deviation from mean assay value. The assay values were within ± 2.0% after 72 h.

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Parameters	DOX		AMB HCL	
	Method A	Method B	Method A	Method B
Label claim(mg)	400mg	400mg	30mg	30mg
% Assay	99.38	100.18	98.67	100.33
SD	0.0173	0.035	0.056	0.050
%RSD	0.130	0.087	1.80	1.66

Table 1 Results of analysis of tablet formulation

Method	Drug	Level of Recovery	Amount Added(µg/ml)	Amount Recovered(µg/ml)	Mean %Recovery	%RSD
A	DOX	80%	25.20	25.12	99.28	0.63
		100%	28.0	27.85	98.92	0.43
		120%	30.80	30.77	99.8	0.56
	AMB	80%	1.8	1.81	101.25	0.07
		100%	2.0	1.98	100.16	0.13
		120%	2.2	2.19	99.16	0.36
B	DOX	80%	25.20	25.21	100.09	0.93
		100%	28.0	28.02	100.14	0.29
		120%	30.80	30.81	100.06	0.17
	AMB	80%	1.8	1.79	98.75	0.38
		100%	2.0	2.01	101.00	0.43
		120%	2.2	2.21	100.83	0.27

Table 2 Results of the Recovery Tests for the Drugs

Parameters	DOX		AMB HCL	
	Method A	**Method B**	**Method A**	**Method B**
λmax (nm)	274	274	247	255
Linearity range (µg/ml)	5-30µg/ml	5-30µg/ml	2-12µg/ml	2-12µg/ml
Correlation coefficient	0.9991	0.9991	0.9986	0.9995
Slop	0.0290	0.0285	0.0266	0.0203
Intercept	0.0195	0.1716	0.0027	0.0018
LOD	0.095	0.119	0.353	0.157
LOQ	0.288	0.362	1.069	0.475
Precision(%RSD)	0.99	0.93	1.8	1.90
Repeatability Precision	0.5-1.2	0.33-0.76	0.8-1.3	0.7-1.27
Intermediate Precision				
Accuracy	99.92-99.28	100.60-100.14	99.16-101	98.75-101
Specificity	No interference	No interference	No interference	No interference
Robustness	Robust	Robust	Robust	Robust

Table 3 Summary of validation parameters

RESULTS & DISCUSSION

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

There were no interference from tablet excipients were observed in these methods. The value of %RSD and correlation coefficient for simultaneous determination (tablet) were found to be (%RSD 0.130-1.8) and Correlation coefficient was 0.9991 for DOX and 0.9995 for AMB HCL. The result of recovery studies for tablet was found to be in the range of (98.92-101.25) for method A, (98.75-100.83) for method B. 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 economical and are validated as per ICH guidelines.

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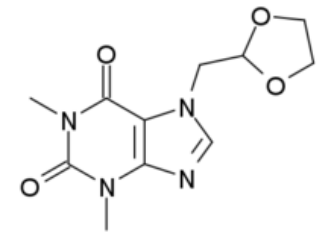

Fig. 1.1: Doxofylline

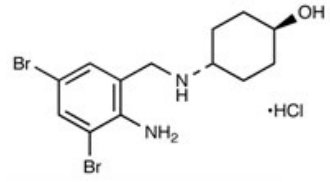

Fig. 1.2: Ambroxol Hcl

Figure 1: Structure

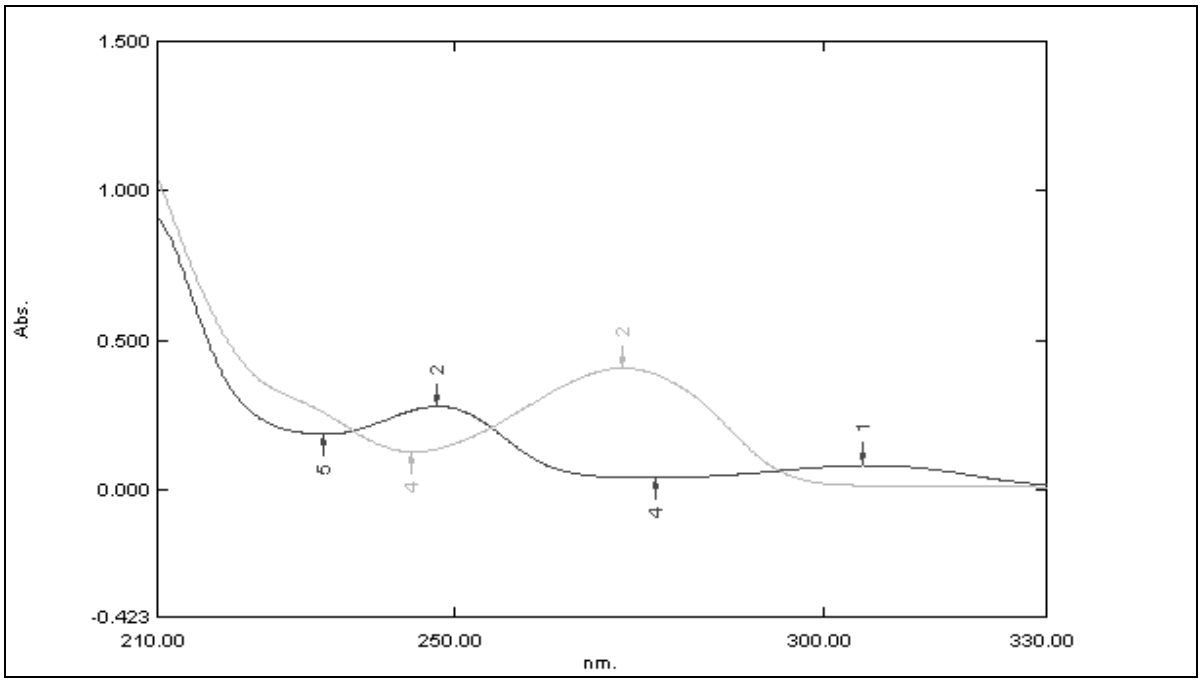

Figure 2 Overlain spectra of Doxofylline(10µg/ml) and Ambroxol Hydrochloride(10µg/ml)

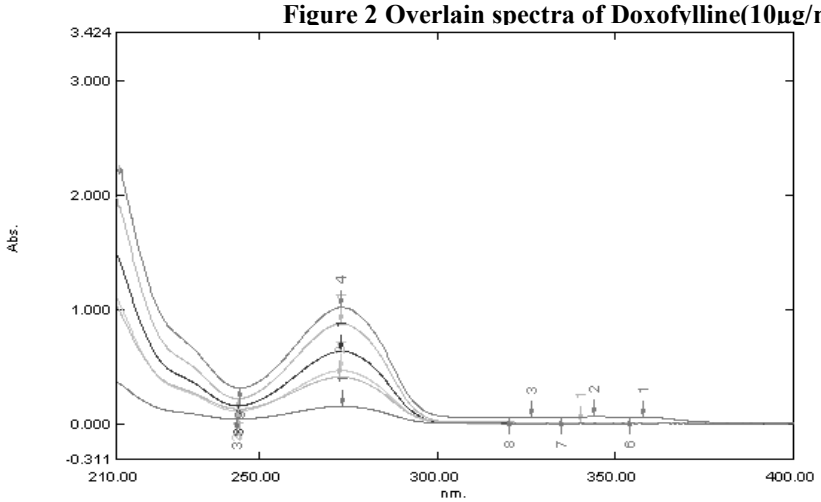

Figure 3. Overlain spectra of Doxofylline(5-30µg/ml) at 274nm

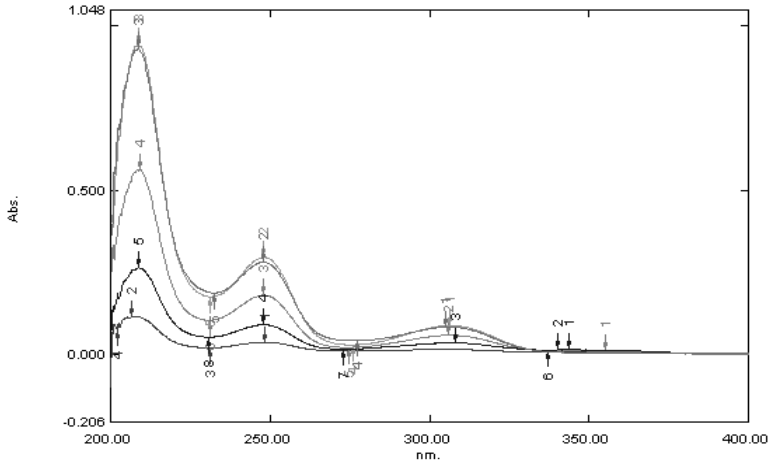

Figure 4. Overlain spectra of Ambroxol Hydrochloride(2-12µg/ml) at 247nm

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CONCLUSION

The results indicate that the proposed UV spectrophotometric methods are simple, rapid, precise, reliable, sensitive and economical. The developed UV spectrophotometric methods were found suitable for determination of DOX and AMB HCL as bulk and its combined dosage form without any interference from the excipients. Statistical analysis proves that, these methods are repeatable and selective for the analysis of DOX and AMB HCL.

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