International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 367-379 www.ijprajournal.com ISSN: 2456-4494

"Development and Validation of Rp- Hplc Method for Estimation of Ivermectin in Tablet Dosage Form"

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Submitted: 01-07-2022 Accepted: 14-07-2022

ABSTRACT: The main aim of this study is to develop and validate the Ivermectin drug substance by reverse-phase liquid chromatography (RP-HPLC).Analytical chemistry is the part of chemistry which has occupy and significant spot in the advancement of pharmaceutical science and technology.The objective of the current work is to develop a simple, efficient, economical and compatible RP-HPLC method for the analysis of Ivermectinin bulk and pharmaceutical dosage formAt present a few analytical strategies are accessible for breaking down analytes viz. spectroscopic and chromatographic. Spectroscopic technique incorporates UV-obvious, infrared, mass, NMR, absorbance spectroscopy while chromatographic strategies incorporate elite liquid chromatography (HPLC), high- performance thin layer chromatography (HPTLC), gas chromatography (GC), supercritical chromatography, gel permiation chromatography techniques so on.

Keywords: Ivermectin, Detection, RP HPLC, Gas Chromatography, Super Critical Chromatography

I. INTRODUCTION:

Analytical chemistry is the part of chemistry which has occupy and significant spot in the advancement of pharmaceutical science and technology .Today ,the discipline of pharmacy has gained huge ground and has developed as a particularly free branch as pharmaceutical science .Pharmaceutical science spread all the stage identified with drug ,from its discovering ,its turn of events , method of activity ,safety, formulation ,use ,quality control ,bundling , storage, marketing, etc.

Analytical Methods:
- Spectroscopic Methods
- UV-Visible Spectroscopic method
- Infrared Spectroscopy
- Mass Spectroscopy
- NMR Spectroscopic method

Chromatographic Methods
- High Performance Liquid Chromatography
- Supercritical Fluid Chromatography
- High Performance Thin Layer Chromatography
- Gas Liquid Chromatography
- GC-MS
- LC-MS

Electrochemical Methods
- Conductometry
- Voltametry
- Potentiometry
- Coulometry
- Atomic Absorption Spectroscopy
- Emission (Plasma) Spectroscopy

Other Conventional Methods:
- Titrimetry
- Gravimetry

The complete analysis of a substance consists of five main steps:
- Sample preparation / sampling,
- Dissolution of the samples,
- Conversion of the analyte into a form suitable for Measurement,
- Measurement,
- Calculation and interpretation of the measurement

HPLC is a condensing for High Performance Liquid Chromatography (It has similarly been depicted as High-Pressure LC). HPLC has been around for concerning 35 years as well just like the greatest separating strategy utilized. HPLC is a partition technique that involves: The infusion of a minuscule volume of liquid model squarely into a cylinder stacked with small amounts (3 to 5 micron (μm) in size called

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the decent stage). Where individual pieces of the example are dropped down the stuffed cylinder (section) with a fluid (portable stage) expected through the segment by high strain conveyed by a siphon. These parts are separated from one another by the section pressing that incorporates different synthetic or potentially actual associations between their atoms as well as the pressing pieces. These separated components are identified at the leave of this cylinder (segment) by a course through gadget (indicator) that decides their amount. A result from this locator is known as a "fluid chromatogram In concept, LC and HPLC work similarly except the speed, effectiveness, sensitivity and simplicity of operation of HPLC is significantly exceptional.

HPLC COMPONENTS

1. Pump: The function of the heart is to require a fluid (called the moveable phase) with the runny chromatograph at a exact flow degree, spoken in mils per minutes (mL/min). Regular flow rates in HPLC remain in the 1-to 2-mL/min variety. Characteristic hearts can reach stress in the series of 6000-9000 psi (400-to 600-bar). During the chromatographic experiment, a pump can supply a consistent mobile phase structure (isocratic) or an enhancing mobile stage composition (slope).

2. Injector: The injector helps to present the liquid example into the circulation watercourse of the moveable stage. Common example volumes are 5- to 20-microliters (µL). The injector necessity also be able to by attitude the tall weights of the fluid system. A car sampler is the automatic variation for when the customer has several examples to assess or when hands-on shot is not sensible.

3. Column: Considered the "heart of the chromatograph" the column's stationary phase separates the example elements of interest utilizing various physical as well as chemical criteria. The little bits inside the column are what trigger the high back pressure at typical flow prices. The pump should press tough to relocate the moveable stage finished the column as well as this resistance triggers a high pressure within the chromatograph. Sorts of columns Analytical [inner size (i.d.) 1.0 - 4.6- mm; sizes 15-- 250 mm] Preparative (i.d.> 4.6 mm; sizes 50-- 250 mm). Capillary (i.d. 0.1 -1.0 mm; different sizes). Nano (i.d.< 0.1 mm, or in some cases stated as < 100 µm).

4. Detector: The identifier can see (recognize) the singular particles that come out (elute) from the segment. An indicator effectively gauges how much those atoms by the goalmouth that the scientist can quantitatively investigate the example parts. The indicator gives a result to a recorder or PC those outcomes in the fluid chromatogram (i.e., the diagram of the identifier reaction).

5. Computer: Often called the information framework, the PC not just controls each ace of the modules of the HPLC instrument yet it takes the sign from the locator and utilizations it to decide the hour of elution (maintenance season) of the example parts (subjective investigation) and how much example (quantitative examination).

Advantages of HPLC¬It gives explicit, delicate, and exact strategy for examination of various muddled examples. ¬ There is simplicity of test planning and test presentation. ¬ Speed of investigation ¬Investigation by HPLC is explicit, exact and exact. ¬ Proposals benefit over gas chromatography in investigation of numerous glacial, ionic materials, metabolic items and thermolabile as well as non-unpredictable substances.

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1.METHOD DEVELOPMENT:

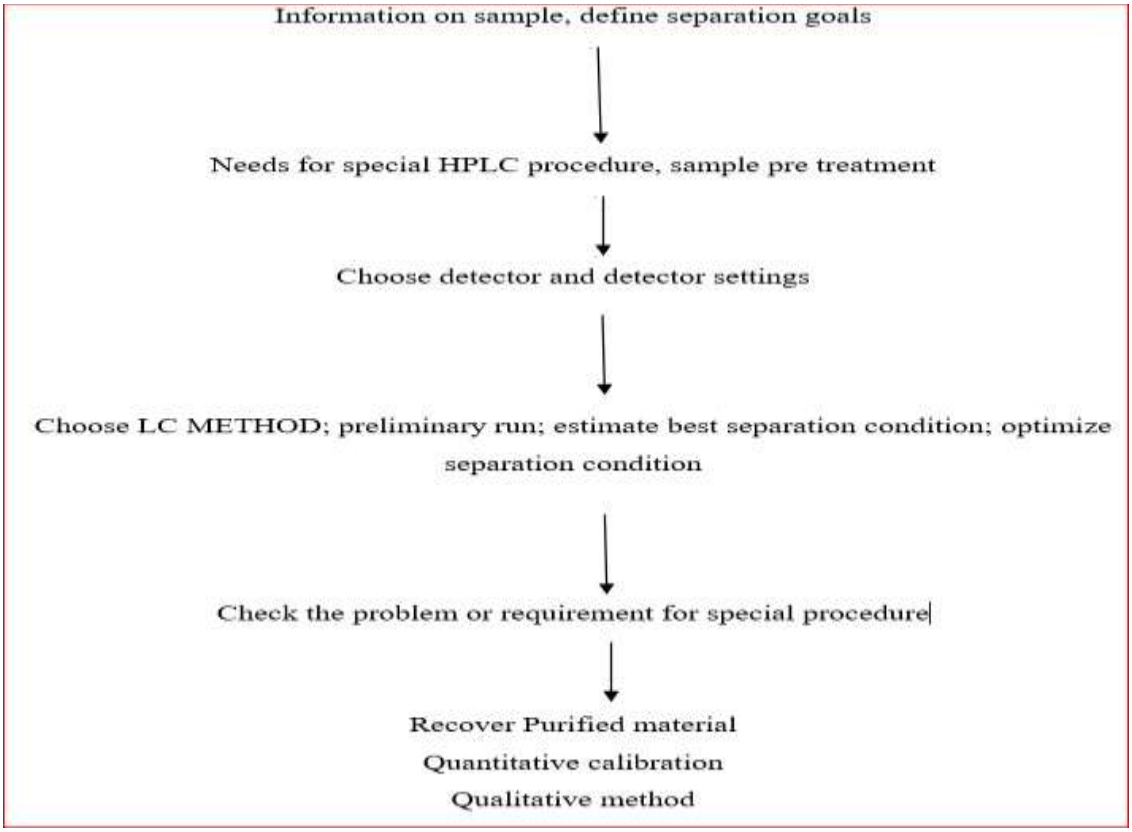

Figure: method of HPLC

1.2.Method validation

is the interaction used to affirm that the scientific strategy utilized for a particular test is reasonable for its expected use. Results from technique approval can be utilized to pass judgment on the quality, dependability, and consistency of logical outcomes; it is a rudimentary part of any great insightful practice. As indicated by ICH Guidelines Authentication of a Logical system is to exhibit that it is reasonable for its expected reason. Endorsement of logical techniques is facilitated to the four most ordinary sorts of legitimate system:

• Recognizing evidence tests;
• Quantitative tests for contaminations' substance;
• Limit tests for the control of contaminations;
• Quantitative preliminary of the powerful moiety in instances of prescription substance or drug thing or other picked component(s) in the medicine thing. Normal approval attributes which ought to be careful are recorded beneath:

- Accuracy
- Precision
- Repeatability
- Intermediate Precision
- Specificity
- Detection Limit
- Quantitation Limit
- Linearity
- Range

1.2.1 ACCURACY:The accuracy of an intelligent procedure imparts the closeness of course of action between the value which is recognized either as a standard certifiable worth or a recognized reference regard and the value found. This is to a great extent named validity.

1.2.2 REPEATABILITY :The Repeatability conveys the exactness under comparable working conditions all through a short stretch of time. Repeatability is moreover named intra-test precision.

1.2.3 REPRODUCIBILITY :Reproducibility imparts the exactness between research focuses (agreeable examinations, ordinarily applied to standardization of approach). Recognition LIMIT The ID farthest reaches of an individual logical

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strategy is the most insignificant proportion of analyte in a model which can be perceived anyway not actually quantitated as an exact worth.

1.2.4 QUANTITATION LIMIT: The quantitation farthest reaches of an individual legitimate technique is minimal proportion of analyte in a model which can in any case hanging out there with proper exactness and accuracy. Quite far is a limit of quantitative analyzes for low levels of blends in model systems, and is used particularly for the confirmation of contaminations and also corruption things.

1.2.5 LINEARITY: The linearity of a quick system is its ability (inside a given reach) to get test results which are clearly relating to the obsession (proportion) of analyte in the model.

1.2.5 RANGE:The extent of a smart procedure is the stretch between the upper and lower center (proportions) of analyte in the model (counting these obsessions) for which it has been displayed that the logical strategy has a healthy level of precision, precision and linearity.

II. REVIEW OF LITERATURE:

Mahmoud Mohamed Ali, (2017)reported new validated RP-HPLC method for determination of ivermectin in its bulk and pharmaceutical dosage.the invented technique envolved the Thermo BDS C-18 (15cm x 4.6mm, 5 µm) column, the utilizing mobile phase decided acetonitrile, methanol and purified water in the concentration range of 60: 30:10 (v/v/v), wavelength detected UV detection at 245 nm. The indicated stratergy found linear over a level of 10- 40µg/ml with a correlation coefficient (r2) of 0.9998 for Ivermectin. Validations of the given procedure were done for its accuracy (%recovery), precision, linearity and range, specificity, LOD and LOQ according to ICH guidelines.

VegadKunjal L, et al. (2017) they developed and validated the new path for the estimation of Ivermectin and Clorsulon in Ivercam injection. (RP- HPLC) technique was invented for routine quantification of Ivermectin and Clorsulon. Column BDS hypersil C18 (5µ, 250 x 4.6 mm) mixture of 60 phosphate buffer (pH 5.5 remaking with 1% O-phosphoric acid) & Methanol (60:40 v/v) with trhe flow rate of 1 mL/min with UV detection of wavelength at 234 nm. The new mwthod became validated for linearity, accuracy and precision. In RP-HPLC techniique, the calibration curves were linear in the proportion range of 2.5-7.5 µg/ml for Ivermectin& 25-75 µg/ml for Clorsulon with % recoveries of 100.34 % and 99.76% for Ivermectin and Clorsulon respectively.

III. PLAN OF WORK

Work was planned on the conventional lines of procedure in development of analytical methods for formulations and is as follows. Selection of Formulation: By literature review and market survey. Development of HPLC Method by using RP-HPLC:

- Procurement of pure drug samples
- Selection of suitable solvent
- Selection of Wavelength
- Selection of mobile phase
- Selection of Stationary Phase
- Optimization of chromatographic condition
- Study of System Suitability Parameter
- Application of proposed method to the marketed formulation Method validation: by ICH guideline
- Linearity
- Accuracy
- %recovery
- Precision
- Robustness
- Limit of detection
- Limit of quantitation

IV. DRUG PROFILE OF IVERMECTIN

IUPAC NAME	22,23-dihydroavermectin B1a + 22,23-dihydroavermectin B1b
EMPIRICAL FORMULA	C47H72O14 (H2B1b)
MOLECULAR WEIGHT	875.1 g/mol
CAS NO-	70288-86-7
APPEARANCE:	white to yellowish-white, non hygroscopic, crystalline powder

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MELTING POINT	155°C.
STORAGE	Store at room temperature below30°C
SOLUBILITY	it is insoluble in water but is freely soluble in methanol and ethanol

4.1Mechanism of Action:
Ivermectin causes influx of ions through the cell membrane of parasites bythe activation ofspecificiivermectin-sensitive ion channels. The resultant hyperpolarization lead to pathogens death.

4.2 MODE OF ACTION:
Metformin is an anti-hyperglycaemic agent, which improve glucose tolerance in patient with type-2 diabetes, lowering both basal and postprandial plasma glucose. Its pharmacological mode of action is different from another oral anti-hyperglycaemic agents[1]. Metformin decrease hepatic glucose production, decrease intestinal absorption of glucose, and improve insulin sensitivity by increasing peripheral uptake and utilization. Unlike sulfonylurea, metformin does not produce hypoglycemia in either patient with type 2 diabetes or normal subjects and does not cause hyperinsulinemia. With metformin therapy, insulin secretion remains unchanged while fasting insulin level and daylong plasma insulin response may actually decrease.[12]

V. LIST OF REAGENTS AND CHEMICALS USED:

Table: List of Reagents and Chemicals Used

Reagents and Chemicals	Details	Make
Water	HPLC grade	MI
Methanol	HPLC grade	Finar

5.1 PHARMACOLOGICAL STUDY
INDICATIONS:
TeneligliptinHBr hydrate used for treatment of Type-2 diabetes mellitus.

5.2 MODE OF ACTION: [18]
Teneligliptin inhibited human dipeptidyl peptidase-4 enzyme activity with the IC_{50}=1 nM, more than 150 fold selectivity against DPP-8 and DPP-9 which suggested little off target skin lesion side effect. By DPP-4 inhibition, teneligliptin prevented the degradation of incretins GIP and promoted insulin release which prevented blood glucose increase after food intake with little hypoglycemia risk during lifetime taken.

VI. MATERIALS AND METHODS USED

INSTRUMENTS

Table: Instrument Used

Sr.No.	Name	Model	Manufacturer/ Supplier
1.	Electronic weighing balance	PGB 100	Wensar
2.	Digital pH meter	pH cal	Analab Instrument
3.	Ultra-Sonicator	WUC-4L	Wensar
4.	UV- Spectrophotometer and Software	UV2450 UV probe v 2.3.3	Shimadzu

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5.	HPLC	HPLC 3000 series P- 3000-M reciprocating (binary pump) UV-3000-M (UV-Visible Detector)	Analytical Technologies ltd.

VII. EXPERIMENTAL WORK:

Preliminary Characterization Identification of drug:Color, odor and appearance

Ivermectin is assessed for different Pre-definition boundaries like shading, smell and appearance and affirmed that they followed official guidelines.

High Performance Liquid Chromatography (HPLC) Method for AnalysisofIvermectin

i. Ultraviolet (UV)spectroscopy:

(Selection of analytical wavelength)

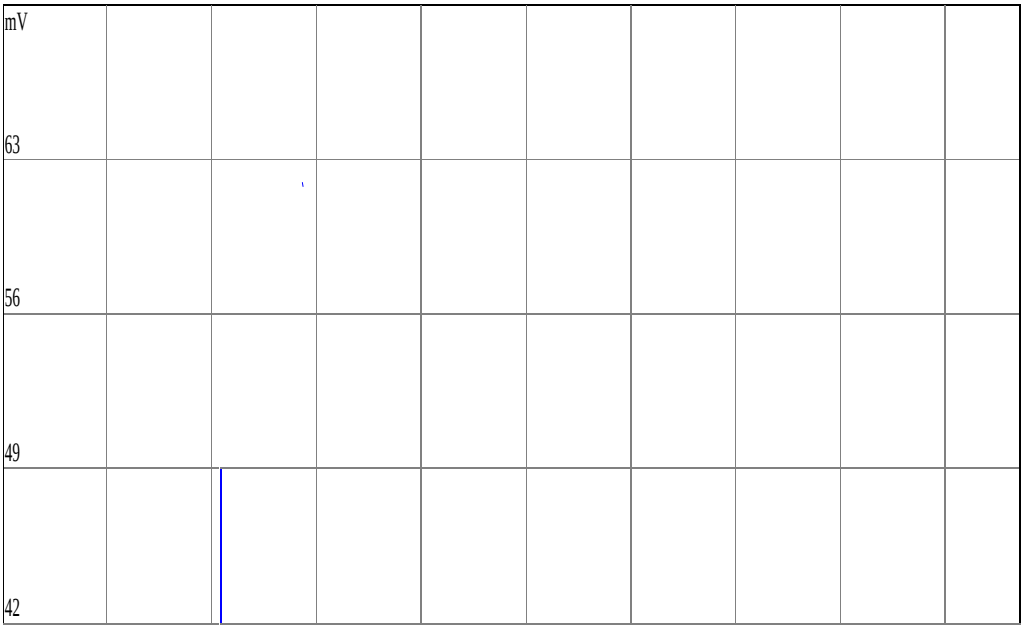
Precisely gauged amount of ivermectin 10 mg disintegrated in 100 ml volumetric flagon utilizing methanol and volume is make sufficient to get 100 µg/ml. From this arrangement 2.5 ml was taken and included 10 ml volumetric flagon and weakened sufficient utilizing methanol. Arrangement was examined utilizing UV- Visible Spectrophotometer in the range mode between the frequency scopes of 400 nm to 200 nm. The frequency chose was 245nm.

ii. Selection of mobilephase:

The unadulterated medication of Ivermectin was infused into the HPLC framework and run in various dissolvable frameworks. Blend of various solvents were attempted so as to decide ideal chromatographic conditions for compelling elution of relative medication. After a few stage and mix, it was discovered that the Methanol: Water gives agreeable outcomes when contrasted with other versatile stages. At long last, the ideal piece of the versatile stage chose according to structure, which gives worthy pinnacle shape and balance of Ivermectin.

iii.Development and Optimization of RP – HPLC MethodIvermectin RP-HPLC Method development:

SampleName : Ivermectin Trial 04 Wavelength :245nm
MobilePhase : Methanol: Water (95:05)
Samplevolume :20µl
Flowrate: 1.5ml/min
Pressure :9-10MPa
Runtime :7.01min



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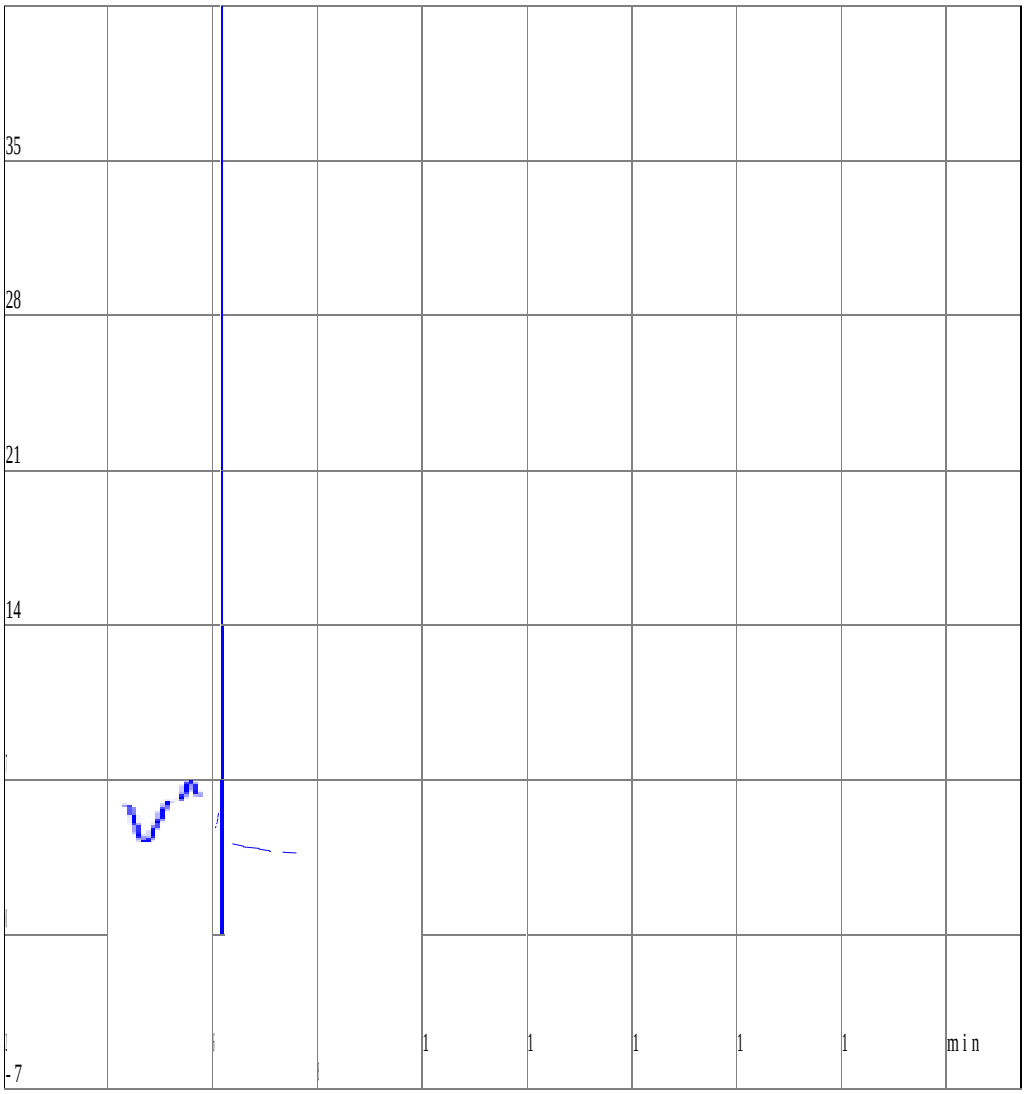

Fig. Chromatogram

RankTime	Area	Resolut	T.PlateNum	Asymmetry
4.558	759157	0.00	8487	1.08

iii. Optimized ChromatographicConditions:

The accompanying chromatographic conditions were built up by experimentation and were kept consistent all throughtechnique.

Table. Optimized Chromatographic Condition

Parameter/ conditions	Description/Values
Column name	Cosmosil C18, (250×4.6mm,5µ)
Detector	245 nm

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Flow rate	1.5 ml/min
Injection volume	20µL
Column Temperature	Ambient
Run time	7.01min
Retention time	4.558
Mobile Phase	Methanol: Water (95:05)

Application of proposed method for analysis of marketed formulation

a) Standard StockSolution

Precisely gauged amount of Ivermectin 10 mg separately broke down in 10 ml volumetric cup utilizing versatile stage and arrangement was sonicated for 20 minutes and volume is make sufficient to get 1000 µg/ml and sifted through 0.45µm film channel. 1ml from every arrangement taken and disintegrated in 10ml volumetric cup separately utilizing portable stage to get 100 µg/ml.

b)Preparations of working standardsolution:

Procedure:

From the standard stock arrangement Ivermectin 0.1 ml taken and included 10 ml volumetric jar and weakened sufficient with versatile stage.

c)Preparation of Samplesolution:

Proedure

20 tablets were gauged and powdered, tablets powder equal to 10 mg of Ivermectin and was moved to 100 ml volumetric cup, adequate measure of versatile stage was included and disintegrated by 20minutes ultrasonication. at that point made the volume upto the imprint with the portable stage and sifted with 0.45µ channel paper. Pipette out 1.5 ml from above arrangement and weakened to 10 ml versatile stage and use for test injection.

Validation of method for analysis of ivermectin:

A. Linearity:

Linearity of a diagnostic strategy is its capacity to evoke test results that are straightforwardly corresponding to the convergence of analyte in tests inside a given range.

The linearity of the systematic technique is dictated by numerical treatment of test outcomes got by investigation of tests with analyte focuses over the claimed range. Region is plotted graphically as a component of analyte focus. Percentage curve fittings aredetermined.

Preparation of sample Solution:

Appropriate volume from the stock solution was diluted to get the final concentration of 5 - 15 µg/ml for Ivermectin and chromatogram was recored for each concentration.

Acceptance criteria:

i. The plot should be linear.

i. Correlation Coefficient should be not less than 0.99999

Observation:

The linear fit of the system was illustrated graphically. The results are presented in figure

B. Accuracy(%recovery):

The accuracy of analyte logical technique is closeness of test outcomesto the geniun true value. It is often expressed as %recovry by assay of recuperated amount ofanalyte.

the accuracy of a diagnostic strategy is dictated by applying the technique to dissected examples, to which known measures of analyte have been included. The accuracy is determined from the test results as the level of analyte recuperated by the measure.

Procedure for Preparation of sample Solution:

Drug Assay was acted in triplicate according to test technique with comparable measure of Ivermectin into each volumetric jar for each spike level to get the focus proportional to half, 100%, and 150% of the marked sum according to the test strategy. The average %recovery of Ivermectin wasdetermined.

$$\%\text{Recovery} = \frac{\text{Amount Recovered}}{\text{Amount Added}} \times 100$$

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Acceptance Criteria:
The mean % recovery at each spike level should be not less than 98% and not more than 102% for both drug
Observation:
The recovery results indicating that the test method has an acceptable level of accuracy. Result of accuracy shown in table No.7.3

Precision:

The precision of an analytical strategy communicates the closeness of understanding (level of disperse) between a progression of estimations got from different testing of similar homogeneous sample under the recommended conditions.

Precision of the strategy was determined by Repeatability (intraday) and Intermediate Precision (interday) examines. Precision study was done by infusing a sample into HPLC without changing the assay strategy and the outcome are demonstrated as the %RSD is under 2% for Ivermectin. The low RSD esteem show that the technique was exactly good work.

E. Limit of Detection (LOD) and limit of quqntitation (LOQ):

Limit of detection: minimum concentration of analyte in the given solution or sample that the process can detect but not necessarily quantify under the stated experimental condition easily shows the sample is below or above certain level. Limit test prescribed as parts per million. Limit of detection will not depend only on procedure but also type ofinstruments.
S/N= 2/1 or 3/1s

Where,

S= Signal N=Noise

It may be calculated based on standard deviation (SD) of the response and slope of the curve(S).
LOD=
Where, SD= Standard deviation S= Slope

Limit of quantitation:
From the linearity data calculate the limit of quantitation, using the following formula LOQ

Where,
SD = standard deviation
S = slope

VIII. RESULT:

Preliminary Characteristics:

Table. Preliminary Characteristics

Drug	Colour	Odour	Appearance
Ivermectin	White	Odourless	Crystalline

System Suitability:

HPLC framework was improved according to the chromatographic conditions. 20 µl of standard arrangements of medications were infused in three-fold into the chromatographic framework. The chromatogram were recorded and measure the reaction for the significant pinnacle. framework reasonableness boundary, for example, maintenance time, hypothetical plate and asymmetry factor.

Table: Data system for suitability of ivermectin

System Suitability parameter	Ivermectin
Retention time	4.558
Resolution	0.00
Theoretical Plate	8487
Asymmetric Factor	1.08

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Assay of the Developed RP-HPLC Method:

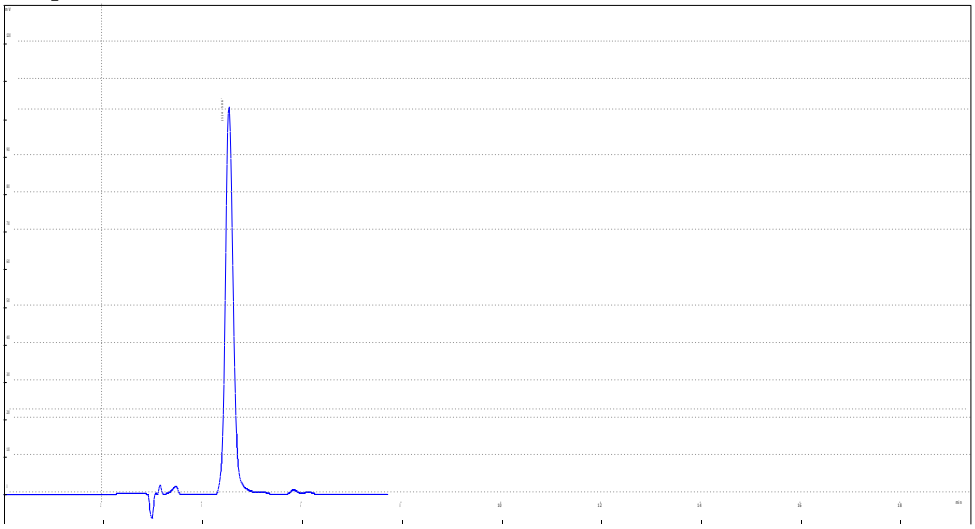
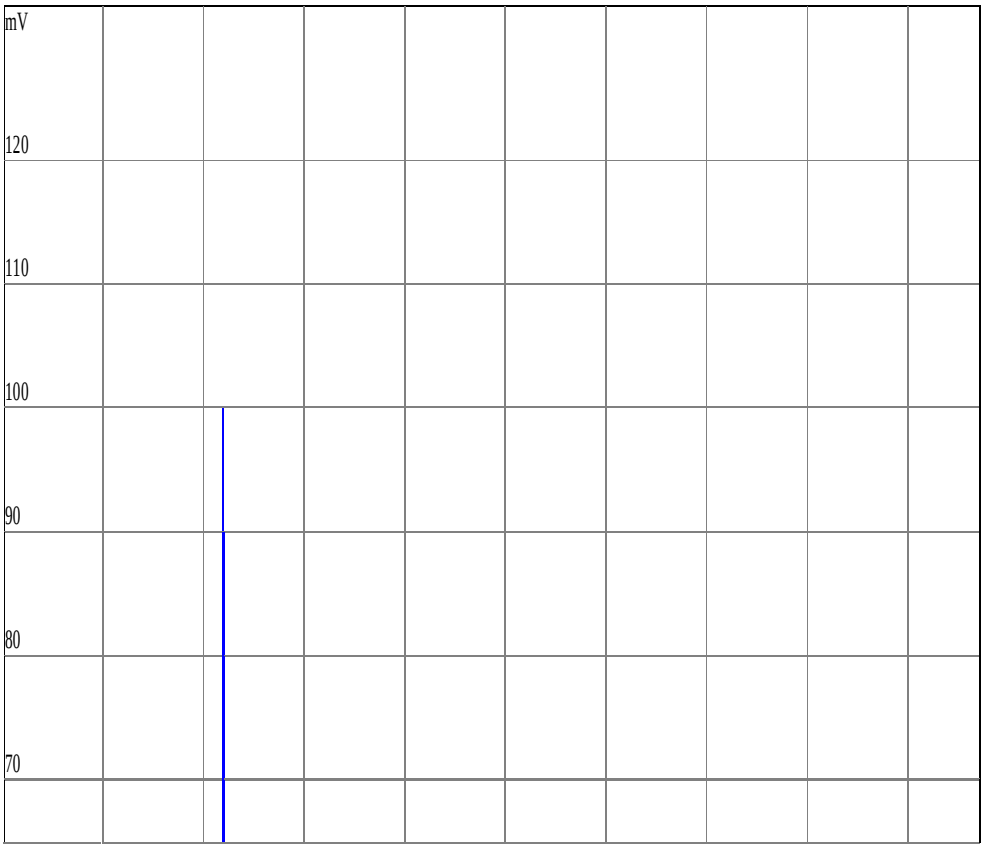


Figure. Chromatogram for Assay standard

Rank time	Area	resolution	t. plate no.	asymmetry
4.506	1443556	0.00	8863	1.11



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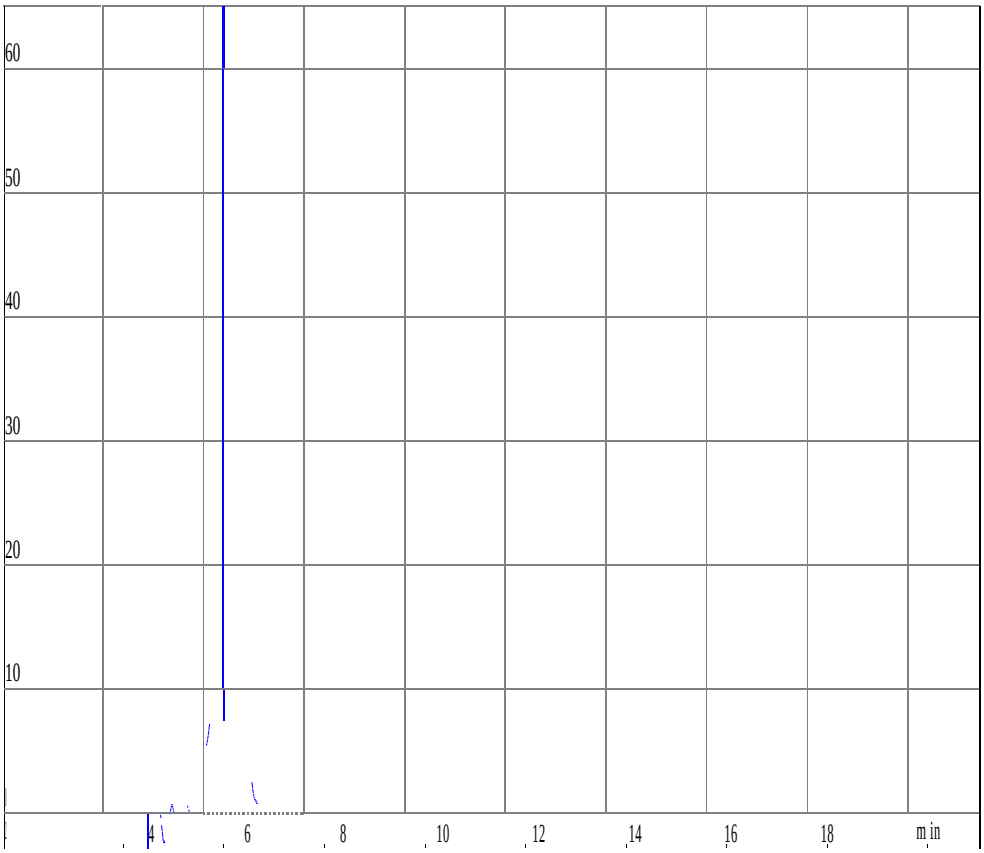

Figure. Chromatogram for Assay sample

Rank time	Area	resolution	t. plate no.	asymmetry
4.362	1440222	0.00	8438	1.08

It is carried out by preparing 30ppm concentration of the formulation. And the resulting %assay is calculated.

Sr. NO.	% Composition	Area of Standard	Area of Sample	% Assay
1	% Assay	1443556	1440222	99.769

The result calculated has the is in the range% assay of 99.75% which,

Validation Parameters	Acceptance criteria	Results
Linearity	Correlation coefficient NLT 0.9990	0.999
Accuracy % Recovery	98 – 102 %	99.96

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Assay		98-102%	99.76
Precision	**Repeatability**	%RSD NMT 2.0	0.26%
	Intermediate	%RSD NMT 2.0	0.22%
Robustness	**Change in wavelength**	%RSD NMT 2.0	0.4547
	Change in flow rate	%RSD NMT 2.0	0.4152
Limit of detection (µg/ml			0.1956 µg/ml
Limit of quantitation			0.5927
Ruggedness		Correlation coefficient NLT0.9990	0.999

IX. CONCLUSION

- Analytical methods were programmed to generate new development and validated for simultaneous estimation of ivermectin tablet dosage Form by RP- HPLC.
- Determination was detected by RP-HPLC using Methanol: Water (95;5) with the flow rate of 1.5 ml/min. column used Cosmosil C18, (250×4.6mm,5µ) as a stationary phase. The retention time were found to be 4.5 min and peak was observed at 245 nm which was selected as a wavelength for quantitative estimation. After development of the method it was validated for linearity, accuracy, precision, robustness' studies according to ICH guidelines**.** The system suitability parameter also reveals that the values within the specified limit for the proposedmethod.
- The calibration curve was linear over the range of 10-50µg/ml. The linearity was observed with correlation coefficient (R2) found to be 0.999 the result of assay were found to be 99.76%. the results of assay found close to100%.
- From the results indicated in the accuracy chart it was invented that recovery value of pure drugs were between 98.0 % to 102% which presented that the method is accurate and also reveals that commonly used excipients and additives present in the pharmaceutical formulations were not interfering in the proposed methods. Mean recovery was found99.96.
- The relative standard deviation values for repeatability and intermediate precision studies were less than 2%. %RSD of repeatability was 0.26% %RSD of intermediate precision was0.22%.
- The result of robustness was found to be satisfactory within range. %RSD of change in wavelength was found 0.4547 and change in flow rate got0.4152
- The LOD(Limit of detection) was found to be 0.1956 for the ivermectin while the LOQ(Limit of Quantification) was0.5927.
- The results of ruggednesss were in the trange that correlation coefficient was 0.999
- The invented RP-HPLC method was specific, easy, close precisie, accurate and robust, for the detection of ivermectin in bulk and table dosageform.

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