ijlpr 2022; doi 10.22376/ijpbs/lpr.2022.12.1.P1-12

International Journal of Life science and Pharma Research

ISSN 2250-0480

Research Article

Allied Sciences

Analytical Method Development And Validation Parameters Of Drug Ivermectin

Sonia goswami*[1], k. Nagarajan*[2], richa goel[3], praveen k. Dixit[4], vidhu saxena[4], sanjeev kumar chauhan[5], vinay kumar[4]

[1]*Department Of Pharmaceutical Quality Assurance, KIET School of Pharmacy, KIET Group of Institutions, Delhi-NCR, Ghaziabad Affiliated to Dr. APJ Abdul Kalam Technical University, Lucknow, India*

[2]*Department Of Pharmaceutical Chemistry, KIET School of Pharmacy, KIET Group of Institutions, Delhi-NCR, Ghaziabad Affiliated to Dr. APJ Abdul Kalam Technical University, Lucknow, India*

[3]*Department of Pharmacognosy, KIET School of Pharmacy, KIET Group of Institutions, Delhi-NCR, Ghaziabad Affiliated to Dr. APJ Abdul Kalam Technical University, Lucknow, India*

[4]*Department of Pharmacology, KIET School of Pharmacy, KIET Group of Institutions, Delhi-NC R, Ghaziabad Affiliated to Dr. APJ Abdul Kalam Technical University, Lucknow, India*

[5]*Department of Pharmaceutics, KIET School of Pharmacy, KIET Group of Institutions, Delhi-NCR, Ghaziabad Affiliated to Dr. APJ Abdul Kalam Technical University, Lucknow, India*

Abstract: An accurate, easy, detailed, selective and fast RP-HPLC stability representative technique was developed and validated for assessment of ivermectin in tablet dosage form. The Reverse Phase High Performance Liquid Chromatographic technique was developed for routine quantification of ivermectin in laboratory prepared mixtures as well as in combined dosage forms. The chromatographic separation was accomplished with INERTSIL C-18 ODS 250×4.6mm, 5µm particle size column along with acetonitrile and methanol as the mobile phase at a flow rate of 1ml/min. Quantification was completed by using a UV detector at 245 nm and the run time was 10 minutes. The retention time was found to be 4.198 min for ivermectin. The linearity was observed in the range of 1-32µg/ml with correlation coefficient r= 0.9798. The % RSD for intraday and interday precision was 1.352 and 1.589 respectively. The LOD and LOQ values were found to be 2.93 and 8.79, respectively. The system suitability parameters for ivermectin such as theoretical plates and tailing factor were found to be 129.949 and 2.0, respectively. Robustness was also studied and there was no significant variation in the system suitability of the analytical method by incorporating small changes in experimental parameters. The technique has been validated for linearity, precision, accuracy and other parameters as approved by ICH guidelines. The results obtained by RP- HPLC methods are found to be fast, detailed, selective and accurate. Therefore, proposed analytical method can be used for regular analysis of ivermectin in injection, tablet and other formulations.

Keywords: Ivermectin, Reverse Phase –High Performance Liquid Chromatography, Methanol, Acetonitrile, ICH

***Corresponding Author**

Sonia goswami , Department Of Pharmaceutical Quality Assurance, KIET School of Pharmacy, KIET Group of Institutions, Delhi-NCR, Ghaziabad Affiliated to Dr. APJ Abdul Kalam Technical University, Lucknow, India

Received On 31 May, 2021
Revised On 1 December, 2021
Accepted On 8 December, 2021
Published On 6 January, 2022

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Sonia goswami, k. Nagarajan, richa goel, praveen k. Dixit, vidhu saxena, sanjeev kumar chauhan, vinay kumar , Analytical Method Development And Validation Parameters Of Drug Ivermectin.(2022).Int. J. Life Sci. Pharma Res.12(1), P1-12 http://dx.doi.org/10.22376/ijpbs/lpr.2022.12.1.P1-12

1. INTRODUCTION

Ivermectin has been the drug of choice to treat various diseases due to pathogenic parasites.[1] This comprises head lice, scabies, river blindness (onchocerciasis), strongyloidiasis, trichuriasis, ascariasis and lymphatic filariasis.[2-4] Ivermectin belongs to the avermectin class of drugs[1]. Ivermectin increases the cell membrane permeability of parasite which results in the paralysis and cidal effect on the microorganism. Ivermectin was found in 1975 and came into therapeutic use in 1981.[5,6] It is an extremely potent semisynthetic subsidiary of an antinematodal standard derived from *Streptomyces avermitilis*.[7] Ivermectin is an FDA approved anti - parasitic agent.[8] Chemically, Ivermectin is 22, 23-dihydro avermectin B1a + 22, 23-dihydroavermectin B1b (Fig 1). Basically, it is a combination of almost 90% avermectin B1a and less than 10% avermectin B1b. In present scenario, this drug is also useful in the management of SARS-COV-2.[9,10] The available literature studies describe utilization of few analytical methods for the evaluation of ivermectin in tablet dosage form. These methods are based on various techniques such as Ultra Violet spectroscopy, diffused reflectance spectroscopy, HPTLC, HPLC with Tandem mass spectroscopy and RP-HPLC.[11] Thus, a validation study of various parameters like linearity, precision, accuracy and robustness was required to study the purity of drug. The major factor of comparison with previous studies was system suitability (resolution, tailing factor, plate height, theoretical plates), LOD and LOQ. These factors can be used for the validation of drug and to find the impurities in tablet dosage form.

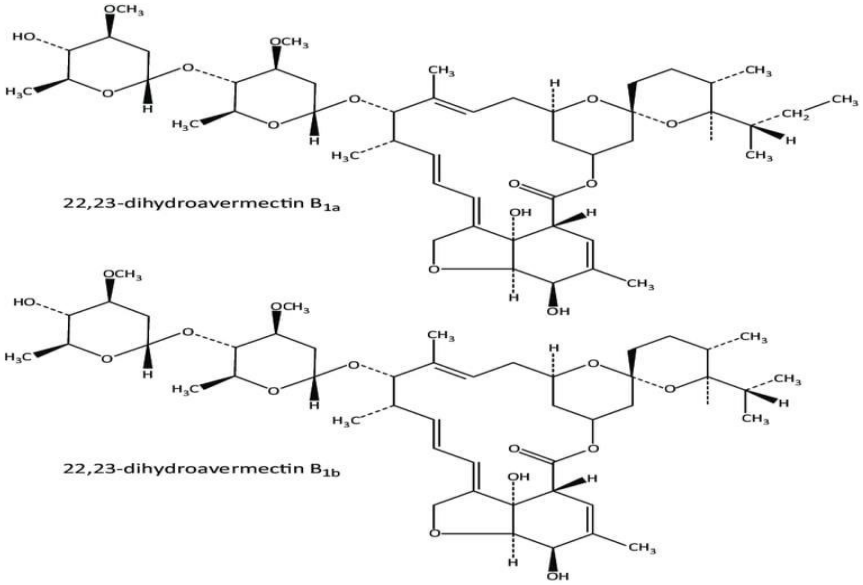

Fig 1: *Chemical Structure of Ivermectin*[12]

1.1 Effects of Ivermectin

Ivermectin is white or light-yellow translucent powder and dissolvable in methyl alcohol, ester and aromatic hydrocarbon. Ivermectin is an antibiotic class of drug which has a cidal impact on nematodes, bugs and vermin. Infusion and lozenges containing Ivermectin as the API are essentially utilized in the medicines of animals' gastrointestinal nematodes, cow-like hypodermosis, calf fly parasite, sheep nasal fly worm, and scabies of sheep and pigs. The most extraordinary element of this insect poison is that it has minimal side effect with an ability to kill various strains of parasites both internally and externally of the host body.[13]

1.2 *Natural pesticide of Ivermectin*

Ivermectin can be classified as plant based pesticide which is made of *Cynanchum komarovii*, *sophora alopecuroides* and various different species. It may be utilized to prevent and kill all types of aphids and defoliators. Whenever diluted with water to 1000～2000 times and sprayed, its control impact can reach above 98%. Ivermectin is a new generation of natural pesticide that has less harm, less residual and has absence of risk to humans, livestock and environment. The insecticidal mechanism of ivermectin is by hindering the synthesis of chitin of insect epicutile.[14]

1.3 Pharmacology and mode of action

Ivermectin belongs to the class of substances known as the avermectins. These are macrocyclic lactones created by maturation of an actinomycete, *Streptomyces avermitilis*. Ivermectin is a broad-spectrum agent against nematodes and arthropods in domestic animals and is thus widely utilized in veterinary medicine.[13] As of now it is viewed as the medication of choice in onchocerciasis. It is a powerful microfilaricide, however it doesn't have any critical macrofilaricidal effect.[15,16] Between 2 to 3 days after oral administration, microfilariae in the skin begin to disappear quickly, while those in the cornea and the interior chamber of the eyes are eliminated more gradually. This is an effect which goes on for up to 12 months.[17-19] The mechanism of activity of ivermectin against onchocerciasis is not clearly understood, however it is presumed to be a GABA-agonist. In susceptible organism, the drug acts by potentiating the release of gamma-aminobutyric acid (GABA) at postsynaptic sites on the neuromuscular junction rendering the nematode paralyzed. [20]

2. MATERIALS AND METHODS

2.1 Chemicals and solvents

HPLC Grade Methanol, HPLC grade Water, Acetonitrile (60%), Methanol (40%), Ivermectin Working Standards and tablet dosage form. The commercial pharmaceutical tablets

of Ivermectin tablet containing 6mg of Ivermectin (manufactured by Micron Pharmaceuticals) was procured from Pradhan Mantri Jan Aushadi medical store, Ganga Nagar, Meerut.

2.2 Instrumentation

The chromatographic separation was carried out by utilizing Waters-HPLC (Model-2489) comprising of an in-constructed autosampler, a column oven and UV detector. The information was obtained throughout Empower-2-programming. The column utilized was Inertsil ODS 250×4.6 mm, 5µm.

2.3 Chromatographic conditions

The mobile phase comprises of Acetonitrile: HPLC grade Methanol (60:40) in isocratic mode. The mobile phase was pumped from solvent reservoir to the column in the flow rate of 1.0 ml/min while run time was set 15 min.[21] The separation was carried out on an Inertsil C-18 ODS 250mm x 4.6mm, 5µm column and the column was maintained at the ambient temperature. The volume of each injection was 10µl. Prior to insertion, the column was equilibrated for at least 30 min with mobile phase flowing throughout the system. The eluents were checked at 245 nm.

2.4 Mobile Phase Preparation[22]

A solution of Acetonitrile (60%) and Methanol (HPLC grade) (40%) was taken and degassed in ultrasonic water bath for 5 minutes. It was then passed through the filter 0.45 micron under vacuum filtration. Methanol was used as the diluent.

2.5 Standard solution preparation

Precisely weighed 6 mg of Ivermectin working standard was transferred into a 10 ml clean dry volumetric flask. Diluent (methanol) was added, sonicated for 10 minutes and final volume was made up to 10ml.1 ml was pipetted out into a 10 ml volumetric flask from the above stock solution and afterwards, made up to the final volume with diluent. Further, 3ml was pipetted out into a 10 ml volumetric flask from above stock solution and afterwards made up to the final volume with diluent (methanol).

2.6 Sample solution preparation

Twenty Ivermectin tablets were weighed and the average weight of every tablet was calculated. The weight comparable to 6 mg of Ivermectin was transferred into a 10 ml clean dry volumetric flask. Methanol was added as diluent, sonicated for 10 minutes and made up to the final volume. 1 ml of above solution was pipetted out into a 10 ml volumetric flask and afterwards made up to the final volume with diluent. Further, 3ml was pipetted out into a 10 ml volumetric flask from the above stock solution and afterwards made up to the final volume with diluent (methanol). The Optimized Chromatographic Conditions and System Suitability Parameters for Proposed HPLC Technique for Ivermectin Instrument High Performance Liquid chromatography (Waters 2489) are presented in Table 1:
analytical conditions. Standard solutions of ivermectin were organized as per procedure and infused for multiple times. Outcomes for responses are mentioned in Table 3.

2.7 Validation of proposed technique

The modifications in the developed method were done in accordance with the International Conference on Harmonization (ICH) rules with respect to system appropriateness, Precision, Specificity, Linearity, Accuracy, Limit of detection and Limit of quantification.[23,24]

2.8 Linearity

For the preparation of the calibration curve of Ivermectin, 1,2,4,8,16,32 µg/ml of stock solution were taken separately in 10ml volumetric flasks and made up the volume with diluent (methanol). Volume of 10 µl of sample was infused multiple times for each concentration level and calibration curve was constructed by plotting the peak area versus drug concentration. A straight connection between peak area versus concentration was observed in the range of study. The calibration curve and observations are mentioned in table 2.

2.9 Precision

2.9.1 System precision

Precision is the measure of the nearness of the data values to one another for a digit of measurements under the similar

2.9.2 Method precision

Method precision of ivermectin was performed by estimating corresponding responses for multiple times on the alternative days. The % RSD (% relative standard deviation) was calculated within the acceptable criteria not more than 2%. The outcomes are mentioned in Table 4.

2.10 Accuracy

The accuracy of Ivermectin was evaluated by using a UV spectrophotometer and the standard working sample of Ivermectin was made in triplicate at various concentration levels (4,6,8 ppm).[25,26] The percentage of analyte recovered by assay from a known added amount is known as accuracy. Thus, data from 9 determinations at three concentrations in triplicate was calculated. The results obtained are given in Table5.

2.11 Limit of detection and limit of quantification

LOD and LOQ were calculated through the method based on the SD (standard deviation) and slope of the calibration curve by using the formula:

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

2.12 Robustness

The assessment of robustness was carried out by modification of method specifications from the optimized chromatographic conditions such as 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 (Not Less Than 2000), tailing factor (Not More Than 2.0) and the % RSD for multiple replicate injections (Not More Than 2.0).[20] All these parameters were found to be within the acceptance criteria. The level of reproducibility of the outcomes demonstrated that the technique is robust. The outcomes are mentioned in Table7.

2.13 System suitability

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

2.14 Specificity and selectivity

The capability of the analytical technique to evaluate the reaction of the analyte in the presence of disruptive substances including impurities and its degradation product is known as specificity.[27] The specificity was assessed by the resolution factor of the drug peak from the nearby peak. The purity of each degradation peak will determine the selectivity of the technique.

3. RESULTS AND DISCUSSION

The chromatographic separation of drug Ivermectin was done using HPLC method. The Optimized Chromatographic Conditions and System Suitability Parameters for Proposed HPLC Technique for Ivermectin Instrument High Performance Liquid chromatography (Waters 2489) is presented in Table 1 below. The chromatogram obtained for standard and sample of Ivermectin are presented in Fig 2 and 3 respectively.

Table 1 *Optimized Chromatographic Conditions*

Flow rate	**1.0ml/min**
Column	Inertsil C-18ODS, 250 x 4.6 mm, 5µm
Mobile phase	Acetonitrile (ACN): HPLC grade Methanol (60:40)
Detector wavelength	245 nm
Column temperature	Ambient
Injection volume	10 µl
Run time	10 min
Diluents	Methanol
Mode of separation	Isocratic mode

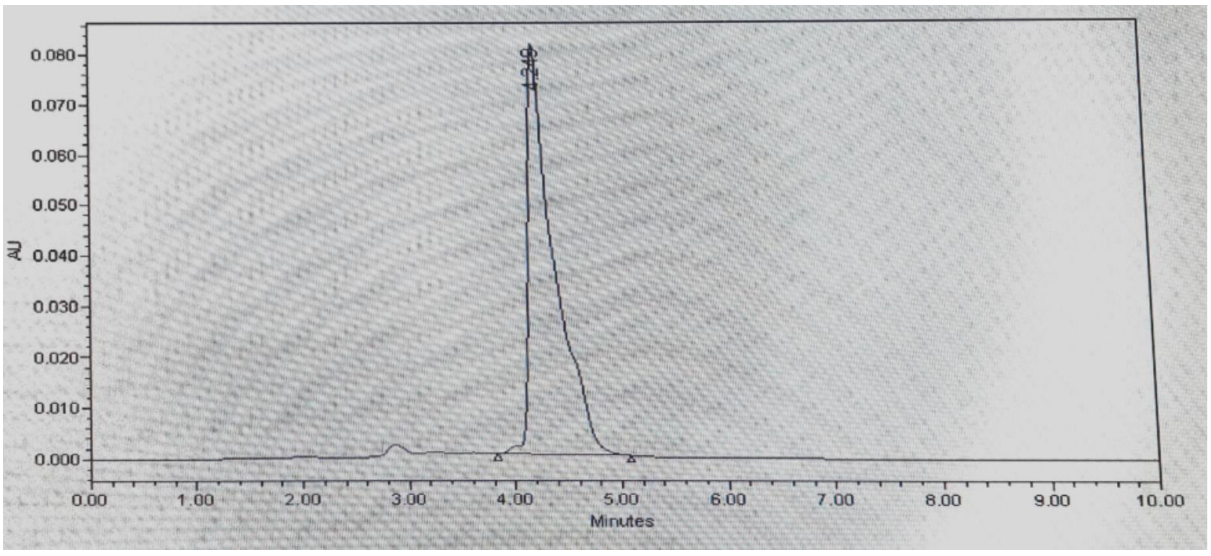

Fig 2: *Standard Chromatogram of Ivermectin*

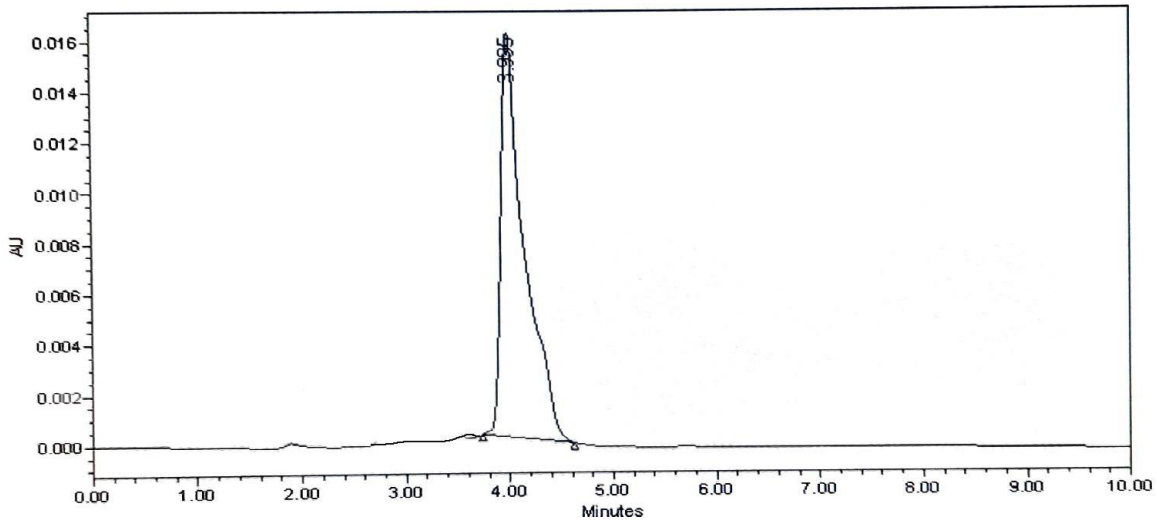

Fig 3: *Sample Chromatogram of Ivermectin*

CALIBRATION CURVE OF HPLC: A straight connection between peak area versus concentration was observed in the range of study. The results of HPLC peak area is expressed in the table 2 and the calibration curve of HPLC by utilizing the same data are expressed in figure 4 with correlation coefficient 0.9798.

Table 2 *Linearity Data of Ivermectin*

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

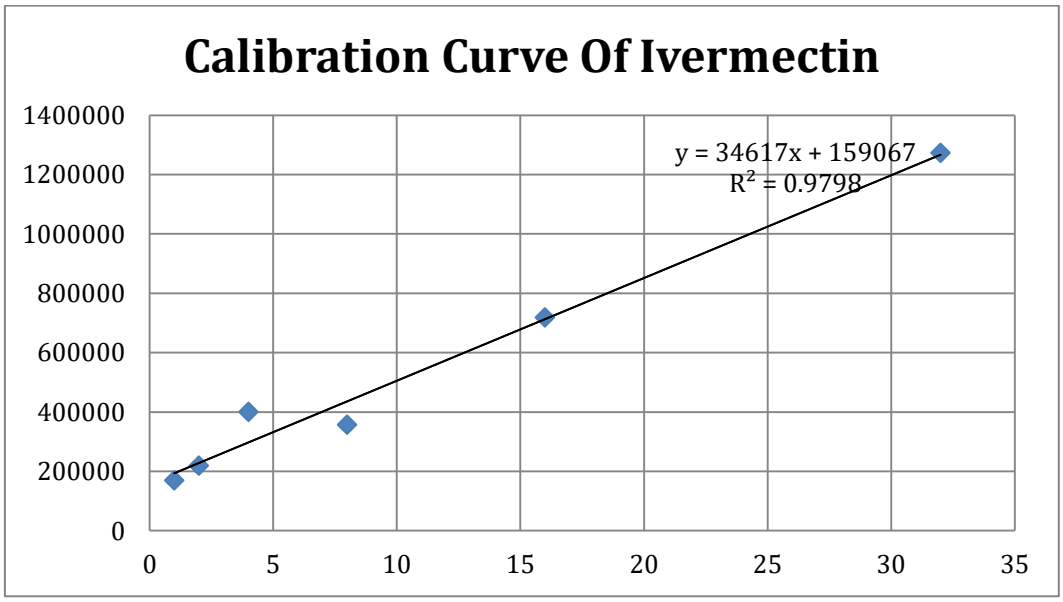

Fig 4: *Calibration Curve of Ivermectin*

PRECISION:It is validated by using the both methods system precision and method precision and the obtained data are expressed in table 3 and 4.

Table 3 *System Precision of Ivermectin*

S.no.	Drug Concentration (µg/ml)	Peak Area	SD %	RSD %
1.	1	1842586	24820.2538	1.352
2.	2	1869521		
3.	4	1833860		
4.	8	1830698		
5.	16	1793472		
6.	32	1844496		

SD: Standard Deviation, RSD: Relative Standard Deviation

Table 4 *Method Precision of Ivermectin*

S.no.	Drug Concentration (µg/ml)	Peak Area	SD %	RSD %
1.	1	248471	4041.34	1.589
2.	2	251774		
3.	4	253255		
4.	8	256293		
5.	16	260132		
6.	32	255793		

SD: Standard Deviation, RSD: Relative Standard Deviation

ACCURACY: Accuracy was done by using UV spectrophotometer and the obtained absorbance are given in table 5.

Table 5 *Accuracy data*

Sample	Absorbance
4ppm	0.155
6ppm	0.226
8ppm	0.355

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION: The LOD and LOQ of the analytes were calculated on the grounds of the standard response deviation and slope, the LOQ being expressed as 3.3 and the LOQ being expressed as 10.

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

Table 6 *LOD & LOQ Values*

Sample	LOD	LOQ
Ivermectin	2.93	8.79

LOD: Limit of Detection, LOQ: Limit of Quantification, S: Slope of the curve, N: Noise ratio.

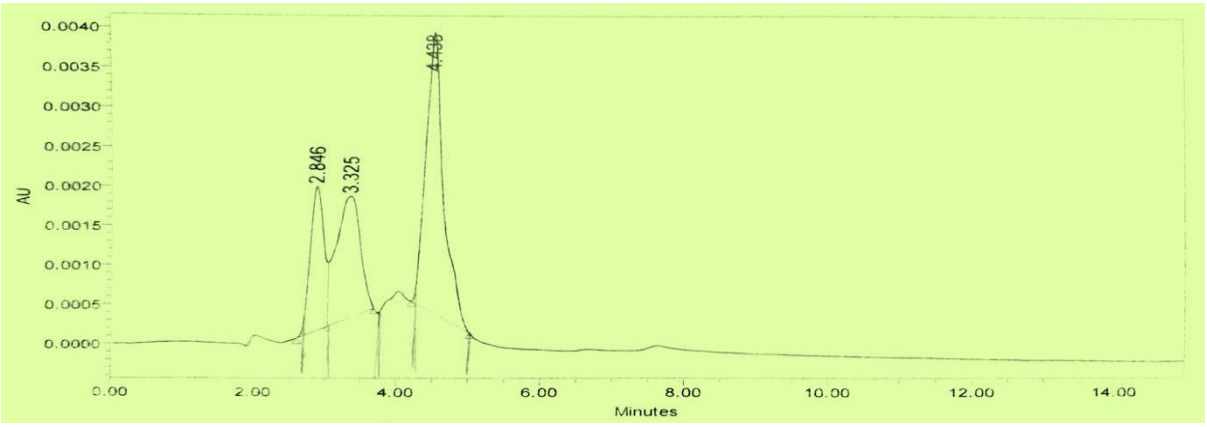

Fig 5: *Chromatogram of LOD& LOQ*

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

Table 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

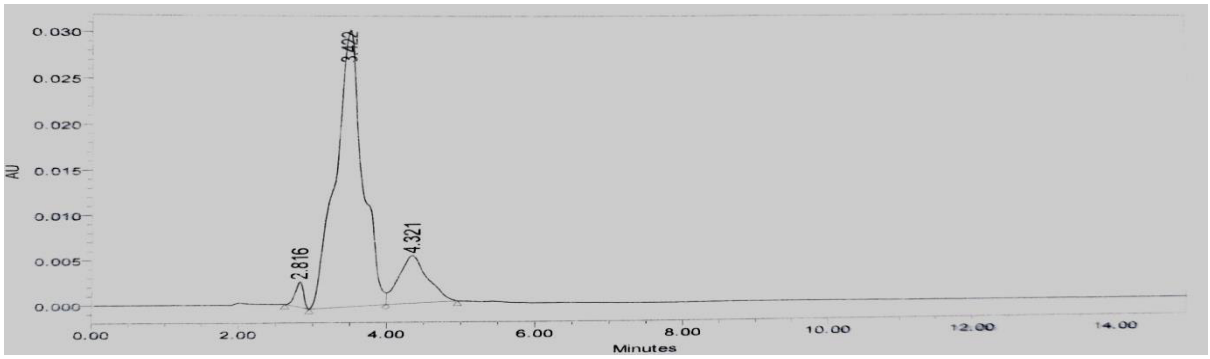

Fig 6: *Chromatogram of THF: H2O*

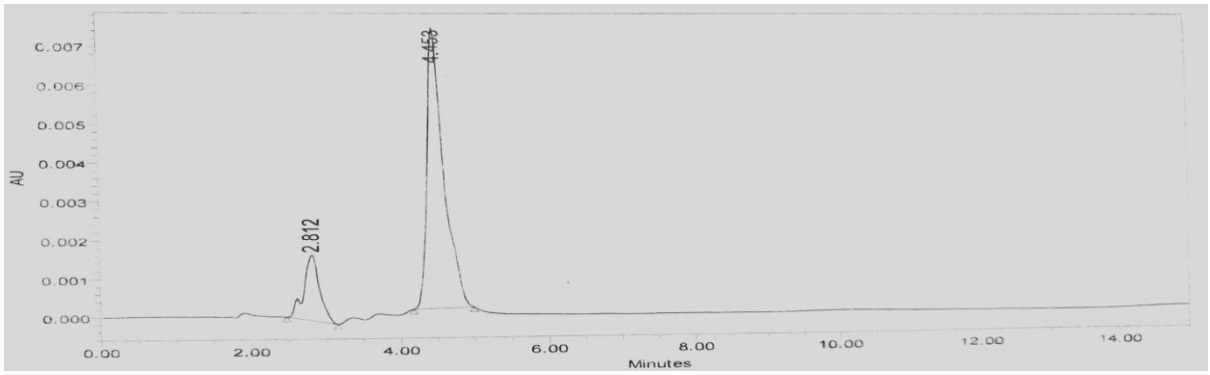

Fig 7: *Chromatogram of METHANOL: H2O*

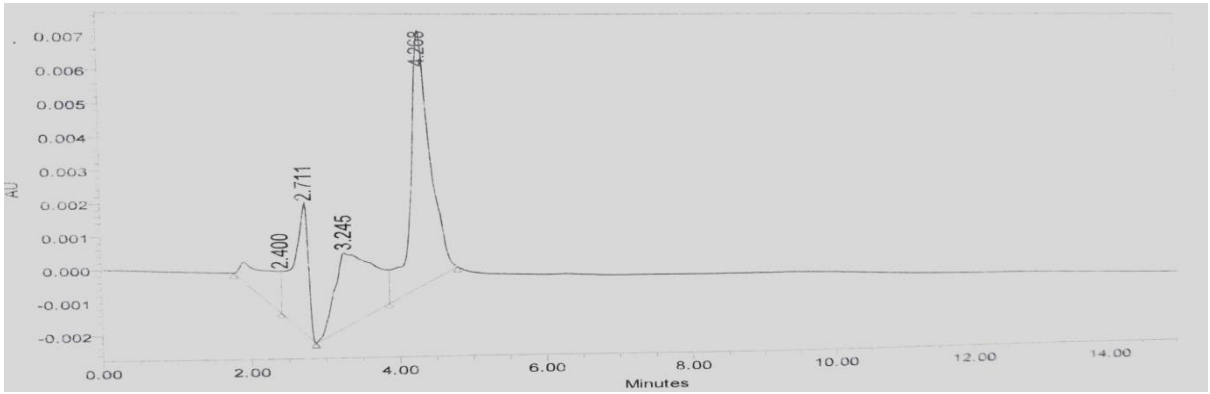

Fig 8: *Chromatogram of ACETONITRILE: H2O*

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

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

RT- Retention Time, AUC- Area Under the Curve, N- Number of Theoretical Plates, TF- Tailing Factor, H- Plate Height

SPECIFICITY AND SELECTIVITY: Ivermectin retention time was 4.198 min, 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.

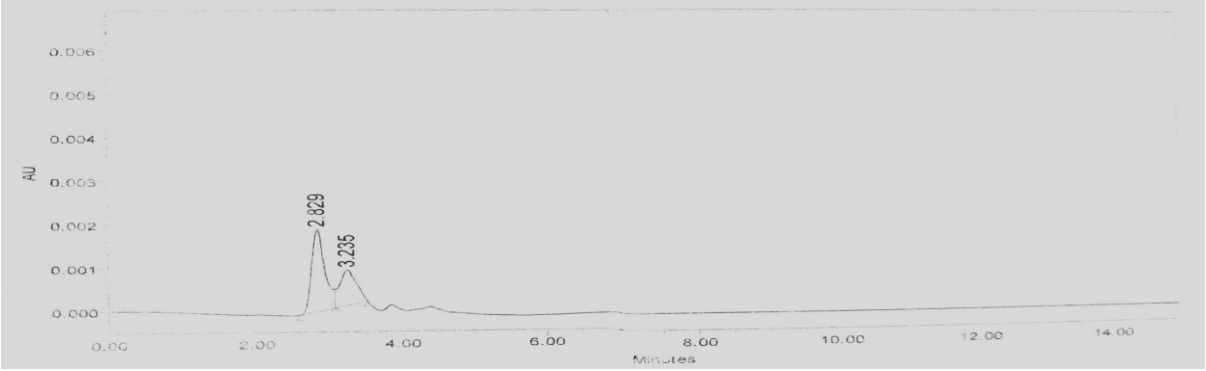

Fig 9: *Chromatogram of Blank*

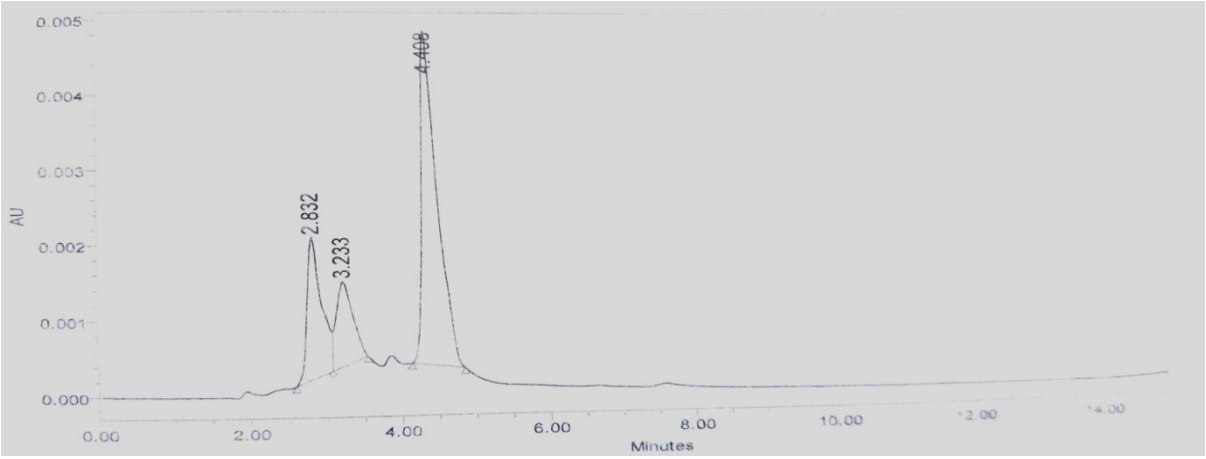

Fig 10: *Chromatogram of Sample*

Table 9 Comparative values of drug Ivermectin by different researchers with corresponding authors

Citations	Linearity (correlation coefficient)	Retention Time	Flow Rate (ml/min)	Precision %RSD Intraday	Interday	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	Inertsil ODS 150*4.6mm*5um	pH sodium phosphate buffer:methanol(25:75v/v)	245
Nischal k *etal.,*	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

2011										
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. Gampa vijay 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
Vegad Kunjal 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	Supel cosil 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**	**1.352**	**1.589**	**-**	**2.93**	**8.79**	**Inertsil C-18 ODS 250*4.6mm*5um**	**ACN:Methanol(60:40v/v)**	**245**

The validation of the drug Ivermectin was done according to parameters given in ICH guidelines. We have compared our results with the previously reported studies on validation of Ivermectin. A comparative chart is mentioned in Table 9.The aim of the present study was the development and validation of an analytical method by utilizing the RP-HPLC technique. The drug Ivermectin being non–polar is preferably analyzed by opposite phase and hence utilization of C18 column was chosen. So, the elution of the column compound was prejudiced by polar mobile phase. Mobile Phase Acetonitrile: HPLC grade methanol (60:40 v/v) was found to give the well resolved, satisfactory separations and good symmetrical peaks. Retention time of drug Ivermectin was found to be 4.198 min, which indicates a good base line. The RSD values for precision and accuracy studies obtained were less than 2% which indicated that developed technique was specific and precise. Method appropriateness and authentication constraints are given in table. The results have been reported and discussed by comparing with the previous reports. In the present study the data were analyzed statistically. It was intended to compare the parameters such as Linearity, precision, accuracy, limit of detection, and limit of quantification, robustness, specificity and selectivity, and system suitability. According to Waldia A. *et al.,* 2008[28] and Patel A *et al.,* 2015[29] (table 9) the linear correlation coefficient (r=0.9798) was comparatively within the range. As per the Nischal k *et al.,* 2011[30], Oltean G. Elena *et al.,* 2011[31] and NVSK Devaka *et al.,* 2019[32] (table 9), the retention time

(4.198 min) of the present study was good and as per Patel A *et al.,* 2015[29] , Konrad P *et al.,* 2013[33] (table 9) the retention time was lesser and according to Bhavya B *et al.,* 2017[34] and Gampa VK *et al*.,2018[35] (table 9*)* the retention time was greater. At the wavelength 245nm the UV spectra has shown the better result. The % RSD of intraday and interday precision for present study was 1.352 and 1.589 while for Ali MM *et al.,* 2017[36] and Shurbaji M *et al*.,2019[37] and it was found to be in range less than 2%. The percentage recovery for the present method and Saidulu B *et al.,* 2015[38] and Vegad kunjal L *et al*., 2017[11] was found in the range 98-102%. Recovery greater than 98% justifies the accuracy of the data. As compared to Gampa VK *et al*.,2018[35] (table 9*)* 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 which gave a sharp peak. In robustness by the alteration of the mobile phase, the developed method was found to be robust. Mobile phase A is tetrahydrofuran: water (70:30v/v) and mobile phase B methanol:water (70:30v/v) and the mobile phase C consisted a mixture of ACN: water (70:30v/v). The data has been illustrated in table 7. 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 8. Finally, the development and validation of an analytical method was performed successfully and the result was obtained satisfactorily.

4. CONCLUSION

The assessment of Ivermectin was conducted by RP-HPLC. The mobile phase comprises of Acetonitrile: Methanol (60:40) % v/v. Inertsil C18 (250x 4.6mm, 5.0mm) or identical was utilized as stationary phase. The identification was done by utilizing a UV detector at 245 nm. The arrangements (solutions) were chromatographed at a constant flow rate of 1.0 ml/min. The analytical method conditions and the mobile phase solvent provided good resolution for Ivermectin. Exclusively, the main features of the developed method are short run time and retention time was 4.198min. System suitability parameters were determined which incorporates efficiency, resolution and tailing factor. The unwavering quality and appropriateness of the technique could be seen from recuperation studies. Further there is no impedance due to excipients. Precision of the techniques were concentrated by making repeated infusions of the samples and values were determined. The strategy was approved for linearity, precision, accuracy, robustness. The strategy is straightforward, explicit and simple to perform and expects short to analyze the samples. Low LOD and LOQ makes this strategy reasonable for Quality control. The technique was discovered to be straight, precise, accurate and robust. Thus, it was concluded that the RP-HPLC method developed was especially appropriate for routine investigation of Ivermectin.

5. ACKNOWLEDGEMENT

The Authors are thankful to KIET School of Pharmacy, KIET Group of institutions, Delhi-NCR, Ghaziabad for providing all the necessary infrastructure for an exhaustive research of this article.

6. AUTHOR CONTRIBUTION STATEMENT

The authors ‘s confirm contribution to the paper as follows: Ms. Sonia Goswami carried out the literature review and experimental work in the lab. Dr. K. Nagarajan conceived the ideas or experimental design of the study and provided guidance for conduction of experiments. Dr. Richa Goel provided guidance in literature review. Mr. Praveen Km. Dixit checked the plagiarism many times. Ms. Vidhu Saxena helped in the drafting of the paper. Mr. Sanjeev km. Chauhan helped in experimental work. Dr. Vinay Kumar helped in the interpretation of the findings of this work. All authors discussed the results and contributed to the final manuscript. All the authors read and approved the final version of the manuscript

7. CONFLICT OF INTEREST

Conflict of interest declared none.

8. REFERENCES

1. The American Society of Health-System Pharmacists. Archived from the original on January 3, 2016. Retrieved January 16,2016.
2. Sneader W (Drug Discovery a History. Chichester: John Wiley & Sons. 2005: 333. ISBN 978-0-470-01552-0.
3. Saunders Handbook of Veterinary Drugs: Small and Large Animal (4 ed.). Elsevier Health Sciences. 2015: 420. ISBN 978-0-323-24486-2. Archived from the original on January 31, 2016.
4. CDC-Centers for Disease Control and Prevention (August 23, 2019). "Ascariasis - Resources for Health Professionals". www.cdc.gov. Retrieved December 28, 2019.
5. Mehlhorn H (2008). Encyclopedia of parasitology (3rd ed.). Berlin: Springer. p. 646. ISBN 978-3-540-48994-8. Archived from the original on June 26, 2020.
6. Vercruysse J, Rew RS, eds. (2002). Macrocyclic lactones in antiparasitic therapy. Oxon, UK: CABI Pub. p. Preface. ISBN 978-0-85199-840-4. Archived from the original on January 31, 2016
7. Dennis J Compatibility of Pharmaceutical Products and Contact Materials: Safety Considerations Associated with Extractables and Leachable 2008 October doi:10.1002/9780470459416
8. Ahmed S, Karim MM, Ross AG, Hossain MS, Clemens JD, Sumiya MK, et al. "A five-day course of ivermectin for the treatment of COVID-19 may reduce the duration of illness". International Journal of Infectious Diseases. 2020 December; 103:214. doi:10.1016/j.ijid.2020.11.191.
9. Bryant A, Lawrie TA, Dowswell T, Fordham EJ, Mitchell S, Hill SR, Tham TC. Ivermectin for Prevention and Treatment of COVID-19 Infection: A Systematic Review, Meta-analysis, and Trial Sequential

Analysis to Inform Clinical Guidelines. Am J Ther. 2021 Jun 21;28(4): e434-e460. doi: 10.1097/MJT.0000000000001402.

10. Jabeen T, M A Khader, And S Jabeen "A Review On The Antiparasitic Drug Ivermectin For Various Viral Infections And Possibilities Of Using It For Novel Severe Acute Respiratory Syndrome Coronavirus 2: New Hope To Treat Coronavirus Disease-2019". *Asian Journal of Pharmaceutical and Clinical Research*, 2020 August;13. (8):21-27, doi:10.22159/ajpcr. 2020.v13i8.38357.
11. Vegad Kunjal L et al. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Ivermectin and Clorsulon in Ivercam Injection. Indo American Journal of Pharmaceutical Research.2017;7(08):523-531.
12. Roz Laing, Victoria G, Eileen D, Ivermectin- Old Drug, New trick? ,Trends in parasitology,2017 March;33(6); doi: 10.1016/j.pt.2017.02.004
13. Campbell WC, Fisher MH, Stapley EO, Albers-Schönberg G, Jacob TA. Ivermectin: a potent new antiparasitic agent. Science, 1983; 221(4613), 823–828.doi:10.1126/science.6308762.
14. Campbell WC. Ivermectin as an antiparasitic agent for use in humans. Annu Rev Microbiol 1991;45: 445–474.doi: 10.1146/annurev.mi.45.100191.002305.
15. Goa KL, McTavish D, Clissold SP. Ivermectin: A review of its antifilarial activity, pharmacokinetic properties and clinical trials in onchocerciasis. Drugs,1991; 42(4): 640–658.doi: 10.2165/00003495-199142040-00007.
16. Awadzi K, Dadzie KY, Schultz-Key H, Haddock DRW, Gilles HM, Aziz MA . Ivermectinin onchocerciasis. Lancet,1984 October; 324(8408): 921.doi: http://doi.org/10.1016/s0140-6736(84)90671-8.
17. Schultz-Key H. Observations on the reproductive biology of Onchocerca volvulus. ActaLeiden,1990; 59(1), 27–43.
18. Taylor HR, Parque M, Munoz B, Greene BM. Impact of mass treatment of onchocerciasiswith ivermectin on the transmission of infection. Science,1990; 250(4977): 116–118.doi: 10.1126/science.2218502.
19. Cupp EW, Bernardo MJ, Kiszewski AE, Collins RC, Taylor HR, Aziz MA, Greene BM. The effects of ivermectin on transmission of Onchocerca volvulus. Science,1986; 231(4739):740–742.doi: 10.1126/science.3753801.
20. Pong SS, Wang CC, Fritz LC. Studies on the mechanism of action of avermectin Bla: stimulation of release of gamma-aminobutyric acid from brain synaptosomes. J Neurochem,1980 February; 34(2): 351–358.doi:10.1111/j.1471-4159. 1980.tb06604. x.
21. Anna Kulik, Wanda Bia Ecka, Marzena Podolska, Barbara Kwiatkowska-Puchniarz and Aleksander Mazurek, HPLC Method For Identification And Quantification Of Benz-Imidazole Derivatives In Antiparasitic Drugs, Acta Poloniae Pharmaceutica ñ Drug Research,2011; 68 (6): 823-829.
22. Harod S S, Manocha Nimita, Hingole Ashwin and Dubey P.K., Development and Validation of Analytical Method for Fluconazole and Ivermectin in Tablet Formulation by Using RP-HPLC, International Research Journal of Pharmacy, 2012; 3 (8): 257-261.
23. Patel JK, Patel NK. Stability-Indicating RP HPLC Method for the Determination of Ambrisentan and Tadalafil in Pharmaceutical Dosage Form. Sci Pharm. 2014 May 22;82(4):749-63. doi: 10.3797/scipharm.1403-22.
24. Pontes Flavia LD, Roberto Pontarolo, Francinete Ramos Campos, Joao Cleverson Gasparetto, Marco Andre Cardoso, Mario Sergio Piantavini and Angela Cristina 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,2013; 6, (2): 191-200.
25. Limbani Rajen K, Modi Jignasa and Pasha T Y, Simultaneous Equation Method for The Estimation of Ivermectin and Clorsulon in Their Combined Pharmaceutical Dosage Form By UV-Visible Spectrophotometry, International Bulletin of Drug Research.,2014; 4(6): 131-139.
26. Chomwal kumar Rajiv, and Goyal Anju, Simultaneous Spectrophotometric Estimation of Albendazole and Ivermectin in Pharmaceutical Formulation, Research and Reviews: Journal of Pharmaceutical Analysis,2014 January-March; 3 (1): 11-14.
27. K Na-Bangchang, V Banmairuroi and A Choemung, High-Performance Liquid Chromatographic Method For The Determination of Ivermectin In Plasma, Southeast Asian J Trop Med Public Health,2006 September; 37 (5) :848-58.
28. Waldia A, upta S, Issarani R and Nagor P Badrii, Validated liquid chromatographic method for simultaneous estimation of albendazole and ivermectin in tablet dosage form, Indian Journal of Chemical Technology,2008 November; 15(16): 617-620.
29. Patel A, Joshi Hirak V, Patel JK, Development and Validation of stability indicating RP-HPLC method for estimation of ivermectin and albendazole in pharmaceutical dosage form, Indian journal of drugs ,2015; 3(3):57-70.
30. Nischal K, Somshekar B, Abhilekha P M, Sharadamma K C, Radhakrishna P M, A Simple RP-HPLC Method for Estimation of Triclabendazole and Ivermectin in a Pharmaceutical Suspension Dosage Form,Current pharma research, 2011;1(4): 306-310.
31. Oltean G. Elena, Development and Validation of a RP-HPLC method for the quantitation studies of Ivermectin in solution Dosage Form, Medicamental Vetrerinar/ Vetrenary Drug 2011.,;5(2):68-70 .
32. NVSK Devaka, Rao VM, Chromatographic Quantification of ivermectin and pranziquantel in the tablets using stability indicating RP-HPLC method, Pharma sciences,2019; 25(3):254-265., doi: 10.15171/PS.2019.41
33. Konrad P, Piotr J, Determnation of ivermectin in medicated feeds byn liquid chromatography with fluroscence detection,The scientific world journal,2013.Doi: http://doi.org/10.1155/2013/362453.
34. Bhavya B, Nagaraju P, Mounika V, G Indira Priyadarshini. Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Albendazole and Ivermectin in Pharmaceutical Dosage form. Asian J. Pharm. Ana. 2017; 7(1): 6-14. doi: 10.5958/2231-5675.2017.00002.3

35. Gampa VK, sravanthi B, Praveen A, Analytical method development and validation for ivermectin and Albendazole in combine dosage form by RP-HPLC, Pharma research library;22 September 2018
36. Ali MM, Elbashir Elfatih, Mohamed N Abdalaziz. Development and Validation for HPLC Method of Assay of Ivermectin and Clorsulon in Combined Pharmaceutical Dosage Form. International Journal of Homeopathy & Natural Medicines 2017; 3 (6): 45-55. doi: 10.11648/j.ijhnm.20170306.11
37. Shurbaji M, Mohamad H Abu Al Rub, Munib M Saket, Ali M Qaisi, Maher L Salim, Eyad S M Abu-Nameh, Development and Validation of a New HPLC-UV Method for the Simultaneous Determination of Triclabendazole and Ivermectin B1a in a Pharmaceutical Formulation, Journal of AOAC INTERNATIONAL,2010 November; 93(6): 1868–1873. Doi: https://doi.org/10.1093/jaoac/93.6.1868
38. Saidulu B, G Usha, Dr. V Uma Maheshware Rao, Analytical method development and validation for simultaneous estimation of albendazole and ivermectin tablet dosage form by RP-HPLC, Journal of pharmaceutical and biomedical Analysis letters,2015; 3(1):193-202.