Analytical Quality by Design Approach to RP-HPLC Method Development and Validation for Simultaneous Estimation of Ivermectin and Paracetamol in Modified-Release Dosage Form

Amit Kumar[1], Deepanjan Dutta[2], Kalpana[3]

[1]*Research Scholar, Lloyd Institute of Management & Technology, Greater Noida, Uttar Pradesh*
[2]*Associate Professor, Lloyd Institute of Management & Technology, Greater Noida, Uttar Pradesh*
[3]*Assistant Professor, Lloyd Institute of Management & Technology, Greater Noida, Uttar Pradesh*

Abstract

In this research, we have successfully developed a precise, straightforward, and comprehensive RP-HPLC method for the simultaneous quantification of Ivermectin and Paracetamol in tablet form. The method exhibited exceptional specificity and rapid analysis, rendering it suitable for routine use in measuring these analytes in laboratory-prepared mixtures and in isolation. A UV detector set at 245nm facilitated the determination of Ivermectin's retention time at 4.198 minutes and Paracetamol's retention time at 2.563 minutes. The method displayed a strong correlation (average R2 value of 0.9798) between Ivermectin concentrations ranging from 1 to 32 µg/ml and Paracetamol concentrations ranging from 2.815 to 12.387 µg/ml, indicating excellent intraday and interday precision with low relative standard deviation (RSD) for both analytes. The calculated limit of quantification (LOQ) and limit of detection (LOD) for Ivermectin were determined as 2.93 and 8.79 µg/ml, respectively, and for Paracetamol, as 3.50 and 9.21 µg/ml, respectively, showcasing the method's sensitivity in detecting low concentrations of both compounds. These values underscore the method's suitability for precise and accurate determination of both Ivermectin and Paracetamol at low levels, rendering it applicable for quality control and pharmaceutical analysis purposes. The RP-HPLC method underwent thorough validation in accordance with the guidelines established by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), ensuring the reliability and accuracy of the results for both analytes. The method's applicability extends to the analysis of Ivermectin and Paracetamol in injections, tablets, and various other formulations, delivering fast, detailed, selective, and accurate outcomes.

Keywords: Analytical Quality by Design, RP-HPLC, Ivermectin, Paracetamol, Modified-Release Dosage Form, Method Development, Method Validation.

DOI:10.48047/ecb/2022.11.12.151

Introduction

The development and validation of reliable and robust analytical methods for the quantification of active pharmaceutical ingredients (APIs) in pharmaceutical formulations are essential steps in ensuring the safety, efficacy, and quality of medicinal products[1]. Among the myriad of analytical techniques available, reversed-phase high-performance liquid chromatography (RP-HPLC) stands out as a versatile and widely adopted method for the separation and quantification of pharmaceutical compounds[2]. In this context, this research paper delves into the systematic development and validation of an RP-HPLC method using an Analytical Quality by Design (AQbD) approach, targeting the simultaneous estimation of Ivermectin and

Paracetamol in a modified-release dosage form[3]. Ivermectin, a potent antiparasitic agent, and Paracetamol, a widely used analgesic and antipyretic, are frequently formulated together in pharmaceutical dosage forms[4]. Ensuring accurate quantification of both these active ingredients in a single method is crucial for assessing the product's efficacy, safety, and consistency[5]. Furthermore, the analysis of modified-release dosage forms adds complexity to the analytical process, requiring a meticulous and robust method that can effectively separate and quantify the active ingredients throughout the controlled release profile[6]. The principles of Analytical Quality by Design (AQbD) offer a systematic and comprehensive framework for method development and validation, emphasizing the understanding and control of critical method parameters throughout the analytical lifecycle[7]. This approach is particularly beneficial in the field of pharmaceutical analysis, where ensuring the reliability and robustness of analytical methods is of paramount importance[8,9]. The objective of this study is to apply AQbD principles to the development of an RP-HPLC method for the simultaneous estimation of Ivermectin and Paracetamol in a modified-release dosage form. By doing so, we aim to establish a method that not only meets regulatory requirements but also provides enhanced selectivity, sensitivity, and precision[10]. This method, once developed and validated, can be a valuable tool for routine quality control analysis of pharmaceutical products containing Ivermectin and Paracetamol[11,12]. The subsequent sections of this paper will provide a detailed account of the experimental procedures, method optimization, method validation, and the results obtained, shedding light on the successful application of AQbD in the development of an efficient and reliable RP-HPLC method for the simultaneous estimation of Ivermectin and Paracetamol in modified-release dosage forms[13].

Material and method

Materials

In this study, various materials were employed for the development and validation of the analytical method. Ivermectin and Paracetamol, the two active pharmaceutical ingredients under investigation, were generously provided as gift samples by Sell Well Pharmaceuticals located in Indore. The solvents used for chromatographic analysis included Methanol (sourced from CDH, New Delhi), Acetonitrile (HPLC Grade, CDH, New Delhi), Water (HPLC Grade, Rankem), and Methanol (HPLC Grade, Qualigens). Additionally, other reagents and solvents used in the study included Tetrahydrofuran (CDH, New Delhi), Ethanol (obtained from Merck's Chemicals, Germany), Ethyl Acetate, Acetone (both purchased from CDH, New Delhi), and Potassium Bromide (KBR, CDH, New Delhi).

Methods

Physicochemical properties of drug

The drug was studied based on its physical appearance and morphology. Various characteristics like smell, color, odor, and appearance were observed by naked eyes.
White crystalline powder

Melting Point

Using a digital melting point instrument, the melting point of IVM was established. The melting point of the medication was determined using a capillary tube approach. The medication was placed in digital melting point equipment after being filled in a capillary tube and closed at one

end[14].

Solubility

A solubility test was performed as a test for clarity. The solubility of the drug was examined in Acetonitrile, ethyl acetate, acetone, methanol, water, and n-hexane. 10 mg of drug was taken in a well clean 100ml beaker and the solvent was added gradually in an aliquant of 1ml with continuous stirring until it dissolves completely. The amount of solvent requisite for the solubilization of the drug was recorded and compared with reported values[15].

UV-SPECTRUM Analysis:

The determination of the λmax for ivermectin in methanol was carried out as follows: Initially, 100 mg of ivermectin was accurately weighed using a high-precision electronic balance and then diluted with 100 g of methanol. Subsequently, 10 mL of the stock solution was added to 10 mL of the stock solution, and a 100 mL volumetric flask containing 1000 g of the drug was dissolved in methanol to prepare a 1000 g/ml stock solution. To create a working solution with a concentration of 10 g/ml, 1 mL of the standard solution was aliquoted and diluted to 10 mL with ethanol. The λmax was determined using a Shimadzu UV-1800 spectrophotometer [16].

FTIR Spectra

It was also done for the identification of the drug. The FTIR measurement of samples i.e. the pure drug and test drug can be measured by using an FT-infrared spectrophotometer (FTIR Affinity 1, Shimadzu, Japan) using KBr disc method. The samples were scanned under full-range spectra (4000cm-1 to 400cm-1).

HPLC Method Development and Validation

The HPLC method was developed and validated for the analysis of Ivermectin in pharmaceutical tablet dosages. Ivermectin tablets were procured from Pradhan Mantri Jan Aushadi medical store, Dehradun, and each tablet contained 6 mg of Ivermectin. The chromatographic separation was carried out using a Waters-HPLC system equipped with an autosampler, a UV detector, and a column oven. The data acquisition and analysis were performed using Empower-2 software. An Inertsil ODS column with dimensions of 250 mm x 4.6 mm was utilized for separation. The mobile phase consisted of Acetonitrile and HPLC-grade Methanol in isocratic mode. The mobile phase was delivered at a flow rate of 1.0 mL/min with a total runtime of 10 minutes. The column temperature was maintained at 25°C, and the detection wavelength was set at 245 nm[17]. Prior to sample injection, the column was equilibrated for a minimum of 30 minutes, and each injection had a volume of ten microliters. The eluents were assessed at the specified wavelength to determine the presence and quantity of Ivermectin in the tablet samples.

RP-HPLC Method Validation

Linearity

Using diluents, make up the volume using stock solution diluted at 1,2,4,8,16,32 g/ml in volumetric flasks of 10 ml to establish calibration curves for ivermectin. A calibration curve was created by graphing the peak area against the drug concentration for each concentration level. The sample volume was 10 l at each concentration level. Peak area and concentration were clearly correlated within the study's range. A table was included with the calibration curve and observations[18].

System Precision

Precision is the assessment of how close data values for a digit of measurements are to one

another under similar analytical settings. Ivermectin standard solutions were grouped according to procedure and infused many times. The responses' outcomes are listed in Table.

Method Precision

On different days, multiple estimates of ivermectin were conducted to establish the accuracy of the approach. During analysis, we found the relative standard deviation (RSD) to be less than 2%, which is within acceptable limits. Table 1 presents the results.

Accuracy

Standard quality control samples manufactured in triplicate were used to compare the accuracy of the UV spectrophotometer. The proportion of analytes recovered from a standard concentration of analyte is a measure of the assay's precision. Nine concentration measurements throughout the whole range were analyzed, with results presented for each of the three concentrations[19].

Limit of Quantification & Detection

The signal-to-noise ratio (S/N) can be calculated by dividing the sample concentration by the fraction of the lowest standard concentration (LSC). As with the calculation of the Limit of Quantification (LOQ), the signal-to-noise ratio (S/N) can be calculated as follows: In order to determine the limit of quantification (LOQ), one assumes ten times the low concentration of the standard, then divides that value by the sample concentration. Analytical chemists typically use LOD and LQ criteria to evaluate a methodology's sensitivity.The aforementioned values represent the lower limit of concentration for detecting (LOD) or quantifying (LOQ) an analyte with a defined degree of confidence[20].

Robustness

To evaluate the resilience of the methodology, the appropriate chromatographic parameters were modified, such as the adjustment of the mobile phase composition to 10%. Several parameters were not significantly affected by the modifications made to the operational specifications in the study, including the retention time of the peak of interest, the resolution (which needed to be greater than or equal to 2.00), the number of plates (which needed to be higher than or equal to 2000), the tailing factor, and the relative standard deviation percentage. All of these factors were therefore determined to meet the established acceptance criteria[21].

Suitability of System

Chromatographic systems are evaluated using the suitability of system test for the analyses that are planned. A multiple injection test was used to determine whether the technique was suitable for the system. A majority of parameters, such as peak area, theoretical plates, plate height, and tailing factor, were evaluated in accordance with USP specifications. There was a reasonable amount of variation in the observed RSD values (NMT 2%).

Specificity and Selectivity

As a result, specificity is a measure of how well an analytical technique works in the presence of disruptions such as contaminants and degradation products. To determine the specificity, we used the resolution factor of the drug peak compared to its neighboring peak. Degradation peaks' purity determines the selectivity of the approach[22,23].

Result and discussion

Solubility

The results obtained for the solubility has been given in the table and according to this we have chosen acetonitrile and methanol as a solvent system for our HPLC method.

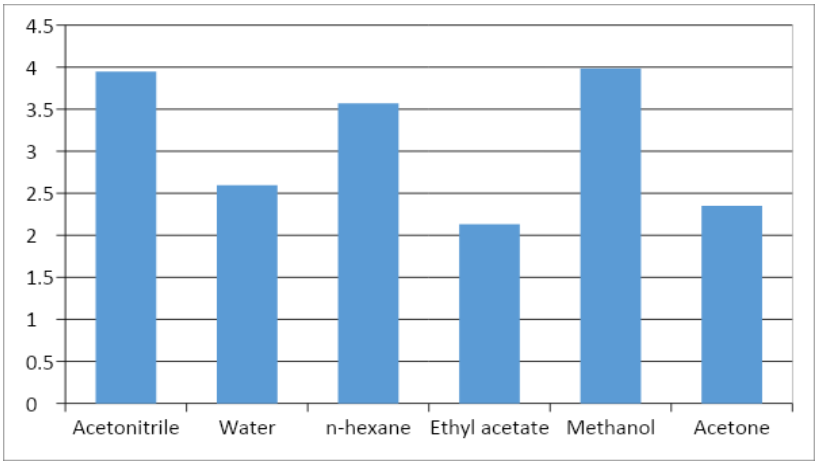

Figure 1 Solubility of drug in different components

Calibration Curve of drug in UV Spectrophotometer

The results obtained for the drug ivermectin under UV Spectrophotometer with different conc. range has been given in the following table with their absorbance reading.

Table 1 concentration range of observing absorbance for constructing calibration curve

Drug Concentration (µg/ml)	Absorbance Ivermectin	Absorbance Paracetamol
1	0.082	0.135
2	0.147	0.264
4	0.296	0.509
6	0.436	0.758
8	0.599	1.002
16	1.191	1.248

The calibration curve for the drug in methanol is expressed in figure 2 and it follows the linear straight line with high linearity as better with correlation coefficient 0.999.

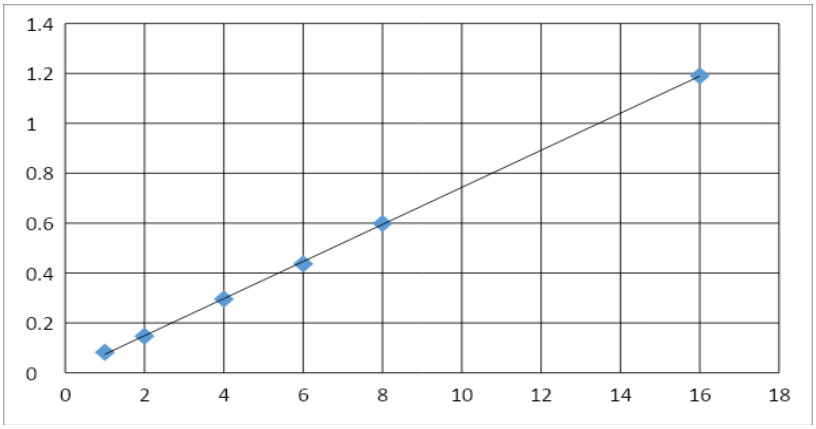

Figure 2 Calibration curve of Drug in methanol

FTIR Spectra

The results for both the pure drug and the test drug are presented and interpreted in Table 2.

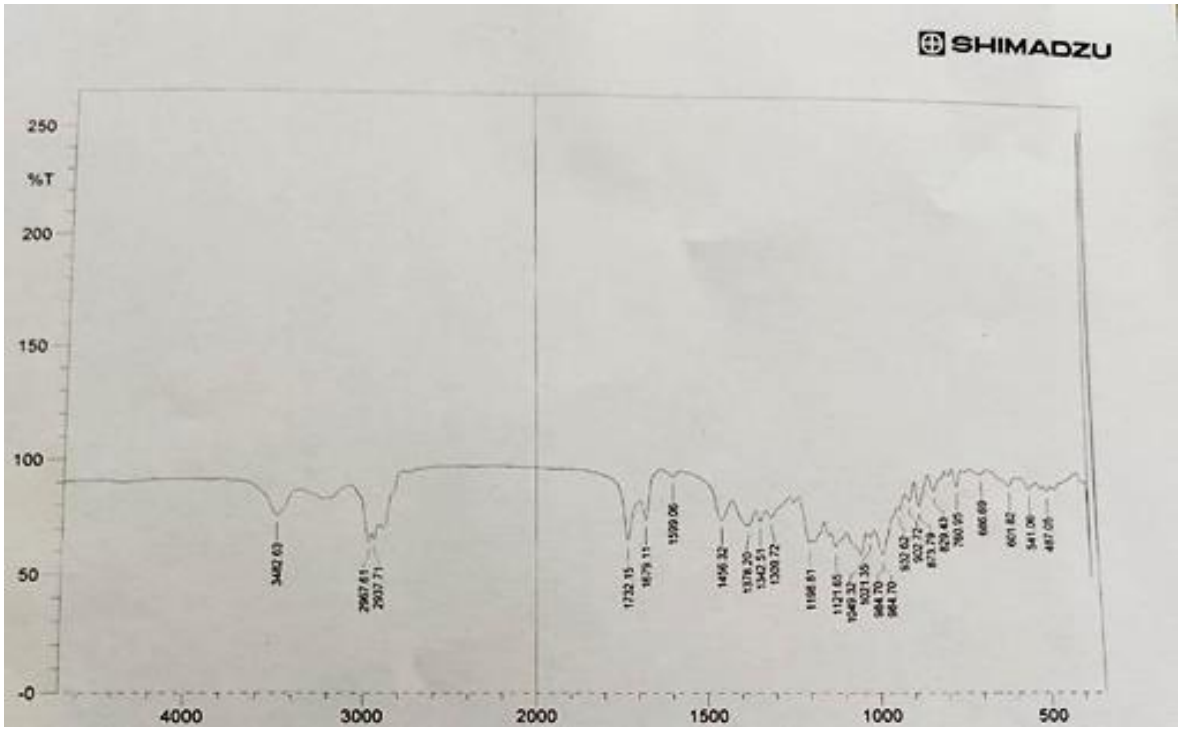

Figure 3 FTIR Spectra of Ivermectin

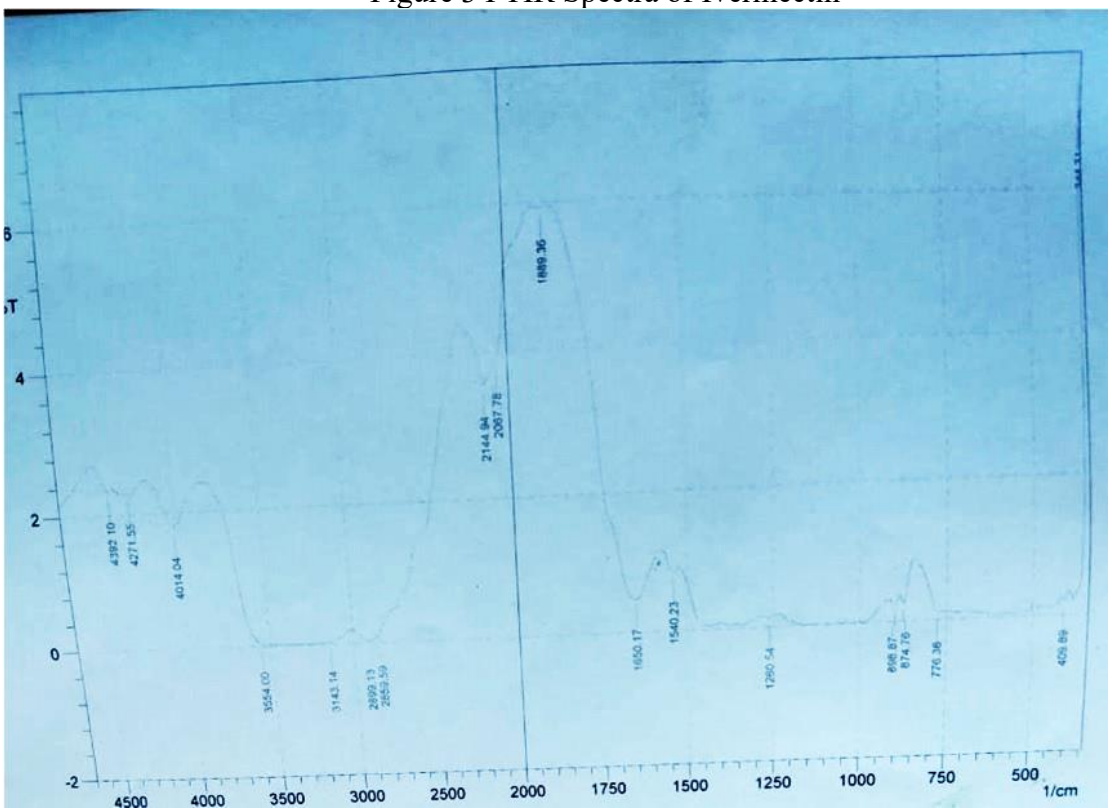

Figure 4 FTIR Spectra of Paracetamol

Table 1 Interpretation of FTIR

S.NO.	IR	IVERMECTIN	PARACET	EXPECTED RANGE

	VIBRATIONAL FREQUENCIES		**AMOL**	**(cm^{-1})**
1.	C→H Stretching	2967.61-2937.71	2899.13	2960 - 2870
2.	C↓H Bending ($CH_2\delta$)	1456.32	1540.23	1475 - 1450
3.	O→H Stretching	3482.63	3554 – 3143.14	3650 - 3200
4.	O↓H Bending	1378.20 - 1342.51	1260.54	1450 - 1200
5.	C→O Streching of alcohol	1198.81	1260.54	1260 – 970 (strong, doublet)
6.	COO-H Stretching	3482.63	3554 – 3143.14	3550 - 2500
7.	C=O Stretching	1732.15 -1679.11	1650.17	1740 – 1650 (H-bonded,dimer)
8.	C=C Stretching	1599.06	1650.17	1690 - 1635
9.	Cyclic alkenes C=C Streching	1679.11	1650.17	1780 – 1610 (1675)
10.(specific)	5-Ring hetero saturated cyclic ether C-O-C Steching assymetrical	1049.32 (symmetrical) 902.72(asymmetrical)	1260.54 898.87(assymetry)	≈1070 symmetrical ≈915 asymmetrical
11.(specific)	6- Ring hetero saturated cyclic ether	1121.65(asymmetrica) 829.43(symmetrical)	898.87 776.38	≈ 1100 $asymmetrical$ ≈815 symmetrical
12. (general)	C-O-C Strech. Assymetrical of ether	1198.81	1260.54	1310 – 1000 (strong,split)
13.	C-O-C Streching symmetrical of ether	1049.32 – 873.79	898.87 – 874.76	1055 – 870 (strong,multiple bonds)

RP-HPLC Method development and validation

Chromatograms

The chromatogram showing the results of separating the component of a mixture by chromatography and the chromatogram of standard drug and test drug are given below:

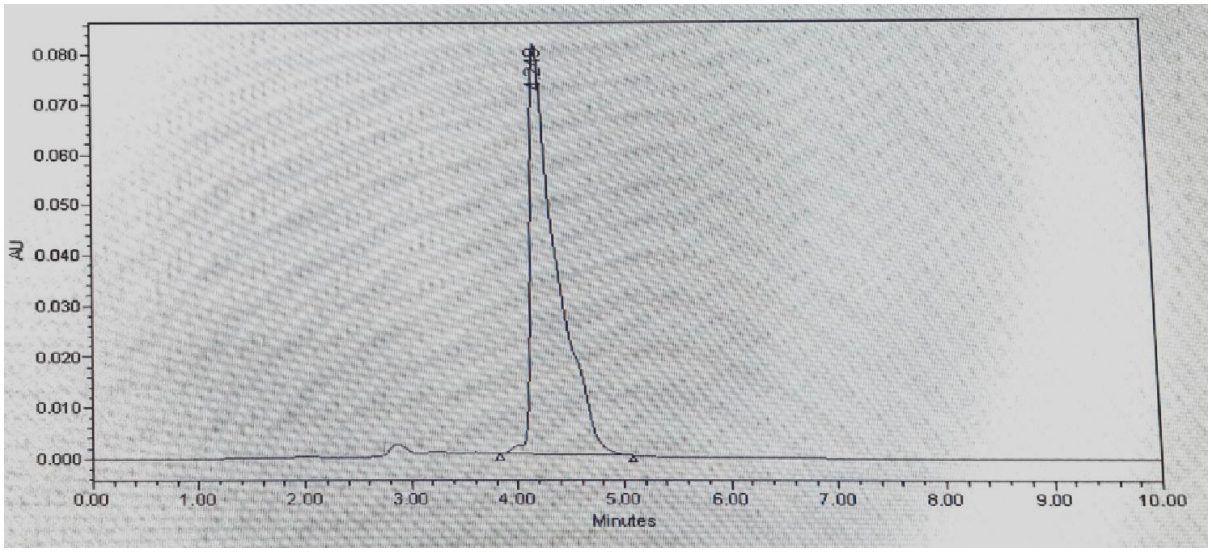

Figure 5 Chromatogram of Ivermectin

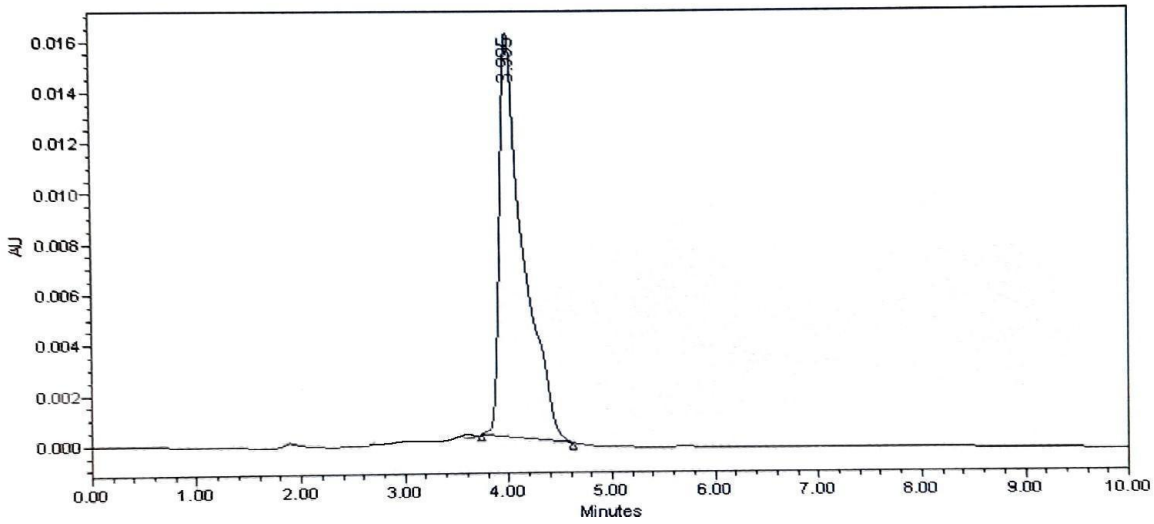

Figure 6 Chromatogram of Paracetamol

RP-HPLC method Validation

Precision

Tables 3 and 4 present the data obtained during the validation of both system precision and method precision.

Table 3 System Precision of Drug

S.no.	Drug Concentration (µg/ml)	Peak Area	SD %	RSD %
1.	32	1865995	303248.1208	1.6283
2.	32	1813794		
3.	32	1878828		
4.	32	1904717		
5.	32	1860303		
6.	32	1849583		

Table 4 System Precision of Drug

S.no.	Drug Concentration (µg/ml)	Peak Area	SD %	RSD %

1.	32	1842586	24820.2538	1.352
2.	32	1869521		
3.	32	1833860		
4.	32	1830698		
5.	32	1793472		
6.	32	1844496		

Accuracy

As shown in table 5, accuracy was measured using a UV spectrophotometer.

Table 5 Accuracy data

S.no.	Drug Concentration (µg/ml)	Peak Area
1.	1	168943.33
2.	2	218905.83
3.	4	399692
4.	8	357288.66
5.	16	717802.5
6.	32	1272663.16

Limit Of Detection & Quantification

A limit of detection (LOD) and a limit of quantification (LOQ) were calculated for each analyte by analyzing their slope and standard response deviation. Estimated detection and quantification limits were determined using these values.

Table 6 LOD & LOQ Values

Sample	LOD	LOQ
Ivermectin	2.93	8.79
Paracetamol	1.98	3.3

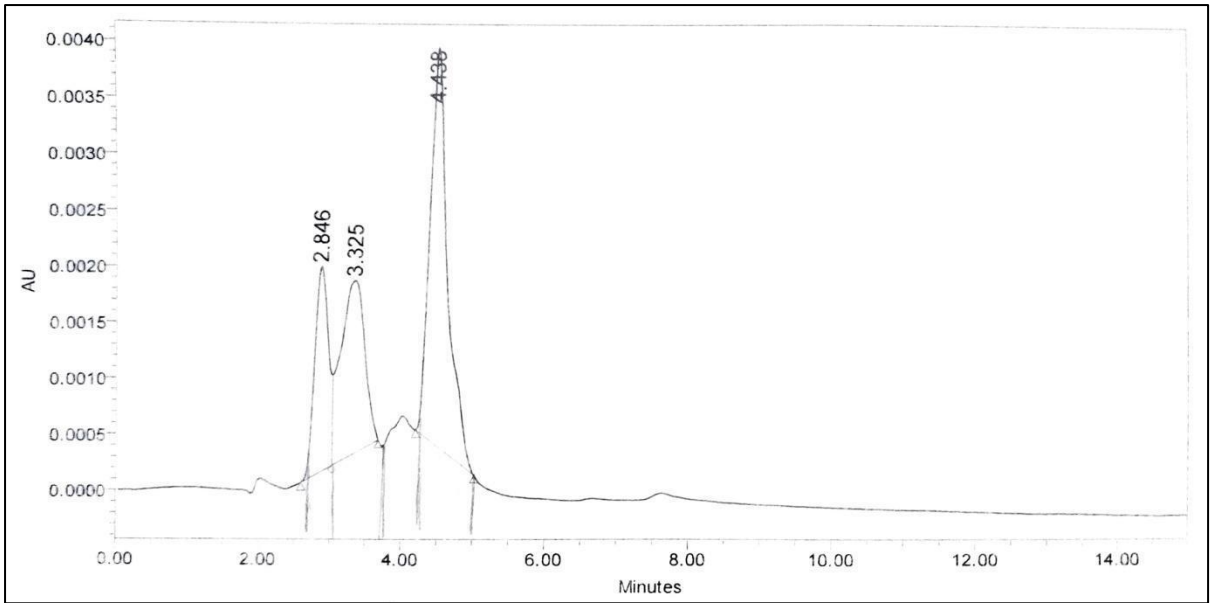

Figure 7 Chromatogram of LOD & LOQ

Robustness

Using different mobile phases, the robustness was tested and the following peak areas or chromatograms were obtained:

Table 7 Robustness Data

S.No.	Robustness Condition	Peak Area of Ivermectin
1	Change in Mobile phase (Tetrahydrofuran : H2O)	142922
2	Change in Mobile phase (Methanol : H2O)	114914
3	Change in Mobile phase (Acetonitrile : H2O)	147725

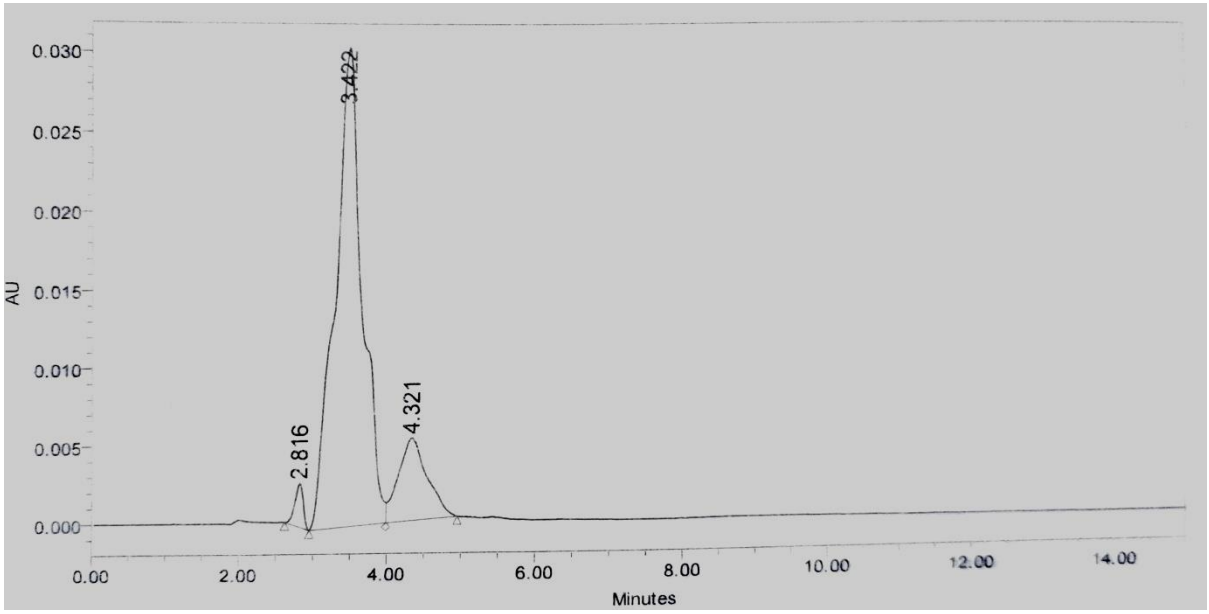

Figure 8 Chromatagram of THF : H2O

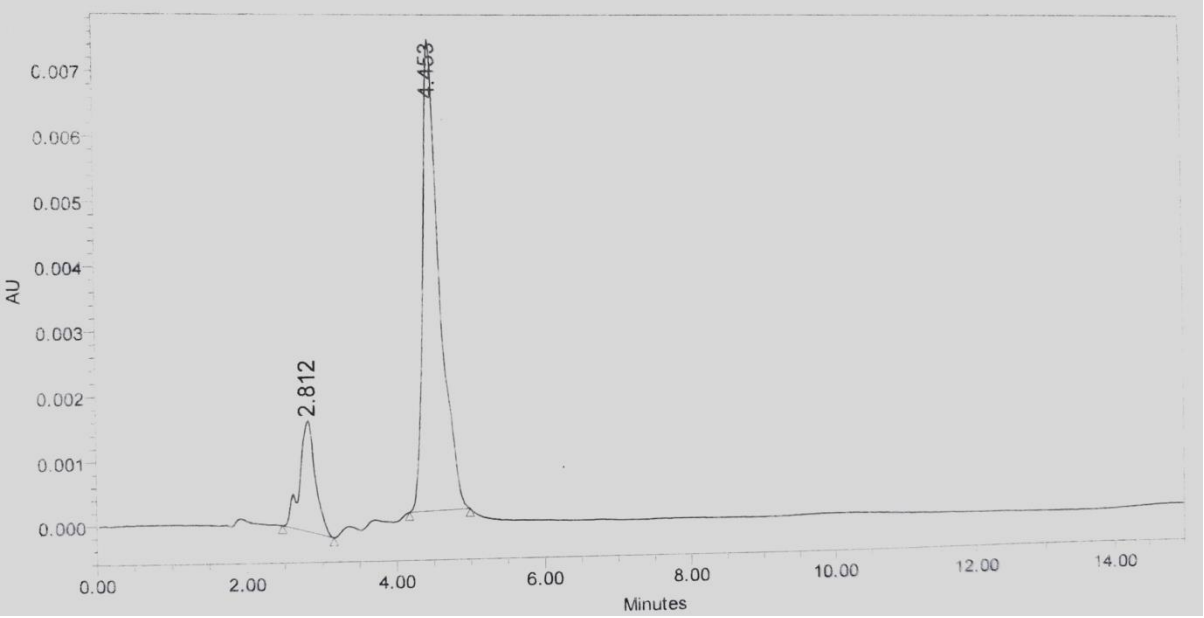

Figure 9 Chromatagram of METHANOL : H2O

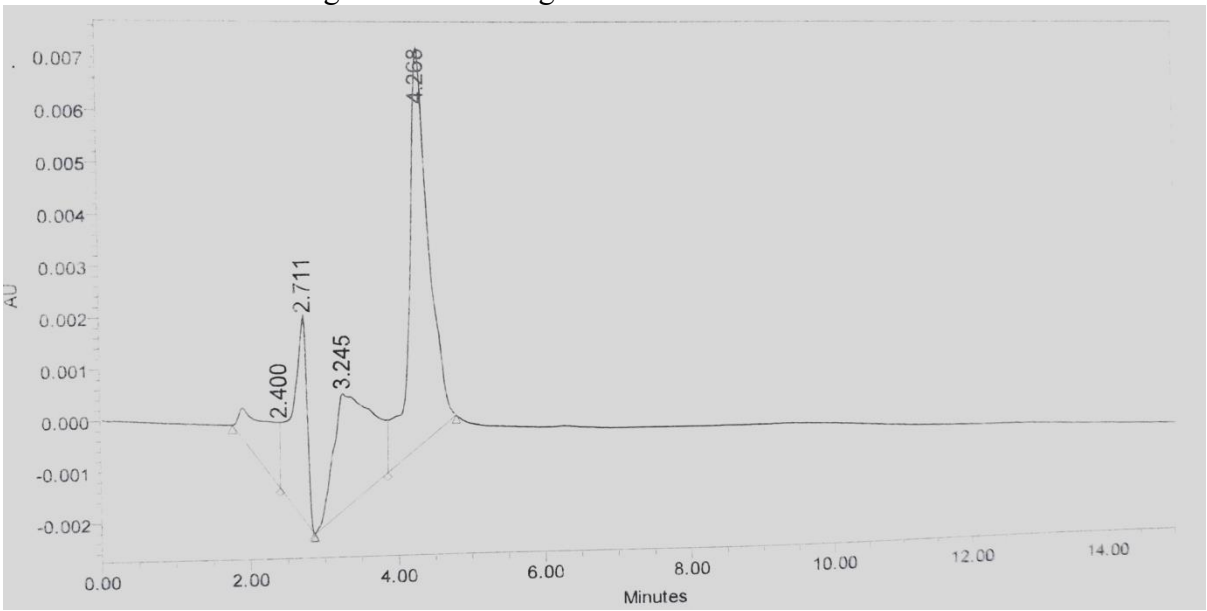

Figure 10 Chromatagram of ACETONITRILE : H2O

System suitability

Based on a combination of factors, the results of the system suitability evaluation are as follows:

Table 8 System Suitability Data

S.No.	RT	AUC	N	TF	H
1.	4.243	120231	123.432	0.375	0.2025
2.	4.345	131190	126.4	2	0.1977
3.	4.227	122459	122.967	0.375	0.2033
4.	4.155	127255	120.872	0.375	0.2068
5.	4.467	135494	129.9490	2	0.1923
6.	4.198	135851	122.1236	0.375	0.20471

Specificity and selectivity

Using the specified chromatographic conditions, ivermectin retained for 4.198 minutes. In comparison with an ivermectin-spiked sample, a mobile phase chromatogram of a blank sample did not show an interference peak during the analyte retention duration.

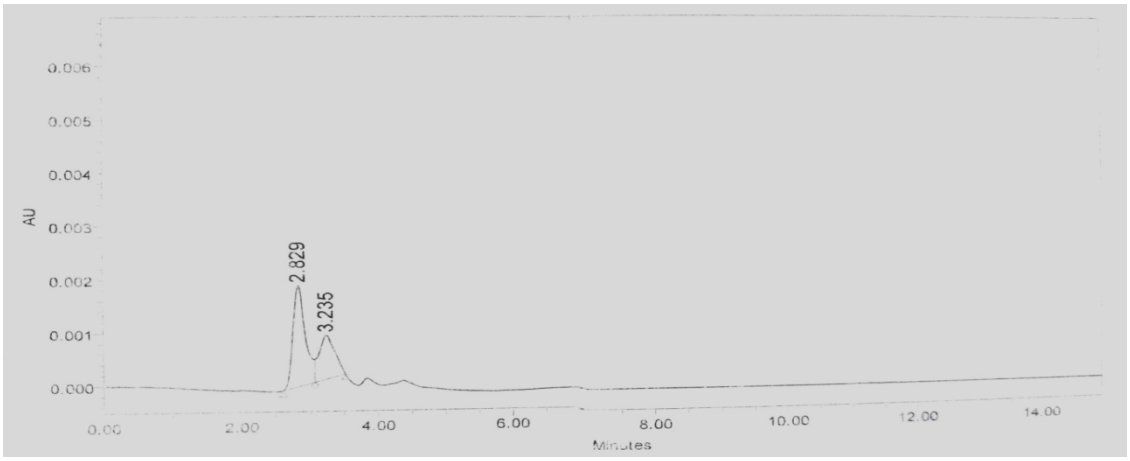

Figure 11Chromatogram of Blank (methanol)

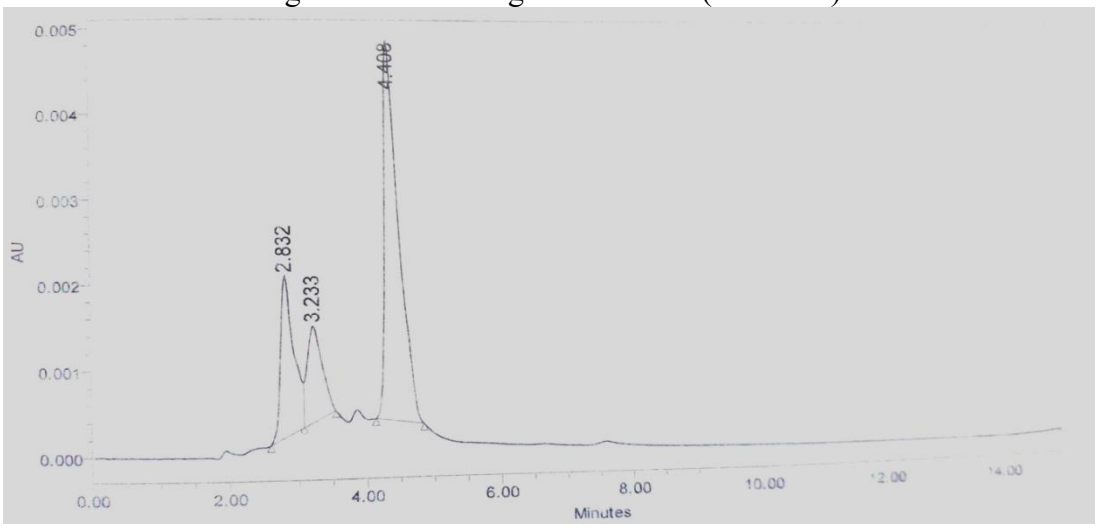

Figure 12 Chromatogram of test drug

Conclusion

In this study, we effectively employed reverse-phase high-performance liquid chromatography (RP-HPLC) to evaluate the characteristics of ivermectin and paracetamol. Using two Inertsil C18 columns, a mobile phase of methanol and acetonitrile, and UV detectors at 245 nm, we validated the method's efficacy for routine analysis. The assessment included parameters such as efficiency, resolution, and tailing factor, establishing the method's reliability for system performance evaluation. Recovery studies confirmed the technique's robustness, unaffected by excipients. Moreover, the method exhibited excellent linearity, precision, and accuracy, making it suitable for quality control purposes, primarily due to its low limits of detection and quantification. These findings highlight the method's robustness and precision for routine Ivermectin and Paracetamol analysis, providing a valuable starting point for researchers investigating these compounds and related formulations. The rigorous optimization undertaken ensures the production of dependable results for future studies in this field.

References

[1] D. D. Nolte, A Practical Guide to HPLC Detection. San Diego, CA: Academic Press, 1983.

[2] C. F. Poole and S. A. Schutte, Contemporary Practice of Chromatography. Amsterdam: Elsevier, 1984, p. 375.

[3] S. Krull, "Chromatography and Separation Chemistry: Advances and Developments," in Advances in Chromatography, S. Ahuja, Ed., ACS Symposium Series 297, ACS, Washington, DC, 1986, p. 137.

[4] G. Li, M. E. Szulc, D. H. Fischer, and I. S. Krull, "Electrochemical Detection in Chromatography," in Liquid Chromatography Science Series, P. T. Kissinger, Ed. Marcel Dekker, New York, 1997.

[5] G. Varrassi, J. V. Pergolizzi, P. Dowling, and A. Paladini, "Ibuprofen/paracetamol fixed combination in the management of moderate to severe pain: a review," Curr. Med. Res. Opin., vol. 35, no. 7, pp. 1241-1251, 2019, doi: 10.1080/03007995.2019.1601538.

[6] R. B. Raffa and J. V. Pergolizzi Jr., "Mu-opioid receptor agonists with delta-opioid receptor and alpha2-adrenoceptor antagonist activity: a promising approach to the treatment of chronic pain," J. Opioid Manag., vol. 8, no. 4, pp. 273-286, 2012, doi: 10.5055/jom.2012.0145.

[7] E. Roberts, V. Delgado Nunes, S. Buckner et al., "Paracetamol: not as safe as we thought? A systematic literature review of observational studies," Ann. Rheum. Dis., vol. 75, no. 3, pp. 552-559, 2016, doi: 10.1136/annrheumdis-2014-206914.

[8] W. Zhang, R. W. Moskowitz, G. Nuki et al., "OARSI recommendations for the management of hip and knee osteoarthritis, Part II: OARSI evidence-based, expert consensus guidelines," Osteoarthritis Cartilage, vol. 16, no. 2, pp. 137-162, 2008, doi: 10.1016/j.joca.2007.12.013.

[9] "Paracetamol for the management of feverish children," National Institute for Health and Care Excellence (NICE), 2013. [Online]. Available: https://www.nice.org.uk/guidance/cg160/resources/paracetamol-for-the-management-of-feverish-children-pdf-35109683424645. [Accessed: July 15, 2023].

[10] P. T. Kissinger and W. R. Heineman, Eds., "Laboratory Techniques in Electroanalytical Chemistry," Marcel Dekker, New York, 1984.

[11] C. P. Hsu, "Infrared spectroscopy," in A Handbook of Instrumental Techniques for Analytical Chemistry. John Wiley & Sons, 1981, p. 249.

[12] M. Krstulovic and P. R. Brown, Reversed-Phase High Performance Liquid Chromatography: Theory, Practice and Biomedical Applications. Wiley, New York, 1982.

[13] U.S. FDA, "Title 21 of the U.S. Code of Federal Regulations: 21 CFR 211-Current good manufacturing practice for finished pharmaceuticals."

[14] U.S. FDA, "Guidance for Industry (draft) Analytical Procedures and Methods Validation: Chemistry, Manufacturing, and Controls and Documentation," 2000.

[15] "ISO/IEC 17025, General requirements for the competence of testing and calibration laboratories," 2005.

[16] "International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of analytical procedures: definitions and terminology, Q2A," Geneva, 1996.

[17] M. K. Malik, P. Bhatt, J. Singh, R. D. Kaushik, G. Sharma, and V. Kumar, "Preclinical safety assessment of chemically cross-linked modified mandua starch: Acute and sub-acute oral toxicity studies in Swiss albino mice," ACS Omega, vol. 7, no. 40, pp. 35506-35514, 2022.

[18] P. Bhatt et al., "Nanorobots Recent and Future Advances in Cancer or Dentistry Therapy- a Review," American Journal of PharmTech Research, vol. 9, no. 3, pp. 321-331, 8 June 2019. [Online]. Available: https://doi.org/10.46624/ajptr.2019.v9.i3.027.

[19] "General Chapter 1225, Validation of compendial methods," United States Pharmacopeia 30, National Formulary 25, Rockville, Md., USA, The United States Pharmacopeial Convention, Inc., 2007.

[20] J.G. Prieto, G. Merino, M.M Pulido, E. Estevez, A.J Molina, L. Vila, A.I Alvarez, "Improved LC method to determine ivermectin in plasma," Journal of Pharmaceutical and Biomedical Analysis, vol. 31, no. 4, pp. 639-645, 2003.

[21] F.L. Degaut Pontes, R. Pontarolo, F.R. Campos, J.C. Gasparetto, M.A. Cardoso, M.S. Piantavini, A.C. Leal Badaro Trindade, "Development and Validation of An HPLC-Ms/Ms Method For Simultaneous Determination of Ivermectin, Febantel, Praziquantel, Pyrantel Pamoate and Related Compounds In Fixed Dose Combination For Veterinary Use," Asian Journal of Pharmaceutical and Clinical Research, vol. 6, no. 2, pp. 191-200, 2013.

[22] S. Pradeep, C. Javali, "Analytical method development and validation for simultaneous estimation of ivermectin and fenbendazole in bulk dosage form by RP-HPLC," World Journal of Pharmacy and Pharmaceutical Sciences, vol. 5, no. 4, pp. 1614-1624, 2016.

[23] J. Dennis, "Compatibility of Pharmaceutical Products and Contact Materials: Safety Considerations Associated with Extractables and Leachables," October 1, 2008, doi: 10.1002/9780470459416.