Original Research Article

Spectroscopic Estimation of Doxylamine Succinate in Tablets and Human Plasma by Formation Ion-Pair Complex

Running title: Spectroscopic Estimation of Doxylamine

ABSTRACT

Aim: To develop a simple spectroscopic method to estimate doxylamine (DOX) succinate in its tablet dosage form and human plasma with an ion-pair complex formation.

Methods: In this method, methyl orange (MO, 0.05 % w/v) dye was used to form an ion-pair complex in acetate buffer (pH.5.00) at temperature conditions 30^0 C ± 2^0C. The ion-pair complex formed was extracted with chloroform. The absorbance of the ion-pair complex was measured at 420 nm.

Results: The method was obeyed Beer's law in the linearity concentrations ranging from 5-25 g/mL of DOX succinate with a correlation coefficient (r^2) of 0.992. The Drug-dye ion-pair complex was formed in a 1:1 ratio demonstrated by Jobs' method of continuous variation. The proposed method satisfied the validation criteria, which was evaluated according to ICH Q_2 (R_1) guidelines. Accuracy studies the percentage recovery was found to be 99.06-100.9 %. The precision and robustness of the method were found within limits, i.e., <2% RSD. The LOD and LOQ values were found to be 0.31 and 0.939 µg/mL, respectively.

Discussion and Conclusion: The developed method was simple, specific, and requires a short analysis time, and it could be easy and economical to perform. Also it could be well suitable for routine analysis of DOX succinate in its pharmaceutical dosage forms and biological matrices.

Keywords: Doxylamine succinate, spectroscopy, ion-pair complex, methyl orange, linearity, method validation, etc.

1. INTRODUCTION

An ion-pair formation strategy works with the concept that ionizable species of both analyte and pairing reagents are more readily extracted with the help of suitable organic solvents as ion-pairs. The separation could affect the type and concentration of the counter ion and the type of organic phase used. An ion-pair composes of oppositely charged ions held together *via* Columbic attraction that behaves as a single unit. The technique has great importance in drug delivery to enhance the permeability of several drugs such that they will be ionized throughout the physiological pH range (Libermann *et al*., 2015). Ion-pairing has a tremendous advantage in enhancing the delivery of various drugs *via* several routes, including transversal, oral, ocular, and parenteral.

Doxylamine (DOX) succinate is a first-generation antihistamine. It is an ethanolamine derivative that reduces the effects of histamine in the body. Chemically, it is *N, N*-dimethyl-2-[1-phenyl-1-(2-pyridinyl) ethoxy]-butanedioate succinate. The drug is freely soluble in water and chloroform and slightly soluble in diethyl ether and benzene. DOX succinate tablets are commercially available under different brand names (Ex.: UNISOM sleep tablets).

A literature survey revealed that the development of various spectrophotometric [1-3] chromatographic [4-12] methods for estimating DOX succinate in various dosage forms in individual and in combination with other drugs. In our attempt, we tried to develop a simple, economical, and sensitive spectroscopic method that that could be useful for quantifying the drug in biological matrices; moreover, there was no such reported spectroscopic method for the targeted drug. In this investigation, we demonstrated the applicability of an ion-pair complex formation of the drug. Estimation of DOX succinate was performed for the pure tablet dosage form and spiked human plasma [13-17]. Further, the method was validated according to the International Conference on Harmonization (ICH) guidelines [18-19]. The present communication describes the direction for the development of an analytical method for quantification of DOX succinate in tablet dosage forms and human plasma.

2. MATERIALS AND METHODS

2.1 Apparatus: The analysis was performed in 10 mm quartz cells using a UV-Visible spectrophotometer (Lab India, UV-3000+) with a fixed 2 nm spectral bandwidth. UV-Win 5 software v5.1.1 was used for all absorbance measurements in the region of 200-800 nm [3]. The pH of the solutions was measured with the help of a digital pH meter (Elico, L1 120).

2.2 Materials and reagents: DOX succinate was procured as a gift sample from Granules India Limited, Hyderabad. Tablet formulations (UNISOM tablets) were obtained from the local market. Solvents and chemicals utilized in the entire work were Analytical Grade (AR), were purchased from Merck India Limited, Mumbai, and SD Fine Chemicals, Mumbai. 1.0 M of acetate buffer solution (pH: 5.00) was used for analysis, the pH was adjusted with glacial acetic acid. 0.05% w/v of methyl orange (MO) dye solution was prepared using 50 mg of MO (Merck India., Mumbai, India) dye in water [13].

2.3 Determination of absorption maxima: The analysis was performed in 10 mm quartz cells using a UV-Visible spectrophotometer (LAB India, UV 3000+) with the specifications as mentioned above; all absorbances were measured because of their advantages of high-resolution capacity and better reproducibility. The absorbance maximum was measured for the standard solution (10 µg/mL) and ion-pair (DOX-MO) complex by taking approximately 3.0 mL of each solution in 10 mm cuvette and scanned under UV-visible region (200-800 nm).

2.4 Optimization of experimental variables: The method was optimized to achieve the degree of completion of the reaction, effective extraction, and sensitivity. To optimize the conditions few preliminary experiments were conducted, such as pH range, extracting solvent, and temperature conditions for the extraction of DOX-MO ion-pair complex.

2.5 General method for ion-pair complex [13]*:* To each separating funnel (six 250 mL separating funnels as a set), aliquots (1-6 mL) of the standard solution (100 µg/ mL) was taken (concentrations in the range of 5-30 µg/mL DOX succinate), and each funnel were diluted with water (10 mL). About 2 mL of acetate buffer solution (pH 5.00) was added to each separating flask, followed by 1 mL of MO (0.05% w/v) dye solution, and the volume was made to 20 mL with water with thorough mixing. The contents of each funnel were allowed to stand for one minute after shaking with 10 mL of chloroform. Two layers were separated, and the chloroform layer's absorbance (dried on anhydrous sodium sulfate) was measured at 420 nm against a blank (reagent).

2.6 Preparation of standard solution of DOX succinate: The standard solution (1000 µg/ mL) was prepared by accurately weighing 10 mg of DOX succinate in a 10 mL volumetric flask containing 3 mL of distilled water and sonicated for about 20 min volume was made up to the mark with the solvent. This 1 mL of solution was transferred into a 10 mL volumetric flask and diluted with distilled water to get a 100 µg/ mL concentration. From this 2 mL was transferred into a 10 mL volumetric flask and distilled water to get a standard working solution (20 µg/ mL).

2.7 Preparation of sample solution: Accurately weighed twenty tablets (Unisom tablets) and powdered. Tablet powder equivalent to 0.01g of DOX succinate was taken in a 100 mL calibrated flask containing 50 mL of water. The contents were shaken for 20 min and diluted with water up to the mark. The resulting solution was passed through a 0.45 µ filter paper to filter insoluble matter, if any. The sample solution was analyzed by taking 5 mL aliquot in five replicates, as described in the general method for ion-pair complex.

2.8 Assay in the spiked plasma sample: 5 mg of DOX succinate was added in plasma (1 mL) in a 50 mL calibrated flask, and the volume made to the mark with water. The contents were mixed well in order to dissolve the drug completely. 1.0 mL of resulted spiked-plasma solution (100 µg/mL of DOX) was considered for analysis using the general method of ion-pair complex in five replicates. The regression equation was applied to know the content of DOX succinate.

2.9. METHOD VALIDATION

The method was validated for various parameters according to the ICH Q2 (R1) guidelines [20-24].

2.9.1 Specificity: The specificity of the method was established by using 20 µg/mL of working standard solution and sample solution as per the general method. Also, a blank extract was performed to know the selectivity of the method.

2.9.2 Linearity: Five concentrations of DOX succinate were freshly prepared for linearity studies and measured the absorbance at 420 nm. The absorbances were recorded and the calibration curve plotted against concentration. The standard solutions (1.0-5.0 mL) were transferred into respective volumetric flasks by using a grade bulb pipette and the remaining procedure was followed to get ion-pair (DOX-MO) complex in the concentration of 5-25 µg/ mL. The solutions were then filtered through a 0.45 µ membrane

filter. Each solution was scanned at 420 nm in triplicate and linearity was evaluated by linear regression analysis.

2.9.3 Accuracy: A known amount of the standard was added to the sample at three levels (50, 100, and 150 % - of the labeled claim). Three determinations were considered for each level at the detection wavelength (420 nm) and were expressed in percent analyte recovered as well as %RSD.

Accuracy level 1 (50%): About 2.5 mL of DOX standard solution (100 µg/mL) was added to a drug-dye complex (DOX-MO) in a 20 mL volumetric flask. After adding all the contents, it was extracted with chloroform and measured for absorbance at 420 nm.

Accuracy level 2 (100%): About 5 mL of DOX standard solution (100 µg/mL) was added to a drug-dye complex (DOX-MO) in a 20 mL volumetric flask. After adding all the content it was extracted with chloroform and measured for absorbance at 420 nm.

Accuracy level 3 (150%): About 7.5 mL of DOX standard solution (100 µg/mL) was added to a drug-dye complex (DOX-MO) in a 20 mL volumetric flask. After the addition of all the contents it was extracted with chloroform and measured for absorbance at 420 nm.

2.9.4 Precision: Precision was determined as repeatability, in which system precision and method precision was established following ICH guidelines. The system precision was established by replicating the standard solution into the spectroscopic system by maintaining the optimized conditions. Six replicates of sample solutions established the method precision.

2.9.5 Robustness: The following changes were made in the spectroscopic system to determine the effect of deliberate variations in the optimized spectroscopic conditions like buffer volume, dye content, etc.

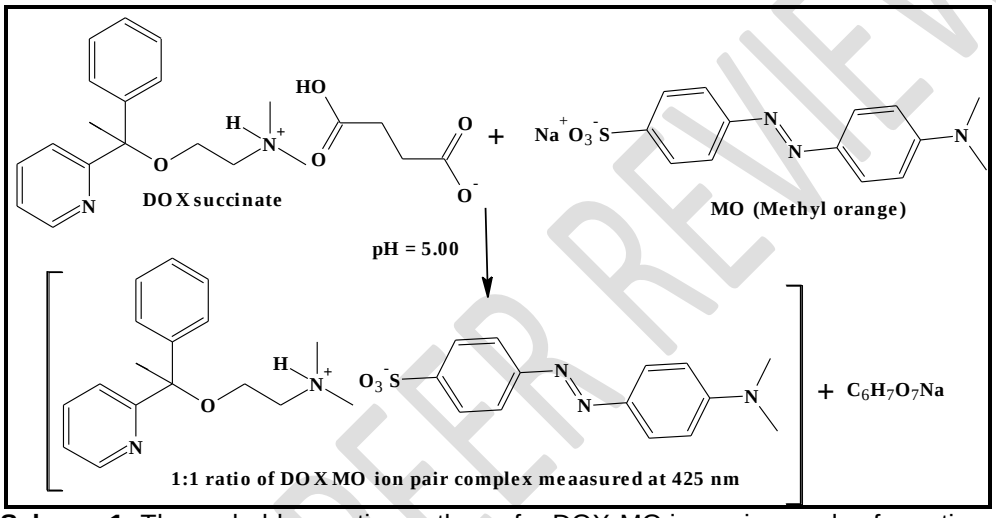
2.9.6 Ruggedness: This parameter was validated with degree of reproducibility of test results. It was obtained by analyzing the samples under varied test conditions such as different analysts, laboratories, instruments, etc. The sample solution of 20 µg/mL of DOX succinate was taken and followed the general method of ion-pair complex in triplicate and their relative absorbance was measured at variable conditions. The % RSD of the results for the absorbances was conveyed.

2.9.7 LOD and LOQ: In general, the limits are expressed with the help of signal to noise ratio (S/N). Analysts often use S/N of 2:1 or 3:1 (LOQ = 10 σ / S) to measure the LOD, while an S/N of 10:1 (LOQ =

10 σ / S) is often considered for the LOQ.

3. RESULTS AND DISCUSSION

3.1 Absorption maxima: The spectroscopic conditions were successfully optimized for assay of DOX succinate in its tablet dosage form and human plasma. DOX succinate was allowed to react with MO dye in a buffer solution (pH = 5.00) which was converted into a yellow-colored product (ion-pair complex) (**Scheme 1**), it was extracted with chloroform. The maximum absorbance for the complex was measured at 420 nm, where the reagent blank had no absorbance in this region (**Fig. 1-3**).



Scheme 1: The probable reaction pathway for DOX-MO ion-pair complex formation

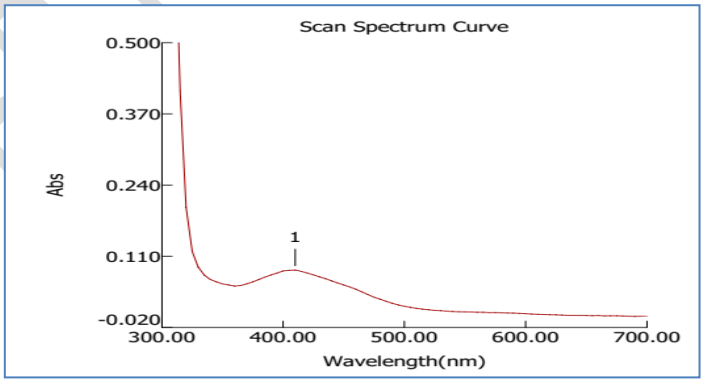


Fig. 1: Absorption spectrums of MO dye (λ_{max} 410 nm).

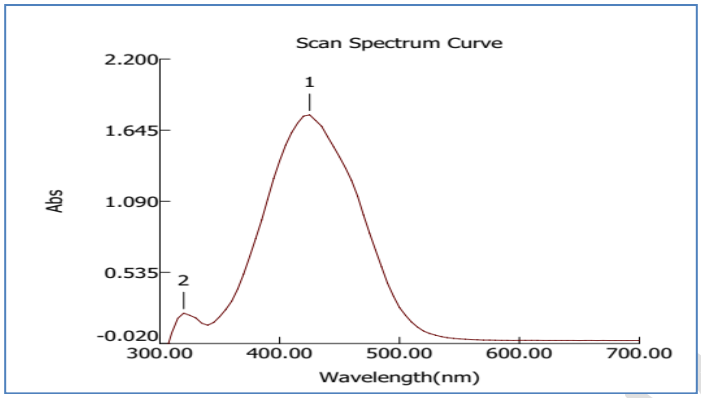


Fig. 2: Absorption spectrum of DOX-MO ion-pair complex (At 50 g/ mL of DOX, λ_{max} 420 nm)

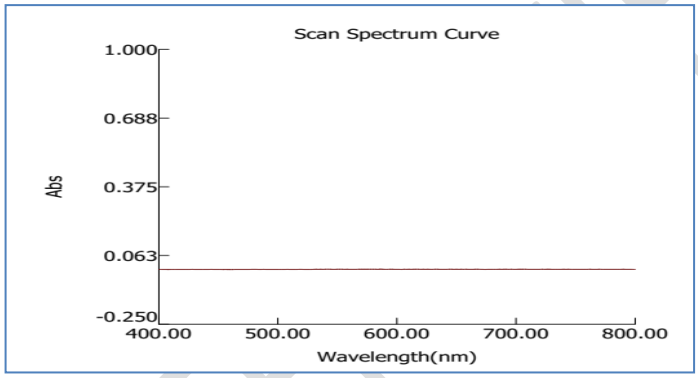


Figure 3: Absorption spectrum for blank (Extracting solvent, $CHCl_3$) without DOX and MO dye

3.2 Optimization of experimental variables: As per the optimized conditions, MO dye is used as an ion-pairing reagent, acetate buffer (pH 5.00) was used as a solvent medium for allowing reaction, and chloroform was used as an extracting solvent. Preliminary experiments were conducted to know the effect of different variables on ion-pair complex formation such as pH, type of extracting solvent, and temperature for extraction of ion-pair (DOX-MO) complex.

3.3 Selection of extraction solvent: The extraction of ion-pair complexes was tested with ethyl acetate, dichloromethane, chloroform, and carbon tetrachloride. In these studies, chloroform was found to better extraction solvent has enhanced efficacy for color intensity and provided selective extraction to that of other solvents. Moreover, as per the standard protocols, single extraction solvent could give the quantitative recovery for all the complexes.

3.4 Effect of pH: The optimum pH conditions for the formation of the ion-pair complex was adjusted with the help of varying pH ranges (pH: 3.2-5.2) of the buffer solution. The pH was optimized at 5.00 as it showed the maximum absorbance.

3.5 Effect of dye concentration: The concentration of MO dye is one of the crucial parameters in adjusting the complexes' colour intensity; it was achieved by varying the dye concentration (0.01-0.1 %) at the detection wavelength (420 nm). The colour intensity of the ion-pair complex was compatible with 0.05% MO dye.

3.6 Effect of shaking mode and time: Deferent approaches were employed to extract the ion-pair complexes using a separating funnel, a magnetic stirrer, and a vortex mixture. Among this vertex, mixture gave a little higher absorbance value. Further, shaking time was optimized (for 1.0 to 5.0 min) and there was no appreciable improvement in extraction after 1.0 min.

3.7 Effect of temperature on the stability: Temperature variation affected the stability of the ion-pair complex, which was tested at 25, 30, and 35°C. The results exhibited that 30°C was found optimum with a minute change in the absorbances. Moreover, as the extracting solvent was volatile at tested temperatures, there was a slight increase (~1–3%) in the absorbance of the drug-dye complex.

3.8 Stoichiometry of DOX-MO complex: It was established by Job's method of continuous variation, in which the composition of the ion-pair (DOX-MO) complex were measured (Job, **1928**). In this study, the test solutions were prepared in identical concentrations of the drug (1.5×10^{-3} M). Both the solutions were mixed in varying volume ratios by adjusting the total volume of each solution as constant. The resulting complexes (with varied volume ratios of drug and dye) were measured for absorbance at 420 nm. In the a plot of absorbance against the mole fraction of the drug, these results showed the 1:1 stoichiometric ratio of drug and dye showed a stable ion-pair complex in **Fig. 4**.

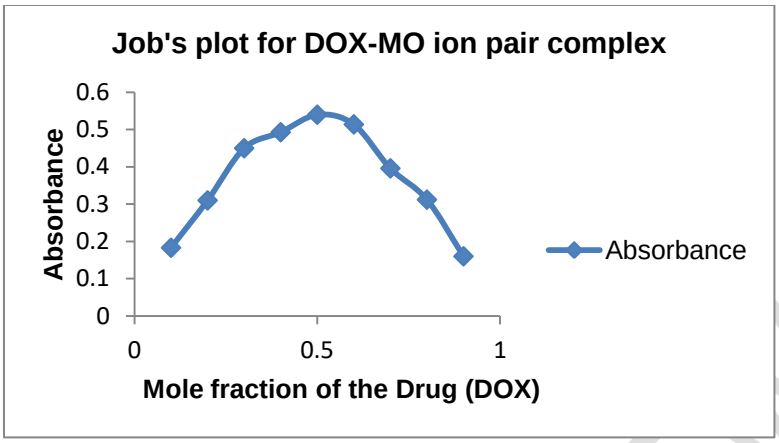


Fig. 4: Job's plot for ion-pair complexes of MO with DOX succinate.

3.9 Assay: The standard and sample solutions of DOX succinate were prepared separately in appropriate concentrations per protocol provided in the ion-pair complex method. The sample solution containing nearly equivalent to the standard amount (as per the label claim) was prepared and scanned to measure absorbances in triplicate at 420 nm (**Table 1**). The mean absorbance values for standard and sample solution were found as 0.826 and 0.822, respectively. The amount of drug present was found to be 99.25 %, which was found as per the label claim amount. The absorption spectrum for standard and sample solution was showed in **Fig. 5** & **6**.

Table 1: The Absorbance values of the DOX-MO ion-pair complex

S. No.	Analyte (DOX-MO ion-pair complex)	Absorbance 420 nm			Mean absorbance
		A_1	A_2	A_3	
1	Standard	0.827	0.827	0.826	0.826
2	Sample	0.822	0.820	0.826	0.822

A = Absorbance

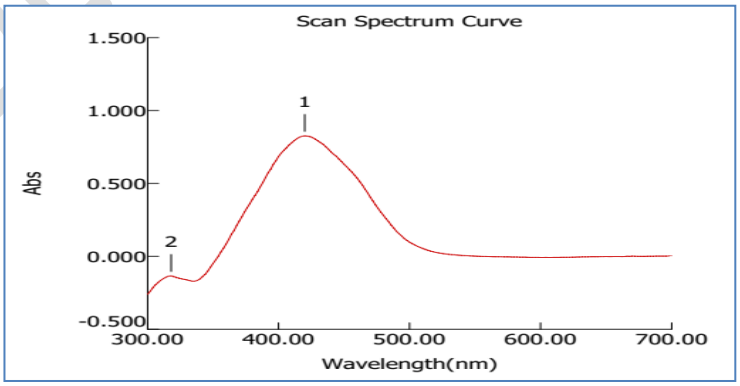


Fig. 5: Absorption spectrum of standard (DOX-MO ion pair complex)
(At 10 µg/ mL of DOX, λ_{max} 420 nm)

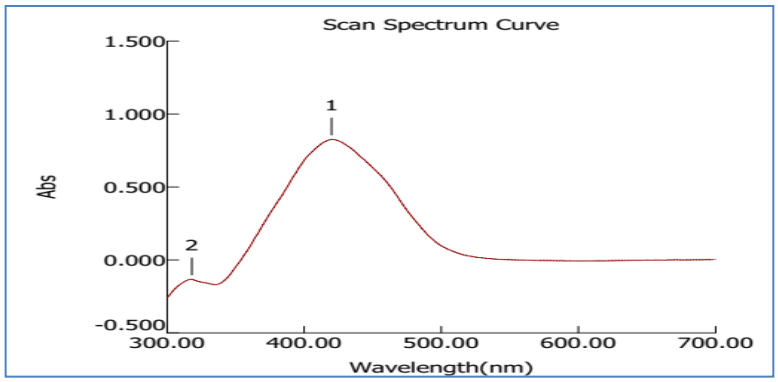


Fig. 6: Absorption spectrum of sample (DOX-MO ion pair complex)

(At 10 µg/ mL of DOX, λ_{max} 420 nm)

3.10 Method validation

3.10.1 Specificity

Identification of DOX-MO dye ion-pair complex: Standard and sample solutions were prepared and analyzed as per test procedure and scanned under UV-visible spectrophotometer (**Fig. 7 & 8**). The absorption spectrum showed that both standard and sample spectrums for DOX-MO dye ion-pair complex were similar and no interferences of excipients were observed. The absorption maximum was observed for the DOX-MO dye ion-pair complex at 420 nm.

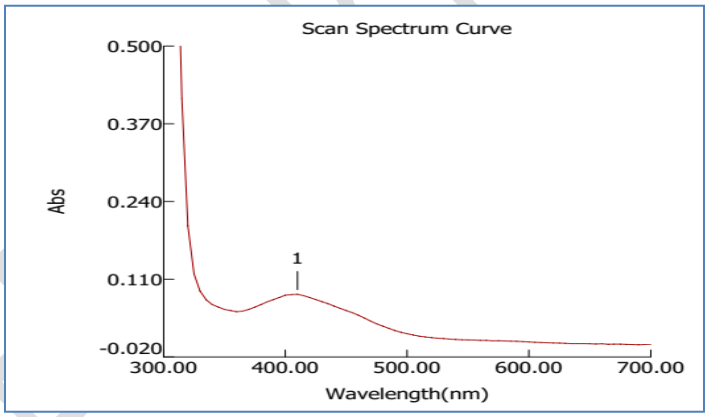


Fig. 7: Absorption spectrum of MO dye (λmax 410 nm)

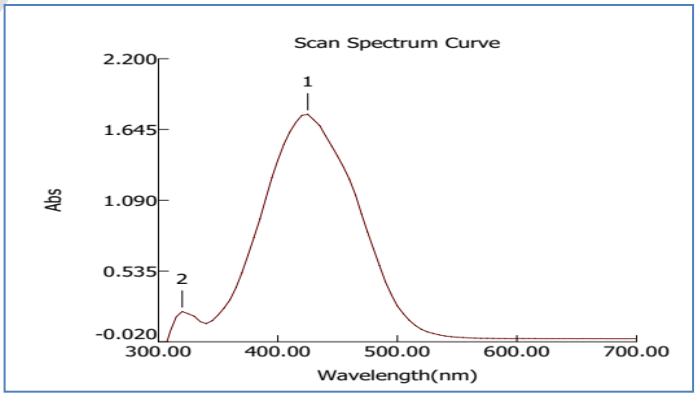


Fig. 8: Absorption spectrum of DOX-MO ion-pair complex (At 50 g/ mL of DOX, λ_{max} 420 nm).

Blank determination: The extracting solvent ($CHCl_3$) with reagent blank (without drug) was scanned in the UV-visible region (200-800 nm) as per optimized conditions (**Fig. 9**). The blank spectrum showed that there was no interference of solvent or dye in absorbance.

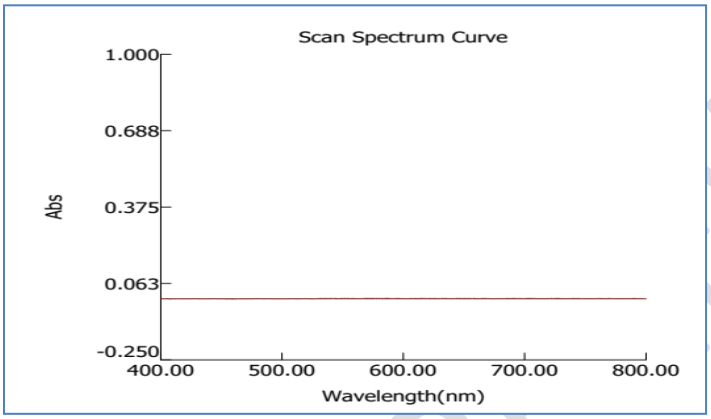


Fig. 9: Absorption spectrum for Blank (extracting solvent, $CHCl_3$) without DOX and MO dye.

3.10.2 Linearity: Five concentrations (5-25 µg/mL) of DOX succinate with 2 mL MO dye were freshly prepared for linearity studies and measured the absorbance at 420 nm. The overlain spectrum was drawn for five concentrations individually (**Fig. 10**). The absorbances were recorded and the calibration curve was plotted against concentration v/s absorbance's (**Fig. 11**). Each solution was scanned in triplicate and linearity results showed a good linear relationship in the calibration curve plot with a regression equation of y = 0.030x + 0.122 for the DOX-MO dye ion-pair complex (**Table 2**). The correlation coefficient (r^2 = 0.992) found was within the prescribed range. Hence the method found to be linear in the concentration over the range of 5-25 µg/mL.

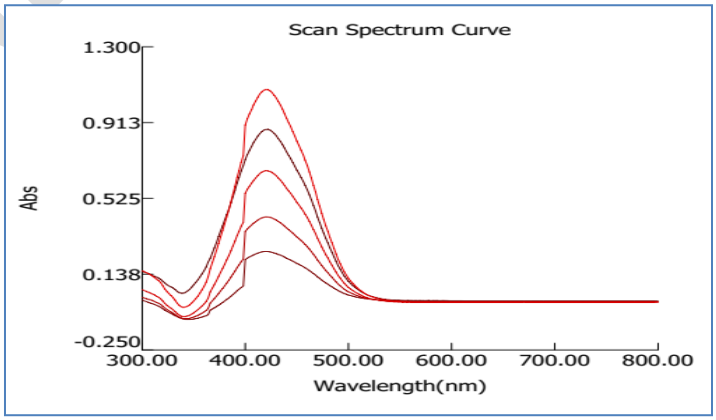


Fig. 10: Overlain spectrum of linearity levels of DOX-MO ion-pair complex in the concentration range.

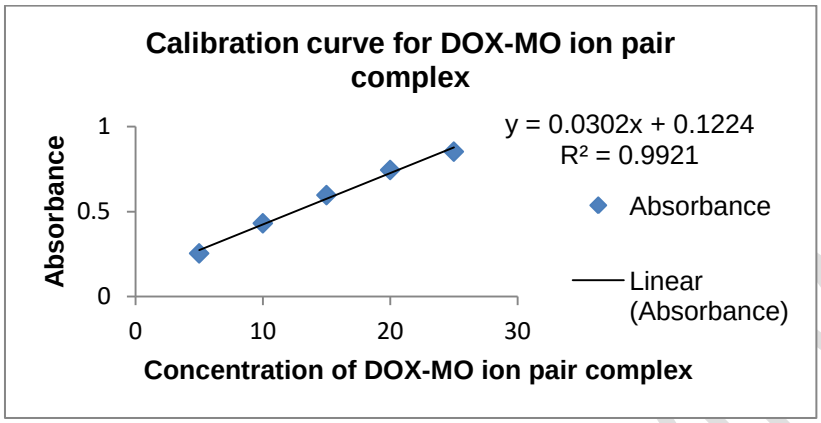


Fig. 11: Calibration curve for DOX-MO dye ion-pair complex (5-25 µg/mL).

Table 2: Linearity data of DOX-MO ion-pair complex

Sample	Concentration of analyte (µg/mL)	Absorbance	Linear regression equation
DOX-MO ion-pair complex	5	0.254	y = 0.030x + 0.122 r² = 0.992
	10	0.431	
	15	0.667	
	20	0.745	
	25	0.853	

3.10.3 Accuracy: The percent analyte recovered was calculated at each level, in which the mean recovery was 98.7-99.7 % (**Table 3**). The % RSD values were within 2.0 % for each recovery, which reveals that good agreement with accuracy **(Fig. 12-14**).

Table 3: Accuracy data of the DOX-MO ion-pair complex (n = 3)

S. No.	Spiked level	Absorbance	% Recovery	SD	% RSD	% Mean recovery ±SD
1	50 %	0.693	101.0			
	50 %	0.686	100.4	0.001	0.324	100.6±0.65
	50 %	0.685	100.6			
2	100 %	0.818	100.3			
	100 %	0.811	99.7	0.002	0.324	100.2±0.45
	100 %	0.809	100.8			
3	150 %	0.942	99.15			
	150 %	0.933	99.12	0.0026	0.21	99.09±0.081
	150 %	0.928	99.01			

n= number of determinations; SD = Standard deviation; % RSD = % Relative standard deviation

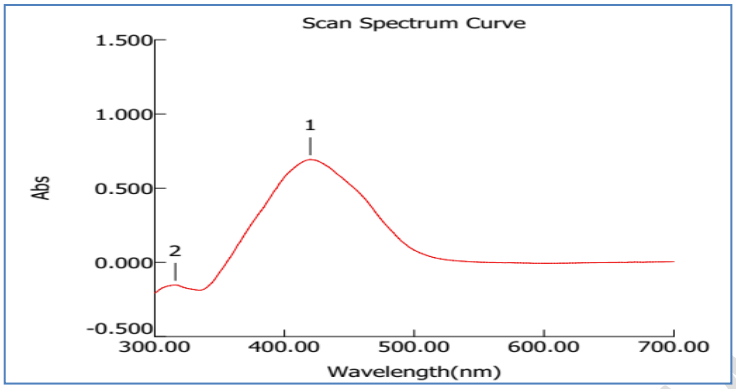


Fig. 12: Accuracy level 50% of the DOX-MO ion-pair complex.

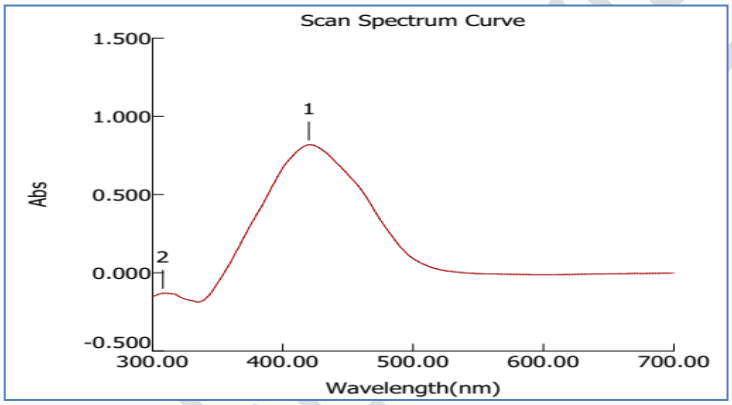


Fig. 13: Accuracy level of 100% of the DOX-MO ion-pair complex.

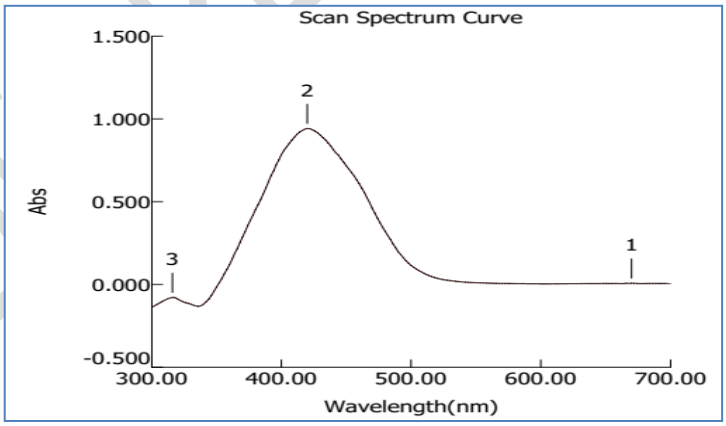


Fig. 14: Accuracy level of 150% of the DOX-MO ion-pair complex.

3.10.4 Precision: Precision was determined as system precision and method precision, and the % RSD results obtained were within acceptance criteria which indicated that the method was highly precise. The system precision was established by measuring the absorbances at 420 nm for five

replicates of standard solutions of the DOX-MO dye ion-pair complex by maintaining the optimized spectroscopic conditions (**Table 4**).

Table 4: System precision data (n =3)

S. No.	Concentration (µg/mL)	Absorbance
1	20	0.839
2	20	0.840
3	20	0.839
4	20	0.836
5	20	0.835
Mean	-	0.837
SD	-	0.0021
%RSD	-	0.25

n= number of determinations; SD = Standard deviation; % RSD = % Relative standard deviation

The system precision was established by measuring the absorbance at 420 nm for five replicates of sample solutions (tablet dosage form) of DOX succinate by maintaining the optimized spectroscopic conditions (**Table 5**). The % assays (98% to 102%) and % RSD (>2%) of the assay was calculated with the proposed method, which showed the results were within the limit. Hence the method called to pass repeatability.

Table 5: Method precision data **(**n = 3**)**

S. No.	Concentration (µg/mL)	Absorbance 420 nm	Assay (%) DOX-MO ion-pair complex
1	20	0.842	99.8
2	20	0.838	99.1
3	20	0.844	99.5
4	20	0.837	99.6
5	20	0.838	99.5
Mean	-	0.839	-
SD	-	0.0031	-
% RSD	-	0.369	-

n= number of determinations; SD = Standard deviation; % RSD = % Relative standard deviation

3.10.5 Robustness: In varying buffer content, the % RSD found as 0.878 for DOX succinate (**Table 6**). Variation in dye content does not show any drastic changes in absorbance. The % RSD was found to be 1.150 (**Table 7**), which shows the method was robust by marginal variation in wavelength and temperature.

Table 6: Robustness data change in buffer content

S. No	DOX succinate taken (µg/mL)	Change in Wavelength	Absorbance of DOX-MO ion-pair complex	% RSD
1	20	Low (1.5 mL)	0.846	
2	20	Original (2 mL)	0.876	0.878

3	20	High (2.5 mL)	0.912	

Table 7: Robustness data change in dye content

S. No	DOX succinate taken (µg/mL)	Change in Temperature	Absorbance of DOX-MO ion-pair complex	% RSD
1	20	Low (0.5mL)	0.858	1.150
2	20	Original (1 mL)	0.876	
3	20	High (1.5 mL)	0.882	

3.10.6 Ruggedness: In the ruggedness studies, the sample solution of 20 µg/mL was analyzed in triplicate, and their relative absorbance was measured at variable conditions. The % RSD of the results found for the different analysts was 1.32 %, for different laboratories as 1.65 %, and for different instruments as 1.59%, which indicates the method follows ruggedness.

3.10.7 LOD and LOQ: The LOD for the ion-pair (DOX-MO) complex was found to be 0.31 µg/mL (**Fig. 15**). The LOQ for the ion-pair (DOX-MO) complex was found to be 0.939 µg/mL (**Fig. 16**). The method gave a good sensitivity for the drug-dye (DOX-MO) complex.

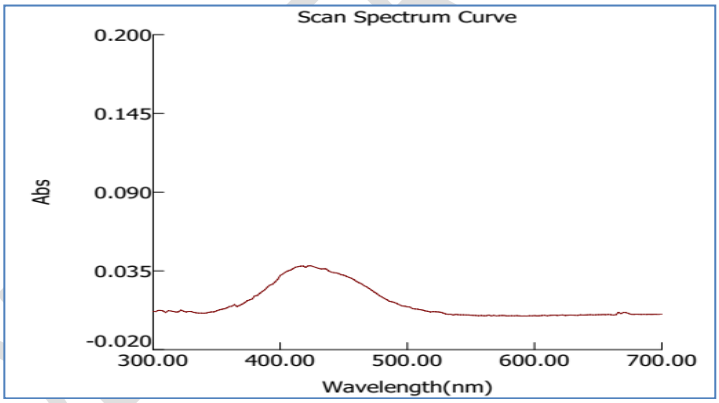


Fig. 15: LOD spectrum for DOX-MO ion-pair complex at 1 µg/mL.

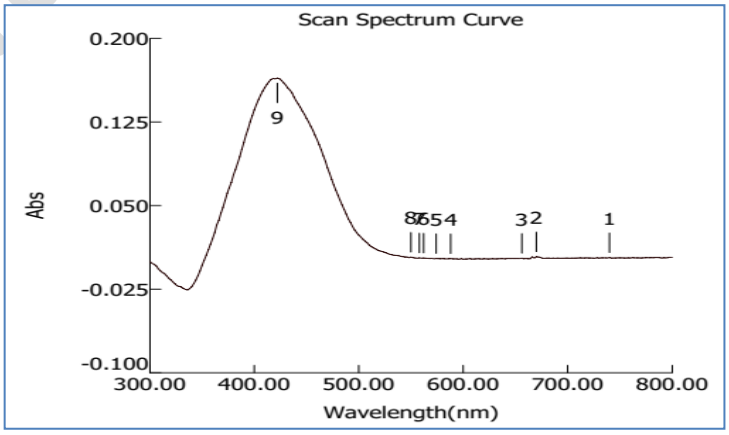


Fig. 16: LOQ spectrum for DOX-MO ion-pair complex at 2.5 µg/mL

4 CONCLUSIONS

An ion-pair formation method was developed to estimate DOX succinate in its tablet dosage form and human plasma using UV-visible spectrophotometry. Acetate buffer (pH.5.00) was used as a solvent system at temperature conditions 30 ^{0}C ±5^{0}C. MO dye (0.05 % w/v) was used to form a complex. An ion-pair complex was formed with the dye used which was measured at 420 nm. The proposed method was validated for specificity, linearity, accuracy, precision, ruggedness, robustness, LOD, and LOQ according to ICH $Q_2(R_1)$ guidelines and was found to be satisfactory. The proposed method was simple, specific, and requires a short analysis time, and it could be easy and economical to perform. Hence it was concluded that the present method developed was well suitability for routine analysis of DOX succinate in its tablets and biological samples.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Secker VR, Baig I, Amulya R, Nagarjuna P. A validated simultaneous estimation of Doxylamine succinate and pyridoxine hydrochloride by UV spectrophotometric method in bulk and formulation. Int J Pharm Res Anal. 2014; 4 (2): 139-43.
2. Avanti SJ, Shivangi JP, Nirali KS, Sandhya N, Prasanna KP, Umesh MU. Development and

validation of analytical methods for the simultaneous estimation of drugs in soft gelatin capsules (cough and cold preparation) by UV-spectroscopy. Int J Pharm Sci Res. 2015; 6(2): 287-98.

3. Narender M, Rao AA, Sreehasa A, Mounika P, Sonekar S, Durga CV, Srivani NN**.** UV-spectroscopic method development and validation for simultaneous estimation of Doxylamine succinate and Pyridoxine hydrochloride in bulk and pharmaceutical dosage form. Res J Pharm Tech. 2020; 13(10): 1-8.
4. Ali MY, Hebatallah ME. Development and validation of a generic high-performance liquid chromatography method for the simultaneous separation and determination of six cough ingredients: Robustross study on core-shell particles. J Sep Sci. 2008; 11562.
5. Donato JL, Koizumi F, Pereira AS, Mendes GD, DeNucci G. Simultaneous determination of dextromethorphan and doxylamine in human plasma by HPLC coupled to electrospray ionization tandem mass spectroscopy: Application to a pharmacokinetic study. J Chromatogr B. 2012; 899: 46-56.
6. Rosa PCP, Jardim ICSF. Simultaneous determination of clobutonol hydrochloride and doxylamine succinate from syrups by RP-HPLC using a new stationary phase containing embedded urea polar groups. Braz J Pharm Sci. 2012; 48: 316.
7. Raja A, Samatha M, David BM, Rao KNV, Sujitha, Vinod J, Kumar SD. Analytical method development and validation of acetaminophen dextromethorphan hydrobromide and doxylamine succinate in soft gel capsule dosage form by using RP-HPLC. World J Pharm Pharm Sci. 2013; 2(6): 5852-62.
8. Ivković B, Marković B, Vladimirov S. Development and validation of RP-HPLC method for analysis of multi-component cough-cold syrup formulation. Arh Farm. 2014; 64:271-84.
9. Giriraj P, Sivakumar T. Development and validation of a rapid chemostatis assisted RP-HPLC with PDA detection method for the simultaneous estimation of pyridoxine HCl and doxylamine succinate in bulk and pharmaceutical dosage form. Chromatogr Res Int. 2014; 2014: 1-8.
10. Bhortake P, Lokhande RS. Analytical development and validation of acetaminophen, doxylamine succinate and dextromethorphan hydrobromide in liquicap dosage form by RP-HPLC. Int J Pharm Sci Invent. 2014; 3 (7): 8-12.

11. Shaikh MSA, Lakhani HJ, Dey S, Pradhan PK, Upadhyay UM. Simultaneous estimation of phenylephrine HCl, doxylamine succinate and dextromethorphan HBr in soft gelatin capsule (cough and cold preparation) by RP-HPLC. Int J Pharm Sci Res. 2014; 5(5): 218-26.
12. Varasala D, Konidala SK. Stability indicating RP-HPLC method development and validation for simultaneous determination of doxylamine succinate and dextromethorphan hydrobromide in pharmaceutical dosage forms. Der Pharmacin Lettre 2015; 7(2): 112-18.
13. Qarah N, Basavaiah K, Abdulrahman SAM. Spectrophotometric assay of diethylcarbamazine citrate in pharmaceuticals and human urine via ion-pair reaction using methyl orange dye. J Pharma Care Health Sys. 2017; 4 (4): 1000182.
14. Adikwu MU, Ofokansi KC, Attama AA. Spectrophotometric and thermodynamic studies of the charge-transfer interaction between diethylcarbamazine citrate and chloranilic acid. Chem Pharm Bull. 1999; 47: 463-66.
15. Ajima U, Onah JO, Ogugua SC. Development and validation of an extractive ion-pair spectrophotometric method for the determination of ciprofloxacin hydrochloride. J Chem Pharm Res. 2015; 7: 470-76.
16. Gouda AA, Sheikh R, Amin ASA, Ibrahim SH. Optimized and validated spectrophotometric determination of two antifungal drugs in pharmaceutical formulations using an ion-pair complexation reaction. J Taibah Univ Sci. 2016; 10: 26-37.
17. Nair SG, Shah JV, Shah PA, Sanyal M, Shrivastav PS. Extractive spectrophotometric determination of five selected drugs by ion-pair complex formation with bromothymol blue in pure form and pharmaceutical preparations. Cogent Chem. 2015; 1 (1): 1-14.
18. ICH, "Q_2 (R_1), Validation of analytical procedures, text and methodology", in proceedings of International Conference of Harmonization (ICH), Geneva, Switzerland, p1, 2005.
19. United States Food and Drug Administration Guidance for Industry, ICH Q6A, and Specifications test procedure and acceptance criteria for new drug substances and new drug products, chemical Substances, 1999.

20. Chawla R K, Panda S, Umasankar K, Panda S P, Damayanthi D. Method development and validation for quantitative estimation of calcium acetate in calcium acetate capsules by RP-HPLC using indirect UV method. Indian J Pharm Edu Res. 2019; 53(3s): s380-s386.
21. Phani R S Ch, Prasad K R S, Mallu U R. New analytical HPLC method development and validation for the simultaneous quantification of paritaprevir, ombitasvir and ritonavirin spiked human plasma. Orient J Chem. 2017; 33(5): 2363-69.
22. Prasad SS, Mohan GVK, Babu AN. Development and validation of stability-indicating RP-HPLC method for the estimation of lenalidomide and its impurities in oral solid dosage form. Orient J Chem. 2019; 35(1): 140-149.
23. Mallu UR, Anna VR, Kasimala BB. Rapid stability indicating HPLC method for the analysis of leflunomide and its related impurities in bulk drug and formulations. Turk J Pharm Sci. 2019; 16(4): 457-65.
24. Malothu N, Kona SB, Muthyala B, Katamaneni P. A simple liquid chromatographic method for simultaneous estimation of azithromycin, fluconazole and ornidazole in bulk and pharmaceutical dosage forms. Int J Pharm Pharm Sci. 2019; 11 (8): 26-34.