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Development and Validation of Solvent Extraction Spectrophotometric Method for Simultaneous Estimation of Doxofylline and Terbutaline sulphate In their Combined Dosage Form

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ABSTRACT

Simple, specific, accurate, precise and reproducible method have been developed and validated for the simultaneous estimation of both drugs in their combined dosage form. UV spectrophotometric method was a determination using the solvent extraction method at 277 nm and 279 nm over the concentration range 10-50 and 20-60 µg/ml for Doxofylline in chloroform and terbutaline sulphate in water respectively. The % recoveries of the both the drugs were found to be 100.34% – 100.72 % and 98.25– 99.19 % respectively. Method was statistically validated for accuracy, precision, specificity, LOQ, robustness and ruggedness according to ICH guidelines and can be used for analysis of combined dosage form.

Key words: Doxofylline, Terbutaline sulphate, Solvent Extraction Spectrophotometric method.

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INTRODUCTION

Doxofylline (DOX) is chemically 7-(1,3-dioxolan-2-ylmethyl)-1,3-dimethylpurine-2,6-dione[1] is bronchodilator used as Antiasthmatic[2]. It is official in IP. IP describes High Performance Liquid Chromatography (HPLC) method[1]. Literature survey also reveals Spectrophotometric and HPLC[3-11] methods for determination of DOX with other drugs.

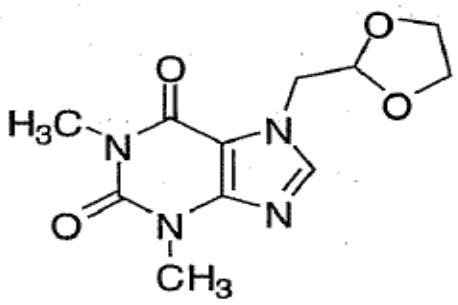

Figure 1. Structure of Doxofylline

Terbutaline sulphate is chemically 5-[2-(tert-butylamino)-1-hydroxyethyl] benzene-1,3-diol[12] is broncho-dilator used as Antiasthmatic[13]. It is official in IP, BP and USP. IP describes Spectrophotometric and High Performance Liquid Chromatography (HPLC) method[12]. BP describes High Performance Liquid Chromatography (HPLC) method[14]. USP describes High Performance Liquid Chromatography (HPLC) method[15]. Literature survey also reveals Spectrophotometric[16] and HPLC[17-23] methods for determination of TBS with other drugs.

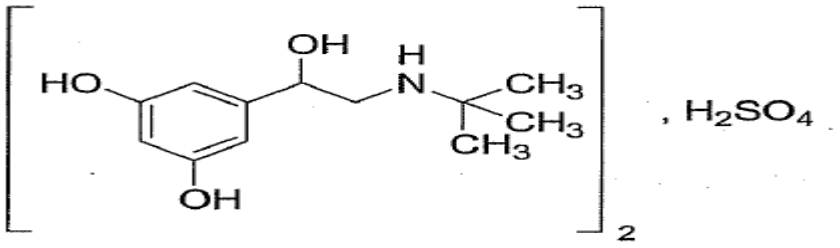

Figure 2. Structure of Terbutaline sulphate

The combined dosage forms of DOX and TBS are available in the market for the Treatment of Asthma. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of DOX and TBS in their combined dosage forms. Literature survey does not reveal any simple HPLC method for simultaneous estimation of DOX and TBS in combined dosage forms. The present communication describes simple, specific, rapid, accurate and precise chromatographic method based on High Performance Liquid Chromatographic method for simultaneous estimation of both drugs in their combined tablet dosage forms.

MATERIALS AND METHODS

Reagents and Materials

DOX and TBS bulk powder was kindly gifted by Zydus Cadila Pharmaceuticals Ltd., Ankleshwar, Gujarat, India and Tuttsan Pharmaceutical Ltd., Santej, Gandhinagar, Gujarat, India respectively. The commercial fixed dose combination product MUCOSMA-T (DOX – 400 mg, TBS – 5 mg) was procured from the local market which is manufactured by Azine Healthcare Private Limited. All chemicals and reagents were of analytical grade and were purchased from Merck chemicals Pvt. ltd, Mumbai, India.

Instrumentation

Analytical technologies Ltd., UV 2080 plus model, silicon photodiode detector controlled by UV Analyst software was used in this method.

Selection of solvent

DOX and TBS both are soluble in water. DOX soluble in chloroform. TBS insoluble in chloroform. Therefore solvent extraction method was performed. Spectrum of TBS in chloroform (10µg/ml, n=5) which does not show any interference of absorbance. Similar procedure was recorded for solution of TBS in chloroform (20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml, 60µg/ml, n=5 determination) and similar responses were obtained (Fig. 3). The overlain spectra of DOX show feasibility of using chloroform as solvent for Spectrophotometric analysis for simultaneous estimation of DOX drug. The overlain spectra of TBS show feasibility of using water as solvent for Spectrophotometric analysis for simultaneous estimation of TBS drug. Therefore chloroform was selected as solvent for DOX and water was selected as solvent for TBS.

Preparation of Standard Stock Solution

Doxofylline (DOX) standard stock solution: (100 µg/ml)

A 10 mg of DOX standard was weighed and transferred to a 100 ml volumetric flask. 70 ml of chloroform was transferred to this volumetric flask and sonicated for 10 min. The flask was shaken and volume was made up to the mark with chloroform to give a solution containing 100 µg/ml DOX

Terbutaline sulphate (TBS) standard stock solution: (100 µg/ml)

A 10 mg of TBS standard was weighed and transferred to a 100 ml volumetric flask. 70 ml of water was transferred to this volumetric flask and sonicated for 10 min. The flask was shaken and volume was made up to the mark with water to give a solution containing 100 µg/ml TBS.

Selection of Analytical Wavelength

10 - 50 µg/ml solutions of DOX were prepared in chloroform and spectrum was recorded between 200-400 nm. Spectrums for above concentration were obtained with n = 5. Similarly 20 - 60 µg/ml solutions of TBS were prepared in water and spectrum was recorded between 200-400 nm. DOX showed λ_{max} at wavelength 277 nm and TBS showed λ_{max} at wavelength at 279 nm. The overlain spectrum of DOX and TBS at different concentration were recorded (Figure. 4, 5). The Wavelength, for detection of DOX was 277 and TBS was 279 selected.

Calibration Curve for DOX and TBS

Calibration curve for the DOX (10 - 50µg/ml)

Appropriate volume of aliquot from standard DOX stock solution was transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with the chloroform to obtain concentration of 10, 20, 30, 40 and 50µg/ml. The curve of each solution against the chloroform was recorded. Absorbance at 277 nm was measured and the plot of absorbance vs. concentration was plotted. The straight-line equation was determined (figure 6).

Calibration curve for the TBS (20 - 60 µg/ml)

Appropriate volume of aliquot from standard TBS stock solution was transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with the water to obtain concentration of 20, 30, 40 50 and 60µg/ml. The curve of each solution against the water was recorded. Absorbance at 279 nm was measured and the plot of absorbance vs. concentration was plotted. The straight-line equation was determined (figure 7).

Determination of Doxofylline and Terbutaline sulphate in their Combined Dosage

Sample preparation (Label Claim: 400 mg DOX and 5 mg TBS per tablet)

Twenty tablets were weighed and finely powered. Powder equivalent to 400 mg DOX and 5 mg TBS was accurately weighed and transferred to volumetric flask of 100 ml capacity. 70 ml of chloroform was transferred to this volumetric flask and sonicated for 10 min. The flask was shaken and volume was made up to the mark with chloroform. The solution was filtered through whatman filter paper (0.45µ). From this solution 1ml was transferred to volumetric flask of 10 ml volume was made up to the mark with chloroform to give a solution containing 400 µg/ml DOX. The solution was filtered through whatman filter paper (0.45µ). From this solution 1ml was transferred to volumetric flask of 10 ml volume was made up to the mark with chloroform to give a solution containing 40 µg/ml DOX.

The residue was transferred to volumetric flask of 50 ml capacity. Volume was made up to the mark with water to give a solution containing 100 µg/ml TBS. From this solution 4ml was

transferred to volumetric flask of 10 ml volume was made up to the mark with water. Volume was made up to the mark to give a solution containing 40 µg/ml TBS.

Volume was made up to the mark to give a solution containing 40 µg/ml DOX and 40µg/ml TBS. This solution was used for the estimation of DOX and TBS.

VALIDATION OF THE DEVELOPED METHOD:

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[24].

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy 20 tablets were weighed and powdered and analysis of the same was carried out. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels taking into consideration percentage purity of added bulk drug samples.

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It provides an indication of random error results and was expressed as coefficient of variation (CV).

Repeatability

Standard mixture solutions of DOX (10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50 µg/ml) and TBS (20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml and 60 µg/ml) were prepared and chromatograms were recorded. Area was measured of the same concentration solution six times and RSD was calculated.

Intra and inter day precision

Variation of results within the same day (intra-day), variation of results between days (inter day) were analyzed. Intraday precision was determined by analyzing DOX and TBS for three times in the same day. Inter day precision was determined by analyzing both the drugs daily for three days.

Reproducibility

The areas were measured at different laboratory using another instrument by another analyst and the values obtained were evaluated using t-test to verify their reproducibility.

Linearity and Range

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is

the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity.

Specificity and selectivity

Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix. While selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix. Commonly used excipients in Tablet preparation were spiked in a pre weighed quantity of drugs and then area was measured and calculations done to determine the quantity of the drugs.

Ruggedness

The solutions were prepared and then analyzed with change in the analytical conditions like different laboratory, different analyst, and different instrument.

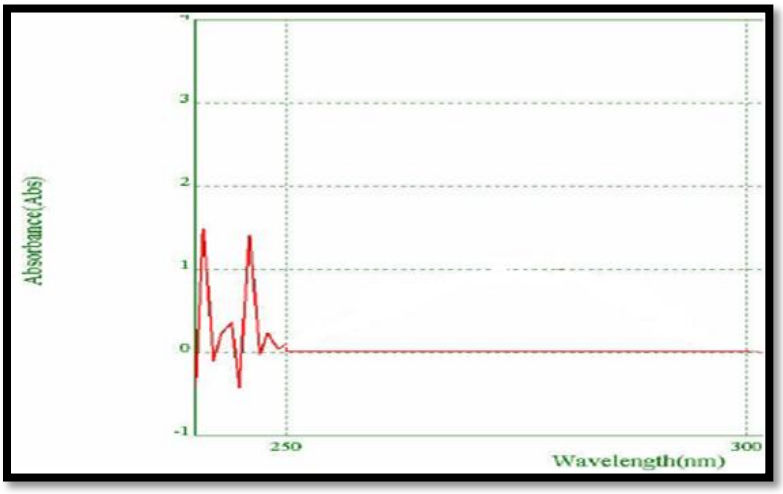

Figure 3. TBS in Chloroform (10µg/ml)

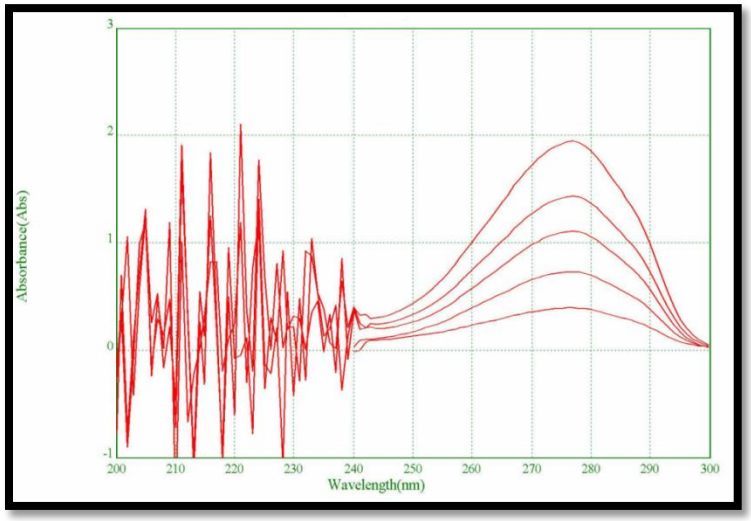

Figure 4. Overlain spectrum of DOX in chloroform

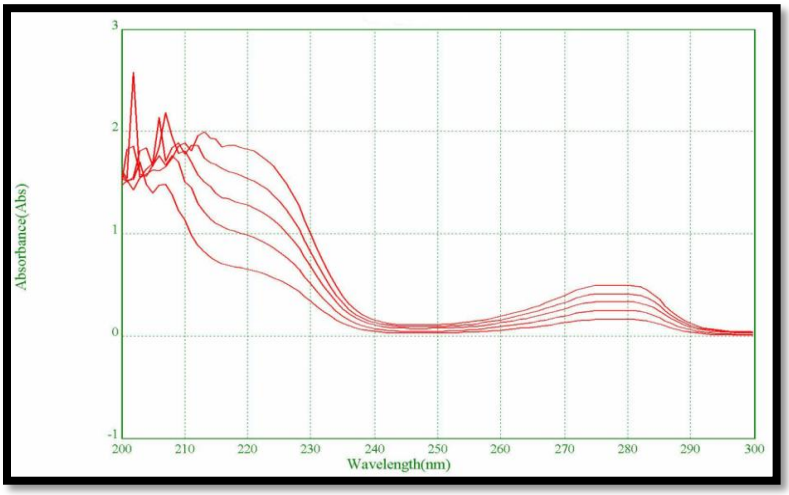

Figure 5. Overlain spectrum of TBS in water

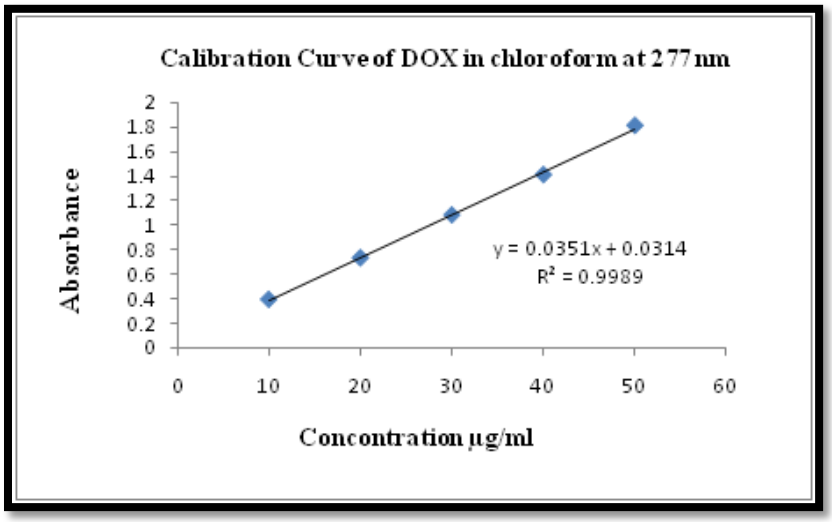

Figure 6: Calibration Curve of DOX in chloroform at 277 nm

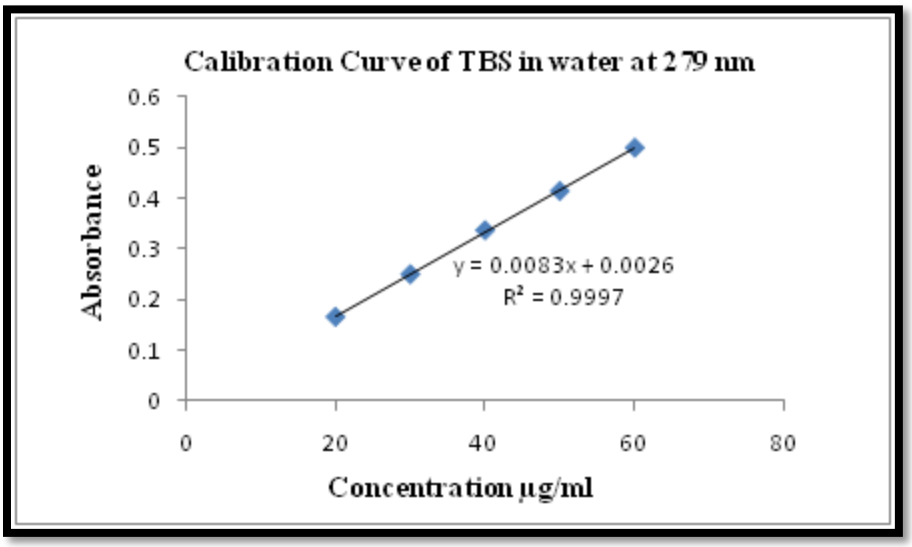

Figure 7: Calibration Curve of TBS in water at 279 nm

RESULTS AND DISCUSSION

Spectrophotometry method for DOX and TBS in their combined tablet dosage form.

In solvent extraction method quantitation of individual drugs in combinations has been performed by separation of individual drugs based on their selective solubility followed by Spectrophotometric measurement. Owing to the solubility of DOX in the chloroform and TBS in the water and also there was no shift in the absorbance maxima of DOX and TBS in above solvent. Hence chloroform for DOX and water for TBS was selected as solvent.

From overlain spectra of DOX exhibits λ_{max} at 277 nm and TBS exhibits λ_{max} at 279 nm. For estimation of DOX and TBS using Spectrophotometry solvent extraction method was decided to be used. In this method two wavelengths are required. Calibration data at 277 for DOX and 279 TBS are shown in respectively (Table 1, 2). Calibration curves for DOX and TBS were plotted between absorbance and concentration. The following equations for straight line were obtained for DOX and TBS.

Linear equation for DOX at 277 nm, **Y = 0.035 xs + 0.031……**Equ. 1

Linear equation for TBS at 279 nm, **Y = 0.008x + 0.002……..……**Equ. 2

The developed Solvent Extraction Spectrophotometry method was validated. The linear range, detection limit and standard deviation for DOX and TBS by Spectrophotometry method are shown (Table 3). Accuracy was determined by calculating the recovery. The method was found to be accurate with % recovery 100.34 – 100.72 % for DOX and 98.25 – 99.19 % for TBS respectively (Table 4). Precision was calculated as repeatability and intraday and interday variation for both the drugs (Table 5-7). The method was found to be precise with C.V. 0.03 – 0.05 for intraday (n=3) at 277 and C.V. 0.03– 0.06 for interday (n=3) at 277 for DOX (Table 8). And C.V. 0.05– 0.18 at 279 for intraday (n=3) and C.V. 0.04– 0.09 at 279 for interday (n=3) for TBS respectively (Table 9). The method was found to be reproducible(Table 10, 11). The method was found to be specific as no interference observed when the drugs were estimated in presence of excipients (Table 12).The method was also rugged as there was no change in area up to 48 hours of preparation of solution in solvent (Table 13). The LOD for DOX and TBS was found to be 2.086 µg/ml at 277 nm and 1.330 µg/ml at 279 nm respectively. Summary of validation parameters are shown in Table 14.

Marketed formulation was analyzed by the proposed method and assay result of marketed formulation is shown in Table 15.

Table 1: Result of calibration readings at 277 nm for DOX in chloroform

Concentrations (µg/ml)	Absorbance at 277 nm Mean ± S.D. (n=5)	Coefficient of variation
10	0.39274 ± 0.00065	0.165
20	0.73184 ± 0.002097	0.286
30	1.08158 ± 0.001028	0.095
40	1.40966 ± 0.000792	0.056
50	1.81026 ± 0.001913	0.105

Table 2: Result of calibration readings at 279 nm for TBS in water

Concentrations (µg/ml)	Absorbance at 279 nm Mean ± S.D. (n=5)	Coefficient of variation
20	0.16658 ± 0.000268	0.161
30	0.25010 ± 0.000187	0.074
40	0.33628 ± 0.000192	0.057
50	0.41310 ± 0.000354	0.085
60	0.49778 ± 0.000239	0.047

Table 3: Statistical data for DOX & TBS by Solvent Extraction Spectrophotometry method

Parameter	DOX (at 277nm)	TBS (at 279nm)
Linear Range (µg/ml)	10-50	20-60
Slope	0.035129	0.008254
Intercept	0.031358	0.002608
Standard deviation of slope	0.00067	0.00734
Standard deviation of intercept	0.022207	0.003328
Limit of Detection (µg/ml)	2.086	1.330
Limit of Quantitation (µg/ml)	6.321	4.031

Table 4: Determination of Accuracy

% Spiking	Amt of drug added		Amt. recovered		% Recovery	
	DOX (µg/ml)	TBS (µg/ml)	DOX (µg/ml)	TBS (µg/ml)	DOX %	TBS %
80 %	32	32	32.11	31.74	100.34	99.19
100 %	40	40	40.29	39.30	100.72	98.25
120 %	48	48	48.26	47.48	100.54	98.92

Table 5: Repeatability data for DOX at 277 nm

Concentration	10 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml
Absorbance	0.3925	0.738	1.0815	1.4083	1.8121
	0.3924	0.7382	1.0822	1.4095	1.8069
	0.3926	0.7379	1.0891	1.4081	1.8069
	0.3927	0.7406	1.0884	1.4091	1.8065
	0.3923	0.7412	1.0897	1.4093	1.8046
Mean.	0.3925	0.73918	1.08618	1.40886	1.8074
Std. Dev.	0.000158	0.001588	0.003987	0.000623	0.002795
% RSD	0.040	0.214	0.367	0.044	0.154

n = 5 determination

Table 6: Repeatability data for TBS at 279 nm

Concentration	20 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml	60 µg/ml
Absorbance	0.1664	0.2511	0.3366	0.4135	0.498
	0.167	0.2514	0.3362	0.4133	0.4974
	0.1641	0.2503	0.3364	0.4133	0.4971
	0.1671	0.2508	0.3384	0.4141	0.4977
	0.167	0.2508	0.3358	0.413	0.4977
Mean.	0.16632	0.25088	0.33668	0.41344	0.49758
Std. Dev.	0.001272	0.000409	0.001006	0.00041	0.000342
% RSD	0.764	0.162	0.298	0.099	0.068

n = 5 determination

Table 7: Repeatability of sample application data for DOX at 277 nm & TBS at 279 nm

Sample	DOX(40 µg/ml)	TBS (40 µg/ml)
Absorbance	1.4414	0.3186
	1.4421	0.3173
	1.4414	0.3175
	1.4425	0.3173
	1.4403	0.3174
Mean.	1.44154	0.31762
Std. Dev.	0.000838	0.000554
% RSD	0.058	0.174

n = 5 determination

Table 8: Precision data for DOX at 277 nm

Conc. (µg/ml)	Intraday (n =3) Absorbance ± S.D.	C.V.	Interday (n =3) Absorbance ± S.D.	CV
30	1.092133 ± 0.000416	0.038	1.093533 ± 0.000666	0.060
40	1.409233 ± 0.000777	0.055	1.409900 ± 0.000458	0.032
50	1.092133 ± 0.000416	0.038	1.905233 ± 0.000709	0.037

Table 9: Precision data for TBS at 279 nm

Conc. (µg/ml)	Intraday (n =3) Absorbance ± S.D.	C.V.	Interday (n =3) Absorbance ± S.D.	CV
30	0.251400 ± 0.000458	0.182	0.251033 ± 0.000115	0.045
40	0.336933 ± 0.000569	0.168	0.336633 ± 0.000306	0.090
50	0.413233 ± 0.000208	0.050	0.413233 ± 0.000208	0.050

Table 10: Reproducibility data for DOX at 277 nm (40 µg/ml)

Instrument 1 (n =3) Absorbance ± S.D.	Instrument 2 (n =3) Absorbance ± S.D.	Result of t test*	Inference
1.464267 ± 0.000551	1.467967 ± 0.000666	0.019	Not significant difference

*** At 95% confidence interval, (t-Tabulated = 4.30)**

Table 11: Reproducibility data for TBS at 279 nm (40 µg/ml)

Instrument 1 (n =3) Absorbance ± S.D.	Instrument 2 (n =3) Absorbance ± S.D.	Result of t test*	Inference
0.3472 ± 0.000794	0.346733 ± 0.000493	0.593	Not significant difference

* At 95% confidence interval, (t-Tabulated = 4.30)

Table 12: Specificity and Selectivity study

Study	DOX	TBS
Specificity	Specific	Specific
Selectivity	Selective	Selective

Table 13: Solvent Suitability Study of DOX (40 µg/ml) and TBS (40 µg/ml)

Time	Absorbance		Result %	
	DOXO	TBS	DOXO	TBS
0 hr.	1.4428	0.3204	100.82	99.49
4.0 hrs.	1.4426	0.3201	100.83	99.41
8.0 hrs.	1.4423	0.3204	100.80	99.50
24.0 hrs.	1.4420	0.3203	100.78	99.47
48.0 hrs.	1.4424	0.3202	100.81	99.44

Table 14: Summary of validation Parameters of Spectrophotometry

Parameters	DOX	TBS
Recovery%	100.34 – 100.72	98.25 – 99.19
Repeatability (%RSD, n=6)	0.058	0.174
Precision(C.V.)		
Intra-day (n=3)	0.038 – 0.055	0.050 – 0.182
Inter-day (n=3)	0.032 – 0.060	0.045 – 0.090
Specificity	Specific	Specific
Solvent suitability	Suitable for 48 hrs.	Suitable for 48 hrs.

Table 15: Assay Results of Marketed Formulation (n=3 determination)

Formulation	Actual concentration µg/ml		Amount obtained µg/ml		% DOX	% TBS
	DOX	TBS	DOX	TBS		
Tablet Mucosma -T	40	40	40.30	39.45	100.75 ± 0.92	98.63 ± 0.12

CONCLUSION

All the validation parameters for all the developed methods were complied with ICH requirements. All the methods were found to be simple, accurate, Specific, Selective, Precise, economic, & Rapid with different analysts. Hence all the methods can be used for routine analysis of both the drugs from their combined solid dosage form.

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