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REVIEW ON METHOD DEVELOPMENT AND VALIDATION OF RP- HPLC TECHNIQUE FOR THE ESTIMATION OF IVERMECTIN FROM ANIMAL BODY FLUIDS

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ABSTRACT

Ivermectin is a potent antiparasitic agent widely used in human and veterinary medicine. Accurate quantification of ivermectin in pharmaceutical dosage forms is essential for ensuring therapeutic efficacy, safety, and regulatory compliance. This review consolidates validated analytical methods—primarily reverse-phase high-performance liquid chromatography (RP-HPLC) and ultraviolet (UV) spectrophotometry—used for the estimation of ivermectin in bulk and combined formulations. It discusses method development strategies, validation parameters, and comparative performance metrics to guide researchers in establishing robust, reproducible, and regulatory-compliant analytical techniques. A selective, accurate, and stability-indicating reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the estimation of Ivermectin in pharmaceutical dosage forms. The method was designed to support routine quality control and formulation analysis, ensuring reliable quantification of Ivermectin in both pure drug and tablet preparations. Chromatographic separation was achieved using a C18 column with a mobile phase composed of acetonitrile and methanol. The system operated under optimized conditions with UV detection at a suitable wavelength for Ivermectin. The method was validated in accordance with ICH Q2(R1) guidelines, covering essential parameters such as specificity, accuracy, precision, robustness, and sensitivity. To confirm the method's stability-indicating capability, Ivermectin samples were subjected to various stress conditions including acidic and basic hydrolysis, oxidative degradation, photolytic exposure, and thermal stress. The method successfully distinguished the active pharmaceutical ingredient from its degradation products, demonstrating its suitability for stability studies. Robustness testing revealed consistent performance despite minor variations in chromatographic conditions, affirming the method's reliability in routine laboratory settings. The simplicity of the mobile phase and ease of sample preparation further enhance its practical utility. In conclusion, the developed RP-HPLC method is simple, specific, and reproducible, making it highly suitable for the regular analysis of Ivermectin in pharmaceutical formulations. Its stability-indicating nature ensures accurate assessment under diverse stress conditions, supporting its application in both research and industrial environments. The method's compliance with regulatory standards and its operational efficiency make it a valuable tool for quality assurance in pharmaceutical analysis.

KEYWORDS: Ivermectin, RP-HPLC method development, Analytical method validation, Dosage form analysis, Forced degradation studies, Chromatographic separation, Reverse-phase chromatography, ICH Q2 (R1) guidelines.

INTRODUCTION

High-Performance Liquid Chromatography (HPLC) has emerged as one of the most powerful and versatile analytical techniques in modern pharmaceutical and biomedical research. It is extensively employed for the separation, identification, and quantification of components within complex mixtures. Unlike classical column chromatography, HPLC offers superior resolution, speed, sensitivity, and reproducibility, making it indispensable in both qualitative and quantitative analysis of pharmaceutical formulations, biological samples, and chemical compounds.

HPLC is particularly suitable for compounds that are thermally unstable, non-volatile, or possess high molecular weights, which are often challenging to analyze using gas chromatography or other traditional methods. The technique operates by pumping a liquid mobile phase at a constant rate through a column packed with a stationary phase. The sample to be analyzed is injected into the mobile phase stream, which carries it into the column. Once inside, the sample components interact with the stationary phase based on their physicochemical properties—such as polarity, molecular size, and affinity—resulting in differential retention times. These interactions allow for the effective separation of individual analytes.

The rate of distribution of analytes between the stationary and mobile phases is governed by diffusion processes. Minimizing diffusion enhances the efficiency and speed of separation, which is a critical factor in high-throughput analytical environments. The eluted components are detected using various detectors, such as UV-Vis, fluorescence, refractive index, or mass spectrometry, depending on the nature of the analytes and the sensitivity required.

The term “high-performance” in HPLC reflects the significant advancements over classical column chromatography. These include the use of high-pressure pumps, fine particle columns, and automated sample injectors, which collectively contribute to faster analysis times, improved resolution, and enhanced reproducibility. HPLC has become the gold standard for routine quality control, stability testing, and pharmacokinetic studies in pharmaceutical industries.

In the context of combination drug therapy, HPLC plays a vital role in the simultaneous estimation of multiple active pharmaceutical ingredients (APIs) within a single formulation. Combination therapies are increasingly used to enhance therapeutic efficacy, broaden the spectrum of activity, and reduce the risk of resistance. One such combination is ivermectin and albendazole, which is widely used as an anthelmintic treatment, particularly for intestinal helminthic infections in pediatric and adult populations.

As the India is majorly Farming country, the farmers hereby use pet animals like cows, dogs, goats, sheeps, etc. for the secondary income source. But nowadays, this secondary becomes primary along with farming. Before animal harvesting, there is need of their proper nurturing and maintain healthy conditions as required. So, the infections and diseases occurring due to environmental conditions should have to treated along with maintaining the desired terms for selling the products from their outputs like milk from cows, buffaloes.

For this treatment from the infection, various medications of antibiotics are used by farmers. In this treatment, now majorly Ivermectin is introduced. This ivermectin is introduced for the anthelmintic for the worm infection which are present in soil, but sometimes infection is spread through the blackflies. So, this is necessary to estimate the bioavailability for the protection from this infection for the animals like dogs, cats, sheeps, goats, etc. Specially this pet animals are majorly found more affected by this type of infections. Also, some side effects of these medications are useful for the other infections like antifungal activity. Majorly it cures the infections from soil-transmitted worm infection.

During medication the findings are not performed well by the physician for the activity of ivermectin as the area for the various pet animals. The ivermectin also can not be maintain if it is flowing through the system route. So, there is need to gain the knowledge of bioavailability and the desired quantity of the ivermectin in the body fluid directly by extraction. We need to find out the actual amount of ivermectin during the dosage for the prevention of animals to reduce adverse effects. These adverse effects may vary from animal to animal but it can be measured by taking samples from various intervals. Also, it is required to do analysis of this samples as the given medication safety and efficacy is to be measured. So, there is requirement of the validated method for estimation for the analysis of ivermectin from various body fluids and comparison with previous batches during production and also after the final production of batches for quality control.

Ivermectin is a chemical agent used for the antiparasitic medication in animals and humans to treat specific infections caused by worms such as river blindness and intestinal threadworms which is occurs by onchocerciasis & strongyloidiasis respectively. It is also used for the treatment of acariasis (mite infestations) and heartworm disease in animals like, cows, sheeps, etc Topical formulations of ivermectin are approved for the headlice and roscea like skin infection. Various dosage forms are available like tablets, lotions, creams for the humans along with emulsions for the animals. Ivermectin interferences the neural and muscle functioning and perform role for the occurrence of paralysis in parasitic worms leads to death.

Ivermectin is an anti-parasitic medication used in humans to treat specific infections caused by parasitic

worms, such as river blindness (onchocerciasis) and intestinal threadworms (strongyloidiasis). Topical forms are also approved for conditions like head lice and the skin condition rosacea.

Ivermectin has been the preferred treatment for several diseases caused by parasitic infections. These include conditions such as head lice, scabies, river blindness (onchocerciasis), strongyloidiasis, trichuriasis, ascariasis, and lymphatic filariasis. Ivermectin belongs to a class of drugs known as avermectins. Its mechanism of action involves increasing the permeability of the cell membrane in parasites, which leads to their paralysis and death. The drug was discovered in 1975 and started being used in therapy in 1981. It is a potent, semisynthetic compound derived from an antinematodal substance originally obtained from Streptomyces avermitilis. Ivermectin is approved by the FDA as an anti-parasitic agent. Chemically, it consists of 22,23-dihydro avermectin B1a and 22,23-dihydro avermectin B1b. Approximately 90% of the compound is avermectin B1a, while less than 10% is avermectin B1b. In recent times, it has also been used in the treatment of SARS-CoV-2. Various analytical methods have been developed to assess the presence of ivermectin in tablet form. These methods include techniques like Ultra Violet spectroscopy, diffuse reflectance spectroscopy, HPTLC, HPLC with tandem mass spectroscopy, and RPHPLC. To ensure the quality and purity of the drug, a validation study was conducted to evaluate parameters such as linearity, precision, accuracy, and robustness. The main factors compared with earlier studies were system suitability parameters like resolution, tailing factor, plate height, and theoretical plates, as well as LOD and LOQ. These factors are essential for validating the drug and identifying any impurities in the tablet dosage form.

Therapeutic Importance of Ivermectin

Ivermectin is a macrocyclic lactone disaccharide derived from the fermentation products of Streptomyces avermitilis. It exhibits potent antiparasitic activity by binding to glutamate-gated chloride channels in the nerve and muscle cells of parasites, leading to paralysis and death. Ivermectin is effective against a wide range of parasitic infections, including onchocerciasis, strongyloidiasis, scabies, and head lice. It has also been investigated for its potential role in managing SARS-CoV-2, although its use in viral infections remains controversial and under study.

Given the widespread use of this combination in clinical practice, there is a pressing need for a validated analytical method to estimate the concentration of both drugs in bulk and formulated dosage forms. Accurate quantification ensures dosage uniformity, therapeutic efficacy, and patient safety, while also supporting regulatory compliance and quality assurance.

MATERIALS AND METHODS

Materials

The analytical studies utilized pharmaceutical-grade compounds and solvents. Fluconazole (FLU), Ivermectin (IVR), and Albendazole were sourced from certified suppliers including Aurobindo Pharma and Sura Labs. Commercial tablet formulations containing Ivermectin in strengths ranging from 5 mg to 10 mg were procured from authorized medical outlets. Analytical-grade solvents such as methanol, water, and acetonitrile were obtained from Merck (LICHROSOLV), suitable for HPLC applications.

Instrumentation

Spectrophotometric analysis was performed using a UV-visible spectrophotometer equipped with quartz cells. Chromatographic separation was carried out using a Waters-HPLC system (Model 2489) integrated with an autosampler, column oven, and UV detector. Data acquisition was managed through Empower-2 software. Column trials included Inertsil ODS and Xterra Phenomenex Luna C18, with dimensions ranging from 150 mm to 250 mm in length and 4.6 mm in diameter, featuring 5 µm particle sizes.

Method Development Techniques

Two analytical approaches were employed: UV spectrophotometry and high-performance liquid chromatography (HPLC). Spectrophotometric methods included simultaneous equation and Q-analysis techniques, utilizing absorbance measurements at selected wavelengths. HPLC method development involved systematic trials with varying mobile phases and column types to optimize separation efficiency and peak resolution. All methods were validated in accordance with ICH guidelines.

Mobile Phase Optimization

Spectrophotometric analysis used methanol as the primary diluent due to its solubility profile for the target compounds. HPLC trials initially explored combinations of methanol: water and acetonitrile: water in varying proportions. Final mobile phases were optimized to methanol: water in ratios ranging from 80:20 to 90:10 v/v, and acetonitrile: methanol in ratios around 60:40 v/v, depending on the compound under analysis.

Column Selection

Column optimization was critical to achieving reliable chromatographic performance. Among the tested columns, C18-based columns with lengths between 150 mm and 250 mm and particle sizes of 5 µm were found to be most effective. The Xterra Phenomenex Luna C18 column provided optimal peak shape and resolution at a flow rate of approximately 1 ml/min.

Proposed Analytical Methods

Ivermectin has been successfully estimated using both RP-HPLC and UV spectrophotometric methods. RP-HPLC provides high resolution and specificity, making it

suitable for regulatory and industrial applications. It typically uses a C18 column with mobile phases such as methanol: water (85:15 v/v) or acetonitrile: methanol (60:40 v/v), a flow rate of 1.0 ml/min, and detection wavelengths between 245–285 nm. UV methods, including Q-absorption ratio and Vierotd's method, are cost-effective and sensitive, especially for simultaneous estimation in combined dosage forms. These methods use spectroscopic-grade methanol as the diluent and detect absorbance at 245 nm for Ivermectin and 261 nm for co-formulated drugs, with an isosbestic point around 260.7 nm.

General Procedure for Sample Preparation

Standard stock solutions were prepared by dissolving approximately 10 mg of each compound in methanol and adjusting the volume to 10 ml. Working solutions were obtained by serial dilution to achieve concentrations ranging from 1 µg/ml to 100 µg/ml. For tablet assays, sample weights were selected within the range of 100 mg to 200 mg, powdered, and dissolved in methanol. Final dilutions were performed to reach target analytical concentrations suitable for UV or HPLC analysis.

Validation Parameters

Method validation is a systematic process used to confirm that an analytical procedure is suitable for its intended purpose. It ensures accuracy, precision, specificity, linearity, sensitivity (LOD and LOQ), robustness, and reproducibility of the method. Validation is essential for regulatory compliance and reliable quality control of pharmaceutical products. By meeting International Conference on Harmonisation (ICH) guidelines, a validated method guarantees consistent and trustworthy results across different laboratories, analysts, and conditions.

Linearity

Linearity ensures that the analytical response is directly proportional to the concentration of the analyte. For Ivermectin, RP-HPLC methods have demonstrated linearity in the range of 1–32 µg/ml, while UV methods have shown linearity from 0.1–4 µg/ml. The correlation coefficients (R^2) consistently exceed 0.9798, with most methods achieving values above 0.999, indicating excellent linearity and reliability across the tested concentration ranges.

Precision

Precision reflects the consistency of results under the same conditions (intra-day) and across different days or analysts (inter-day). Ivermectin methods have shown %RSD values between 0.27% and 1.58%, well within the acceptable limit of ≤2%. This confirms that the methods are repeatable and reproducible for routine analysis.

Accuracy

Accuracy is evaluated through recovery studies at multiple concentration levels (typically 50%, 100%, and 150%). For Ivermectin, recovery rates have ranged from 98.14% to 99.84%, demonstrating that the methods can accurately quantify the true content of the drug. These values fall within the ICH-accepted range of 98–102%.

Sensitivity (LOD & LOQ)

Sensitivity is measured by the limit of detection (LOD) and limit of quantification (LOQ). RP-HPLC methods report LOD values between 1.2 and 2.93 µg/ml and LOQ values between 3.8 and 8.79 µg/ml. UV methods show superior sensitivity, with LOD values as low as 0.0185 µg/ml and LOQ values down to 0.0553 µg/ml, making them ideal for detecting trace levels of Ivermectin.

Specificity and Selectivity

Specificity ensures that the method can distinguish Ivermectin from other components, including excipients and degradation products. No interfering peaks were observed at Ivermectin's retention time or absorbance maxima. Forced degradation studies under acid, base, oxidative, thermal, UV, and freeze-thaw conditions confirmed that the method could selectively quantify Ivermectin even in stressed samples.

System Suitability

System suitability tests confirm that the chromatographic system is functioning properly. Parameters such as tailing factor (≤2.0), theoretical plates (>2000), and resolution (>2.0) were consistently within acceptable limits. These results validate the system's readiness for accurate and precise analysis.

Robustness and Ruggedness

Robustness tests the method's stability under small, deliberate changes in conditions such as flow rate, wavelength, and mobile phase composition. Ruggedness confirms consistent results across different analysts and instruments. Both parameters showed %RSD values below 0.4%, indicating that the method is reliable and unaffected by minor variations.

RESULT AND DISCUSSION

Reverse-phase high-performance liquid chromatography (RP-HPLC) has emerged as a powerful and widely accepted analytical technique for the estimation of ivermectin in animal body fluids due to its high sensitivity, specificity, and reproducibility. The reviewed literature demonstrates that method development typically begins with the selection of a suitable stationary phase, with C18 columns being the most commonly employed owing to their non-polar characteristics that effectively retain the lipophilic nature of ivermectin. Mobile phase optimization is crucial, with mixtures of acetonitrile and water or methanol combined with buffers such as phosphate buffer (pH 3–5) being frequently used to achieve sharp, symmetrical peaks and optimal resolution. Detection is generally carried out using UV spectrophotometry at wavelengths around 245–250 nm, where ivermectin exhibits significant absorbance. Sample preparation from biological matrices like plasma, serum, and milk involves protein precipitation using

organic solvents such as acetonitrile or methanol, followed by liquid-liquid extraction (LLE) or solid-phase extraction (SPE) to enhance recovery and minimize matrix interference, with reported recovery rates ranging from 85% to 98%. Method validation, as per ICH guidelines, encompasses parameters such as linearity (typically 0.1–10 µg/mL with correlation coefficients >0.999), accuracy (98–102% recovery), precision (%RSD < 2% for intra- and inter-day studies), and sensitivity (LOD ~0.03 µg/mL; LOQ ~0.1 µg/mL). Robustness studies confirm the method's stability under slight variations in chromatographic conditions, including flow rate, pH, and temperature. These validated methods have been successfully applied in pharmacokinetic studies across various animal species, residue monitoring in milk and meat to ensure compliance with regulatory standards, and therapeutic drug monitoring in veterinary practice. Despite its advantages, RP-HPLC faces challenges such as complex biological matrices requiring rigorous cleanup and occasional need for derivatization to enhance UV detection. Recent trends suggest a shift toward LC-MS/MS for even greater sensitivity and specificity, although RP-HPLC remains a cost-effective and accessible choice for routine analysis. Future directions may include the development of miniaturized, high-throughput platforms and the incorporation of green analytical chemistry principles to reduce solvent usage and environmental impact. Overall, the review underscores RP-HPLC's critical role in ensuring accurate, reliable, and regulatory-compliant estimation of ivermectin in animal body fluids, supporting both research and clinical applications in veterinary pharmacology.

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