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# Comparative analysis of α-mangosteen in developed nutraceutical with market sample

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**Abstract**
**Background Information**: Mangosteen or Garcinia mangostana L. belongs to the family Guttiferae and its fruit, whose pericarp contains a family of tricyclic isoprenylated polyphenols referred to as xanthones. Xanthones possess anti-oxidant, anti-proliferative, pro-apoptotic, anti-inflammatory and anti-carcinogenic activities.
**Objectives**: Quantitative analysis of α-mangosteen content in the developed nutraceutical product and comparing with purchased market sample with the validated HPLC method.
**Materials & Methods**: High performance liquid chromatography (HPLC) was used to characterize the α-mangosteen content of developed nutaceutical product.
**Results**: The content of α-mangosteen, 8.689% was conformed in the sample which is developed as the nutraceutical product and in market samples A & B was 8.97% & 9.04% respectively.
**Conclusion**: It has been estimated there is minimum loss in the developed nutraceutical product when compared with market Sample A & B which conforms the quality of the product.

**Keywords:** comparative, α-mangosteen, developed nutraceutical, market sample

## Introduction
Today the trend being exploration and exploitation of the disease fighting properties of a multitude of phytochemicals found in both food and non-food plants have created a renaissance in human health and nutrition research. At the same time, many opportunities for the development of novel dietary products have been created. With all new fields of study come new term knew as "Nutraceuticals", a term combining the words “nutrition” (a nourishing food or food component) and “pharmaceutical” (a medical drug), is a food or food product that provides health and medical benefits, including the prevention and treatment of disease. Such products may range from isolated nutrients, dietary supplements and specific diets to genetically engineered foods, herbal products and processed foods. (Singh *et al.*, 2012) [2]

At present, few plants and underutilized plant parts like rind, seed owing to their Nutraceutical importance has now progressed from being a mere concept representing an area within biomedical research, to a multibillion dollar industry with a very bright future ahead. Plants having health benefits including the prevention and treatment of diseases are employed throughout the industrialized and developing world as home remedies and ingredients for the pharmaceutical products (Pothitirat *et al.*, 2009) [11].

Mangosteen or *Garcinia mangostana* Linn. belongs to the family Guttiferae and it is widely cultivated throughout Southeast Asian countries, especially in eastern and southern parts of Thailand. It is used as traditional medicine to treat skin infections, wounds, and diarrhea (Mahabusarakam, *et al.*, 1987) [8].

Mangosteen (Garcinia mangostana L.) is a tropical tree native to Southeast Asia that produces a fruit whose pericarp contains a family of tricyclic isoprenylated polyphenols referred to as xanthones. Numerous in vitro studies have shown that these xanthones possess anti-oxidant, anti-proliferative, pro-apoptotic, anti-inflammatory and anti-carcinogenic activities. Aggressive marketing of such health promoting benefits has resulted in mangos teen’s classification as a “super fruit. (Orozco, F.G *et al.*, 2013) [6]

Mangosteen has become recently popular as an alternative medicinal product. There are over 50 natural xanthones (Pedraza, *et al.*, 2008) [9] reported in mangosteen (Zarena *et al.*, 2009) [13].

Recently, the pharmacological properties of xanthones in the cardiovascular system have attracted great interest. Xanthones and xanthone derivatives have been shown to have beneficial effects on some cardiovascular diseases, including ischemic heart disease, atherosclerosis, hypertension and thrombosis. The protective effects of xanthones in the cardiovascular system may be due to their antioxidant, anti-inflammatory, platelet aggregation inhibitory, antithrombotic and/or vasorelaxant activities. In particular, the antagonism of endogenous nitric oxide synthase inhibitors by xanthones may represent the basis for improved endothelial function and for reduction of events associated with atherosclerosis (Jiang, D.J *et al.*, 2004) [5].

Compounds isolated from the fruit peel of mangosteen contain abundant xanthones (especially -mangosteen). It has been used as traditional medicine such as anti-inflammatory and antibacterial and is popularly applied to cosmetic and pharmaceutical products. However, there is little information for quality and quantity determination of mangosteen in Mangosteen (Yodhnu, *et al.*, 2009) [12]. Because α-mangosteen (Figure. 1) represents the majority of the clinical benefits of this herbal medicine, it is reasonable and logical to determine the concentration of α-mangosteen as a chemical marker for the quality control of *G. mangostana* and its products, which usually is the only xanthone ingredient quantity-marked in label.(Zarena *et al.*, 2009) [13].

Mangosteen xanthones are found in the whole mangosteen fruit, alpha-mangosteen and gamma-mangosteen have been the main subject of many studies by mangosteen researchers around the world. (Chung, M.I, *et al.*, 2002) [4]

α- and γ-Mangosteen are the most abundant prenylated xanthones present in the fruit of the mangosteen tree. These compounds have been reported to possess numerous bioactivities that have provided the impetus for use of mangosteen products as nutraceuticals and in functional foods and dietary supplements. The health-promoting benefits of mangosteen are dependent on delivery of the xanthones to target tissues (Bumrungpert, A *et al.*, 2009) [1].

Various analytical methods to quantitative analysis α-mangosteen have been reported such as gas chromatography (GC) and high performance liquid chromatography (HPLC) (Jefferson A, *et al.*, 1966, Pothitirat, W *et al.*, 2008) [7, 10]. This study focused on identifying presence of -mangosteen through HPLC Analysis.

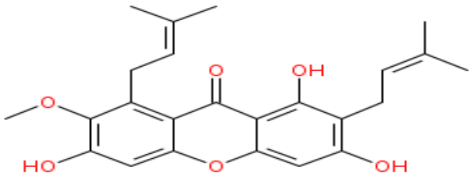



**Fig 1:** Chemical structure of α-mangosteen.

Due to its pharmacological activities, it is popularly applied to herbal cosmetics, pharmaceutical products and also sold as nutraceuticals. However there are different brands of nutraceutical in the market with alpha mangosteen. There is limited information for quality and quantity determination of α-mangosteen in mangosteen extract. So, analytical methods play an important role in the quality control of its raw materials and products (Pothitirat, *et al.*, 2009) [11]. For the quality control of the products the quantitative analysis of specific compounds is necessary. Therefore, the aims of this study were to quantitatively analysis α-mangosteen content in the developed nutaceutical products and compared with purchased market sample with the validated HPLC method.

## Materials and Methods

### Chemicals and Reagents

α- Mangosteen was purchased from Chroma Dex. The other chemicals and solvents used in this experiment were analytical grade which were purchased from Fisher Scientific (Mumbai, India). Acetonitrile 99.8% purity, Potassium Dihydrogen orthophosphate 99% purity.

### Selection of Nutraceutical

The different brands of nutraceutical product in the market with Mangosteen extract were analyzed. And a better product was developed with the combination of Mangosteen extract and Grape seed extract. The other two products in the local market with mangosteen extract were purchased for analysis. Because α-mangosteen represents the majority of the clinical benefits of this herbal medicine, it is reasonable and logical to determine the concentration of α-mangosteen as a chemical marker for the quality control of *Garcinia mangostana* and its products, which usually is the only xanthone ingredient quantity-marked in label (Yodhnu, 2009) [12]. So, α- Mangosteen was analyzed in the Developed Nutraceuticals and the local market products named as Local market sample A and Local market sample B. Alpha – Mangosteen was analyzed for these three products by proposed HPLC method.

### Instrumentation and Chromatographic Condition

HPLC method was performed on a Shimadzu SPD-M20A HPLC system, equipped with a model LC-20 AD pump equipped with a PDA Detector, Rheodyne injector fitted with a 20 µL loop and manual injector (7725 i). A Phenomenex Luna C18 column, 4.6 X 250 mm (5 micron) with a C-18 Reverse Phase column was used. The elution was carried out with gradient solvent systems with a flow rate of 1mL/min$^{-1}$ at room temperature (23 ± 2). The mobile phase was consisted of 0.02 M Potassium dihydrogen Ortho Phosphate (Solvent A) and Acetonitrile (Solvent B).The mobile phase was prepared freshly and filtered through a 0.45µm and sonicated before use. Total running time was 35 min and the gradient programme was as follows: 50% B for 0-5 min, 50% B to 80% B for 5-20 min, 80% B to 50% B for 20-35 min, 50% B for 35 min. Analyzed data processed in post-run analysis. The sample injection volume was 20 µl while the wavelength of PDA detector was set at 272nm.The compounds was quantified with LC solution software.

### Preparation of Standard solutions

A stock solution of α- Mangosteen reference standard (Purity 94%) was prepared by dissolving an accurately weighed 10 mg of α- Mangosteen in 10 ml of acetonitrile in a volumetric flask. Various concentrations of the standard solution were diluted to obtain final concentrations at 0.01, 0.05, 0.1, 0.5, 1, 10, 20 and 30 µg/mL$^{-1}$ with Acetonitrile. Sonicate the standard for 2 mins and then injected.

### Preparation of Sample Solutions

For the determination of α- Mangosteen content from the Developed Nutraceutical capsule and purchased market samples. Each capsule was opened separately 10mg of sample was weighed accurately and transferred carefully to a

100ml Volumetric flask. Acetonitrile was added to volume (final concentration 1000 µg/mL$^{-1}$). Prior to analysis, the solutions were filtered through 0.45 µm membrane filters. Sonicated the sample for 2 mins and then injected.

**Quantitative Analysis of α- Mangosteen Content**

Determination of α- Mangosteen content was carried out by HPLC under the same condition as the proposed method. α-Mangosteen content in the Developed and local market products was calculated using its calibration curve with regarding to the dilution factor. The contents of α-Mangosteen in the Developed product is 8.689%, the Market Sample product A is 8.97%.and the Market sample product B is 9.04%. Each determination was carried out in triplicate.

**Linearity**

Linearity was determined by using α- Mangosteen standard stock solution of 1000µg/mL$^{-1}$ in 10 ml acetonitrile. Serial dilution was performed thereafter to get calibration standard solutions of 0.01, 0.05, 0.1, 0.5, 1, 10, 20 and 30 µg/mL$^{-1.}$ All standard solutions were injected in triplicate and the linearity was assessed based on calibration equation which was calculated from plotting the mean peak areas versus corresponding concentrations

**Limit of detection (LOD) and Limit of quantitation (LOQ)**

In conformity with the "International conference on Harmonization of Technical Requirement for the Registration of Pharmaceuticals for Human use"(Baber N, 1994), the approach based on the standard deviation of the response and the slope was applied here in order to assess both the detection (LOD) and quantitation (LOQ) limits using the following equation:

$$LOD = \frac{3.3\sigma}{S}$$

$$LOQ = \frac{10\ \sigma}{S}$$

σ represents standard deviation of the response whereas S represents slope of the calibration curve.

**Results and Discussion:**

HPLC method with gradient elution was used for the quantification of α-mangosteen in the developed nutraceutical capsules compared with purchased market Sample A & B. The mixture of 0.02M potassium dihydrogen ortho phosphate and acetonitrile gave optimum chromatographic separation of α-mangosteen with the other peaks in the mixture (Figure 2). The wavelength of PDA detector was set at 272nm and the same wavelength was used for all measurements due to its maximum absorption. It is well documented that efficient and fast chromatographic separation of any compound can be achieved by reduction in the particle size of HPLC's column packing material.

The method was validated for its linearity, LOD and LOQ. The calibration graph for α-mangosteen was within the concentration range of 10-30 µg mL$^{-1}$ with a correlation coefficient ($r^2$) of 0.999 (Table 1). The LOD and LOQ for α-mangosteen were found to be 0.01 and 0.02 µg mL$^{-1}$ respectively, which indicate a high sensitivity of the method (Table I)

**Table 1:** Method validation parameters for the quantification of α-mangosteen by the proposed HPLC method.

| Parameters | Results |
|---|---|
| Linear Range (µg mL$^{-1}$) | 10-30 |
| Regression equation (µg mL$^{-1}$) | y= 44067x - 26717 |
| Correlation coefficient ($r^2$) | 0.999 |
| LOD (µg mL$^{-1}$) | 0.01 |
| LOQ (µg mL$^{-1}$) | 0.02 |

X is the concentration of α-mangosteen in µg mL$^{-1}$

Y is the peak area at 272nm

The standard was prepared in three concentrations 10 µg/mL$^{-1}$, 20 µg /mL$^{-1}$, 30 µg/mL$^{-1}$ (Fig. 3, 4 & 5) and the replicates of every concentration were injected. Assay was calculated for every concentration and the average assay was reported.

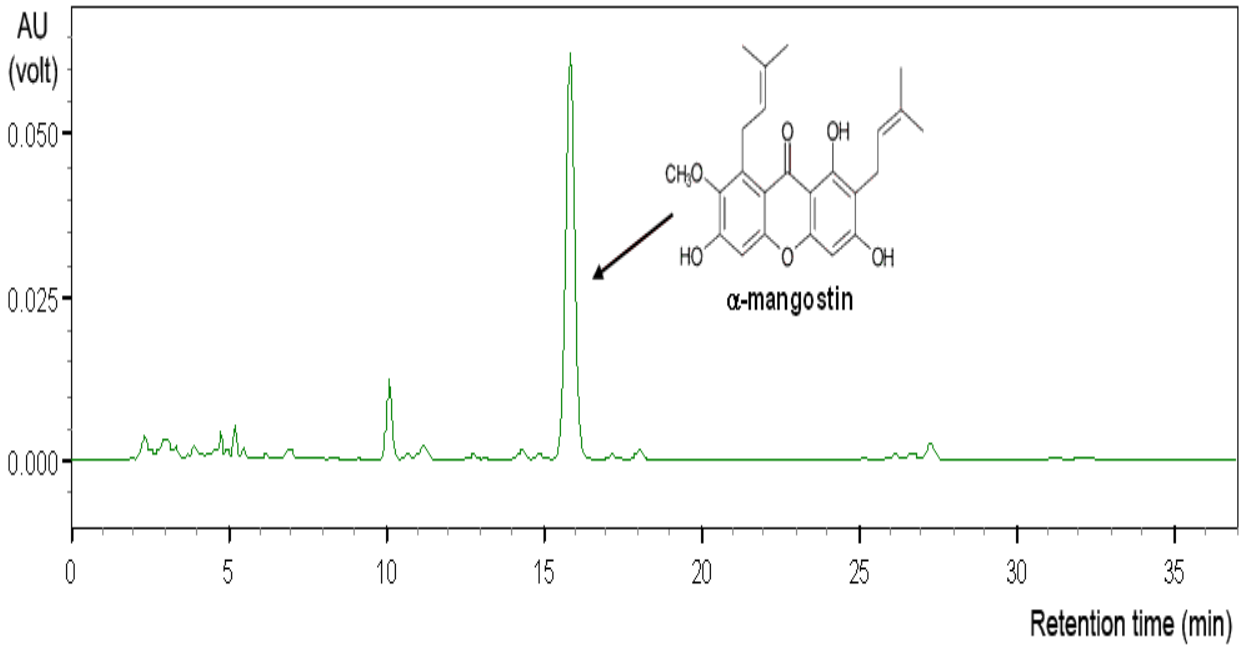



**Fig 2:** HPLC fingerprint of *G. mangostana* fruit rind extract

**Table 2:** Calibration values of Standard Mangosteen

| Concentration | Area |
|---|---|
| 10 µg/mL$^{-1}$ | 404915 |
| | 403168 |
| | 409281 |
| 20 µg/mL$^{-1}$ | 865564 |
| | 871316 |
| | 875985 |
| 30 µg/mL-1 | 1297586 |
| | 1274991 |
| | 1288808 |

The content of α-mangosteen was analyzed by HPLC and 8.689% of α-mangosteen was conformed in the sample HPMC developed capsules and followed by the market samples A & B contain 8.97% & 9.04% of α-mangosteen (Figure 6). The HPLC chromatograms of standard and sample coincide with each other. The identity of the peak of α-mangosteen in the sample chromatograms was confirmed by spiking with its standard and determination of retention time.

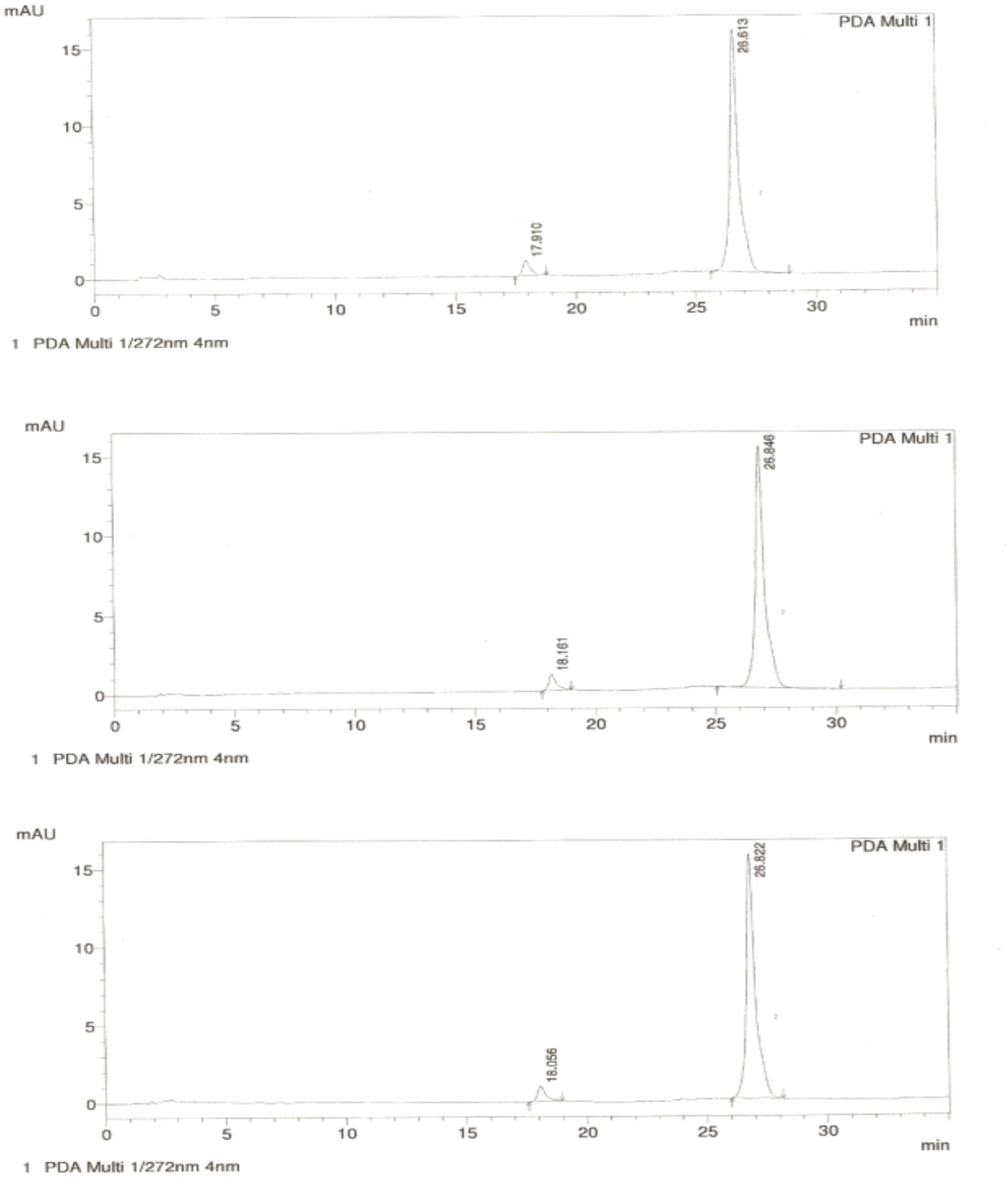



**Fig 3:** Triplicate of α-Mangosteen Reference standard (10 µg mL$^{-1}$) graph

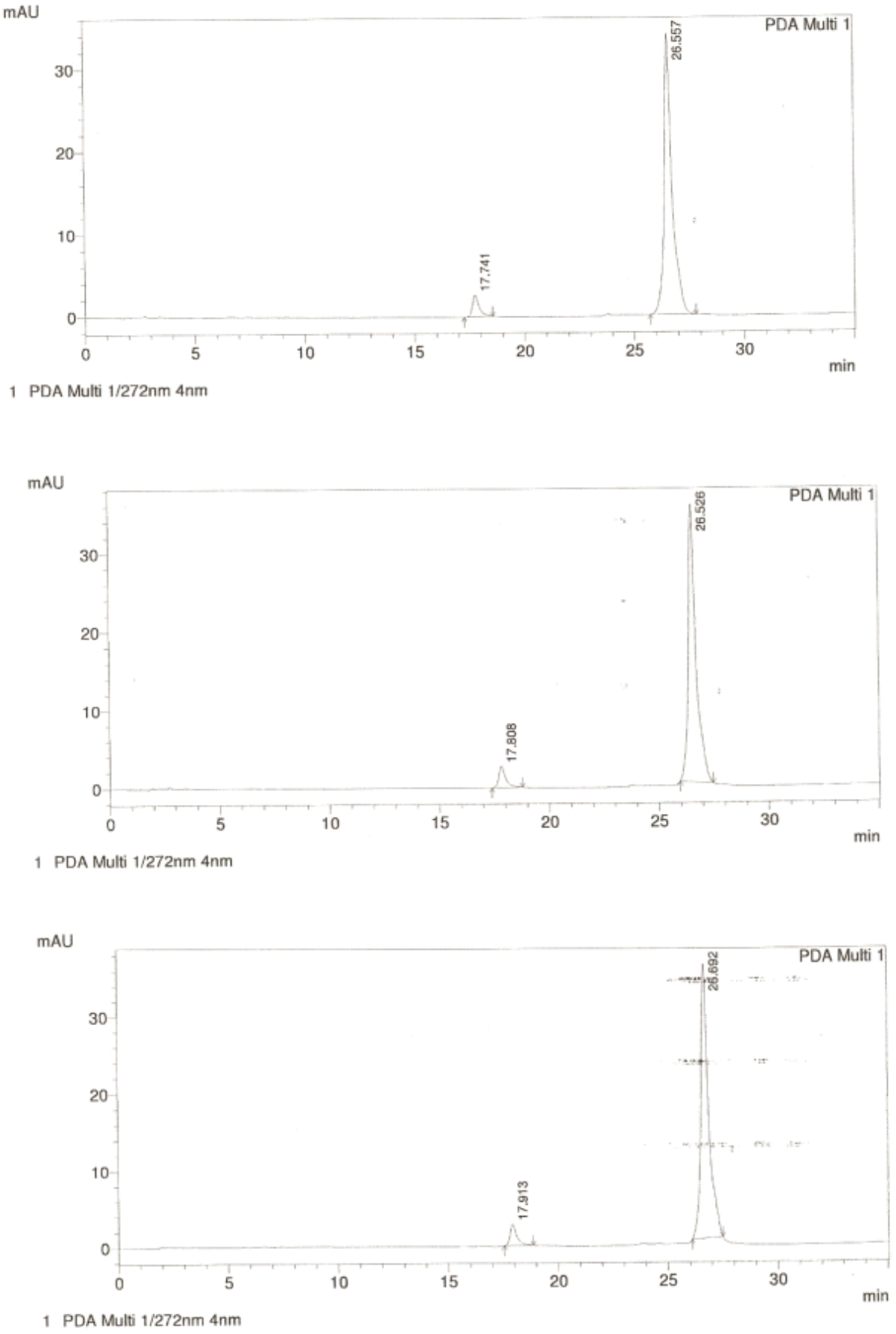



**Fig 4:** Triplicate of α-Mangosteen Reference standard (20 µg $mL^{-1}$) graph

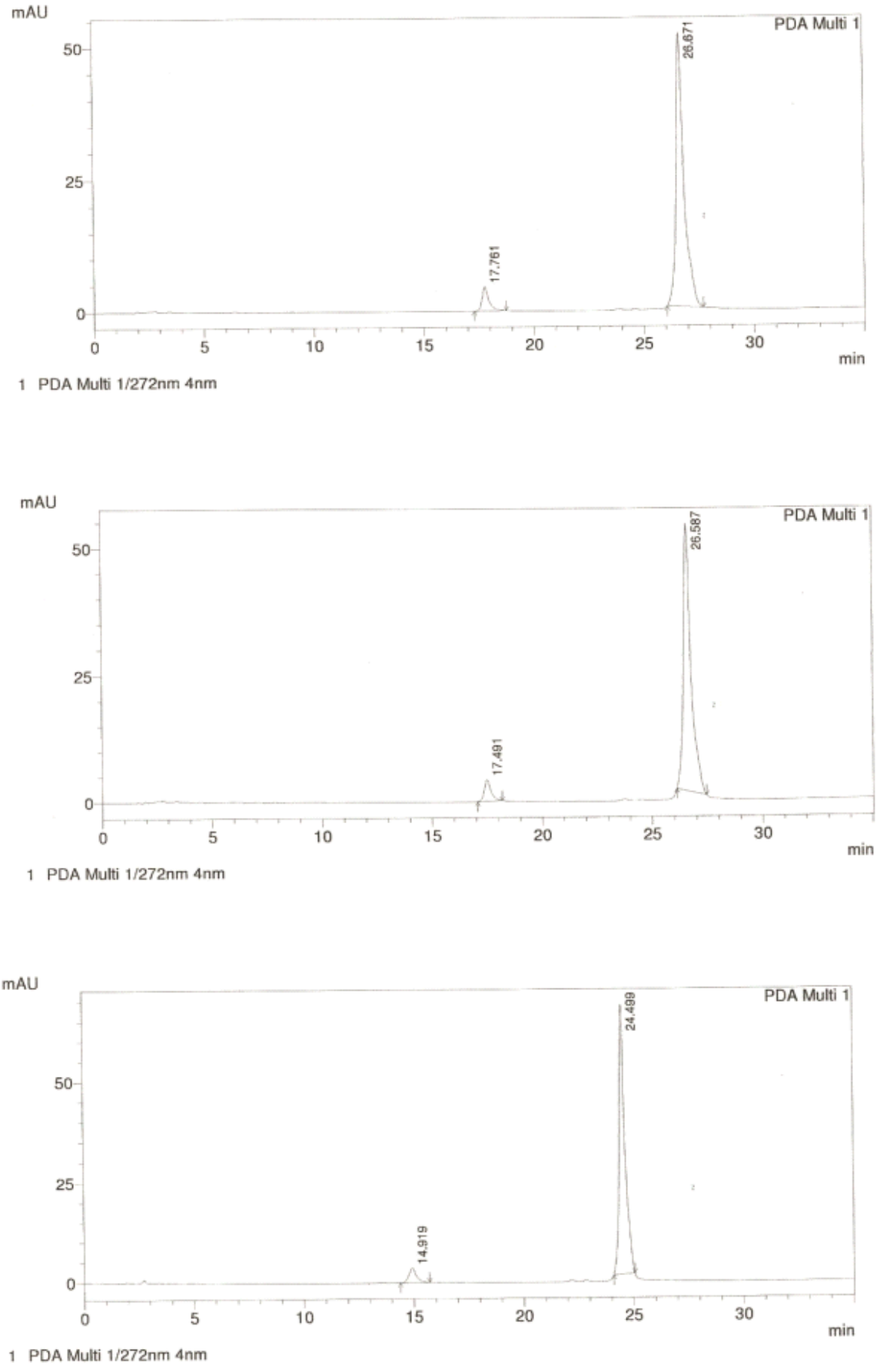
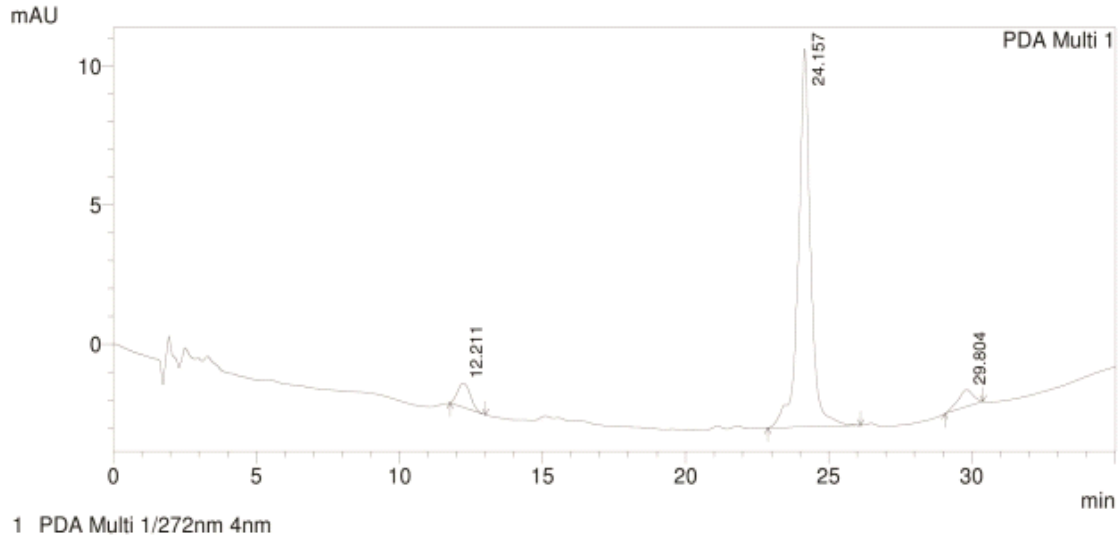
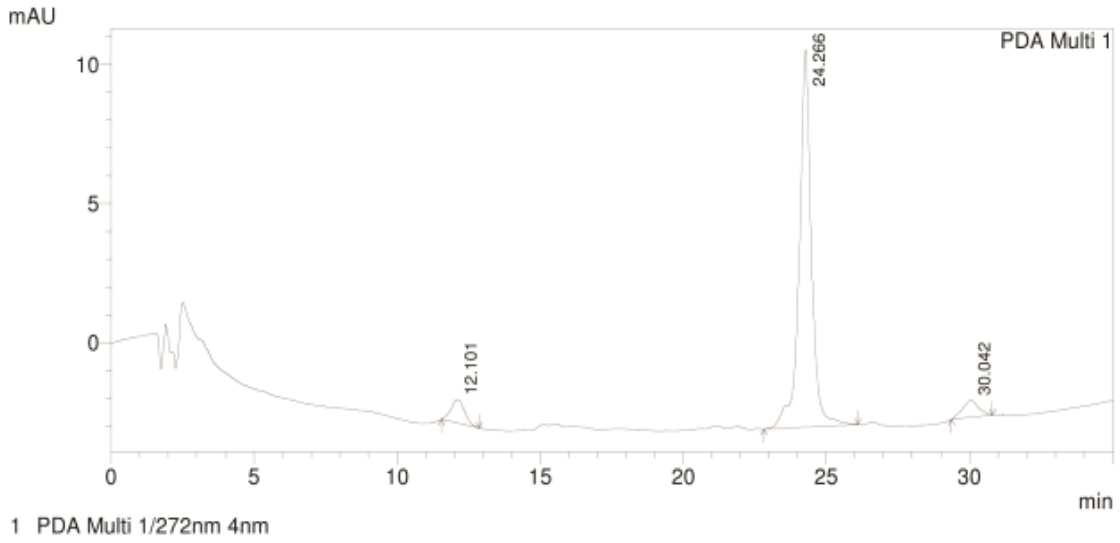



**Fig 5:** Triplicate of α-Mangosteen Reference standard (30 µg $mL^{-1}$) graph



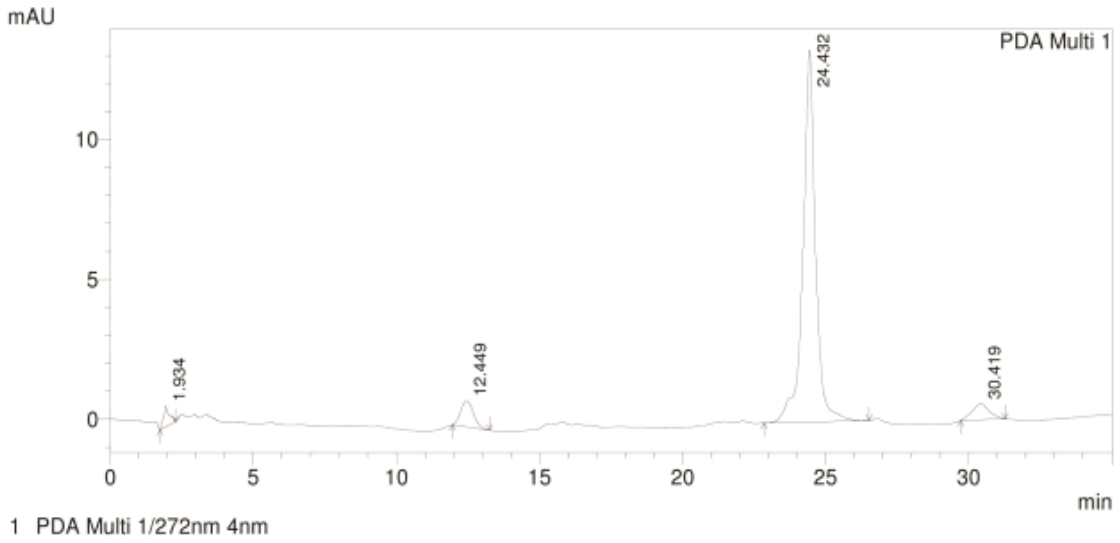




**Fig 6:** Typical HPLC chromatograms of α-mangosteen present in the Samples

**Analysis of α-mangosteen content in the developed nutraceutical**

The nutraceutical supplement was developed with a special combination of mangosteen extract and grape seed extract by reviewing the research literature. The COA of the mangosteen extract from the supplier Energesia denotes 11% α-mangosteen in the purchased material.

The mangosteen extract = 400 mg

11 % α-mangosteen are present in mangosteen extract = 44 mg

The Grape seed extract = 100 mg

The Developed Nutraceutical supplement with the combination of Mangosteen & Grape seed extract contain = 500 mg

Amount of α-mangosteen in the developed nutraceutical supplement (500 mg) = 44 mg

10 mg of sample was dissolved in 100 ml of Acetonitrile (Mobile phase B) and prepared sample solution for HPLC analysis

The amount of α-mangosteen in the sample solution will be 0.88 mg

The Percentage of α-mangosteen content in the developed nutraceutical which contain the purchased mangosteen extract will expected to be 8.8%

The developed nutraceutical were estimated by HPLC method to standardize the amount and percentage of α-mangosteen content.

The Percentage of α-mangosteen content in the mixture of mangosteen & grape seed extract analyzed by HPLC is 8.689%

**Table 3:** Developed nutraceutical product

| Material | Content | α-mangosteen (mg) | α-mangosteen (%) |
|---|---|---|---|
| Mangosteen Extract | 400 mg | 44 mg | 11% |
| Grape Seed Extract | 100 mg | -- | --- |
| Developed supplement (Mangosteen Extract and Grape Seed Extract) | 500 mg | 44 mg | ---- |
| Expected HPLC analysis | 11 mg | 0.88 mg | 8.8% |
| Estimated HPLC analysis | 11 mg | 0.8689 mg | 8.68% |

**Sample A**

The Sample A contains a combination of mangosteen and policosanol purchased from the market specified with 10 % α-mangosteen.

The mangosteen extract = 390 mg

10 % α-mangosteen are present in mangosteen extract = 39 mg

Policosanol = 10 mg

The purchased market sample A Nutraceutical supplement with the combination of Mangosteen & Policosanol contain = 400 mg

Amount of α-mangosteen in the Purchased market sample A nutraceutical supplement (400 mg) = 39 mg

10 mg of sample was dissolved in 100 ml of Acetonitrile (Mobile phase B) and prepared sample solution for HPLC analysis

The amount of α-mangosteen in the sample solution will be 0.975 mg

The Percentage of α-mangosteen content in the developed nutraceutical which contain the purchased mangosteen extract will expected to be 9.75%

The developed nutraceutical were estimated by HPLC method to standardize the amount and percentage of α-mangosteen content.

The Percentage of α-mangosteen content in the mixture of mangosteen extract & Policosanol analyzed by HPLC is 8.97%

**Table 4:** Market sample A

| Material | Content | α-mangosteen (mg) | α-mangosteen (%) |
|---|---|---|---|
| Mangosteen Extract | 390 mg | 39 mg | 10% |
| Policosanol | 10 mg | -- | --- |
| Market Sample A (Mangosteen Extract and Policosanol) | 400 mg | 39 mg | --- |
| Expected HPLC analysis | 10 mg | 0.975 mg | 9.75% |
| Estimated HPLC analysis | 10 mg | 0.897 mg | 8.97% |

**Sample B**

The Sample B is a Triple standardized Mangosteen product with 10 % α-mangosteen purchased from the market.

**Table 5: Market sample B**

| Material | Content | α-mangosteen (mg) | α-mangosteen (%) |
|---|---|---|---|
| Mangostαin Extract | 500 mg | 50mg | 10% |
| Expected HPLC analysis | 10 mg | 1 mg | 10% |
| Estimated HPLC analysis | 10 mg | 0.904 mg | 9.04% |

The mangosteen extract = 500 mg

10 % α-mangosteen are present in mangosteen extract = 50 mg

Amount of α-mangosteen in the Sample B (500 mg) = 50 mg

10 mg of sample was dissolved in 100 ml of Acetonitrile (Mobile phase B) and prepared sample solution for HPLC analysis

The amount of α-mangosteen in the sample solution will be 1 mg

The Percentage of α-mangosteen content in the Sample B market sample will expected to be 10%

The Sample B product purchased from market were estimated by HPLC method to standardize the amount and percentage of α-mangosteen content.

The Percentage of α-mangosteen content in the Sample B analyzed by HPLC is 9.04%

**Table 6:** Comparison of α-Mangosteen in Developed Nutraceuticals with Market Sample A & B

| Sample ID | Mangosteen Extract (mg) | α-Mangosteen (mg) | α- Mangosteen (%) | Expected HPLC analysis | Estimated HPLC analysis |
|---|---|---|---|---|---|
| Developed Nutraceutical | 400 mg | 44 mg | 11% | 8.8% | 8.689% |
| Sample A Purchased market sample | 390 mg | 39mg | 10% | 9.75% | 8.97% |
| Sample B Purchased market sample | 500 mg | 50mg | 10% | 10% | 9.04% |

## Conclusions

The alpha-mangosteen in developed nutraceuticals product and Purchased Sample A & B mangosteen extract were confirmed by spiking their peaks areas with three concentration levels of standard alpha-mangosteen. By comparing the developed nutraceutical capsules with market Sample A & B it has been estimated there is minimum loss in the developed nutraceutical which conforms the quality of the developed products.

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