IJBPAS, August, 2026, 15(8): 3923-3935

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

DEVELOPMENT OF VALIDATED UV SPECTROSCOPIC AND HPLC METHODS FOR SIMULTANEOUS DETERMINATION OF DOXYCYCLINE HYCLATE AND COLISTIN SULPHATE FROM A MIXTURE

TAMBE VS*, KAKADE T, BAGUL K, VICHARE V AND DHOLE S

Department of Pharmaceutical Chemistry, Modern College of Pharmacy (for Ladies), Moshi, Pune, Maharashtra, India

***Corresponding Author: Dr. Vrushali S. Tambe: E Mail: vrushalitambe99@gmail.com**

Received 26th July 2025; Revised 24th Sept. 2025; Accepted 31st Jan. 2026; Available online 1st Aug. 2026

https://doi.org/10.31032/IJBPAS/2026/15.8.10410

ABSTRACT

The aim of the present research work is to develop and validate methods for the simultaneous estimation of doxycycline hyclate and colistin sulphate from a mixture, by using UV spectroscopy and High-Performance liquid chromatography. UV spectroscopic Absorption correction method using water as a solvent was developed. Chromatographic separation was performed on a Zorbax XDB C_{18} column packed with particles of 5µ and dimensions of 150*4.6 mm. The acceptable separation was achieved using a mobile phase gradient for a run time of 18 minutes. The mobile phase A consist of water: acetonitrile: formic acid (90:10:0.08 v/v/v) while mobile phase B was water: acetonitrile: formic acid (95:05:0.1 v/v/v) and was delivered at a flow rate of 1ml/min. The detection was made at 210 nm. The linearity of the method was found to be 0.9996 and 0.9995 for doxycycline hyclate and colistin sulphate respectively for HPLC. It was 0.9998 at 338nm and 0.9992 at 202nm for UV. The limit of detection and limit of quantitation values of doxycycline hyclate and colistin sulphate found to be 30.921 µg/ml, 93.701 µg/ml and 17.544 µg/ml, 53.164 µg/ml, respectively for HPLC. Both the methods were found to be accurate, robust and precise.

Keywords: Doxycycline hyclate, Colistin sulphate, UV-visible spectroscopy, HPLC, method development

List of Abbreviations

Abbreviation	Full Form
mg	Microgram
µL	Micro litre
µg/ml	Microgram per millilitre
mg/ml	Milligram per millilitre
ACN	Acetonitrile
Conc.	Concentration
DOH	Doxycycline hyclate
COS	Colistin sulphate
PDA	Photodiode Array
HPLC	High Performance Thin Layer Chromatography
ICH	International Council for Harmonisation

INTRODUCTION

Doxycycline hyclate (DOH) is an antibiotic that belongs to the group of tetracyclines. It has a bacteriostatic effect by inhibiting the protein synthesis of pathogens. It is employed in the treatment of a wide range of bacterial infections, including acne, sinusitis, and urinary tract infections **[1, 2]**. Colistin sulphate (COS) is also an antibiotic from the group of Polymyxins which is synthesized by aerobic spore-forming *Bacillus polymyxa*. COS has a bactericidal effect against Gram-negative bacteria such as *E. coli*, *Salmonella spp.*, and *Pseudomonas spp.* Colistin, also referred to as polymyxin E, are cationic peptides. It primarily consists of two main components polymyxin E1 and E2, also known as colistin A and B. The mixture of both drugs is recommended in the treatment of animals with septicaemia, respiratory diseases (bronchopneumonia, pleuropneumonia, atrophic rhinitis, etc.), and in digestive tract diseases (colibacillosis, salmonellosis) **[3-5]**. Their marketed combination powder formulation contains 250mg of DOH and 117.07mg of COS. DOH is soluble in water with pKa of 3.37 and Log P of 3.3. Its molecular weight is 513.0 and its molecular formula of $C_{46}H_{58}Cl_2N_4O_{18}$. COS has a molecular weight of 2801.27 and has a molecular formula of $C_{53}H_{102}N_{16}O_{17}S$. It is also soluble in water with a Log P of 11. The structures of both drugs are shown in **Figure 1**.

Based on the literature survey, a few UV **[6-8]**, HPLC **[9-11]** and other analytical methods **[12-14]** were developed for DOH. Similarly, for COS, there are some UV **[15]**, HPLC, and other methods **[16]** reported. These methods can quantify drugs alone but not in combination. Hence, the development of new methods is necessary to explore and analyze these compounds simultaneously.

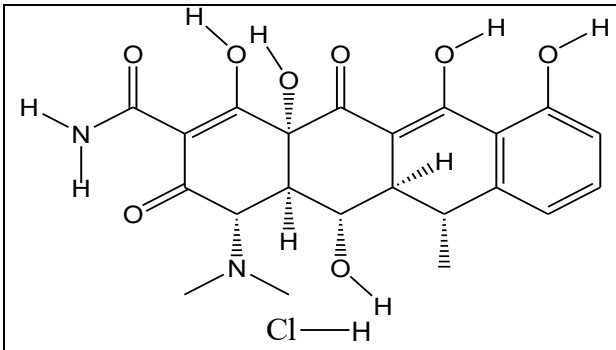
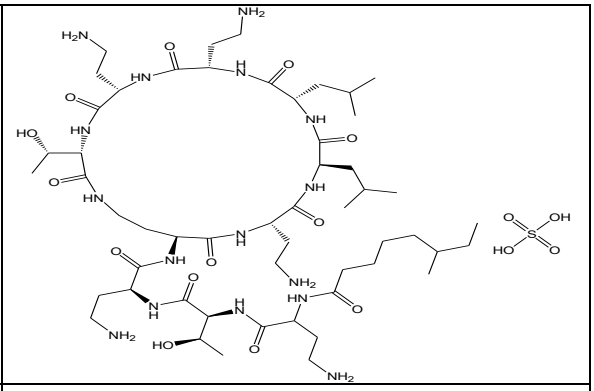


Figure 1- a) Structure of DOH



b) - Structure of COS

MATERIALS AND METHODS

Chemicals and Reagents

DOH (% w/w) was provided as a gift sample from Nulife Pharmaceuticals, Pune. While, COS (%w/w) was supplied by ChromeIn laboratory, Pune. All compounds, including HPLC-grade acetonitrile (ACN), water and formic acid (FA), were procured from the chemical division of Merck located in Mumbai.

Instrumentation

UV-Visible Spectrophotometer of Shimadzu (1800) with UV Probe 3.43 software and matched quartz cuvette with 1 cm pathlength was used for all absorbance measurements. All weighing were done on single pan balance (Shimadzu). HPLC of Waters Alliance 2695 HPLC with PDA detector and Empower 2 software was used

UV-Spectroscopic method development

To prepare standard stock solutions of DOH and COS, accurately weighed 10 mg of each drug and transferred to separate clean 10 mL volumetric flask. The drugs were dissolved in distilled water to get a concentration of 1000 µg/ml. To determine analytical wavelength, 10 µg/mL solutions of DOH and COS were produced separately by diluting the standard stock solution with distilled water and scanned in the spectrum mode from 200 to 400 nm. From the spectra of these drugs, wavelengths 338 nm (λmax DOH), and 202 nm λmax (COS) were selected for analysis.

Absorption correction method was used to determine the concentration of drug using following equations.

$A_{total(338)} = a_{2(338)}. C_2$ ……(I)

$A_{total(202)} = a_{1(202)}. C_1 + a_{2(202)}. C_2$ …….(II)

Where, $A_{total(202)}$ and $A_{total(338)}$ are total absorbance of test solution at 202 and 338nm respectively, $a_{1(202)}$ $a_{2(202)}$ absorptivity of COS and DOH at 202 nm respectively, $a_{2(338)}$ is absorptivity of DOH at 338nm, C_1 and C_2 are concentrations of COS and DOH respectively.

UV Spectroscopic Method Validation

The suggested spectrophotometric approach was validated using ICH recommendations for the parameters mentioned below. Calibration curves have been developed from concentrations range of 5-30µg/ml for DOH and 5-50µg/ml COS. LOD and LOQ were calculated. Linearity of the mixture of DOH and COS was performed in the concentration range of 4-24µg/ml and 2-12µg/ml respectively. Results are shown in **Table 1**. Accuracy was determined in terms of recovery studies. The proposed method's precision was estimated in terms of repeatability, intermediate precision, intra-day and inter-day precision. Results are shown in **Tables 2 and 3**. The repeatability was determined by preparing multiple aliquots of a standard solution containing 6µg/ml of COS and 12µg/ml of DOH. % Relative standard deviation (%RSD) of the response was determined. For intermediate precision, test solution containing 6µg/ml of COS and 12µg/ml of DOH were evaluated by different analysts. The robustness study was performed for the prepared formulation mixture by changing the assay method parameters, which include change in the detection wavelength. Absorbance was observed at 337 and 201nm, 339 and 203nm. The standard deviation and % RSD were calculated. Results are shown in **Table 4**.

The accuracy studies were carried out at three distinct levels- 80%,100%, and 120%. The resulting solutions were scanned under UV and absorbance was recorded. % Recovery was calculated.

Weighed powder equivalent to 0.08/0.1/0.12gm of DOH and 0.04/0.05/0.06gm of COS Poured it into 100 mL of a volumetric flask containing 50ml of water and sonicated it for 15min. Then the volume was made up to 100ml water. Then these solutions were filtered and analysed. This study was performed in triplicate. Results are shown in **Table 5**.

Assay of mixture using UV Spectroscopy

Weighed powder equivalent to 0.1gm of DOH and 0.05gm of COS and transferred it into 250ml volumetric flask, sonicated for 15-20min and then filtered to get a solution with theoretical content of 400µg/ml for DOH and 200µg/ml for COS. About 10ml of this solution was diluted to 100ml with distilled water (40 µg/ml for DOH and 20µg/ml for COS). Further, 3 ml of the solution was diluted with water. The final solution used for analysis has 12 µg/ml DOH and 6 µg/ml of COS as theoretical content. This solution was scanned and the absorbance was recorded at 202 and 338nm. The practical contents were determined.

High-performance liquid chromatography method development

COS has poor absorptivity and lower concentration as compared to DOH. Hence, in order to optimize the signal of COS,

210nm was selected for HPLC analysis. Methanol, ACN and water in the ratio of 10:10:80 (v/v/v) was used as diluent based on the solubility and response of both drugs.

Weighed accurately 100mg of COS and 200mg of DOH and dissolved separately in a 100 mL volumetric flask using a diluent to achieve a concentration of 2000 µg/mL of DOH and 1000 µg/mL of COS. This solution was further diluted to get a concentration of 2000 µg/mL of DOH and 1000 µg/mL of COS. The resulting solutions were injected into the chromatographic system. After doing numerous trials, the mobile phase A (Water: ACN: FA 95:05:0.0.8%, v/v/v) and mobile phase B (Water: ACN: FA, 90:10:0.01% v/v/v). The gradient program of (T/%A) 0.01/0; 1/0; 11/70; 13/70; 13.5/0 and 18/0 with a run time of 18min was used to achieve good peak shape and resolution.

Method validation

Different aliquots of the mixture of DOH and COS were prepared and injected. Linearity was established in the range of 500-1500 µg/mL for COS and 250-750 µg/mL for DOH in the mixture. Results of linearity, LOD and LOQ are shown in **Table 6**. For the repeatability study, the sample mixture was run repeatedly 6 times and the %RSD of the response was determined.

Accuracy was determined for various concentrations of mixture equivalent at 50, 100, and 150 % levels of the test procedure. Weighed powder equivalent to 0.05/0.1/0.15gm of DOH and 0.025/0.05/0.075gm of COS. Poured it into 100 mL of a volumetric flask containing 50ml of water and sonicated it for 15min. Then the volume was made up to 100ml diluent. Then these solutions were filtered through an HPLC filter and analysed. This study was performed in triplicate. Results are shown in **Table 7**.

For the robustness study, the effect of variation at a change in flow rate and temperature was checked. A test solution containing 1000µg/mL of DOH and 500 µg/mL of COS was used. The chromatograms were obtained by varying the flow rate by ±0.1ml/min i.e. 0.9ml/min, 1.0ml/min and 1.1ml/min and changing the column temperature by ±2°C i.e. 28°C, 30°C, 32°C. Results are shown in **Tables 8 and 9** respectively.

Assay of mixture using HPLC

Weighed and transferred powder equivalent to 0.1gm of DOH and 0.05gm of COS into a 100 mL volumetric flask containing 50ml of water and sonicated for 15min. The volume was made up to 100ml using diluent to achieve a concentration of 1000 µg/mL of DOH and 500 µg/ml COS. Then this solution was filtered through a syringe filter and analysed by the proposed method.

RESULT AND DISCUSSION

UV-Spectrophotometric Method

DOH and COS exhibited significant absorbance in water at 338 and 202nm respectively as shown in **Figure 2**. Hence, those were chosen as detection wavelengths. Colistin sulphate did not show any signal at the detection wavelength of DOH, hence absorption correction method was selected for analysis. The proposed method for the simultaneous estimate of both drugs was validated according to ICH guidelines. The linearity was seen in the concentration range of 5-30µg/ml for DOH and 5-50µg/ml for COS. The slope and correlation coefficient values of DOH at 338nm and 202nm are 0.0261, 0.9974, and 0.0374, 0.9932, respectively, while for COS at 202nm, it is 0.0229, 0.9954. Results of the linearity of DOH at 338nm and 202nm and COS at 202nm are shown in **Table 1**. The slope and correlation coefficient values of the mixture of DOH and COS at 338nm and 202nm are 0.028, 0.9998 and 0.114, 0.9992 respectively. The results of linearity of a mixture is shown in **Figure 3**. The accuracy of the method was assessed by recovery experiments. Recovery was close to 100%w/w for both drugs. The precision of the method was studied as repeatability, intra-day and inter-day variations; the %RSD less than 2, indicates the precision of the proposed method. The amount of drugs estimated by the proposed method was in good agreement with the label claim.

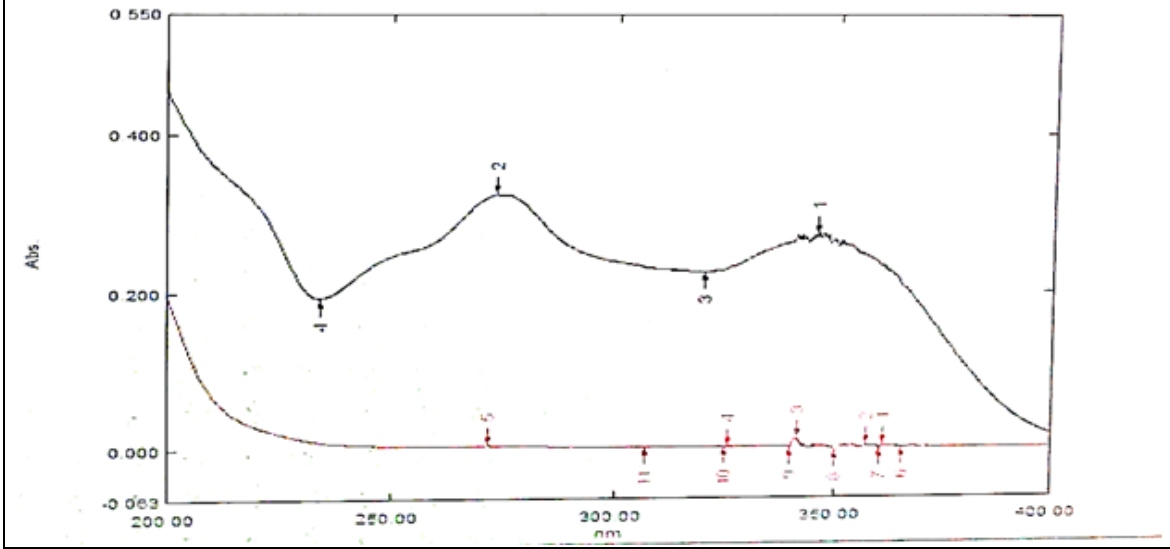


Figure 2: Overlay of DOH and COS at a concentration of 10 µg/mL

Table 1: Results of linearity of DOH and COS (n=3)

DOH			COS	
Concentration	Absorbance at	Absorbance at	Concentration	Absorbance at

(µg/ml)	338nm	202nm	(µg/ml)	202nm
5	0.16	0.298	5	0.167
10	0.26	0.434	10	0.27
15	0.412	0.636	20	0.524
20	0.524	0.811	30	0.793
25	0.666	0.985	40	0.943
30	0.809	1.242	50	1.2

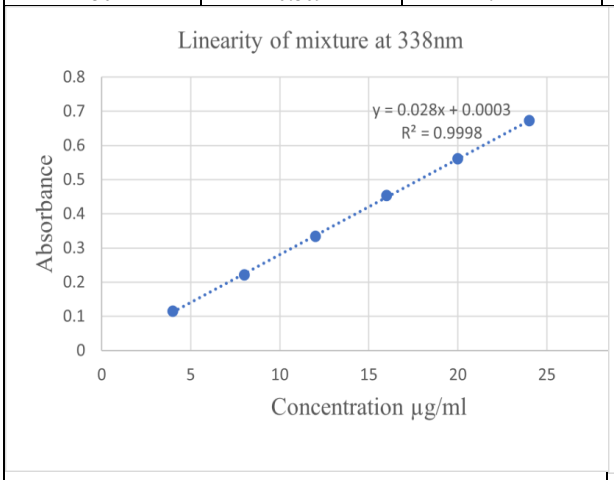


Figure 3 (a): Calibration curve plot of linearity of the mixture at 338nm

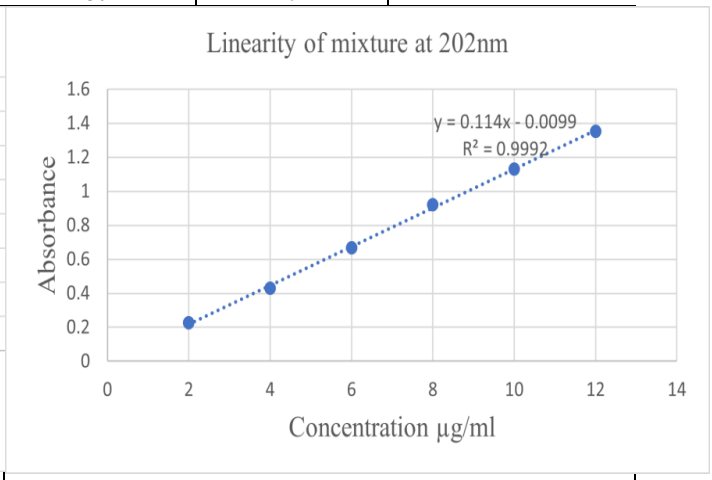


Figure 3 (b): Calibration curve plot of linearity of the mixture at 202nm

Table 2: Results of Intraday and Interday precision

Intraday Precision			Interday Precision		
Sr. No.	Absorbance of Mixture at 202nm	Absorbance of Mixture at 338nm	Sr. No.	Absorbance of Mixture at 202nm	Absorbance of Mixture at 338nm
(Day 1) 1	0.682	0.340	(Morning) 1	0.646	0.330
2	0.675	0.338	2	0.684	0.346
3	0.679	0.342	3	0.678	0.339
(Day 2) 1	0.678	0.334	(noon) 1	0.684	0.341
2	0.670	0.339	2	0.681	0.347
3	0.679	0.345	3	0.675	0.343
(Day 3) 1	0.685	0.344	(Evening)1	0.681	0.336
2	0.680	0.341	2	0.684	0.340
3	0.683	0.346	3	0.673	0.335
Average	0.679	0.339889	Average.	0.676222	0.339667
SD	0.0044	0.0042	SD	0.120	0.005
%RSD	0.65	1.24	%RSD	1.77	1.59

Table 3: Results of Intermediate Precision

Sr. No.	Absorbance of mixture at 202nm	Absorbance of mixture at 338nm
(Analyst 1) 1	0.716	0.339
2	0.693	0.338
3	0.69	0339
(Analyst 2)1	0.672	0.327
2	0.674	0.327
3	0.676	0.328
(Analyst 3) 1	0.693	0.338
2	0.69	0.34
3	0.691	0.336
Average	0.688	0.334
SD	0.013	0.005
%RSD	1.953	1.677

Table 4: Results of Robustness

Drug	Conc (µg/ml)	Amount found (µg/ml) mean	% Assay ± %RSD
λ Changed to 201nm for COS and 337nm for DOH			
COS	6	6.75	112.5 ± 0.161

DOH	**12**	**12.23**	**101 ±0.173**
λ Changed to 202nm for COS and 338nm for DOH			
COS	**6**	**6.55**	**109 ±0.0931**
DOH	**12**	**12.24**	**102±0.299**
λ Changed to 203nm for COS and 339nm for DOH			
COS	**6**	**6.52**	**104 ±0.162**
DOH	**12**	**12.23**	**101 ±0.457**

Table 5: Results of Accuracy/Recovery

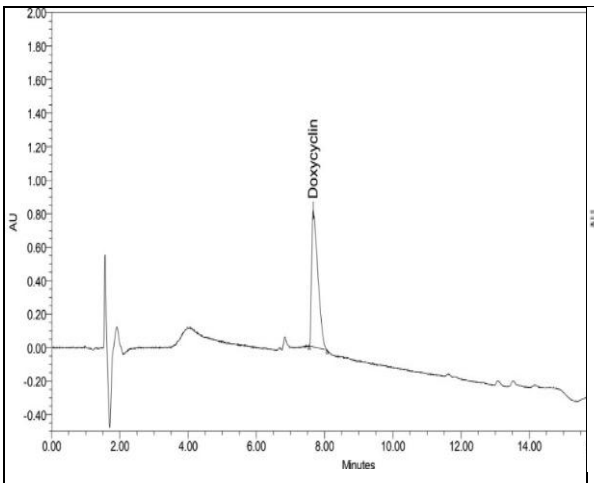
Wavelength	**COS**			**DOH**		
	202 nm			**338nm**		
% Recovery Level	**80 %**	**100 %**	**120 %**	**80 %**	**100 %**	**120 %**
Amt. added (µg/ml)	**4.8**	**6**	**7.2**	**9.6**	**12**	**14.4**
Amt. Recovered	**5.54**	**6.84**	**8.41**	**9.72**	**12.24**	**14.66**
	5.54	**6.84**	**8.26**	**9.68**	**12.24**	**14.62**
	5.52	**6.64**	**8.39**	**9.72**	**12.28**	**14.67**
Avg. of amt. recovered	**5.54**	**6.77**	**8.35**	**9.70**	**12.25**	**14.67**
Avg. % recovery	**115.4**	**112.6**	**116**	**101.1**	**102**	**101.8**
SD of drug content recovered	**0.02**	**0.115**	**0.081**	**0.023**	**0.023**	**0.055**
% RSD of drug content recovered	**0.36**	**1.70**	**0.97**	**0.237**	**0.188**	**0.379**

Results of assay

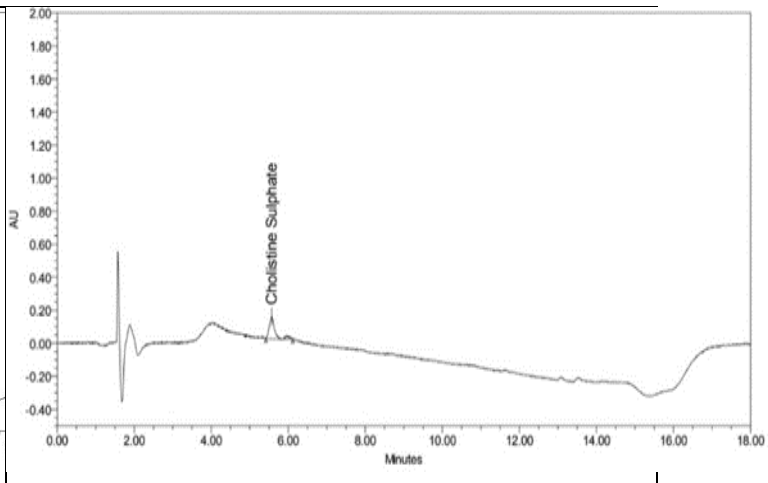
The assay findings for COS and DOH show that for COS with a label claim of 117 mg, the theoretical content was 6 µg/ml, while the practical content measured was 7.11 µg/ml, resulting in a percentage assay of 116.3% (w/w). For DOH with a label claim of 250 mg, the theoretical content was 12 µg/ml, whereas the practical level was 12.09 µg/ml, yielding a percentage assay of 101.4% (w/w).

High-performance liquid chromatography

DOH showed a peak at 7min whereas COS showed a peak at 5min. A Chromatogram of the mixture is shown in **Figure 4** indicating good separation of both drugs.



(a) Chromatogram of DOH



(b) Chromatogram of COS

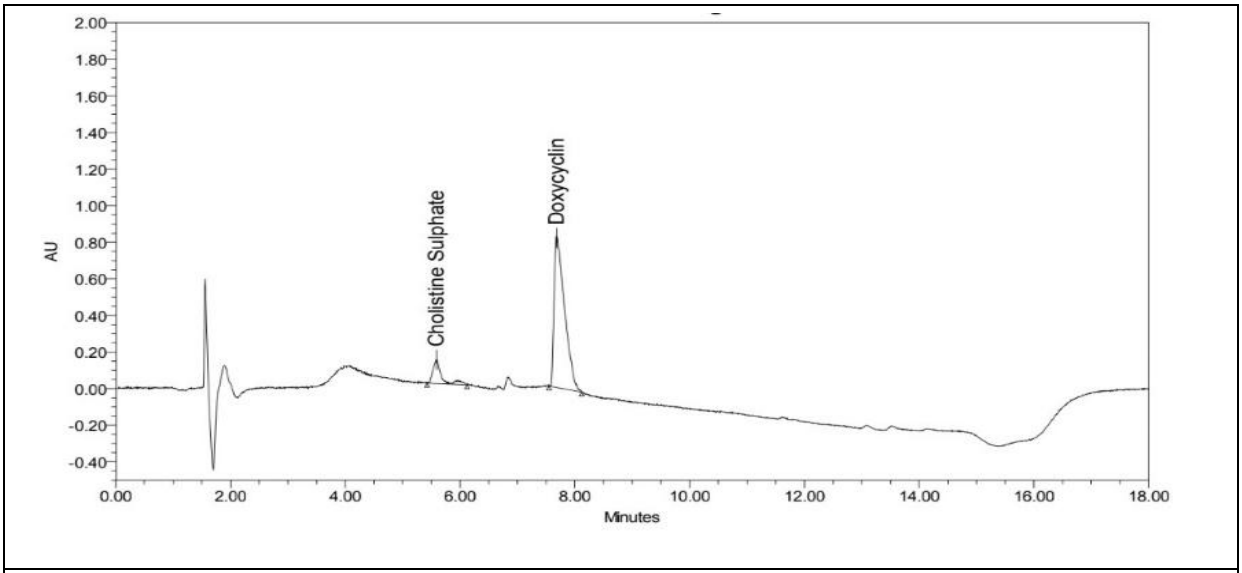


Figure 4 (c): Chromatogram of the mixture of DOH and COS

Table 6: Result of Linearity of DOH and COS in a mixture

Concentration (µg/ml)	Peak area of DOH (Avg)	Concentration (µg/ml).	Peak area of COS (Avg)
1500	15336503	750	1967741
1250	12943017	625	1662199
1000	10303444	500	1336291
750	7654075	375	969525
500	5261229	250	645725
250	2868383	125	321925
DOH	LOD [µg/ml]: 30.92 LOQ [µg/ml]: 93.70	COS	LOD [µg/ml]: 17.54 LOQ [µg/ml]: 53.16

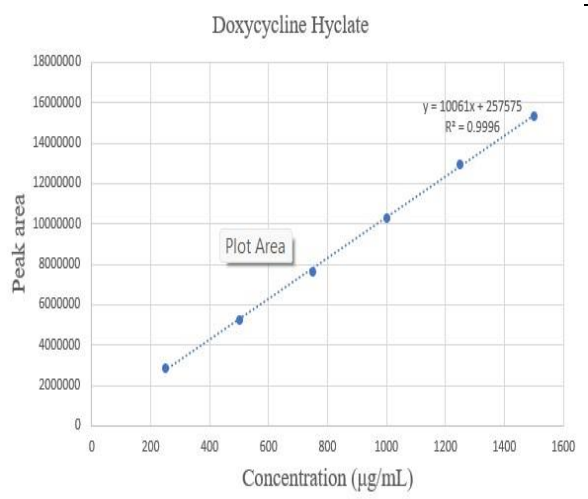
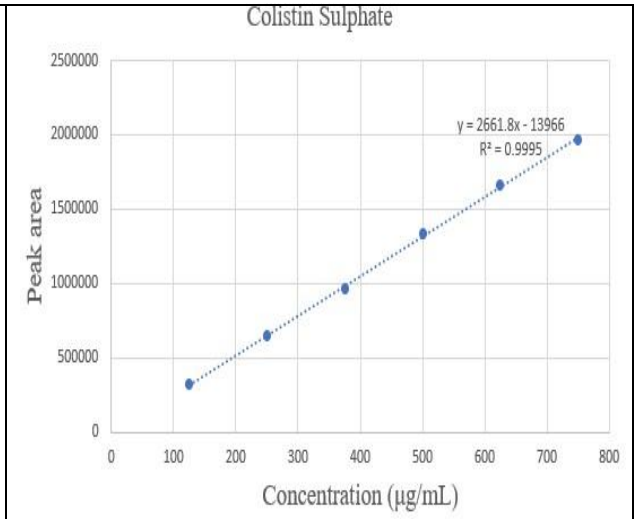


Figure 5: (a) Calibration Curve of DOH in mixture



(b) Calibration Curve of COS

Table 7: Results of Accuracy/Recovery

Wavelength	COS			DOH		
% Recovery Level	50 %	100 %	150 %	50 %	100 %	150 %
Amt. added (µg/ml)	250	500	750	500	1000	1500
Amt. Recovered	249.4	505.7	752.7	497.3	998.4	1498.7
	249	508.3	749	497.1	1006.4	1500
	249.4	505.7	751.3	499.1	997.4	1501
Avg. of amt. recovered	249.2	507	751	497.8	1000.7	1501.1
Avg. % recovery	99.7	101.3	100	99.56	100	100
SD of drug content recovered	0.208	1.30	1.86	1.09	4.97	3.1
% RSD of drug content recovered	0.083	0.257	0.248	0.218	0.492	0.206

Results of Repeatability

The repeatability test findings for DOH and COS showed that the peak areas measured across six trials averaged 10,285,561.3 for DOH and 1,392,359 for COS. The standard deviations (SD) for DOH and COS were 97,958.8 and 10,839.4, respectively. The percentage relative standard deviation (%RSD) was calculated to be 0.952% for DOH and 0.778% for COS, both of which are within the acceptability standards of not exceeding 2% (%RSD ≤ 2%). As a result, the assay's repeatability is confirmed to be within acceptable limits.

Table 8: Results of Robustness by change in flow rate

Drug	Peak area (Average)	Drug content (µg/ml) mean	% Assay ± %RSD
(Changed in flow rate 1.0ml/min to 0.9ml/min)			
COS	1374759	521.7	104.3 ± 0.375
DOH	10603869	1028.3	102 ± 0.876
(Actual flow rate 1.0ml/min)			
COS	1338831	502.2	101.6 ± 0.224
DOH	10326400	1000.7	100± 0.559
(Changed in flow rate 1.0ml/min to 1.1ml/min)			
COS	1271492	482.9	96.5 ± 0.148
DOH	9828869	951.3	95 ±0.535

Table 9: Results of Robustness by change in column Temperature

Drug	Peak area (Average)	Drug content (µg/ml) mean	% Assay ± %RSD
(Changed in Column Temp 30°C to 28°C)			
COS	1268878	481.9	96.3 ± 0.516
DOH	9947519	963.1	102 ± 0.876
(Actual Column Temp 30°C)			
COS	1335854	507.1	101.4 ± 0.296
DOH	10330062	1001.1	96.3± 0.367
(Changed in Column Temp 30°C to 32°C)			
COS	1426414	541	108.2 ± 0.159
DOH	9813395	953.1	95.3 ±0.301

Acceptance Criteria: %RSD should be NMT 2%

Conclusion: %RSD for Robustness was found to be less than 2% which is within the limit.

Results of Assay

The assay results show that COS with a label claim of 117 mg had a theoretical content of 500 µg/ml and a practical content of 506 µg/ml, yielding a percentage assay of 101.2% (w/w). DOH with a label claim of 250 mg had a theoretical content of 1000 µg/ml and a practical content of 1007.9 µg/ml, resulting in a percentage assay of 100.7% (w/w).

DISCUSSION

This study successfully developed and validated UV spectroscopy and High-Performance Liquid Chromatography (HPLC) methods for the simultaneous estimation of doxycycline hyclate (DOH) and colistin sulphate (COS) in a mixture. Both methods demonstrated robust, precise,

and accurate quantification, making them suitable for routine analysis.

UV-Spectroscopic Method

The UV method employed absorption correction at 338 nm and 202 nm for DOH and COS, respectively. The method exhibited excellent linearity with correlation coefficients close to 1, and precision with %RSD values below 2% for both intra-day and inter-day studies. Recovery rates near 100% further validated the accuracy, confirming its suitability for routine analysis, especially in resource-limited settings.

HPLC Method

The HPLC method, utilizing a Zorbax XDB C18 column and gradient elution, provided higher sensitivity and specificity, particularly for COS. The method showed strong linearity with correlation coefficients above 0.999 and low LOD and LOQ values. The %RSD values remained within acceptable limits, underscoring the method's reliability.

Comparison and Applicability

Both methods are effective for analyzing DOH and COS in pharmaceutical formulations. The UV method is advantageous for its simplicity and cost-effectiveness, while the HPLC method is preferred for detailed analysis due to its higher sensitivity. These methods are essential tools for quality control in pharmaceutical industries, ensuring the efficacy of combination therapies involving these antibiotics.

REFERENCES

[1] Talaro KP, Talaro AJ. Foundations in Microbiology: Basic Principles. 10th ed. McGraw-Hill Education; 2017

[2] Benet LZ, Yoo MS. Doxycycline: A review of its pharmacokinetics, mechanisms of action, and clinical uses. J Clin Pharmacol. 2022;62(2):220-231.

[3] Landman D, Georgescu C, Martin DA, Quale J. Polymyxins revisited. Clin Microbiol Rev. 2008;21(3):449-465.

[4] Lim LM, Ly N, Anderson D, Yang JC, Macander L, Jarkowski A III, et al. Resurgence of colistin: A review of resistance, toxicity, pharmacodynamics, and dosing. Pharmacotherapy. 2010;30(12):1279-1291.

[5] Li J, Nation RL, Turnidge JD, Milne RW, Coulthard K, Rayner CR, et al. Colistin: The re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. Lancet Infect Dis. 2006;6(9):589-601.

[6] Ramesh PJ, Basavaiah K, Divya MR, et al. Simple UV and visible spectrophotometric methods for the determination of doxycycline

hyclate in pharmaceuticals. J Anal Chem. 2011;66:482-489.

[7] Md K, Hasan K, Haque M, Kobir SM, Hossain M. Quantification of doxycycline hyclate in different pharmaceutical samples by UV assay. Eur J Biol Res. 2016;6:275-286. doi:10.5281/zenodo.163091.

[8] Sahoo P, Sahoo P, Mohapatra J, Panigrahi D, Patra A, Mishra A. Development and validation of stability indicating assay method of doxycycline hyclate by using UV-spectrophotometer. J Drug Deliv Ther. 2023;13:89-94. doi:10.22270/jddt.v13i6.6087.

[9] Mileva R. Determination of free doxycycline concentrations in the plasma and milk of sheep and in the plasma of rabbits by using the HPLC method. Maced Vet Rev. 2019;42(2):123-130. doi:10.2478/macvetrev-2019-0016.

[10] Ghidini L, Kogawa AC, Salgado HRN. Eco-friendly green liquid chromatographic method for determination of doxycycline in tablets and in the presence of its degradation products. Drug Anal Res. 2018;2:49-55. doi:10.22456/25272616.89412.

[11] Skúlason S, et al. Development of a simple HPLC method for separation of doxycycline and its degradation products. J Pharm Biomed Anal. 2003;33(4):667-672. doi:10.1016/s0731-7085(03)00316-9.

[12] Pippalla S, Nekkalapudi AR, Jillellamudi SB, Reddy MP, Kumar CV. A stability-indicating, reversed-phase HPLC method for quantification of assay and organic impurities in doxycycline hyclate bulk and parenteral dosage forms. Biomed Chromatogr. 2023;37(6):5626. doi:10.1002/bmc.5626.

[13] Pourmoslemi S, Mirfakhraee S, Yaripour S, Mohammadi A. Development and validation of a stability-indicating RP-HPLC method for rapid determination of doxycycline in pharmaceutical bulk and dosage forms. Pharm Sci. 2016;22:96-104.

[14] Injac R, Djordjevic-Milic V, Srdjenovic B. Thermostability testing and degradation profiles of doxycycline in bulk, tablets, and capsules by HPLC. J Chromatogr Sci. 2007;45(9):623-628. doi:10.1093/chromsci/45.9.623

[15] Elimam M, Shantier S, Gadkariem E, Mohamed M. Derivative spectrophotometric methods for the analysis and stability studies of colistin sulphate. E-J Chem.

2015;1-5.
doi:10.1155/2015/624316.

[16] Cancho-Grande B, Rodríguez-Comesaña M, Simal-Gándara J. Simple HPLC determination of colistin in medicated feeds by pre-column derivatization and fluorescence detection. Chromatographia. 2001;54:481-484.