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Review Article

Antitubercular Drug-Loaded Solid Lipid Nanoparticles Formulation and Validation by HPLC

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ABSTRACT

Tuberculosis (TB) remains a significant global health challenge, and the development of effective drug delivery systems is crucial for improving treatment outcomes. The purpose of this work was to develop prolonged-release solid lipid nanoparticles (SLNs) of Rifampicin, Pyrazinamide, Isoniazid for oral drug delivery and to improve the bioavailability of RIF. Solid lipid nanoparticles were designed using lipid core materials, with surfactant and co-surfactant as a stabilizer. The SLNs were prepared by the o/w micro emulsion technique and RP-HPLC method was validated in accordance with the International Conference on Harmonization guideline, Q2 (R1) criteria.

INTRODUCTION

Tuberculosis (TB) is a chronic communicable disease caused by Mycobacterium tuberculosis that infected over billions of people world-wide. India is the second most populous country in the world where one/fourth of global TB cases are reported. 10.6 million People fell ill with tuberculosis (TB) world-wide. The advent of multidrug-resistant tuberculosis (TB) has brought attention to the need for new TB medication therapies while also drawing attention to the shortcomings of the present conventional TB treatment, particularly the lengthy treatment regimens and patient compliance issues. Formulation scientists are now more focused on creating Nanoparticulate delivery carriers in order to improve the management of medical disorders. These delivery systems offer unique ways for targeted drug administration within the host, enhancing therapeutic effects while minimizing systemic side effects, and prolonged drug release, which may avoid high dose frequency. For "Category-III" tuberculosis treatment, the first-line antitubercular medicines (ATDs) rifampicin

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(RIF), isoniazid (INH), and pyrazinamide (PYZ) were utilized. The dosage in accordance with the brief course of treatment that has been specifically monitored (DOTS) For category III, three doses of INH, RIF, and PYZ per week for two months was the recommended oral administration in the first phase. There are several drawbacks to these first-line antitubercular medications, including hepatotoxicity, gastrointestinal disturbances, rashes, and drug resistance. [1] Introduced in 1991, solid lipid nanoparticles (SLN) offer an alternative to conventional colloidal carriers such emulsions, liposomes, and polymeric micro- and nanoparticles. SLN combine advantages of the traditional systems but avoid some of their major disadvantages like drug explosion upon storage, microbial growth upon storage, active targeting is difficult to achieve.[2] Solid lipid nanoparticles (SLNs) encapsulated by ATDs have been created as a drug delivery system due to its many advantages over other innovative colloidal carriers, including controlled release, physical stability, high tolerability, and protection of labile pharmaceuticals. Pharmaceutical scientists throughout the world have been paying more and more attention to the creation of SLNs because of their advantages and superior tolerability over other polymeric nanoparticles. Compared to treatments that are delivered freely, several studies have demonstrated that encapsulating TB medications in nanoparticles can enhance their effectiveness. This may significantly boost the therapy's effectiveness and decrease the frequency of doses. In particular, the loading of hydrophobic drugs into SLNs have given promising therapeutic results in various studies and, in some studies, the use of antibiotic loaded nanoparticles has even achieved complete eradication of Mycobacterium tuberculosis.

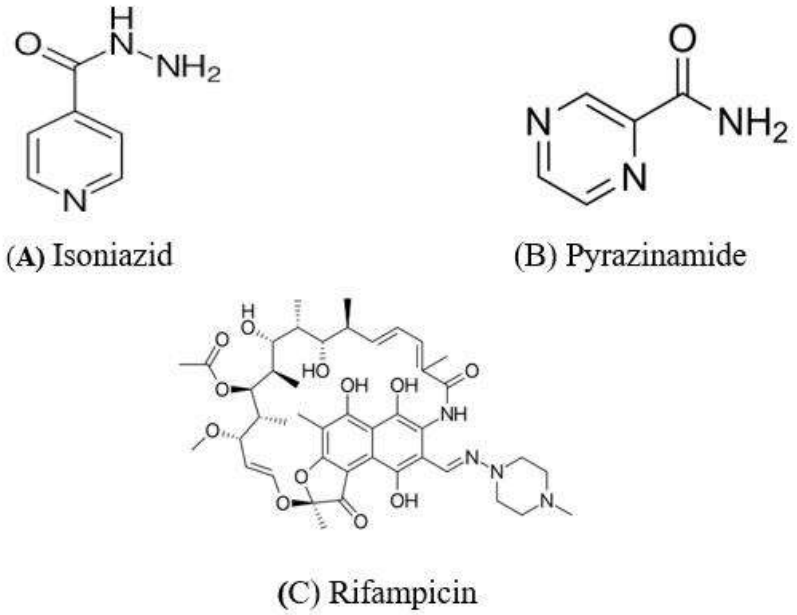

Fig 1: Chemical structures of rifampicin (RIF), isoniazid (INH), pyrazinamide (PYZ)

For the analysis of pharmaceutical preparations including many components, HPLC is the preferred approach because to its high sensitivity, repeatability, and specificity. the issues related to chromatographic condition optimization, including choosing the right column type, temperature, and injection volume, as well as the make-up of the mobile phase. Despite this, this approach surely offers a more sensitive determination than UV spectroscopic techniques.

It is currently the preferred approach for the majority of drugs and their mixtures. An RP-HPLC technique for the simultaneous measurement of INH, PYZ, and RIF was reported by Gaitonde and Pathak.

Background

Despite being as old as humanity, tuberculosis (TB) remains one of the most common and deadly illnesses.More than two billion people worldwide—one-third of the global population—are afflicted with mycobacterium tuberculosis. Due to the rising prevalence of AIDS, it is still a big worry in wealthy countries even though it is more prevalent in underdeveloped and developing nations. An estimated 1.77 million people died from tuberculosis (TB) in 2007, and there are an estimated 9.27 million new cases of the disease each year.. The majority of the time, multidrug resistant forms of tuberculosis make complete eradication of the disease impossible. Additionally, patients may decide to stop their treatment due to adverse effects, to extend their therapy, or because their symptoms have subsided. By extending the duration of medication residence time and minimizing side effects, nano-particulate drug delivery devices can enhance the efficacy of antibacterial treatment. Solid lipid nanoparticles (SLNs) are drug delivery vectors that are nanoscale in size and possess the benefits of polymeric nanoparticles, lipid emulsions, and liposomes while eschewing many of its drawbacks. Since physiological lipids are used to make SLNs, concerns regarding their safety and biocompatibility are minimal. They also have a solid matrix to regulate the released drug's rate of release and can transport both hydrophilic and lipophilic medicines. They can be made using standard equipment used to create lipid-based infusions on a large scale, and they can be sterilized using various methods. [3]

Delivery of SLNs by different route of administration

- Parenteral route of administration
- Oral route of administration
- Transdermal route of administration
- Nasal route of administration
- Pulmonary administrations
- Ocular route of administration

Preparation of Solid Lipid Nanoparticles:

The various methods used for the preparation of solid lipid nanoparticles are listed below [4].

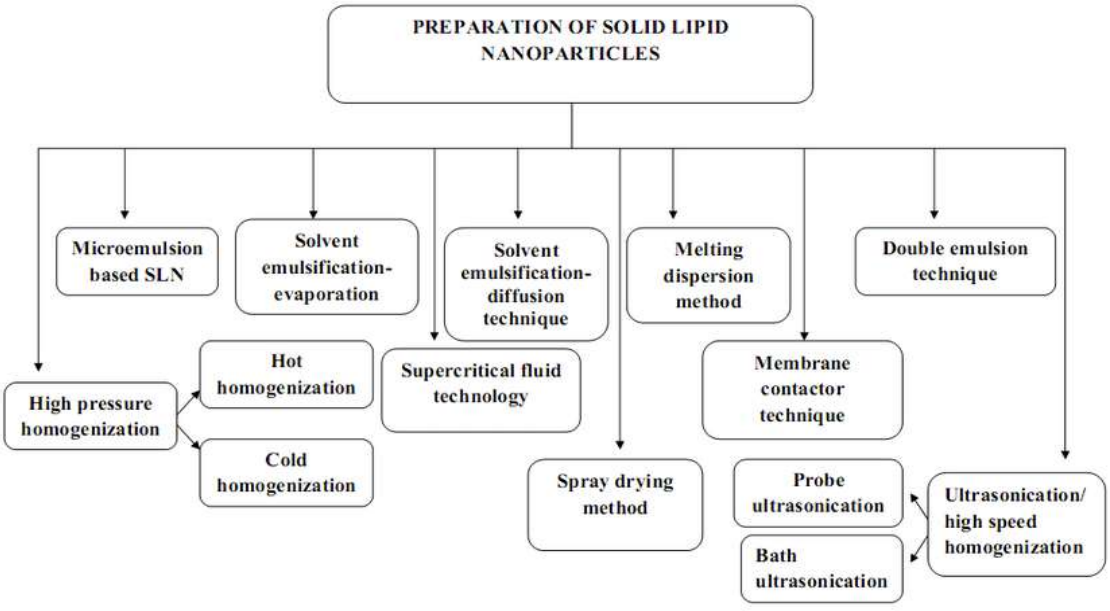

Fig 2: Methods used for the preparation of solid lipid nanoparticles

1. High Pressure Homogenization:

HPH is an appropriate approach for SLN, NLC, and LDC preparation. It can be carried out at room temperature (cold HPH technique) or at a higher temperature (hot HPH technique). Turbulence and cavitation reduce the particle size. Lipids are forced through a small gap of a few micrometer ranges at high pressure (100–200 bars) in the high pressure homogenization procedure. Thus, the forces responsible for the disruption of particles in the submicron range include shear stress and cavitation (resulting from an abrupt drop in pressure). Lipid contents typically vary from 5 to 10%. The homogenizer does not present any issues at this concentration. Homogenization at high pressure exhibits no scaling issues. There are essentially two methods for producing SLN by high pressure homogenization: hot and cold homogenization processes.

2. Micro-emulsion Based SLN Preparation:

Microemulsion, which is usually made up of water, an emulsifier, a co-emulsifier, and a low melting lipid, is an optically transparent mixture that is 65 to 70°C or a slightly bluish solution. Lipid phase precipitation occurs when the heated microemulsion is dissolved in cold water (2–3°C) while being constantly stirred, resulting in the formation of tiny particles smaller than 300 nm. The hot microemulsion to cold water volume ratio typically ranges from 1:25 to 1:50. The To eliminate surplus emulsifier residue and boost particle concentration, excess water is removed via ultra-filtration. When it comes to microemulsions, the pH level and temperature gradient determine the product quality in addition to the microemulsion's content. Elevated temperature gradients speed up the crystallization of lipids and stop them from aggregating.

3. Solvent Emulsification-evaporation Technique:

The lipophilic substance and hydrophobic drug were dissolved in a water immiscible organic solvent (such as cyclohexane, dichloromethane, toluene, or chloroform) and then emulsified in an aqueous phase using a high-speed homogenizer in the solvent emulsification-evaporation process. The microfluidizer was used to move coarse emulsion through in order to increase the efficiency of fine emulsification. Lipid precipitates of SLNs were then left behind when the organic solvents were mechanically stirred at room temperature and under low pressure (using a rotary evaporator, for example) and evaporated. Here, the amount of lipid in the organic phase determines the mean particle size. Low lipid load (5%) might produce incredibly small particle sizes. in connection with organic solvent. This method's main benefit is that it avoids any heat stress, which makes it ideal for including medications that are extremely thermolabile. The usage of organic solvent has a definite drawback in that it may interact with drug molecules and reduce the lipid's solubility in the organic solvent.

4. Solvent Emulsification-diffusion Technique:

The solvent (such as benzyl alcohol, butyl lactate, ethyl acetate, isopropyl acetate, or methyl acetate) used in the solvent emulsification-diffusion technique must be somewhat miscible with water. This procedure can be performed in either an aqueous phase or an oil. Initially, to maintain the initial thermodynamic equilibrium of both liquids, the solvent and water were mutually saturated. After dissolving the lipid in the water-saturated solvent, the solvent-saturated aqueous surfactant solution is used to emulsify the lipid at high temperatures. When additional water is added (usually in a ratio of 1:5 to 1:10), the organic solvent diffuses from the emulsion droplets into the continuous phase, causing the SLN to precipitate.

5. Ultrasonic or High-Speed Homogenization:

To make SLN, sonication or fast stirring were employed. The primary advantages stem from the

fact that all of the equipment used in this lab is very conventional. One limitation of this approach is the broader particle size distribution, which can exceed micrometer diameters. This leads to physical instabilities such as particle growth with aging. The potential for ultrasonication to contaminate with metal is another significant problem with this method. As a result, numerous research organizations have carried out studies combining high-speed churning and high-temperature ultrasonication in order to make a formula that is stable. This can be achieved with a lipid concentration of less than 1% and a considerable surfactant concentration.

6. Melting Dispersion Method (Hot Melt Encapsulation Method):

Melting and dispersing a pharmaceutical is done by heating the water phase to the same temperature as the oil phase while simultaneously melting the drug and solid lipid in an organic solvent, which is referred to as the oil phase. Subsequently, the oil phase was mixed with a small quantity of water phase and the resulting emulsion was agitated at a higher speed for many hours. SLNs could finally be administered after it was brought to room temperature. Similar to the solvent emulsification evaporation process, this was the final step. However, there was no need to evaporate any organic solvent when using the melting dispersion technique. Reproducibility was worse to the solvent emulsification-evaporation method but higher than the ultrasonication strategy.

7. Double Emulsion Technique:

In order to prepare hydrophilic loaded SLN, a unique solvent emulsification evaporation procedure has been utilized. To prevent the drug from partitioning into the outer water phase of the w/o/w double emulsion during solvent evaporation, the drug is stabilized within the drug encapsulation in this case. For the purpose of achieving a high hydrophilic molecule integration, this approach is particularly helpful.

8. Membrane Contactor Technique:

The SLN preparation process is novel. Using the membrane contactor approach, the liquid phase was driven through the membrane pores (Kerasep ceramic membrane with an active ZrO2 layer on an AlO2-TiO2 substrate) at a temperature greater than the melting point of the lipid, forming small droplets. The droplets that were accumulating at the pore outlets were swept away by the constant agitation and tangential circulation of the aqueous phase within the membrane module. SLNs were produced as the preparation cooled to room temperature. Here, the liquid phase's pressure was produced by nitrogen, and the required temperature was maintained for both phases by immersing them in a thermostate bath.

9. Supercritical Fluid Technology:

The advantage of this relatively new SLN production technology is solvent-free processing. There are various variations of this platform technology available for making powders and nanoparticles. SLN can be made using the Rapid Expansion of Supercritical Carbon Dioxide Solutions (RESS) method. Carbon dioxide (99.99%) was a great solvent choice for this process.

10. Spray Drying Method:

A pharmaceutical product is made from an aqueous SLN dispersion by a procedure other than lyophilization. The cost of this method is lower than that of lyophilization. This method's high temperature, shear forces, and partial melting of the particle lead to particle aggregation. Freitas and Mullera recommend utilizing lipids with melting points higher than 70 °C for spray drying. The optimal outcome was attained when 1% SLN was added to a trehalose solution in water or 20% SLN was added to ethanol-water combinations (10/90 v/v). [6]

Applications of Solid Lipid Nanoparticles

lipid that is solid When it comes to manufacturing scale upgradability and stability, nanoparticles

outperform liposomes. This characteristic could be crucial for a variety of targeting strategies. Colloidal drug delivery systems, which are biodegradable and have a minimum one-year shelf life, are based on solid leaf nanoparticles (SLNs). They have the ability to transport medications to actively phagocytic cells in the liver both in vivo and in vitro.

There are several potential applications of SLNs some of which are given below:

1. **SLNS as Gene vector Carrier:**

It is possible to formulate the gene vector using SLN. In one study, the SLN gene vector was modified to include a diametric HIV-1 HAT peptide (TAT 2) in order to maximize gene transfer. Recent reports have shown that SLN can contain nucleic acids, plasmid DNA, and other genetic/peptide elements. Lipid nuclic acid nanoparticles were synthesized by dissolving both lipid and DNA separately in a liquid nanophase including water and a water-miscible organic solvent. After the organic solvent was removed, stable and uniformly sized lipid-nuclic acid nanoparticles, measuring between 70 and 100 nm, were produced. It is known as a genosphere. By inserting a conjugated antibody-lipo polymer into the particle, it is specifically targeted.

2. **SLNS for Topical Use:**

Many medications, including tropolide, imidazole antifungals, anticancer agents, vitamin A, isotretinoin, ketoconazole, DNA, flurbiprofen, and glucocorticoids, have been applied topically using SLNs and NLCs. The epidermal targeting was caused by podophyllotoxin-SLN penetrating both the stratum corneum and the skin's surface. Vitamin A-loaded nanoparticles can be made with glyceryl behenate. The techniques are beneficial for increasing penetration with long-term release. The lipid nanoparticles loaded with isotretinoin were designed for topical medication delivery. Tween 80 and soy lecithin are utilized in the heat homogenization process for this. The increased accumulative absorption of isotretinoin in skin makes the approach helpful. The creation of SLN gel loaded with flurbiprofen for topical administration offers the possible benefit of delivering the medication straight to the site of action, resulting in increased tissue concentrations. Water, glycerol, and polyacrylamide were utilized to make this kind of SLN gel.

3. **SLNS as Cosmeceuticals:**

The SLNs have been used as an active carrier agent for UV blockers and molecular sunscreens as well as in the formulation of sunscreens. According to the in vivo study, adding 4% SLN to a regular cream will boost skin moisture by 31% after 4 weeks. It has been demonstrated that SLN and NLCs are novel occlusive topicals with regulated release. Glyceryl behenate SLNs have improved vitamin A localization in the higher layers of skin as compared to traditional formulations.

4. **SLNS for Potential Agriculture Application:**

When added to SLN, the essential oil from Artemisia arboreseens L. was able to slow down the rate of evaporation more quickly than emulsions. This allowed the systems to be employed in agriculture as an appropriate vehicle for environmentally safe pesticides. Here, the SLN were created utilizing poloxamer 188 or Miranol Ultra C32 as the surfactant and Compritol 888 ATO as the lipid.

5. **SLNs as a Targeted Carrier for Anticancer Drug to Solid Tumors:**

It has been observed that SLNs can be helpful in treating neoplasms as medication transporters. Tamoxifen is an anticancer medication that is added to SLN to improve permeability and retention while also extending the drug's release following intravenous delivery in cases of breast cancer. Drugs like methotrexate and camptothecin-loaded SLNs have been used to target tumours. [7]

MATERIALS AND METHODS

The Indian Pharmacopoeia Commission (IPC) in Ghaziabad, Uttar Pradesh, India, provided the working standards used for INH, PYZ, and RIF. We bought the Pluronic F-68, also known as the Poloxamer 118, from HiMedia Laboratory Pvt. Ltd. in India. We bought sodium taurocholate from LOBA Chemie in India. We bought mannitol and stearic acid (octadecanoic acid) from Qualigens Fine Chemicals (a division of GSK Pharmaceutical Ltd.). Thermo Fisher Scientific India Pvt. Ltd. provided the analytical grade orthophosphoric acid, sodium hydroxide, methanol, and acetonitrile. A Milli-QTM Integral-3 water purification system (M-Millipore, Fisher Scientific India Pvt. Ltd.) was used to deionize the water.An HPLC instrument (Agilent Technologies 1260 Infinity with EZ Chrome Elite software) was used for the chromatographic experiments. It featured a stainless steel C-18 column (Nucleodur®) packed with octadecylsilane linked to porous silica (5 µm), 250 × 4.6 mm (Macherey-Nagel GmbH and Co, Düren, Germany), an autosampler, and an ultra-violet/photodiode-array detector (PDA).. The temperature of the column was kept at 30°C.

CHROMATOGRAPHIC CONDITIONS:

Using mobile phases A and B with a flow rate of 1.5 ml/min, several researchers conducted and reported chromatographic analyses in a linear gradient program (Table 1). The spectrophotometer was calibrated at 238 nm using the chromatographic system, and a constant column temperature of 30° was maintained. After diluting the samples with a pH 6.8±0.02 orthophosphoric acid (OPA) buffer solution, 20 µl of the mixture was injected into the column. On a 250 x 4.6 mm, 5 µm, C-18 stationary phase (column), separation was accomplished with a 1.5 ml/min linear gradient flow rate. The determination of isoniazid, pyrazinamide, and rifampicin was made by comparing their retention times to established benchmarks..[5]

Preparation of the solutions:

A diluted solution of sodium hydroxide was used to adjust the pH to 6.8±0.02 after 1 milliliter of OPA was diluted in 1000 milliliters of water to create the buffer solution. 17.85 mmol of OPA buffer solution was equal to this buffer solution. A combination of 96 volumes of buffer and 4 volumes of acetonitrile was utilized as the mobile phase. A combination of 45 volumes of buffer and 55 volumes of acetonitrile was utilized as mobile phase B.

Preparation of stock and standard solution:

In methanol, a stock solution was made using 0.04 percent w/v of INH, 0.22 percent w/v of PYZ, and 0.08 percent w/v of RIF. Before being used, the prepared stock solution was kept in storage at 4°C. After being moved to a volumetric flask, 5 ml of the stock solution was diluted with buffer to make 25 ml. INH, PYZ, and RIF were produced to final concentrations of 80, 400, and 160 µg/ml, respectively.

Calibration solutions:

In 25-ml volumetric flasks, the stock solution was diluted appropriately using buffer. Prepared and subjected to linearity analysis were the diluted quality control samples (QCS) of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, and 150%. Following the establishment of the chromatographic and experimental conditions, the RP-HPLC method was validated in accordance with the International Conference on Harmonization guideline, Q2 (R1) criteria.

Method validation:

Once chromatographic and experimental conditions were established, the RP-HPLC method was validated in accordance with the International Conference on Harmonization guideline, Q2 (R1) criteria.

1. **System Suitability:**

A system suitability test (SST) was created to guarantee the analytical procedure's validity. The selection criteria for SST were the area's percent

relative standard deviation (% RSD), the RSD of the retention time (RT), theoretical plates, USP tailing factor, and resolution. The standard solution was injected six times in order to analyze these parameters. It is possible to see that the system suitability results are summarized since the parameters that were examined met the requirements for acceptance. The functional solution was injected six times to verify the system's appropriateness.

2. Specificity:

To make sure that the lipid(s) and surfactant(s) in the SLNs dispersion do not affect the drug's quantification, the method's specificity was established. By comparing the chromatograms of ATDs extracted from SLNs and of blank nanoparticles to ascertain the peak purity, the specificity of the procedure was assessed.

3. Linearity

Definition The ability of analytical methods to yield results that are directly proportional to the analyte concentration range in samples that fall within the necessary concentration ranges is referred to as linearity.

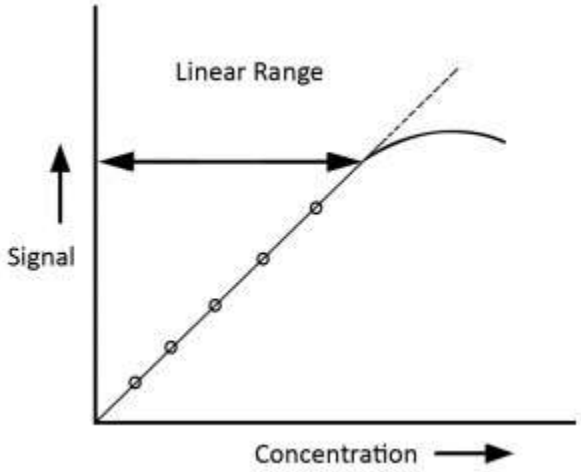

Fig 3: Linearity Graph

To test for linearity, triplicate injections of stock solutions containing isoniazid, pyrazinamide, and rifampicin at concentrations of 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%,130%, 140%, and 150% were made. Plotting the analyte peak area vs percentage drug concentration allowed for the creation of calibration curves for standard doses of isoniazid, pyrazinamide, and rifampicin. If the slope and intercept's percentage RSD values are less than 1%, linearity is verified. [10]

4. Precision

Definition degree of scatter (closeness of agreement) between a set of measurements taken from different samplings of the same homogenous sample.

Precision including:

- Repeatability
- Intermediate precision
- Reproducibility

By completing intra-day analysis at 10% 50% 100% 150% concentration and inter-day analysis by injecting the QCS in triplicate at 10% 50% 100% 150%, the system precision of the method was ascertained. The % RSD

%RSD=SD/Mean X100

And % accuracy

%Accuracy=(Mean Area of Test)/(Mean Area of Standard) X100

Were calculated by comparing the theoretical and measured concentration for each case. According to the ICH guideline, %RSD not more than 2% As per ICH Guidelines. [11]

5. Accuracy

Definition: The degree of agreement between the value found and the value acknowledged as either a conventional true value or an approved reference value indicates the accuracy of an analytical method. Recovery studies were carried out to evaluate the suggested method's accuracy. By contrasting three independently extracted sample preparations (SLNs with ATDs and excipients) with a standard solution, the extraction process of the method was measured. The average recovery of the extracted ATDs from SLNs in comparison to the reference solution served as the acceptability criterion for the extraction investigation. [12]

6. LOD and LOQ

Definition

a. LOD It is the quantity of analyte in a sample that can be identified but not always measured.
b. LOC It is the smallest concentration of analyte in a sample that can be quantified with appropriate accuracy and precision.

The lowest amount of analyte in a sample that could be found under the specified experimental conditions was known as the limit of detection, or LOD. The lowest amount of an active ingredient in a sample that can be identified with reasonable accuracy and precision is known as the limit of quantification, or LOQ. The method for calculating the detection and quantitation limits was based on the slope (m) and standard deviation (SD) of the response, in accordance with the ICH guidance. LOD can be computed using the following formula:

$$\mathbf{LOD=3.3\ SD/M}$$

LOQ can be calculated according to the formula,

$$\mathbf{LOQ=10\ SD/M}$$

S.D = Standard Deviation

M = Slope. [13]

7. Ruggedness

The degree of consistency of test results obtained by analyzing the same sample under various conditions, such as different laboratories, different analysts, different instruments, different days, etc., is defined as the ruggedness of an analytical process.

Some might consist of [14].

a. Source
b. Concentration and stability of solution
c. Heating rate
d. Column temperature
e. Humidity

CONCLUSION

In accordance with International Conference on Harmonization criteria, Q2 (R1), a new simple, fast, and sensitive reversed-phase high-performance liquid chromatography method was designed and validated as an HPLC method of analysis for antituberculosis. The brief retention periods suggested that a large number of samples may be finished quickly. As a result, the technique can be applied to the analysis of several samples. Therefore, it can be said that the developed approach is quick, easy to repeat, speedy, and selective. For the simultaneous measurement of pyrazinamide, rifampicin, and isoniazid in solid lipid nanoparticles. On a 250×4.6 mm, 5µm, C-18 column, separation was accomplished with a linear gradient flow rate of 1.5 ml/min. The accuracy, reproducibility, repeatability, linearity, precision, selectivity, and reliability of the Method have been demonstrated. Based on their retention periods in comparison to standards, isoniazid, pyrazinamide, and rifampicin were identified. This was verified by distinctive spectra obtained using a spectrophotometer set at 238 nm, which eluted the compounds linearly and precisely with percentage RSD values for isoniazid, pyrazinamide, and rifampicin. Because of its simplicity and precision, this method works well for routine quality control and stability monitoring of solid lipid nanoparticles loaded with antitubercular medicines.

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