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DEVELOPMENT AND VALIDATION OF AN ANALYTICAL METHOD FOR QUANTIFICATION OF IVERMECTIN ANALYSIS

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ABSTRACT

Ivermectin is a widely used drug in the treatment and prevention of various parasitic infections in humans and animals. To ensure its quality, safety, and efficacy, it is essential to develop and validate robust analytical methods for its analysis. This research paper aims to provide an overview of the analytical method development and validation parameters for the drug Ivermectin. We developed and validated an accurate, simple, comprehensive, selective, and rapid RP-HPLC technique for the determination of ivermectin in the tablet dosage form. The reverse phase HPLC method was developed for routine measurement of ivermectin in laboratory-prepared mixtures and combination dose forms. Waters model 2489, Software-Empower 2 was utilized as the instrument. The chromatographic separation was carried out using an INERTSIL C-18 ODS 2504.6mm,5um particle size column with a mobile phase of acetonitrile and methanol flowing at a rate of 1ml/min. Quantification was performed using a UV detector set at 245nm for a 10-minute run period. Ivermectin had a retention time of 4.198 minutes. For ivermectin, linearity was found in the concentration range of 1-32g/ml with a correlation value of 0.9798. Intraday precision had a percent RSD of 1.6283 and interday precision had a percent RSD of 1.352. The LOQ and LOD values were determined to be 2.93 and 8.79, respectively, whereas the theoretical plates and tailing factor for ivermectin were determined to be 129.949 and 2.0, respectively. Robustness was also investigated, and it was shown that minor changes in experimental parameters had no substantial effect on the analytical method's system appropriateness. The method has been verified against ICH standards for linearity, precision, accuracy, and other characteristics. The findings produced by RP-HPLC techniques are rapid, detailed, selective, and accurate. As a result, the suggested method may be used to analyse ivermectin in injectable, tablet, and other formulations regularly. The paper discusses various

aspects such as sample preparation, instrumental analysis, method validation, and quality control measures. The development and validation of accurate and reliable analytical methods for Ivermectin are crucial for its pharmaceutical and clinical applications.

KEYWORDS: Ivermectin, analytical methods, method development, method validation, quality control, HPLC, pharmaceutical analysis.

INTRODUCTION

Ivermectin is a widely utilized drug for the treatment and prevention of various parasitic infections in both humans and animals. It belongs to the macrocyclic lactone class of drugs and has exhibited broad-spectrum activity against nematodes, arthropods, and ectoparasites. Due to its efficacy, safety, and affordability, Ivermectin has gained significant importance in the field of veterinary medicine and has also been explored for potential applications in human health. The quality, safety, and efficacy of Ivermectin products are of utmost importance to ensure their therapeutic effectiveness and minimize potential risks. Therefore, it is crucial to develop and validate analytical methods that can accurately determine the concentration and purity of Ivermectin in various pharmaceutical formulations, including tablets, injectable, and other dosage forms. These analytical methods play a vital role in the quality control and regulatory compliance of Ivermectin products. Analytical method development involves selecting suitable techniques and parameters for the reliable analysis of Ivermectin. It encompasses aspects such as sample preparation, selection of appropriate instrumentation, and optimization of separation conditions to achieve accurate and precise results. The developed analytical methods should be sensitive, specific, and capable of providing reliable quantification of Ivermectin in the presence of potential matrix interferences. Furthermore, the validation of analytical methods is essential to demonstrate their suitability for routine use. Method validation ensures that the analytical procedures meet predefined acceptance criteria for various parameters, including specificity, linearity, precision, and accuracy, robustness, and system suitability. Validation studies are conducted in accordance with established guidelines provided by regulatory authorities such as the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH).This research paper aims to provide an overview of the analytical method development and validation parameters specific to Ivermectin analysis. It focuses on the development of a robust and validated reverse-phase high-performance liquid chromatography (RP-HPLC) method for the determination of Ivermectin in tablet dosage

forms. The paper discusses the experimental details, results, and validation parameters of the developed method. Additionally, it emphasizes the importance of accurate and reliable analytical methods for Ivermectin in ensuring the quality, safety, and efficacy of the drug in pharmaceutical and clinical applications.

MATERIAL AND METHOD

Material

Ivermectin, a gift sample from Sell Well Pharmaceuticals located in Indore, was provided for analysis.

Methods

Physicochemical properties of drug

The drug was studied based on its physical appearance and morphology. Various characteristics like smell, colour, odour, and appearance were observed by naked eyes.

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.

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 solubilisation of the drug was recorded and compared with reported values.

Sample Preparation

The sample preparation process is a critical step in the analysis of Ivermectin. It involves the extraction of the drug from the matrix and the removal of interfering substances. Various techniques such as liquid-liquid extraction, solid-phase extraction, and solid-phase micro extraction can be employed for efficient sample preparation.

Instrumental Analysis

High-performance liquid chromatography (HPLC) is the most used technique for the analysis of Ivermectin. It offers excellent separation and quantification capabilities. Other techniques such as gas chromatography (GC), capillary electrophoresis (CE), and spectrophotometry can also be utilized depending on the specific requirements of the analysis. The choice of the analytical technique depends on factors such as sensitivity, selectivity, and the nature of the sample matrix.

Method Precision

Method precision of ivermectin was performed by estimating corresponding responses multiple times on the alternative days. The percent RSD (percent relative standard deviation) was determined within the permitted range of less than 2%. The outcomes were mentioned in the Table.

Accuracy check

The accuracy of the UV spectrophotometer was evaluated using a standard quality control sample manufactured in triplicate at various concentration levels. The percentage of analyte recovered by assay from a known added amount is known as accuracy. Data from 9 determinations at three concentration levels, encompassing the entire range of concentrations. The outcomes were mentioned in the Table.

Limit of Quantification & Detection

LOQ and LOD were computed through the method positioned on the SD (standard deviation) and slope of the calibration curve by utilizing the formula as shown

Limit of Detection = (3 x lowest conc. of the standard / sample) x S/N

Limit of Quantification = (10 x lowest conc. of the standard / sample) x S/N

Robustness

The assessment of robustness was performed by modification of method specifications from the optimized chromatographic conditions like making changes in the mobile phase (+_10%). It was seen that the changes in these operational specifications didn’t prompt extreme changes of retention time of the peak of interest, resolution (Not Less Than 2.00), plate count (> 2000), tailing factor (< 2.0) and the % RSD for multiple replicate injections (< 2.0) were found to be within the acceptance criteria25. The level of reproducibility of the outcomes demonstrated that the technique is robust. The outcomes were mentioned in the Table.

Suitability of System

The suitability of system test is used to check whether or not the chromatographic system is suitable for the analyses planned. The system suitability of the technique was checked by injecting multiple injections. Most of the parameters like peak area, theoretical plates, plate height, tailing factor were checked according to USP criteria. The noticed RSD values were well inside typically accepted limits (NMT 2%).

Specificity and Selectivity

Specificity refers to an analytical technique's capacity to assess analytic reactivity in the presence of disruptive substances such as contaminants and degradation products. The resolution factor of the drug peak from the neighbouring peak was used to determine the specificity. The selectivity of the approach will be determined by the purity of each degradation peak.

RESULTS 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.

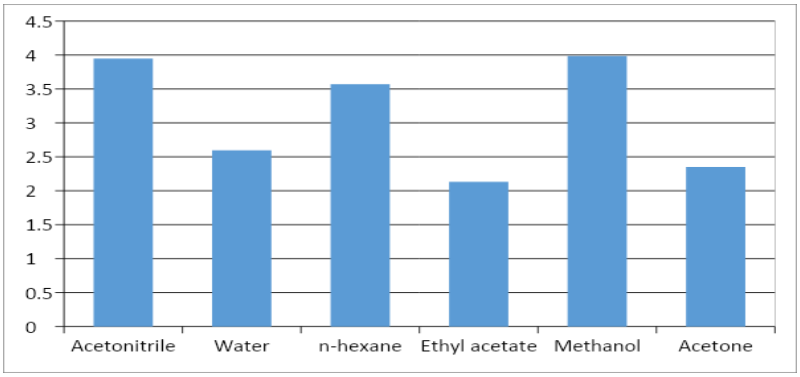

Figure 1: Solubility of drug in different components.

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
1	0.082
2	0.147
4	0.296
6	0.436
8	0.599
16	1.191

The calibration curve for the drug in methanol is expressed in figure 7.2 and it follows the linear straight line with high linearity as better with correlation coefficient 0.999.

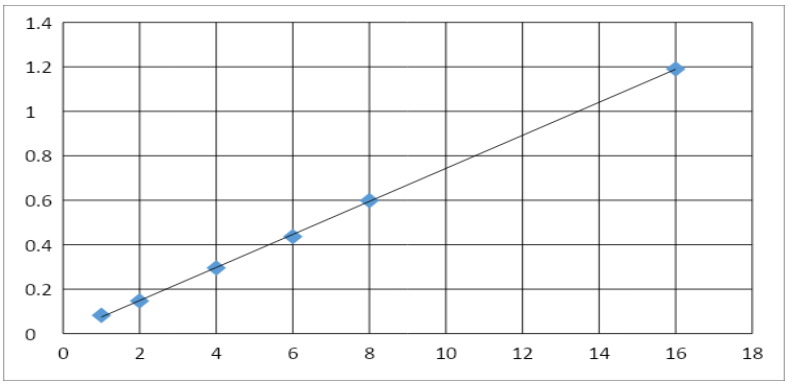

Figure 2: Calibration curve of Drug in methanol.

FTIR Spectra

The results for the pure drug as well as for the test drug are explained in the following table with the interpretation.

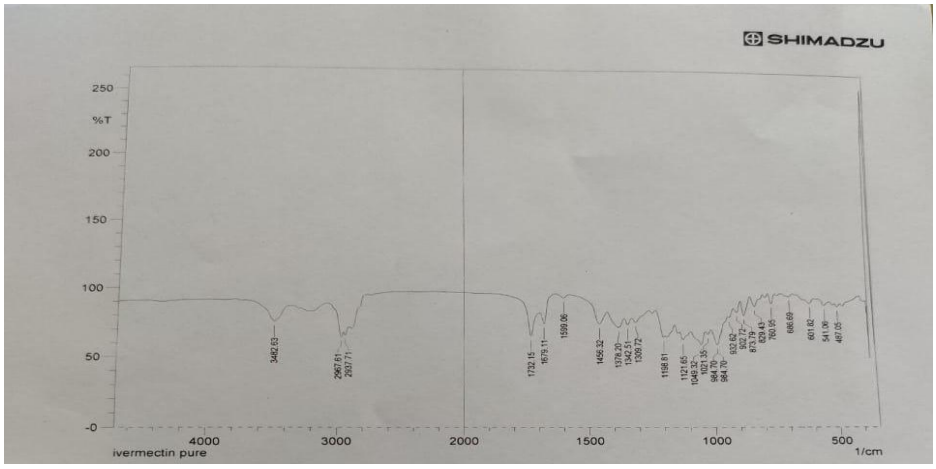

Figure 3: FTIR Spectra of Pure Drug.

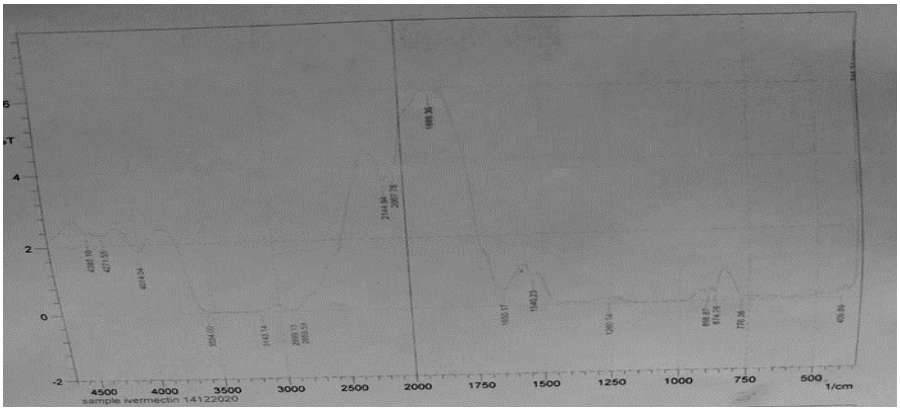

Figure 4: FTIR Spectra of Test Drug.

Table 2: Interpretation of FTIR.

S.No.	Ir Vibrational Frequencies	Pure Standard Observed	Test Drug Observed	Expected Range (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 Stretching	1679.11	1650.17	1780 – 1610 (1675)
10.(specific)	5-Ring hetero saturated cyclic ether C-O-C Stanching asymmetrical	1049.32 (symmetrical) 902.72(asymmetrical)	1260.54 898.87(asymmetry)	≈1070 symmetrical ≈915 asymmetrical
11.(specific)	6- Ring hetero saturated cyclic ether	1121.65(asymmetrical) 829.43(symmetrical)	898.87 776.38	≈ 1100 *asymmetrical* ≈815 symmetrical
10,11. (general)	C-O-C Stretch. Asymmetrical of ether	1198.81	1260.54	1310 – 1000 (strong, split)
12.	C-O-C Stretching symmetrical of ether	1049.32 – 873.79	898.87 – 874.76	1055 – 870 (strong, multiple bonds)

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.

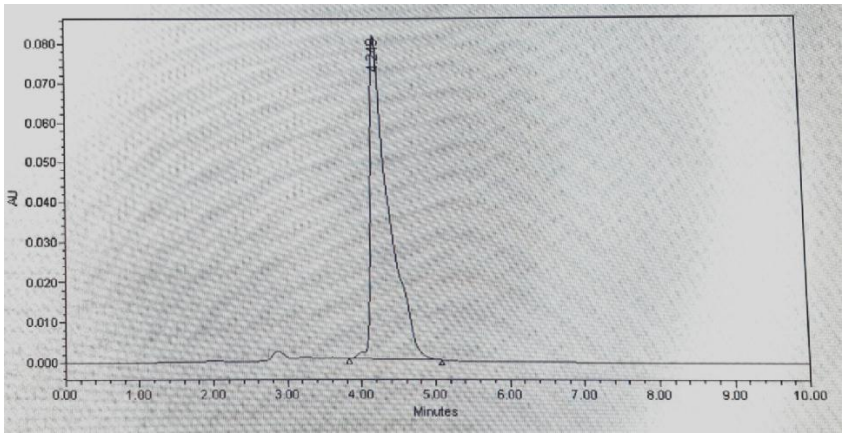

Figure 5: Standard drug Chromatogram of Ivermectin.

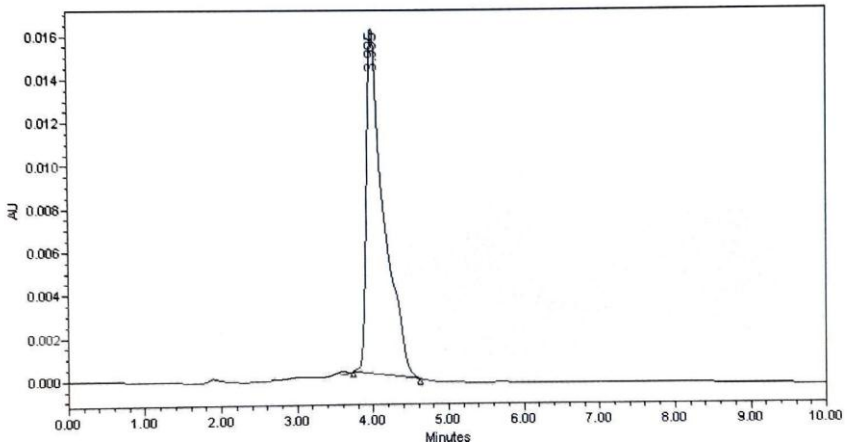

Figure 6: TEST drug Chromatogram of Ivermectin.

Calibration Curve in HPLC

The results of HPLC peak area are expressed in the following table 7.2 and the calibration curve of HPLC by utilizing the same data are expressed in figure 7.7 with correlation coefficient 0.9798.

Table 3: Conc. Range of observing peak area for constructing calibration curve.

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

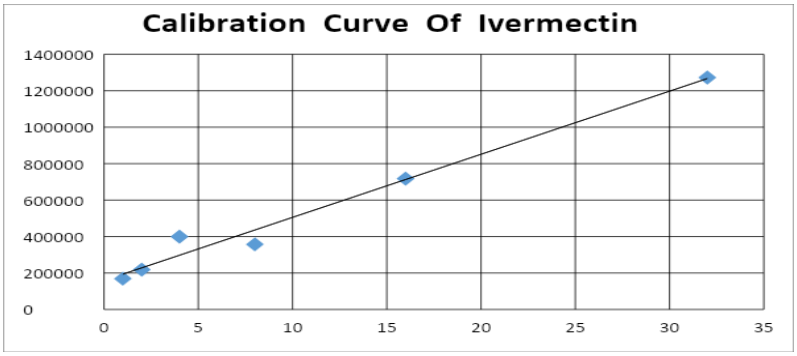

Figure 7: Calibration Curve of ivermectin.

The absolute residue data are obtained by using the regression equation given in table 8.4.

Table 4: Absolute residue data.

Drug Concentration (µg/ml)	AUC	Y=mx+c	Absolute Residue
1	168943.33	193684	-24741
2	218905.83	228301	-9395.17
4	399692	297535	102157
8	357288.66	436003	-78714.34
16	717802.5	712939	4863.5
32	1272663.16	1266811	5852.16

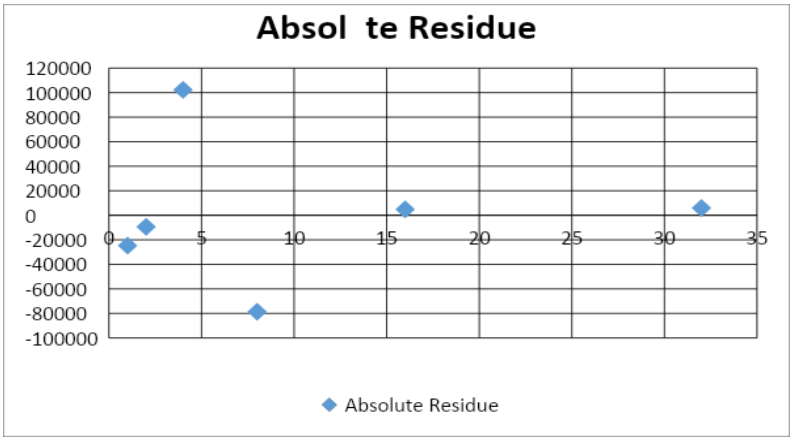

Figure 8: Absolute residue graph.

HPLC method Validation

Precision

The precision is validated by using the both methods system precision and method precision and the obtained data are expressed in table 7.4 & 7.5.

Table 5: 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 6: 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

Accuracy was done by using UV Spectrophotometer and the obtained absorbance are given in table 8.7.

Table 7: Accuracy data.

Sample	Absorbance
4ppm	0.155
6ppm	0.226
8ppm	0.355

Limit of Detection & Quantification

The LOD and LOQ of the analytes were calculated on the grounds of the standard response deviation and slope, the LOD being expressed as 3.3 σ/S and the LOQ being expressed as 10 σ/S .

Table 8: LOD & LOQ Values.

Sample	LOD	LOQ
Ivermectin	2.93	8.79

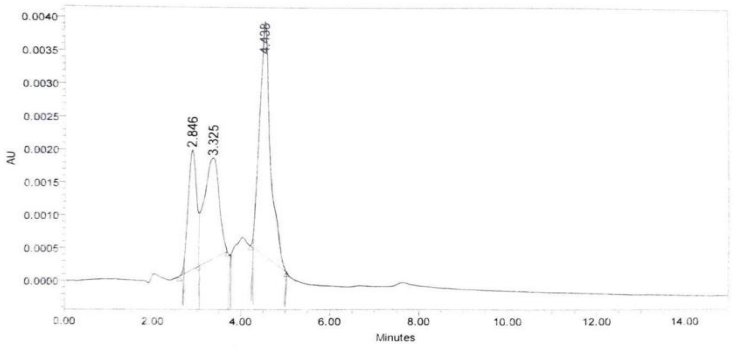

Figure 9: Chromatogram of LOD & LOQ.

Robustness

The robustness was done by using the different mobile phase and obtained peak area or chromatogram are given below.

Table 9: 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

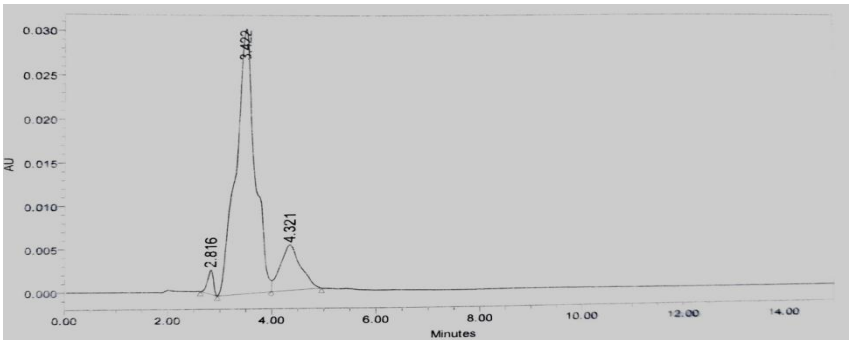

Figure 9 Chromatogram of THF: H2O.

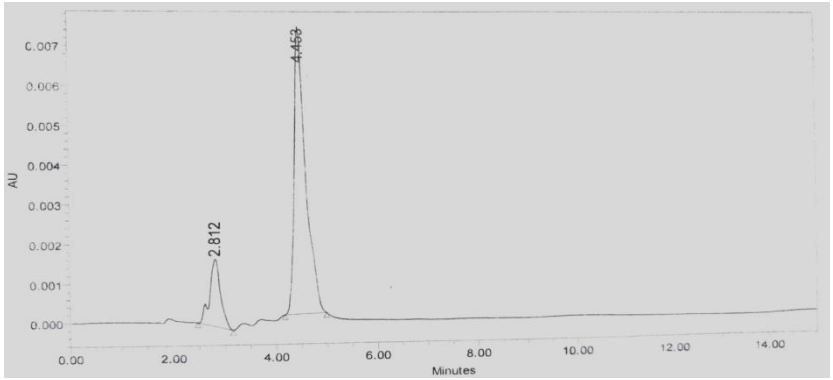

Figure 10 ChromatogramofMETHANOL: H2O.

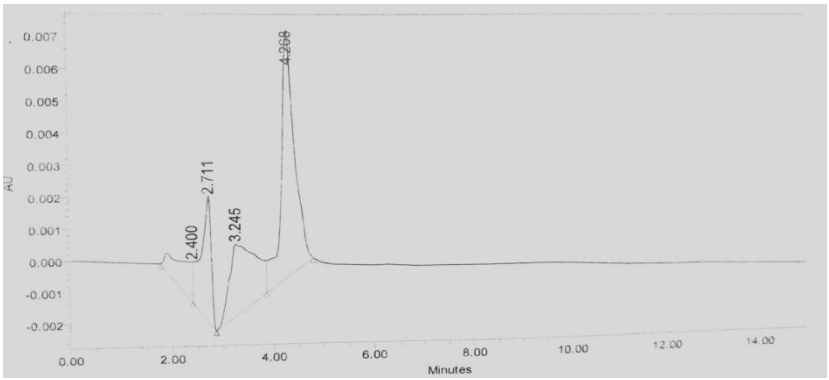

Figure 11 Chromatogram of ACETONITRILE: H2O.

System suitability

The system suitability was completed by using the multiple factors and the results are given below:

Table 10: 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

8.5.6 Specificity and selectivity

Ivermectin retention time was 4.198min, under the chosen chromatographic conditions. The interferences were measured by contrasting blank mobile phase chromatogram with that of mobile phase sample spiked with ivermectin. There were no interfering peaks found during analyte retention time.

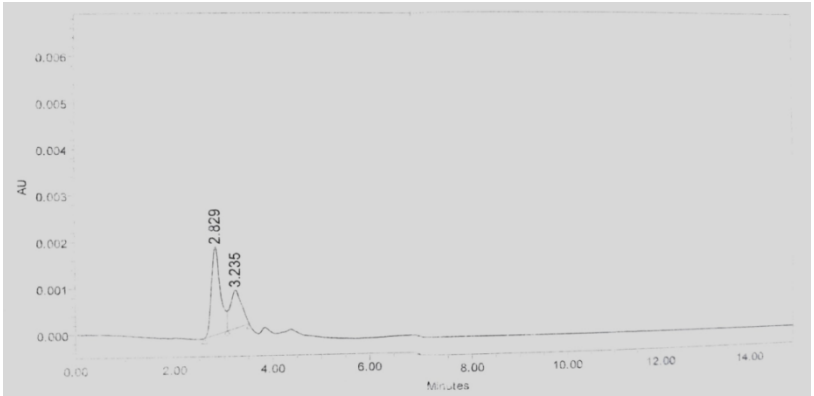

Figure 12: Chromatogram of Blank (methanol)

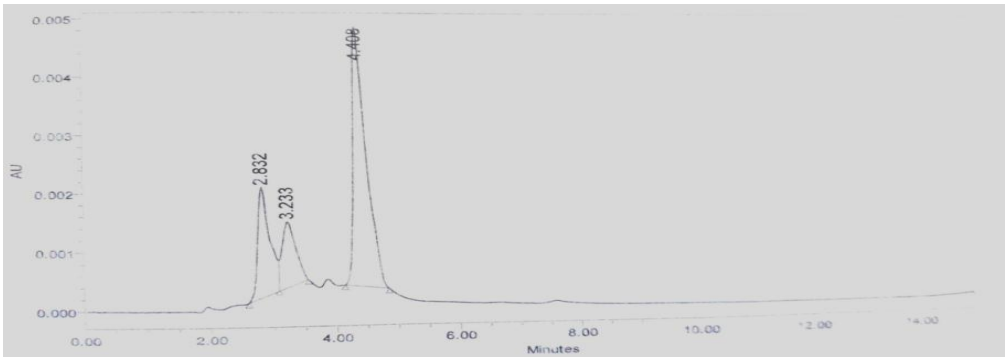

Figure 13: Chromatogram of test drug.

TRIAL & ERROR STUDY BEFORE OPTIMIZATION OF CHROMATOGRAPHIC PARAMETERS

The errors which obtained during the research work are given below.

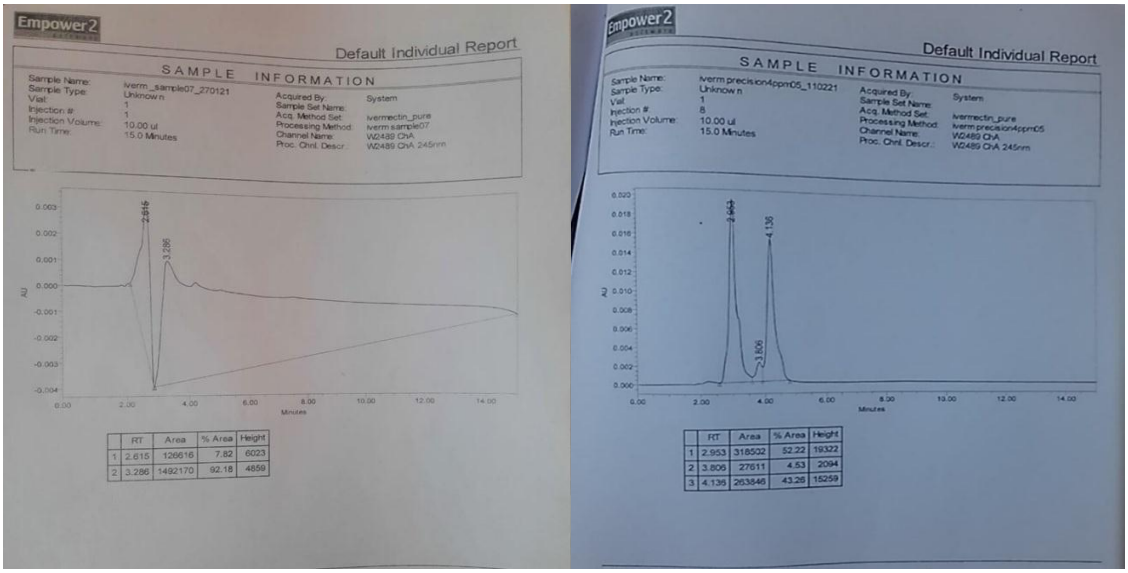

Table 10 Comparative values of drug Ivermectin by different researchers with corresponding authors.

Citations	Linearity (correlation coefficient)	Retention Time	Flow Rate (ml/min)	Precision %RSD Intraday		Accuracy	LOD	LOQ	Column	Mobile phase	Wavelength (nm)
B.Bhavya*etal., 2017*	0.999	2.897	1	1.7	1.6	101.22%	0.31	0.966	Inertial ODS 150*4.6mm*5um	pH sodium phosphate buffer: methanol(25:75v/v)	245
Nischal k *etal* 2011	0.999	5.66	1.5	0.33	0.33	98-102%	-	-	Vydac C-18 250*4.6*5um	ACN:Methanol:water(60:30:10v/v/v)	254
B.Saidulu*etal.,* 2015	1.0	2	1	Less than 2%		98%	0.012	0.038	Inertsil C-18 BDS 250*4.6*5um	ACN:Methanol:water (40:60v/v)	280
M.Shurbaji*etal.,* 2019	0.9999	-	1	0.73	0.59	-	0.07	0.020	-	ACN:methanol:water:acetic acid)56:36:7.5:0.5v/v/v/v/v)	245
NVSK Devaka*etal.*, 2019	0.9998	3.465	1	Less than 2%		99.60%	0.010	0.033	YMC C-18 250*4.6*5um	0.1disodiumhydrogen phosphate:CAN(55:45v/v)	242
Dr. Gampavijay km. *etal.*, 2018	0.999	2.344	1.2	0.2	0.2	99.56%	3.17	0.0172	ACE C-18 150*4.6*5um	Methanol:Phosphate buffer Ph3(70:30v/v)	240
VegadKunjal L *etal.*, 2017	0.9999	-	1	0.203	0.20	100.34%	0.06	0.20	BDS hypersil C-18 250*4.6*5um	Phosphate buffer: methanol (60:40v/v)	234
Patel Asmita *etal.*, 2015	0.9966	7.733	-	0.9286	0.088	99.63%	0.0116	0.0353	Supelcosil TM 150*4.6*5um	ACN:methanol:buffer (51:25:24v/v/v)	-
A.Waldia*etal.*, 2008	0.9969	10.08	1.8	Less than 2%		>98%	-	-	Nucleodur C-18 RP column250*4.6*5um	ACN:methanol:water (60:30:10v/v/v)	245
M.M Ali *etal.*, 2017	0.9998	1.6-2	-	0.591	1	99.92%	0.61	1.80	Thermo C-18 BDS 15cm*4.6mm*5um	ACN:Methanol:purified water(60:30:10v/v/v)	245
Sonia Goswami *etal.*, 2021	0.9798	4.198	1	1.6283	1.352	-	2.93	8.79	Inertsil C-18 ODS 250*4.6mm*5um	ACN: Methanol (60:40v/v)	245

The current study's goal is to design and validate an analytical method by utilizing the RP-HPLC technique. The results were reported and discussed by comparing with the previous reports. In the present study the data were analyzed statistically. It was intended to find out the data of methods used in the study such as Linearity, accuracy, precision, limit of detection, limit of quantification, robustness, specificity and selectivity, and system suitability. According to A.Waldiaetal. 2008 and Patel asmitaetal., 2015(above table) the linear correlation coefficient(r=0.9798) was comparatively within the range. As per the Nischal k et.al, 2011and Naga venkatasureshkumardevaka et.al, 2019 (above table) the retention time (4.198 min) of the present study was good and as per Patel et.al, 2015 (above table) the retention time was lesser and according to B.bhavya et.al, 2017 and Gampavijay et.al, 2018(above table) the retention time was greater. At the wavelength 245nm the UV spectra has shown the better result. The %RSD of intraday and interlay precision for present study was 1.6283 and 1.352 while for M.M Ali et.al, 2017 and M.shurbaji et.al 2019 it was found to be in range less than 2%. The% recovery for the present method and B.saidulu et.al. 2015 and Vegadkujal L et.al, 2017 was found in the range 98-102%. Recovery greater than 98% justifies the accuracy of the data. As compared to Gampavijaykumar et.al, 2018(above table) the LOD and LOQ values (2.93 and 8.79) of the present method were within the range. As compared to all previously used method, in the present method the different column was used that is INERTSIL C- 18 ODS 250×4.6mm×5 um particle size column. In present study the different mobile phase was used i.e., ACN: Methanol (60:40 v/v), 1ml/ min flow rate was optimized resulting in a sharp peak. In robustness by the modification of the mobile phase, the developed approach was found to be robust. Mobile phase A is tetrahydrofuran : water (70:30v/v) and mobile phase B methanol :water (80:30v/v) and the mobile phase C consisted a mixture of ACN : water(70:30v/v).The data has been illustrated in table. The specificity and selectivity parameters were evaluated and found to be within the limit and the data obtained from the system suitability studies exemplified in table. Finally, the development and validation of an analytical approach was performed successfully and the result was obtained satisfactorily.

CONCLUSION

The development and validation of analytical methods for Ivermectin are essential to ensure its quality, safety, and efficacy. These methods provide valuable tools for the pharmaceutical industry and regulatory agencies to assess the quality of Ivermectin products the analysis of Ivermectin was performed using RP-HPLC (Reversed-Phase High-Performance Liquid

Chromatography) technique. The mobile phase consisted of a combination of Acetonitrile and methanol in the ratio of 60:40 (v/v). Inertsil C18 column (250x4.6mm, 5.0mm) or an equivalent column was utilized as the stationary phase. The identification of Ivermectin was achieved using a UV detector set at a wavelength of 245 nm. The samples were chromatographed at a constant flow rate of 1.0 ml/min. To assess the reliability and suitability of the method, system suitability parameters such as efficiency, resolution, and tailing factor were determined. Recovery studies were conducted to demonstrate that the method is unaffected by excipients. The precision of the technique was evaluated by injecting the samples multiple times and calculating the corresponding values. The method was validated for linearity, precision, accuracy, and robustness. This RP-HPLC method is straightforward, specific, and easy to perform, requiring a short analysis time for the samples. The method's low limit of detection (LOD) and limit of quantification (LOQ) values make it suitable for quality control purposes. The developed method exhibited linearity, precision, accuracy, and robustness, indicating its suitability for routine analysis of Ivermectin.

Conflict of interest

None.

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