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FORMULATON AND EVALUATION OF SOLID LIPID NANOPARTICLES CONTAINING DOXORUBICIN AND CURCUMIN-PREFORMULATION STUDIES AND ACTIVITY

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Abstract

Many controlled delivery strategies have been developed in recent years in an effort to increase the solubility, stability, and bioavailability of medications that are poorly absorbed. Lipid nanoparticles have particularly intriguing qualities when it comes to delivering drugs or genes. These systems, made up of lipids stabilized with surfactants. A flexible drug delivery method called solid lipid nanoparticles (SLNs) has been formulated with homogenization process and formulation was optimized .This work aimed to synthesize solid lipid nanoparticles containing curcumin and doxorubicin. Results were found satisfactory from the preformulation examination consisting of solubility studies, particle size and LOD with no incompatibility issues with the excipients. Entrapment of both drugs in SLNs have increased the pharmacological activity compare to that available in crude drug form. In-vitro release kinetics shows sustained release with initial burst effect. The linearity was established in the concentration of 0.2- 10µg/ml for curcumin and 0.015 - 62.50µg/mL for doxurubicin.

Keywords: Chemotheraphy, Chromatogram,Solid lipid Nanoparticles,Doxorubicin, Anticancer agent, Curcumin

Introduction

With a broad spectrum of antitumor activity, anthracycline antitumor antibiotics are among the most potent treatments for solid cancers. The most widely used medication for treating carcinomas of the breast, lung, thyroid, ovary, and soft tissue sarcomas is doxorubicin hydrochloride, also known as adriamycin . However, myelosuppression, mucositis, and cardiac toxicity are among the serious general organ toxicity associated with anthracycline therapy.[1]

Even with the extensive application of anthracyclines in cancer treatment, considerable amounts of research have been conducted due to their limitations. The ongoing synthesis of novel anthracycline analogues to reverse drug resistance and reduce cardiotoxicity is one of the main areas of anthracycline research; another active area is improving anthracycline drug delivery . Doxil® (Caelyx®) [4] and Myocet® , two liposomal formulations, represent a significant turning point in the field. In fact, doxorubicin-loaded liposomes show less cardiotoxicity while exhibiting efficiencies that are on par with traditional anthracycline cytostatic agents. However, if the chemotherapy cycle is not postponed, the administration of long-circulating liposomes in clinical practice has been linked to palmar-plantar erythrodysesthesia, also known as "hand-foot" syndrome, which can progress to ulceration and epidermal necrosis.[2][3].[4]

Another strategy for enhancing activity against multidrug resistance and/or raising cardiac tolerability is to produce prodrug derivatives of doxorubicin . Recently, a long lipophilic acyclic isoprenoid chain derived from squalene was synthesized and tested to create an intriguing doxorubicin prodrug. Solvent polarity has a significant effect on squalene's conformation; in polar solvents like water, squalene adopts a tightly-coiled conformation. SQ-Dox, a squalenoyl derivative of doxorubicin, is capable of self-organizing in water to form distinctive, highly stable "loop-train" elongated nanostructures. Couvreur and colleagues have recently provided an in vivo fate description of squalenoyl nanoassemblies (NAs), emphasizing the important role endogenous lipoproteins (LDL) play in squalenoyl NA delivery in the bloodstream; LDL adsorb onto the NAs, which subsequently disassemble, releasing squalenoyl bioconjugates that insert into lipoproteins. The LDL route, therefore, determines the pharmacokinetics and biodistribution .[5][6]

The rhizomes of the traditional herb turmeric, scientifically known as Curcuma longa Linn, are used to extract curcumin, a naturally occurring bioflavonoid. Because of its diverse pharmacological characteristics, this phyto-extract has been used as a medicine to treat a wide range of illnesses since ancient times. Its anti-inflammatory, antioxidant, wound-healing, anti-microbial, and anti-cancer properties have been investigated and confirmed. Research has examined curcumin's potent anti-cancer properties in relation to a number of cancers, including skin, breast, colon, brain, blood, and prostrate cancers. The research showed that curcumin inhibits cancer cells through a variety of mechanisms. Curcumin's anti-cancer action includes apoptosis, cell cycle arrest, and disruption of multiple signal transduction pathways. Additionally, it prevents NF-kB activity and anti-apoptotic proteins, such as Bcl-2, from growing cancer cells and causing cancer.[7][8][9]

Dispersed systems with sizes ranging from 1 to 1000 nm are known as solid lipid nanoparticles (SLNs), which are a substitute for polymeric particulate carriers. They are typically appropriate for intravenous delivery and are made up of physiological or biocompatible lipids or lipid molecules with a track record of safe usage in therapy. According to certain authors, doxorubicin-loaded SLNs were able to defeat Pgp-mediated multidrug resistance (MDR) in resistant leukemia cells both in vitro and in vivo in a murine leukemia mouse model. The findings indicated that SLNs, which have garnered significant attention in the field of site-specific drug targeting,

including brain delivery, in recent decades, may have the ability to deliver anticancer medications for the the treatment of Pgp-mediated MDR.[10][11]

Because SLNs can improve drug uptake by cells and circumvent the Pgp efflux system, they have even been suggested for use as novel chemotherapeutic agent vehicles for experimental glioblastoma multiforme (GBM) treatment. It is well known that SLNs are subjected to endocytosis by endothelial cells and that they can also be exploited for passive and, if opportunely surface-modified, active targeting to the brain . Indeed, surface-engineering drug delivery systems for molecular targeting is one tactic to boost cancer cell-selective cytotoxicity. Recently, this approach which takes advantage of the distinctions between cancerous and healthy cells—has been used to deliver anticancer drugs via SLN. A lipid nanoparticle system that targets the folate receptor has been created to deliver a prodrug called paclitaxel. Targeted nanoparticles with the folate receptor marker demonstrated increased antitumor activity in mice with the M109 tumor. To target SLNs to the VLDL receptor, functionalization with a chimera peptide (Apo E) was utilized. Apo E has an aminoacidic sequence for binding to the VLDL receptor and a lipophilic moiety. When compared with non-functionalized SLNs, ApoE-conjugated SLNs enhanced the brain accumulation of lipophilic methotrexate prodrug, particularly at longer post-administration times . Furthermore, as documented in the literature, loading drugs into SLNs frequently results in an improvement in their physico-chemical and hydrolytic stability, as was previously observed in curcumin entrapped in fatty acid SLN.[12][13][14][15]

The Reasoning Behind the Research Project

Thousands of studies on a variety of activities show that curcumin has enormous therapeutic potential.The most extensively researched therapeutic activity of curcumin is its ability to prevent cancer. It has also been shown to act on nearly all known cancer treatment targets, and stem cell activity has been demonstrated. Despite these findings, curcumin has never been commercialized because of issues with stability and bioavailability. Curcumin is a BCS class IV drug, meaning that its poor systemic bioavailability limits its use as an anticancer agent, making it suitable only for local action. In the last five years, there have been more than 50 reports on curcumin nanoformulations. All of these reports point to the same conclusion, which is that curcumin's bioavailability can be significantly increased by forming it into nanoparticles. The encapsulation of Curcumin in polymeric nanoparticles has demonstrated a significant increase in oral bioavailability; however, the inherent toxicities of polymers have made polymeric nanoparticles unsuitable as a safe method. Few studies have documented the encapsulation of curcumin within biodegradable and biocompatible lipid nanoparticles. In order to capitalize on curcumin's exceptional therapeutic potential in the treatment of cancer, a decision is made to formulate solid lipid nanoparticles of curcumin mixed with doxurubicin. The cases of acute lymphoblastic leukemia, acute myeloblastic leukemia, Wilms tumor, neuroblastoma, soft tissue and bone sarcomas, breast, ovarian, transitional cell bladder, thyroid, gastric, Hodgkin's disease, malignant lymphoma, and bronchogenic carcinoma where the small cell histologic type is the most responsive in comparison to other cell types doxorubicin is approved by the US Food and Drug

Administration for these conditions. There are two forms of doxorubicin available on the market: injectable and pegylated liposomal injection. Doxorubicin is a BCS class II medication, however its oral bioavailability is poor. A patient's lifetime exposure to doxorubicin is limited to 900 mg/m2 due to the cardiotoxicity it produces. Several studies on the new method of delivering doxorubicin to produce an oral bioavailable form have been published. Oral administration also lessens the financial burden and makes it possible for patients to receive "chemotherapy at home," which eliminates the need for hospitalization for each cycle of chemotherapy.

While injectable doxorubicin is proving to be a successful treatment option, there is still a need for an oral doxorubicin. Polymeric doxorubicin nanoparticles have effectively improved the drug's oral bioavailability.[16]

EXPERIMENTAL

MATERIAL AND METHODS

From Sunpure Extracts Pvt. Ltd. in New Delhi, India, curcumin was purchased. From Sun Pharmaceuticals Industries Ltd doxorubicin was purchased. Compritol® 888 ATO and Glyceryl Monostearate (GMS) was acquired from Gattefosse India Pvt. Ltd, and phospholipon 90G was obtained from Fisher Scientific and Tween 80 and polyethylene glycol 600 were procured from nearby merchants (CDH, New Delhi, India). Product CurcuWIN was bought from OmniActive Health Technologies.

From Fisher Scientific acetonitrile (ACN), chloroform, and methanol (HPLC grade) was purchased and syringe filters was purchased from Waters India Pvt. Ltd. The research also used several reagents, all of which were of analytical quality.

Formulation aspects

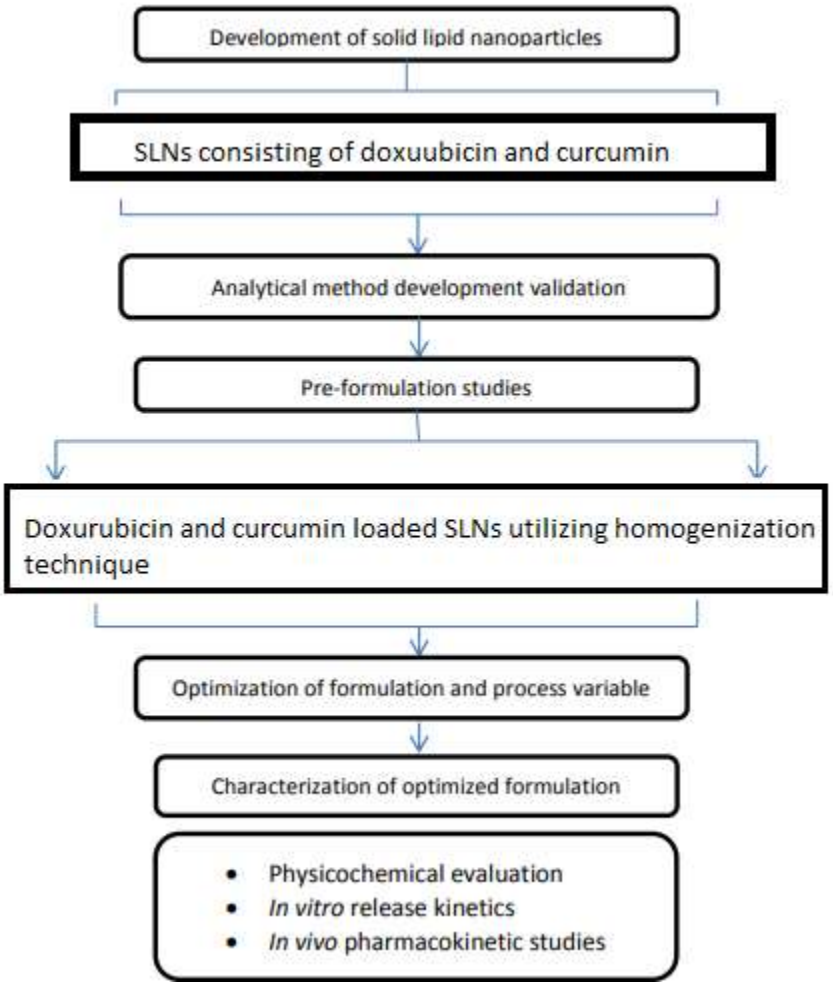

Figure .1 Formulation strategy of Doxurubicin and curcumin loaded solid lipid nanoparticles

Approaches: Findings and Conversations

Development of analytical techniques for in vitro estimation of Curcumin Using HPLC to estimate curcumin in vitro.A HPLC analytical technique was created and approved for use in the in vitro examination of curcumin. The method's specifics are given below.

Preparing a standard Curcumin solution

12.5 mg of curcumin was dissolved in 25 mL of acetonitrile to create a 500 µg/mL stock solution of the drug. After the stock solution was appropriately diluted with the mobile phase, a number of standards were created.

Curcumin estimation calculations

Plotting the peak area of the standards against their concentrations yielded the standard curve. By extrapolating from the regression equation pertaining to the peak area, which was derived from the standard curve, the unknown curcumin concentration was found.

HPLC method's specificity for curcumin's in vitro estimation

The United States Pharmacopeia (USP) defines specificity as the ability of a method to discriminate the analyte from all the potentially interfering substances like impurities, degradation products etc. By contrasting the chromatograms obtained from the injection of mobile phase without drug and the chromatogram of the mobile phase containing drug, the specificity of this HPLC method was examined. The three main curcuminoids found in the commercial grade of curcumin were successfully separated using the HPLC method. Curcumin, demethoxycurcumin, and bisdemethoxycurcumin showed distinct peaks with retention times of 4.8, 3.90, and 3.49 minutes, respectively. There was not a single mobile phase peak that interfered with the drug peak.[7][17]

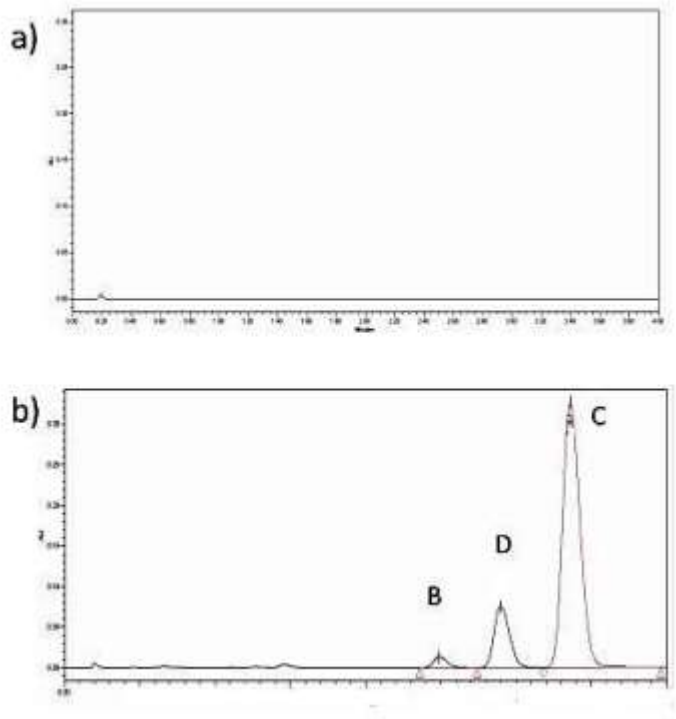

Figure.2 The HPLC method's specificity for curcuminoids

HPLC method's linearity for curcumin estimation in vitro

According to the USP, an analytical method is considered linear if it can yield test results that are exactly proportionate to the analyte concentration in samples falling within a specified range. Using curcumin standards, linearity was examined by injecting seven distinct standard solutions at various concentrations. Plotting the peak area against the concentrations allowed for the construction of calibration curves. Using least squares regression, the linearity was evaluated by determining the slope, y-intercept, and Spearman rank coefficient (r2). It was discovered that the curcumin standard curve was linear throughout the concentration range of 0.015 - 62.50µg/mL.

The relationship between the curcumin concentration (X) and the peak area (Y) of the detector response was determined through the creation of a linear equation for the standard curve, which can be expressed as Y = 166185 X + 9097.5, r2.=1. For an analytical procedure, a spearman rank

coefficient (r2) of value > 0.99 is appropriate. A high correlation coefficient (r2) indicates a robust relationship between the concentration in the specified concentration range and the peak area.

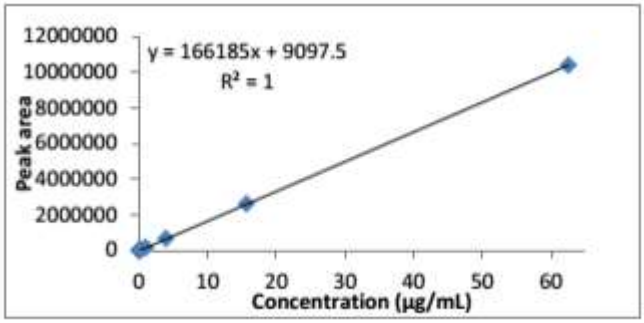

Figure 3: Curcuminoids' analytical method's linearity

Precision of HPLC method for Curcumin In vitro Estimation

The USP states that the degree of agreement between a set of measurements made through repeated sampling of a homogenous sample is expressed by the precision of an analytical method. When repeated measurements are made under the same experimental conditions, the values become closer to one another for the precision. The within-day and day-to-day precision were used to evaluate the precision of the current analytical method. Four injections of a set of standard curcumin solutions were made on the same day in order to achieve within-day precision.

Over the course of thirty days, daily precision was achieved by injecting a series of standard curcumin solutions on five different days. Calculated for both within-day and day-to-day precision, the relative standard deviation (RSD) values were found to be within acceptable bounds.

The HPLC method's accuracy in estimating curcumin in vitro

According to the USP, an analytical method's accuracy is determined by how closely the test results it produced matched the actual value. An analytical method's accuracy needs to be proven across its whole range. By injecting three quality control samples (0.49 µg/mL, 7.81 µg/mL, and 31.25 µg/mL) five times along with the curcumin standards, the accuracy of the provided HPLC method was examined. Using the following formula, the theoretical concentration and the measured concentration value were compared in order to assess the assay's accuracy.

$$\%\ Accuracy = \frac{Measured\ concentration}{Theoritical\ concentration} x\ 100$$

Using HPLC to estimate curcumin in plasma

Curcumin's in vivo quantification was accomplished using a previously documented technique . Waters HPLC system with C-18 analytical column (100 mm x 4.6 mm, 2.6 m) from.For in vivo

curcumin quantificationwavelenght mainly of 425nm. Curcumin was eluted using a gradient flow at a rate of 1 ml/min and a mobile phase consisting of HPLC grade acetonitrile and HPLC grade water. Acetate of 17-estradiol was employed as the internal standard. 17-estradiol acetate was measured to be at 280 nm and curcumin to be at 425 nm.

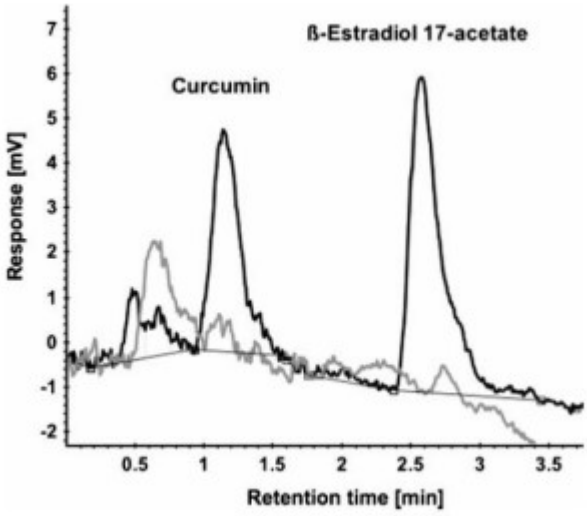

Figure 4: Chromatogram of Curcumin and Internal Standard following HPLC method detection in plasma

The international conference on harmonization of technical requirements for registration of pharmaceuticals for human use established guidelines for the validation of the analytical method. These guidelines were followed. Precision (repeatability (intraday) and intermediate precision (interday)) and accuracy were among the parameters that were validated. Next, the stock solution was diluted with water to create standard solutions for calibration curves, with final concentrations of 0.2 µg/ml, 0.5 µg/ml, 1 µg/ml, 2 µg/ml, 5 µg/ml, and 10 µg/ml.

Doxorubicin estimation calculations

Plotting the peak area of the standards against their concentrations yielded the standard curve. By interpolating from the regression equation relating to the peak area, which was obtained from the standard curve, the unknown concentration of Doxorubicin was found.

Specificity of HPLC technique for Doxorubicin estimation

The United States Pharmacopeia (USP) defines specificity as the ability of a method to discriminate the analyte from all the potentially interfering substances like impurities, degradation products etc. By contrasting the chromatograms obtained from the injection of mobile phase without drug and the chromatogram of the mobile phase containing drug, the specificity of this HPLC method was examined. There was not a single mobile phase peak that interfered with the drug peak. As a result, it was verified that this HPLC method for quantifying doxorubicin was specific.[4]

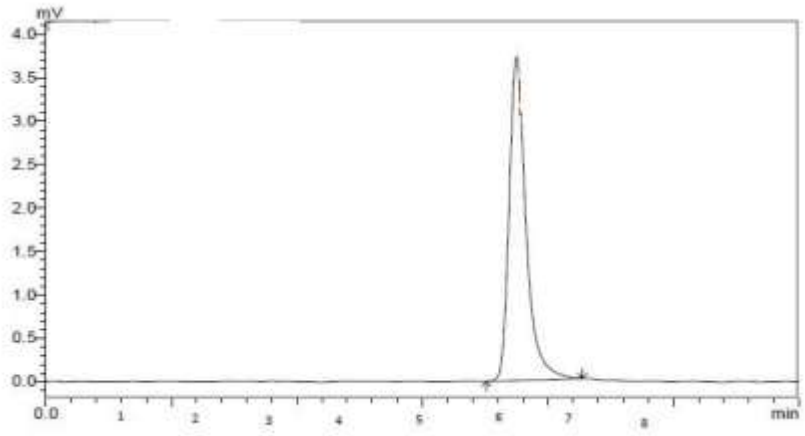

Figure 5: Doxorubicin specificity of HPLC method

HPLC method's linearity in estimating doxorubicin

According to the USP, an analytical method is considered linear if it can yield test results that are exactly proportionate to the analyte concentration in samples falling within a specified range. By injecting seven distinct standard solutions at various concentrations, linearity was examined on doxorubicin standards. Plotting the peak area against the concentrations allowed for the construction of calibration curves. Using least squares regression, the linearity was evaluated by determining the slope, y-intercept, and Spearman rank coefficient (r2).

The HPLC method's accuracy and precision in estimating doxorubicin

The USP states that the degree of agreement between a set of measurements made through repeated sampling of a homogenous sample is expressed by the precision of an analytical method. When repeated measurements are made under the same experimental conditions, the values become closer to one another the higher the precision. The within-day and day-to-day precision were used to evaluate the precision of the current analytical method.

An analytical technique to measure Doxorubicin levels in plasma

A previously published technique was employed to quantify doxorubicin in vivo. Waters HPLC system with C18 analytical column (100 x 4.6 mm, 2.6µm) was used for Doxorubicin quantification in vivo. Using mobile phase,consisting of HPLC grade water and HPLC grade acetonitrile doxorubicin was eluted at a rate of 1 ml/min via an gradiant flow. In ten minutes, doxorubicin was eluted. The internal standard utilized was methyl paraben. The

Analyte is passed through a UV chamber as part of the detection system before being detected using a detector.

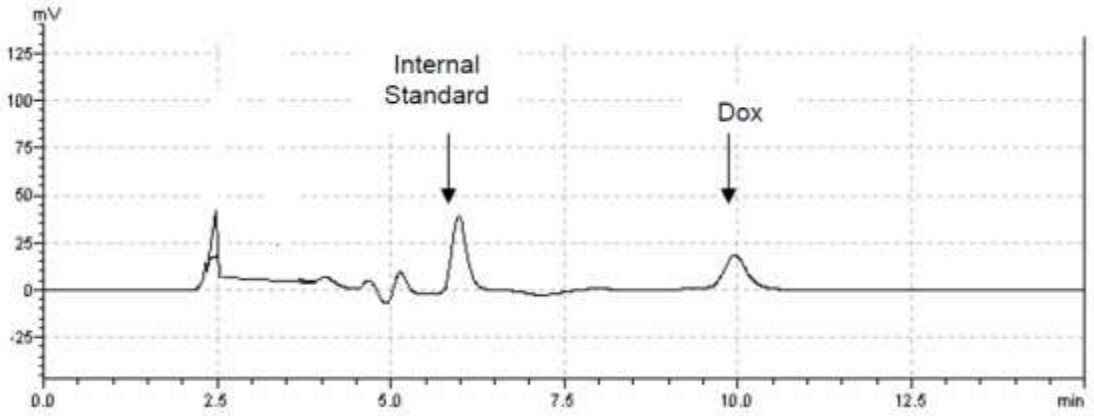

Figure 6: Doxorubicin and internal standard chromatogram in rat plasma

Sensitive analytical methods for estimation of Dox. and Cur. were successfully developed and validated. While the analytical method for Doxorubicin mixed with Curcumin clearly eluted the peak without any interference, the HPLC method reported for Curcumin mixed with Doxorubicin was able to provide a faster elution of Dox. and Cur. with good resolution of the three major curcuminoids. Compared to other analytical techniques, the methods used less organic solvent and were able to detect concentrations as low as 15ng/mL with accuracy.

When the results were checked for accuracy, precision, linearity, and specificity, they were all within acceptable bounds. The analytical techniques can be used to measure doxorubicin and curcumin both in vivo and in vitro.

Cur and Dox Preformulation Studies

A literature review was used to examine background data such as chemical name, structure, solvent of recrystallization, purity, therapeutic category, appearance, color, and odor.

Cur and Dox loss upon drying

Place the filled bottle in the drying chamber (drying oven), remove the stopper, and leave the bottle in the chamber after transferring about 100 mg of the samples to the weighing bottle. Dry the sample for three hours at 105°. Once the drying process is finished, let it cool to room temperature, weigh the contents of the bottle, and use the formula to determine the LOD.

$$\% \text{ Loss on drying} = \frac{\text{Loss in Weight}}{\text{Sample Weight}} \times 100$$

Size of particles of Cur and Dox measurement

A mechanical sieve shaker was used to mechanically shake 200 mg of the sample after it had been weighed and placed on top of the sieve. After removing the sieves, the Cur and Dox that remained

on each sieve was weighed. The findings are tabulated based on the percentage weight of powder retained on each sieve.

Cur and Dox solubility studies

Using an orbital shaker and the equilibrium solubility method, solubility studies for Cur and Dox were carried out in PBS.

Preformulation study

To determine whether a drug ingredient was suitable for formulation into solid lipid nanoparticles, preformulation studies were carried out. The following is a summary of the Preformulation studies findings.

FTIR analysis

After the sample mixture was triturated using KBr (IR grade), FTIR analysis was performed.

FTIR spectra were taken in to observation (Shimadzu) to investigate the possible chemical interactions between the curcumin and the lipid matrix. The above sample's FTIR spectra were produced by averaging 32 interferograms in the 1000–4000 cm–1 range, with a resolution of 2 cm–1.

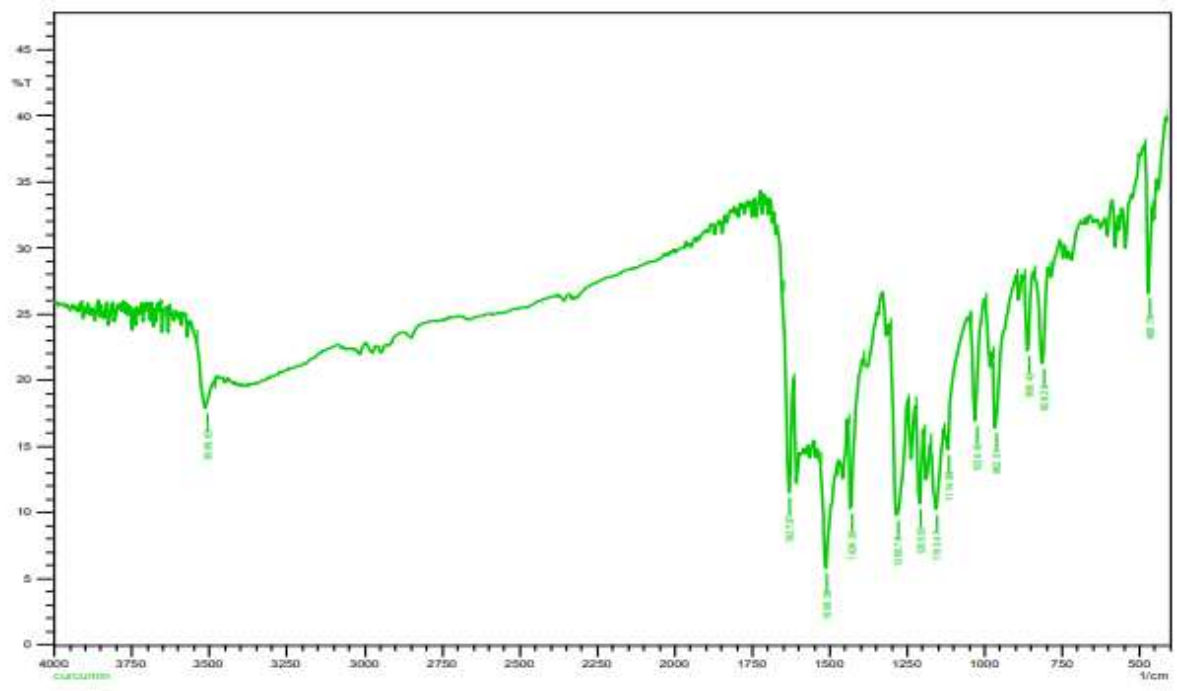

Figure 7: Curcumin's FTIR spectrum

Studies on the compatibility of drug excipients for Dox. and Cur.

Dox. and Cur. was placed in glass vials and physically combined with the formulation's essential excipients in the same ratio as suggested by the formulations. In accordance with ICH guidelines, various storage conditions were applied to the vials. Periodically, samples were taken out and

examined for any alterations in composition or physical state. FTIR spectra were used to validate the samples.

Making a standard Doxorubicin solution

To prepare a 500 µg/ml stock solution, an equivalent quantity of doxorubicin was precisely weighed and dissolved in water.

Pharmacokinetic studies in vivo

Pharmacokinetic studies in vivo for SLNs loaded with Dox. and Cur.

Male Albino rats (200-250 gms) M/F weighing 250-300 g were used for in vivo pharmacokinetic studies. The protocol was duly approved by the Institutional Animal Ethics Committee (IAEC) of LLRM medical college meerut Two groups of animals were created (n12). Group 2 received 50 mg/kg of free cur.and free Dox. orally via an oral dosing cannula, while Group 1 (VH) received 50 mg/kg body weight (bw) of Dox. and Cur.-SLNs.

Plasma was separated by centrifuging the blood samples at 4000 rpm for 10 min at 41 ºC. The obtained plasma was centrifuged and then kept at 20 °C until analysis.

The findings support the theory that by Dox. and Cur.encapsulation in solid lipid the drug's oral bioavailability is enhanced by nanoparticles. The formulation that has been optimized to have the desired physicochemical properties and particle size range can increase the plasma concentration of Dox. and Cur when taken by mouth. Dox. and Cur appears to have the potential to overcome its poor bioavailability when used in conjunction with solid lipid nanoparticles.

Released in vitro Solid lipid nanoparticle kinetics

CurDox-loaded SLNs' in vitro release kinetics

To release the drug from the nanoparticulate (CurDox- SLN), 100 mg of nanoparticles were dissolved in 15 ml of PBS (0.01 M, pH 7.4), and 30 eppendorf tubes (500 µl each) were filled with the solution. This experiment was conducted in triplicate.

The tubes were kept in a shaker at 37 ºC at 150 rpm (Wadegati Lab equip, India). Since free curcumin is totally insoluble in water, the solution was centrifuged for 10 minutes at 3000 rpm (Remi, Germany) at predefined intervals to separate the released (pelleted) Cur and Dox from the SLN. Resolving the liberated CurDox in one milliliter of methanol,and the HPLC was filled with 20 ml of this solution to ascertain the amount of the release of CurDox at various time intervals.[19]

Studies on the in vitro release kinetics of SLNs loaded with CurDox showed a pattern of sustained release.

For seven days, there was a sustained release. The release of CurDox from nanoparticles is responsible for the sustained release of Curcumin over a period of 7 days, while the initial burst release can be attributed to the dissociation of surface absorbed CurDox into lipid matrix. In PBS, native curcumin was clearly insoluble, and after 7 days, there was only 6% drug release.

Since more dose-ranging studies are required to determine the exact amount of drug in the plasma at any given time, the release profile does not suggest that the medication will be effective for up to seven days after the date of administration.

RESULTS AND DISCUSSION

For doxorubicin, the limits of quantification and detection were 13 ng/ml and 1.23 ng/ml, respectively.In compliance with the international conference's guidelines for harmonizing technical requirements for pharmaceutical registration for human use, the analytical method was validated. A sustained release with an initial burst effect is demonstrated by in vitro release kinetics. For curcumin, the linearity was determined in the concentration range of 0.2–10µg/ml, and for doxurubicin, the range was 0.015–62.50µg/mL.

Accuracy and precision (repeatability (intra-day) and intermediate precision (inter-day)) were among the parameters that were verified. The QC standards' intra- and inter-day RSDs were both less than 5% outside of the chosen range. The method's good accuracy was confirmed with recovery values between 96% and 101%. For curcumin, the limits of detection and quantification were 19.5 and 1.95 ng/ml, respectively. The validation of the analytical method followed the guidelines of the international conference on standardization of technical specifications for human use pharmaceutical registration.

Accuracy and precision (repeatability (intra-day) and intermediate precision (inter-day)) were among the parameters that were verified. The QC standards' intra- and inter-day RSDs were both less than 5% outside of the chosen range. The method's good accuracy was confirmed with recovery values between 96% and 101%.

Preformulation research was conducted, and the results showed that the raw materials provided by for the research work were found to be appropriate for carrying out additional work toward developing the formulation into solid-lipid nanoparticles. The drug used is found to be compatible with every excipient used to prepare the formulation, according to preformulation studies and studies of excipient compatibility. Compatibility parameters were then shown over a period of time at various stability conditions. A drug to lipid ratio of roughly 1:20 was found to be optimal for producing particles smaller than 300 nm in size and a reasonably high entrapment efficiency after SLN at different drug:lipid ratios were prepared and observed.It was discovered that all subsequent attempts to achieve a lower drug to lipid proportion were futile.

Abbreviations-Dox-Doxorubicin,Cur-Curcumin

Conflict of interest

Authors declare no conflict of interests.

CONCLUSION

CurDox solid lipid nanoparticles were synthesized and characterized with success. In vivo pharmacokinetic study results support the validity of the hypothesis being investigated. Both CurDox exhibit a notable rise in oral bioavailability in solid lipid nanoparticle form. SLNs that were created are stable.Preformulation studies was found satisfactory for the synthesis of SLNs.

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