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SIMULTANEOUS EQUATION METHOD FOR THE ESTIMATION OF AMBROXOL HYDROCHLORIDE AND DOXYCYCLINE HYDROCHLORIDE BY UV SPECTROPHOTOMETRY

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ABSTRACT

The simple, accurate, and precise UV spectrophotometric method has been developed for simultaneous estimation of Ambroxol Hydrochloride and Doxycycline Hydrochloride in bulk tablet formulation. Two wavelength 247 nm and 274 nm were selected for estimation of Ambroxol Hydrochloride and Doxycycline Hydrochloride by simultaneous equation method respectively. UV spectrophotometric method was developed as per ICH guideline using Methanol and Water as a Mobile phase. Ambroxol Hydrochloride and Doxycycline Hydrochloride individually follows the Beer-Lamberts Law over concentration range 2-10 & 20-100 µg/mL, regression of coefficient was found to be r^2=0.997 & r^2=0.999 respectively. The percentage recovery was found to be 94.42 to 101.52 % at three different levels. The proposed method was successfully applied for determination of Ambroxol Hydrochloride and Doxycycline Hydrochloride in tablet dosage form as per ICH guideline the result of the analysis were validated statistically and were found to be satisfactory.

KEYWORDS: Ambroxol Hydrochloride, Doxycycline Hydrochloride, Method Development, UV spectrophotometer, validation.

INTRODUCTION

Ambroxol Hydrochloride chemically 4-[(2-amino-3, 5dibromophenyl)methylamino]cyclohexan-1-ol;hydrochloride which is semi-synthetic derivative of Vasicine from the Indian shrub " *Adhatodavasica*" . It is used as mucolytic agent. Ambroxol is indicated as Serolytic therapy in bronchopulmonary diseases associated with abnormal mucus secretion[1] .there are many different formulations developed since the first marketing authorisation in 1978. Ambroxol is available as syrup, tablet, dry powder, inhalation solution, drops and ampules as well as effervescent tablets.[2]

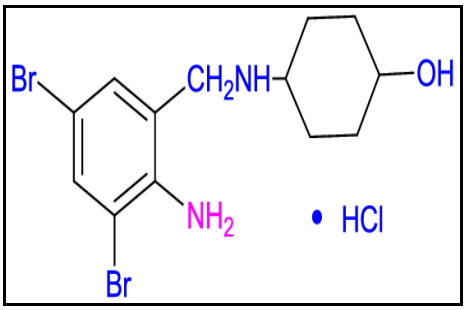

Fig. 1: Structure of Ambroxol hydrochloride.

Molecular formula: - $C_{13}H_{19}Br_2ClN_2O$
Molecular Weight: - 414.56 g/mol [2]

Doxycycline Hydrochloride is a 4-(dimethylamino)-1,5,10,11,12*a*-pentahydroxy-6-methyl-3,12-dioxo-4*a*,5,5*a*,6-tetrahydro-4*H*-tetracene-2-carboxamide; hydrochloride[4] It is a Tetracycline antibiotic used to treat many kinds of infection like skin, dental, urinary track, and respiratory infections, also useful in treatment of malaria, acne and sexually transmitted diseases.[3,4]

Molecular Formula:-C14H11ClNO4S
Molecular Weight: - 338.99g/mol[4]

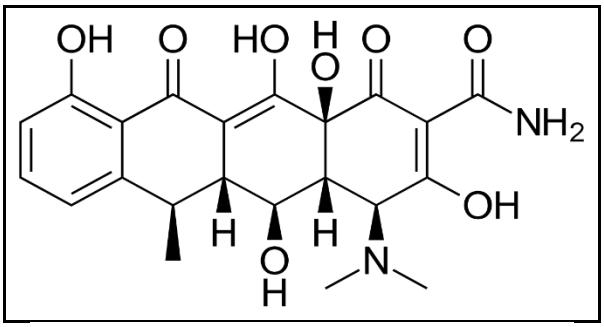

Fig. 2: Structure of Doxycycline Hydrochloride.

From literature survey it was found that no any UV method has been reported on this combination respectively. In this present work, it was proposed to developed and validate a new, simple and accurate UV

method for simultaneous estimation of Ambroxol Hydrochloride and Doxycycline Hydrochloride in tablet formulation.[5 6 7 8]

MATERIALS AND METHODS

- **Instruments**

For weighing, a calibrated weighing balance was used. A double beam UV spectrophotometer (Shimadzu-1800) was used. All the glassware which was used made up of Borosilicate glass and they were calibrated.

- **Chemicals**

Analytical pure sample of Ambroxol Hydrochloride and Doxycycline Hydrochloride were received as a gift sample from Cipla Ltd, Goa and laboratory, Pune Pharmaceuticals, Pune used in study. A pharmaceutical tablet dosage form used in this study was purchased "Doxylab" labelled to contain Ambroxol Hydrochloride and Doxycycline Hydrochloride 7.5:100

Methanol got from Research lab fine chem industry; Mumbai and Distilled Water were used as a mobile phase in this work.

- **Selection of Wavelength**

UV Spectra of Ambroxol Hydrochloride and Doxycycline Hydrochloride at 247 nm and 274 nm respectively Mobile phase Methanol: Water (70:30%) was used for good peaks, good absorbance and better sensitivity.

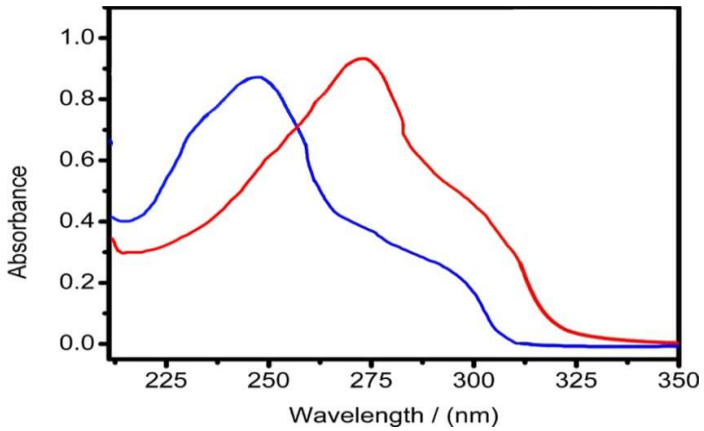

Fig. 3: Overlain spectra of Ambroxol Hydrochloride and Doxycycline Hydrochloride.

- **Preparation of Mobile phase**

500 mL mobile phase was prepared by mixing 400 mL of Methanol and 100 mL of water (90:10% v/v).

- **Preparation of Stock solution of Ambroxol hydrochloride**

Prepare standard solution of Ambroxol hydrochloride by adding 10 mg of Ambroxol hydrochloride in 100 mL of mobile phase in 100 mL volumetric flask, pipet out 0.2 mL of solution from volumetric flask and add in another 10 mL volumetric flask and make up volume upto 10mL using mobile phase. So prepared stock solution was 2µg/mL.

- **Preparation of Stock solution of Doxycycline Hydrochloride**

Prepare standard solution of Doxycycline Hydrochloride by adding 10 mg of Doxycycline Hydrochloride in 100 mL of mobile phase in 100 mL volumetric flask, pipet out 2 mL of solution from volumetric flask and add in another10 mL volumetric flask and make up volume up to 10mL using mobile phase So prepared stock solution was 20µg/mL.

Simultaneous equation method

In order to observe feasibility of proposed method for simultaneous estimation of Ambroxol Hydrochloride and Doxycycline Hydrochloride in formulations, the method was tried on standard mixture of different concentrations of both the drug were prepared in mobile phase. Absorbance of Ambroxol hydrochloride (2µg/mL) and Doxycycline hydrochloride 20µg/mL) were recorded at wavelengths 247 nm and 274 nm respectively using simultaneous estimation method.

$$Cx = \frac{A2ay1 - A1ay2}{Ax2ay1 - ax1ay2}$$

$$Cy = \frac{A1ax2 - A2ax1}{Ax2ay1 - ax1ay2}$$

Where,

Cx= concentration of Ambroxol hydrochloride

Cy= concentration of Doxycycline hydrochloride

ax1=absorptivity value of Ambroxol hydrochloride at 247 nm

ax2= absorptivity value of Ambroxol hydrochloride at 274 nm

ay1= absorptivity value of Doxycycline hydrochloride at 247 nm

ay2= absorptivity value of Doxycycline hydrochloride at 274 nm

A1= absorbance of standard sample at 247 nm

A2= absorbance of standard sample at 274 nm

Analysis of marketed formulation

Ten tablet of brand name "Doxylab" were used. From that select 5 tablets and accurately weighed powdered equivalent to single tablet .10 mg of Ambroxol hydrochloride and 10 mg of Doxycycline hydrochloride were transferred into 100 mL of volumetric flask and make up volume up to 100 mL with mobile phase and pipet out 0.1 mL and transfer in to 10 mL volumetric flask and make up volume up to 10 mL using mobile phase and then sonication for 10-15 min on ultra – sonicator then filtered through Whatmann filter paper from this aliquot portion of filtrate was further diluted to get(2µg/mL) of Ambroxol hydrochloride and (20µg/mL) of Doxycycline hydrochloride respectively. The results obtained are shown in table no (1).

Table 1: Analysis of marketed formulation.

Sr. No.	Ambroxol hydrochloride		% Recovery	Doxycycline Hydrochloride		% Recovery
	Absorbance	Amount recovered in µg/ml		Absorbance	Amount recovered in µg/ml	
1	0.418	0.98	98.48	0.136	0.89	89.41
2	0.415	0.95	95.46	0.135	0.84	84.12
3	0.417	0.97	97.47	0.136	0.89	89.41
Mean	0.417	0.974	97.87	0.135	0.87	87.64
% RSD	0.36	1.55	1.56	0.42	3.31	3.48

Method Validation

Validation of an analytical method is a process to establish the performance characteristics of the developed method to meet the requirement of the intended analytical application. The UV method is validated in terms of linearity, accuracy, precision, LOD and LOQ.[10]

1. Linearity

Linearity method was developed by preparing a series of standard solution of Ambroxol hydrochloride were prepared in the concentration range about 2-10µg/mL and Doxycycline Hydrochloride were prepared in concentration in range of 20-100µg/mL. Linearity curve was plotted; linear regression equation was found for both the drug is shown in below table no.(3).

Table 2: Concentration Range & Absorbance.

Sr. No.	Concentration of Ambroxol hydrochloride in µg/mL	Concentration of Doxycycline Hydrochloride in µg/mL	Absorbance of Ambroxolhydrochloride at 247 nm	Absorbance of Doxycycline Hydrochloride at 274 nm
1	2	20	0.537	0.500
2	4	40	0.698	0.870
3	6	60	0.910	1.241
4	8	80	1.107	1.641
5	10	100	1.324	2.000

Table 3: Linearity values of Ambroxol Hydrochloride and Doxycycline Hydrochloride.

Parameter	Ambroxol hydrochloride	Doxycycline Hydrochloride
Range	2-10 µg/mL	20-100µg/mL
Slope	0.0992	0.0189
Intercept	0.3203	0.1191
Correlation Coefficient	0.997	0.998

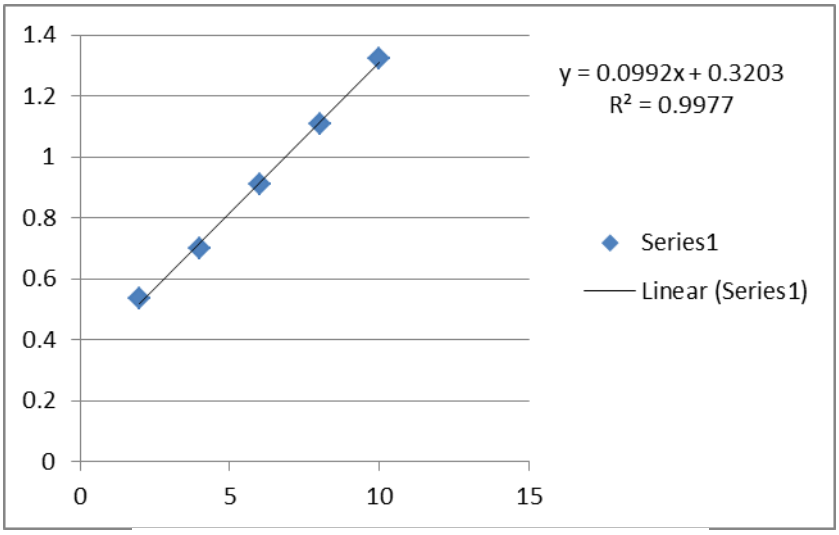

Fig. 4: linearity graph of Ambroxol hydrochloride.

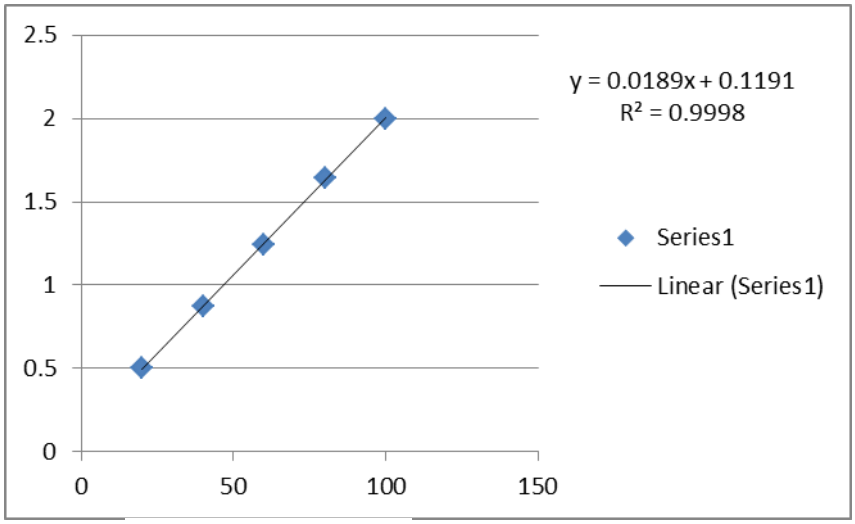

Fig. 5: linearity graph of Doxycycline Hydrochloride.

2. Precision

Precision studies were carried in terms of intra-day and inter-day. The solution containing 2 µg/mL of Ambroxol Hydrochloride and 20 µg/mL of Doxycycline Hydrochloride was injected 3 times for each standard solution and result was observed. The % relative standard deviation (% RSD) values were found to be less than 2 which indicate the method is accurate. The result for intra-day precision is shown in below (table no.4&6) and for inter-day precision is shown (table no.5&7).

Table 4: Intra-day precision study of Ambroxol Hydrochloride.

Conc. µg/ mL	Absorbance			Mean Absorbance	SD	% RSD
	Trial -1	Trial -2	Trial -3			
2	0.362	0.696	0.366	0.3656	0.003512	0.96
24	0.493	0.481	0.488	0.4873	0.006028	1.23
36	0.688	0.671	0.686	0.6816	0.009292	1.36

Table 5: Inter-day precision study of Ambroxol Hydrochloride.

Conc. µg/ mL	Absorbance			Mean Absorbance	SD	% RSD
	Trial -1	Trial -2	Trial -3			
2	0.206	0.204	0.201	0.203	0.002517	1.23
4	0.501	0.511	0.507	0.506	0.005033	0.99
6	0.914	0.901	0.915	0910	0.007753	0.85

Table 6: Intra-day precision study of Doxycycline Hydrochloride.

Conc. µg/ mL	Absorbance			Mean Absorbance	SD	% RSD
	Trial -1	Trial -2	Trial -3			
20	0.300	0.306	0.307	0.304	0.003786	1.24
40	0.486	0.489	0.493	0.489	0.003512	0.71
60	0.819	0.811	0.819	0.813	0.005292	0.65

Table 7: Inter-day precision study of Doxycycline Hydrochloride.

Conc. µg/ mL	Absorbance			Mean Absorbance	SD	% RSD
	Trial -1	Trial -2	Trial -3			
20	0.250	0.256	0.251	0.252	0.003215	1.27
40	0.443	0.446	0.425	0.447	0.004583	1.02
60	0.703	0.712	0.720	0.711	0.008505	1.19

3. Accuracy (recovery study)

The parameter is performed to determine the closeness of the test result with that of the true value which is expressed as % recovery. This study perform at 3 different level (50%, 100% &150%) The % recovery values were shown in the below table no. (8&9).

Table 8: Accuracy study of Ambroxol hydrochloride.

Level	Conc. (µg/mL)		Absorbance	% Recovery	Mean % Recovery ±RSD
	sample	Std.			
50%	2	1	0.617	99.69	100.48±0.84
			0.622	101.36	
			0.619	100.36	
100%	2	2	0.689	92.91	94.43±1.60
			0.695	94.43	
			0.701	95.94	
150%	2	3	0.823	101.35	101.21±0.60
			0.819	100.54	
			0.825	101.75	

Table 9: Accuracy study of Doxycycline Hydrochloride.

Level	Conc. (µg/mL)		Absorbance	% Recovery	Mean % Recovery ±RSD
	sample	Std.			
50%	20	10	0.698	102.098	101.52±0.72
			0.696	101.77	
			0.690	100.68	
100%	20	20	0.821	92.84	93.85±0.93
			0.830	94.29	
			0.833	94.43	
150%	20	30	1.007	93.95	94.55±0.55
			1.012	94.48	
			1.019	95.22	

4. Robustness

The analytical technique's robustness is a measure of its ability to remain unaffected by tiny but deliberate modification in method of parameters, and it gives an indicator of its depend ability in routine use. For Ambroxol Hydrochloride and Doxycycline Hydrochloride the method robustness was investigated.

5. LOD & LOQ

LOD & LOQ were calculated as 3.3 σ/S and 10 σ/S respectively. Whereas σ is the standard deviation of the response (y-intercept) and (S) is the mean of the slop of calibration plot. LOD&LOQ values of Ambroxol Hydrochloride and Doxycycline Hydrochloride is shown in (Table-10)

Table 10: LOD &LOQ values.

Sr.no.	Name of Drug	LOD in µg/mL	LOQ in µg/mL
1.	Ambroxol Hydrochloride	0.60 µg/mL	1.88 µg/mL
2.	Doxycycline Hydrochloride	1.84 µg/mL	0.70 µg/mL

RESULT AND DISCUSSION

The present work provides an accurate, rapid, sensitive method for simultaneous estimation of Ambroxol Hydrochloride and Doxycycline Hydrochloride in bulk tablet formulation. Linear relationship between drug concentrations was obtained over the range of at 2-10 µg/mL for Ambroxol Hydrochloride and 10-100 µg/mL for Doxycycline Hydrochloride respectively. The correlation coefficient, slope and intercept obtained for each drug is shown in table no.(3) The proposed method was also successfully applied to a pharmaceutical formulation. The precision of the method with intra-day and inter-day was found to be good % RSD less than 2 , indicates that the method was precise and the results presented in table no(4&6) &(5&7) . Recovery studies results are tabulated in table no (8&9) for Ambroxol Hydrochloride % recovery range from 100.48 to 101.21with % RSD 0.84 to 0.60. For Doxycycline Hydrochloride % recovery ranges from 101.52 to 94.55 with %RSD 0.72 to 0.55 .The % assay was found to be 97.87 for Ambroxol hydrochloride & 87.64 for Doxycycline Hydrochloride respectively. The LOD values of Ambroxol Hydrochloride and Doxycycline Hydrochloride was found to be 0.60µg/mL and 1.84µg/mL & LOQ values of Ambroxol Hydrochloride and Doxycycline Hydrochloride was found to be 1.88µg/mL and 0.70µg/mL respectively. No interference was found in the spectrogram of formulation within the absorbance indicating that excipients used in tablet formulation did not interfere with simultaneous estimation of Ambroxol Hydrochloride and Doxycycline Hydrochloride in tablet formulation.

CONCLUSION

The recently developed UV spectrometric method for determination of Ambroxol Hydrochloride and Doxycycline Hydrochloride simultaneously is simple, specific, accurate, precise, rapid and economical which indicates its competence for routine pharmaceutical analysis of Ambroxol Hydrochloride and Doxycycline Hydrochloride in bulk tablet formulation. It is conclude that HPLC method is successfully utilise for estimation of Ambroxol Hydrochloride and Doxycycline Hydrochloride this new method has been successfully applied for routine analysis.

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REFERENCE

1. M Sumithra, P. Yuvanesh and Anamika Mistry, Analytical method development and validation Ambroxol Hydrochloride by UV spectroscopy and forced degradation study and detection of stability; Research jornal of Pharmacy and Technology, 9(7): 794-800.
2. https://en.m.wikipedia.org/wiki/Ambroxol.
3. Satish D Pawar, Vaishali Mistry, Stability indicating UV spectrophotometric Method development and validation for estimation of Doxycycline Hyclate in bulk and Pharmaceutical Formulation International Journal of Pharmacy and Pharmaceutical Research. 11(1): 291-300.
4. https://en.m.wikipedia.org/wiki/Doxycycline.
5. Sunil More, Ashpak M Tamboli, Snehal Patil, UV Spectrophotometric method for simultaneous estimation of Pregabalin and Amitriptyline Hydrochloride, International Journal of Pharmacy & Pharmaceutical Research, 2019; 15(3): 16-24.
6. ICH Q2A Text on validation of analytical procedure, International Conference on Harmonisation. Tripartite guideline, 1994; 1-5.
7. Willard HH, Instrumental Method of Analysis 7th ed. CBS publisher and Distributers New Delhi, India, 1986; 1-5.
8. ICH Q2B Text on validation of analytical procedure, International Conference on Harmonisation. Tripartite guideline, 1994; 1-10.
9. Bekett AH, Stenlake JB, Practical Pharmaceutical Chemistry, CBS Publisher and Distributers, New Delhi, India Part-2, 2002; 237-275.