RP-HPLC Analytical Method Development And Validation For Quantification Of Alpha-Mangostin Entrapped In Solid Lipid Nanoparticles

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Abstract

The present study established and validated a quantitative determination of alpha-mangostin extracted from solid lipid nanoparticles of Garcinia mangostana extract, a reverse phase fast, high-pressure liquid chromatographic technique. According to the international conference on harmonization recommendations, a quick and cost-effective approach for estimating and quantifying alpha-mangosteen was designed and verified precisely and correctly. The stationary phase was activated silica with a particle size of 3.5µm based on an X-Bridge C18 guard column (4.6*100mm), resulting in quicker and more accurate separation of eluents due to the smaller particle size of silica. APhoto diode array detector was used to detect acetonitrile and 0.1 % v/v orthophosphoric acid in 65:35 volumes at a flow rate of 1ml/min ata wavelength of 317nm. With an r^2 value of 0.999, linearity was confirmed in the concentration range of 17.38-260.90 g/ml. Interday and intraday variation with a relative standard deviation of 0.048 %-0.165 % and 0.036 %-0.182 %, respectively, were used to achieve precision. The linearity results revealed that the detection and quantification limits were 0.000121 µg/ml and 0.000365 µg/ml, respectively. The elution time for α- mangosteen was 7.82 min.

Keywords:- Alpha-Mangostin, Method development and validation, ICH recommendations, Solid lipid nanoparticles, Reverse phase-high performance liquid chromatography.

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INTRODUCTION

Garcinia mangostan (G. mangostana) fruit rind extract is a tropical fruit queen because of its abundance of xanthones. Alpha (α)-mangostin is the most prevalent xanthone derivate in the pericarp extract, compared to all other xanthones [1, 2]. There are several health advantages linked with this extract, which have been shown to have anti-inflammatory properties, anti- oxidation properties, anti-cancer properties and the ability to modulate immunological responses [3-10].

Biosynthetic xanthan derivatives are tetra-oxygenated di-prenylated[11]. Schemed is credited with discovering α-mangostin in 1855[12]. Explanation of the chemical structure of α- mangostin was provided by Yates et al.[13]. Analytical marker for quantification and certification of detectable xanthones for quality control analysis was suggested to be α-mangostin[14, 15]. α mangosteen has curren-tly been used as an ingredient of choice in several nutraceutical supplements and nutria-cosmeceuticals, despite its long history as

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a pharmaceutical mainstay[16].

A lack of oral bioavailability of α-mangostin has been shown in several pharmacokinetic investigations in rats owing to non-specific tissue distribution and hepatic first-pass metabolism[17]. Solid Lipid Nanoparticles (SLPs) of G. mangostana were developed to improve oral bioavailability by reducing tissue distribution and preventing first-pass metabolic breakdown. With this new technique, it is possible to attain tissue-targeted site- specificity.

For quantitative xanthone extraction, α-mangostin's solubility qualities in SLPs are inadequate. The concentration of α-mangostin in the plasma of people and rats was verified using Liquid Chromatography-Mass Spectrometry (LC-MS) techniques in addition to the High-Performance Liquid Chromatography (HPLC) methods established for this purpose

There is no established procedure for testing the lipid nanoparticle formulation of α- mangostin. So, the study was conducted to show a novel, quick, exact and accurate approachfor the quantification of α-mangostin, which is simple, rapid, precise and accurate.

MATERIALS AND METHODS

MATERIALS

The sample α-mangostin (98 %) pure standard was procured from Sigma-Aldrich (St Louis, USA) and SLPs were prepared from hot melt homogenization followed by Ultra sonication. All chemicals used like orthophosphoric acid, acetonitrile, methanol and water were ultrapure water HPLC grade purchased from Merk, United Kingdom. Degassed and ultra-filtered solvents were filtered through a 0.45 µmfilter and then utilized in the study.

METHOD

Estimation of λmax

In a 100 ml volumetric flask, 100 µg/ml of α-mangostin was diluted with methanol to generate 10 µg/ml standard solutions. These solutions were scanned using an Ultra Violet (UV) spectrophotometer in the 200-400 nm wavelength range.

Equipment details

HPLC Instrument Alliance e2695 (Empower-3 analysis software) used an auto sampler for analysis with a photodiode array detector. The stationary column (100×4.6 mm) with an X-Bridge C18 Colum packed with porous 3.5 µm particles separated eluents. The column iskept at 40°±2°, whereas the sample is kept at 10°±1°.

Chromatographic condition

HPLC grade Acetonitrile (Mobile phase-A) and HPLC grade 0.1 % Orthophosphoric acid (Mobile phase-B) were employed as mobile phase in chromatographic tests with a flow rate of 1 ml/min and an isocratic protocol. Throughout the experiment, the column and sample temperature were kept at 40°±2° and 10°±1°, respectively. All measurements were recordedat 317 nm. Methanol is used to dilute the samples and a volume of 20 µl is injected throughout the experiment. The mobile phase in 65:35 volumes with 20 min of run time was set up.

Preparation of orthophosphoric acid solution (0.1 %)

1 ml of orthophosphoric acid dissolves in 1000 ml of distilled water to get 0.1 % orthophosphoric acid. Buffer pH is adjusted to 6.8±0.1 using sodium hydroxide dilute solution, which has a concentration of 17.80 mol.

Stock solution and standard solution preparation

To obtain stock solutions, 10 mg of α-mangostin was precisely weighed and transferred to10 ml amber-colored volumetric flask and diluted with methanol to get the final 1 mg/ml solution and maintained at 4° until further. To achieve the standard solution, 10 ml of 1mg/ml solution was transferred to a separate volumetric flask (100 ml) and diluted to a final concentration of 100µg/ml with methanol.

Preparation of calibration curve

From a 100µg/ml standard stock solution, concentrations ranging from 17.38 to 260.90 µg/ml were produced. The absorbance peak area of the concentrations was measured using HPLC equipment at 317nm wavelength. By graphing the findings of the different concen-

trations (µg/ml) and peak area values, a calibration curve was generated.

HPLC validation

The Reversed Phase-HPLC (RP-HPLC) technique was designed and validated in accordance with the International Conference on Harmonization (ICH)-Q2R1 requirements. The approach's experimental and chromatographic parameters had been created and evaluated according to conventional practice[19]

System suitability

To validate the RP-HPLC analytical method, the system suitability is established by initially injecting 6 times the standard solution and evaluating parameters such as percentage Relative Standard Deviation (% RSD) of the peak area, tailing factor and no theoretical plate's relative standard deviation of retention time[19]

Specificity of methodology

The components of formed solid lipid nanoparticles, such as solid lipid, surfactants and emulsifiers, should not obstruct the quantification of an active medicinal molecule. To be more specific, the approach was tested for both blank nanoparticles and solid lipid nanoparticles containing active by comparing the purity of both chromatogram peaks.

Linearity

To establish linearity, several concentrations of α-mangostin in the range of 17.38 to 260.690 g/ml were produced from the standard stock solution and tested in triplicates. A calibration curve was generated from the concentration range data by taking concentration terms on the X-axis and peak areas of corresponding concentrations on the Y-axis. The linearity of the concentration terms was validated by slope values with a relative standard deviation of 1% and an intercept of <1%.

Precision

Samples are analyzed in triplicates for both interday and intraday analysis to ensure precision in the procedure. The correctness and precision of the measurements were checked by comparing the actual findings to theoretical ones and calculating % RSD and percentage accuracy. ICH recommends a maximum tolerance of 5% for coefficient variation. % RSD=SD/Avg. divided by 100

Accuracy

Recovery experiments were conducted to confirm whether the suggested approach is accurate. Quantification was measured by comparing standard solutions with samples of solid lipid nanoparticles extracted separately from the drug and excipients

% Accuracy= (Avg. area of test/Avg. area of standard) *100

Limit of Detection (LOD) and quantification (LOQ)

The LOD is the concentration below which the analytic can no longer be detected. The Limit of Quantification (LOQ) is the lowest concentration of a pharmacological ingredient that can still be accurately and precisely quantified. As suggested by ICH, the slope response and standard deviation data from the calibration plot may be used to estimate detection and quantification limits[20] LOD=3.3*(SD/m) LOD= 10.0*(SD/m)

Applicability for the determination of encapsulation efficiency of prepared solid lipid nanoparticles:

Hot melt homogenizations followed byultrasonication were used to create the solid lipid nanoparticles of α-mangostin. The freeze-dried lipid nanoparticles were kept at 4° for further processing. By dispersing 10mg of lyophilized lipid nanoparticles in 10 ml of methanol, the entrapment effectiveness of the produced nanoparticles was assessed in terms of a percentage of the total weight. The supernatant was separated by centrifugation at 3500 rpm for 10 min. After diluting and testing the recovered supernatant, it was devoid of any drugs. The data was put into the equation below to calculate the percentage entrapment efficiency.

% Entrapment efficiency=(Total conc. of drug-Free drug conc. separated) *100/Total conc. of drug.

RESULTS AND DISCUSSION

10 µg/ml solutions scanned in a UV-Visible spectrophotometer between the ranges of 200-400 nm should have the most absorption

at 243 nm, 317 nm and 320 nm. Literature backs up that the absorption peaks happen at this wavelength range. 317 nm is selected for theestimation of α-mangostinby HPLC method. The chromatographic settings were tuned to get peaks that separated well over a range of pH buffers. To achieve satisfactory peak separation, the mobile phase and its ratios were acetonitrile HPLC grade and 0.1 % orthophosphoric acid in 65:35 volumes. The flow rate is set to 1ml/min and the analysis is performed at 317 nm. In (Figure 1), the chromatogram obtained by injecting standard stock solution is shown.

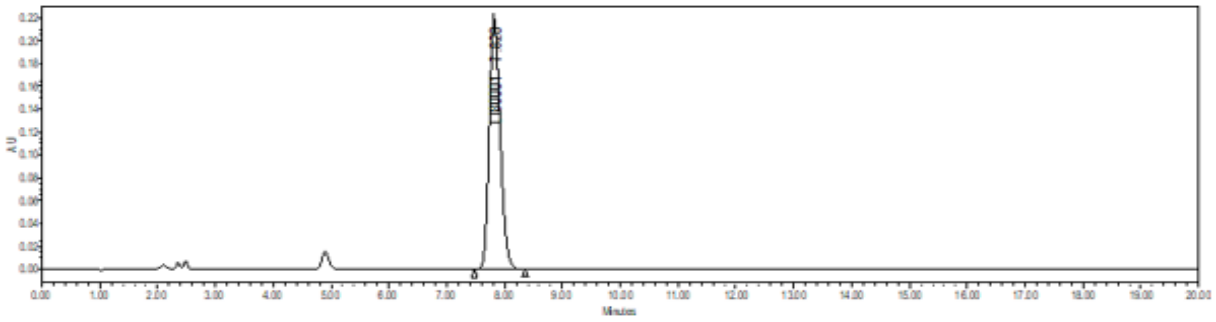

Figure 1: HPLC profile of standard α-mangostin

The chromatographic profiles of solid lipid nanoparticles without and with activities were determined by extracting and injecting the corresponding samples. (Table 1) contains information on the system's suitable characteristics, including retention time, tailing factor, number of theoretical plates and resolution of optimum standards.

Table 1: System suitability test results

System suitability parameters	*Standard limits*	*Experimental results*
Peak area % relative standard deviation	<2.0	0.05
Retention time relative standard deviation	<1.0	0.07
Tailing factors	<2.0	1.01±0.011
No of theoretical Plates	>2000	11346±116

As an essential element of the method development process, system suitability testing verifies the efficacy and appropriateness of the system. The study's outcomes, such as the tailing factor and % RSD, were within the specification's <2.0 % and <2 % respectively limitations, respectively.

The method's specificity is defined by its ability to identify the analyses' reaction even in the presence of contaminants or degradants. The retention time of α-mangostin was 7.82 min in a typical chromatogram, which is equivalent to the standard. Due to the lack of a peak appearing at the same retention period, the specificity investigation proved the absence of excipient interaction in the formulation of SLPs. The specificity findings are shown as a chromatogram in the following (Figure 2 and 3).

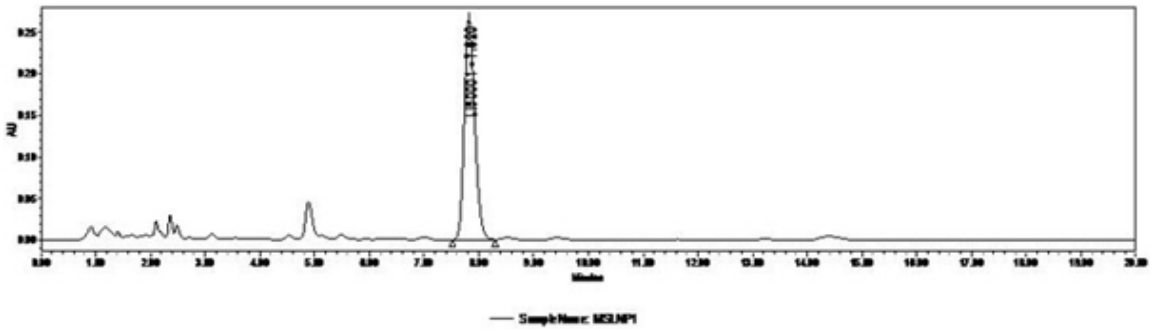

Figure 2: Analytical profile of α-mangostin loaded solid lipid nanoparticles

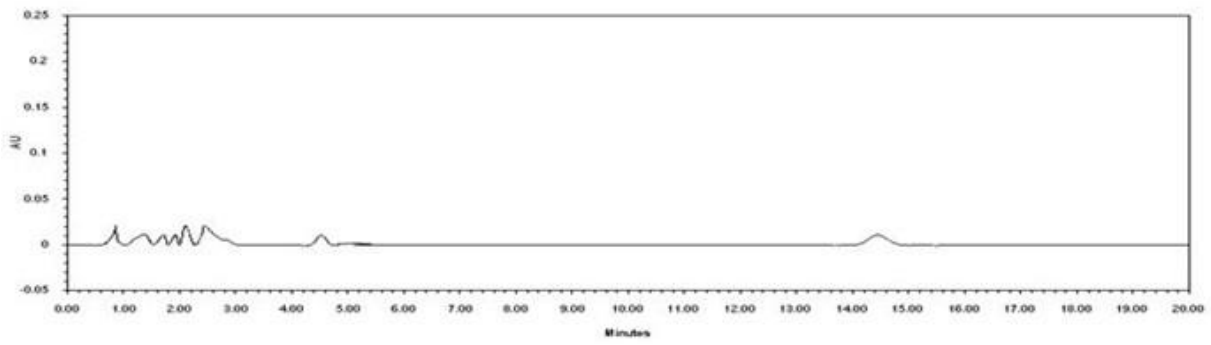

Figure 3: Analytical profile of solid lipid nanoparticles without α-mangostin (blank)

Linearity was confirmed within the concentration range of 17.38 to 260.690 g/ml. The following (Table 2) has the replies to the relevant concentration terms.

Table 2: Calibration curve data of α-mangostin by rp-hplc method

Concentration (µg/ml)	Average peak area±SD (n=3)	% RSD
17.38	613358±327.2	0.043
34.759	1220915±2019.2	0.202
69.518	2418960±2568.3	0.086
130.346	4623080±3197.2	0.061
217.243	7736922±3796.8	0.04
260.9	9413360±4257.4	0.032

Calculate the calibration curve by plotting the concentration terms on the X-axis and the corresponding peak areas on the Y-axis (Figure 4).

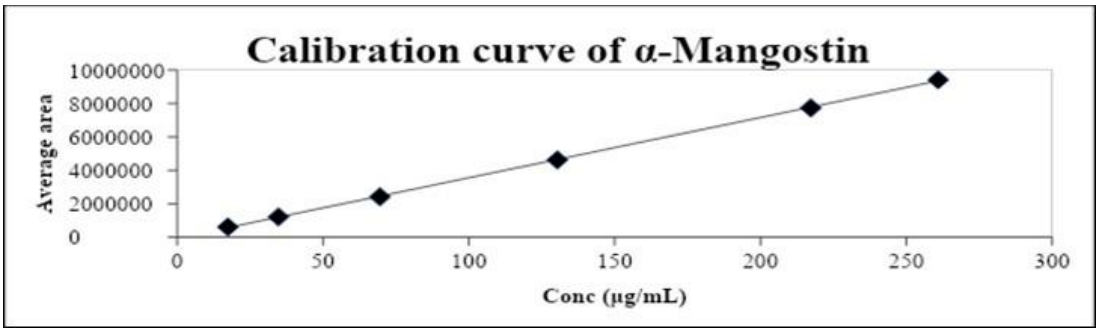

Figure 4: HPLC method calibration curve of α-mangostin

Within the concentration range, satisfactory linearity is obtained with a correlation value of 0.999. Data on linearity and correlation coefficient are shown in (Table 3) below.

Table 3: Regression analysis data of α-mangostin

Drug	Linearity concentration Range	Slope±% RSD	Intercept±% RSD	Regression coefficient (R^2)
α-mangostin	17.380-260.90 (µg/ml)	36051±0.0101	- 49401±0.214	0.999

Experiments established inter-and intraday precision and accuracy. The % RSD for the interand intraday precision of α-mangostin were determined to be 0.18 % and 0.38 %, respectively. The technique that was devised was exact. Additionally, the method's accuracy is proved to be greater than 99.10 % and 100.18 % and greater than 98.60 % and 100.64 %, respectively. The findings indicate that the procedure was precise and accurate. The results are summarized in (Table 4).

Table 4: Inter and intraday precision and accuracy values of α-mangostin

Concentration (µg/ml)	*Observed concentration (µg/ml)*	*% Accuracy*	*% Relative deviation (Precision)*
	Intraday standards results		
34.5	34.42	99.76	0.182
130.5	129.27	99.05	0.058
260.51	262.63	101.81	0.036
	Inter day standards results		
34.5	34.28	99.36	0.165
130.5	128.27	98.29	0.067
260.51	260.63	99.24	0.048

The LOD and LOQ values were found to be 0.000121 and 0.000365 µg/ml respectively. The results were given in (Table 5) below.

Table 5: Limits of detection and limit of quantification

Drug	*Slope*	*LOD(µg/ml)*	*LOQ(µg/ml)*
α-mangostin	36051±0.0101	0.000121	0.000365

The mean recovery data of the α-mangostin were 101.2% which is within the acceptable range of ±3%. The results are given in (Table 6) below.

Table 6: Recovery analysis of α-mangostin loaded solid lipid nanoparticle

Drug	Assay		Recovery		
	Found (µg/ml)	% RSD	Mean recovery (%)	±SD	% RSD
α-mangostin	1.453	0.058	99.69	186029	0.16

The produced SLPs were effectively analyzed using the RP-HPLC technique after being homogenized in hot melt followed by ultrasonication. The findings of the specificity test technique revealed no influence with the measurement of resolved peaks. Recovery yields, on average, were determined to be 98.97 %. The findings demonstrate that the approach devised is adequate for assessing the drug concentration in solid lipid nanoparticles. Not only is the presented approach practical for establishing percent assays, but it is also effective for measuring entrapment efficiency, in vitrorelease profile and stability studies of solid lipid nanoparticles.

The development of the HPLC process depends on several stage gates, including column type and temperature, mobile phase volume and flow rate, the volume of sample injected and its specific wavelength. Regardless of these considerations, HPLC analysis is preferred due to its sensitivity, specificity, precision and accuracy compared to less sensitive spectrophotometric analysis.

The new HPLC approach is cost-effective and easy for determining α-mangostin concentrations informed solid lipid nanoparticles. The technique was verified for accuracy, precision, system compatibility, specificity, quantification and detection lower limits following ICH recommendations (Q2-R1). As the study's run duration is just 20 min, it saves money on solvents. It minimizes environmental impact by examining a more significant number of samples in a shorter amount of time. As a result, it can be stated that the conventional approach is excellent for estimating α-mangostin in solid lipid nanoparticles thathave been created.

CONCLUSION

As a result, it can be stated that the conventional approach is excellent for estimating α- mangostin in solid lipid nanoparticles that have been created.

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REFERENCES

Ruan J, Zheng C, Liu Y, Qu L, Yu H, Han L, et al. Chemical and biological research on herbal medicines rich in xanthones. Molecules2017;22(10):1698.

Chin YW, Kinghorn AD. Structural characterization, biological effects, and synthetic studies on xanthones from mangosteen (Garciniamangostana), a popular botanical dietary supplement. Mini Rev Org Chem 2008; 5(4):355-64.

Kasemwattanaroj P, Moongkarndi P, Pattanapanyasat K, Mangmool S, Rodpai E, Samer J, et al. Immunomodulatory activities of α-mangostin on peripheral blood mononuclear cells. Nat Prod Commun 2013;8(9):1257-60.

Obolskiy D, Pischel I, Siriwatanametanon N, Heinrich M. Garciniamangostana L.: A phytochemical and pharmacological review. Phytother Res 2009; 23(8): 1047-65.

Chin YW, Shin E, Hwang BY, Lee MK. Antifibrotic constituents from Garciniamangostana. Nat Prod Commun 2011; 6(9):1267-8.

Chen SX, Wan M, Loh BN. Active constituents against HIV-1 protease from Garciniamangostana. Planta Med 1996; 62(04):381-2.

Chin YW, Jung HA, Chai H, Keller WJ, Kinghorn AD. Xanthones with quinonereductase-inducing activity from the fruits of Garciniamangostana (Mangosteen). Phytochem 2008; 69(3):754-8.

Devi Sampath P, Vijayaraghavan K. Cardioprotective effect of α-mangostin, a xanthone derivative from mangosteen on tissue defense system against isoproterenol-induced myocardial infarction in rats. J BiochemMolToxicol 2007;21(6):336-9.

Gutierrez-Orozco F, Failla ML. Biological activities and bioavailability of mangosteen xanthones: A critical review of the current evidence. Nutrients 2013; 5(8):3163-83.

Jung HA, Su BN, Keller WJ, Mehta RG, Kinghorn AD. Antioxidant xanthones from the pericarp of Garciniamangostana (Mangosteen). J Agric Food Chem2006; 54(6):2077-82.

Arunrattiyakorn P, Suksamrarn S, Suwannasai N, Kanzaki H. Microbial metabolism of α-mangostin isolated from Garciniamangostana L. Phytochemistry 2011; 72(8):730-4.

Schmid, W. Ueber das Mangostin. Justus Liebigs Ann. Chem 1855; 93:83-88.

Yates P, Stout GH. The structure of mangostin1. J Am ChemSoci 1958; 80(7):1691- 700.

Pedraza-Chaverrí J, Reyes-Fermín LM, Nolasco-Amaya

EG, Orozco-Ibarra M, Medina-Campos ON, González-Cuahutencos O, et al. ROS scavenging capacity and neuroprotective effect of α-mangostin against 3-nitropropionic acid in cerebellar granule neurons. ExpToxicolPathol 2009; 61(5):491-501.

Pothitirat W, Gritsanapan W. HPLC quantitative analysis method for the determination of α-mangostin in mangosteen fruit rind extract. Thai J AgriSci 2009; 42(1):7-12.

Rassameemasmaung S, Sirikulsathean A, Amornchat C, Maungmingsook P, Rojanapanthu P, Gritsanaphan W. Topical application of Garciniamangostana L.pericarp gel as an adjunct to periodontal treatment. Complement Ther Med 2008;16(5):262-7.

Li L, Brunner I, Han AR, Hamburger M, Kinghorn AD, Frye R, et al.

Pharmacokinetics of α-mangostin in rats after intravenous and oral application. MolNutr Food Res 2011; 55(S1):S67-74.

Kondo M, Zhang L, Ji H, Kou Y, Ou B. Bioavailability and antioxidant effects of a xanthone-rich Mangosteen (Garciniamangostana) product in humans. J Agric Food Chem 2009; 57(19):8788-92.

International Conference on Harmonization (ICH) harmonized tripartite guideline.

Validation of analytical procedures: Text and methodology Q2 (R1). International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use; 2005. p. 1-13.

Kumar A, Sharma R, Nair A, Saini G. Development and validation of RP-HPLC method for simultaneous estimation of nimesulide, phenylephrine hydrochloride, chlorpheniramine maleate and caffeine anhydrous in pharmaceutical dosage form.Acta Pol Pharma 2012; 69(6):1017-22.