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Research Article

# Method for determination of gamma oryzanol in dietary supplement by high performance liquid chromatography

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## *Abstract*

Gamma oryzanol (GO), a phytosterol found most abundantly in rice bran oil as well as corn, barley, rye, vegetable oils and wheat bran. Thanks to its ability to slow down the development of melanin pigments, prevent the effects and spread of ultraviolet rays on the skin's surface, limiting freckles and darkening of the skin, in recent years, GO has been added to dietary supplements to help reduce melasma, brighten and smooth the skin. For the purpose of evaluating and controlling product quality, the GO analysis method was developed and validated. GO was analyzed by HPLC using a PDA detector with a C18 chromatography column (250 mm × 4.6 mm, 5 µm) and a mobile phase consisting of methanol and acetonitrile in isocratic mode 35: 65 (v/v). The test sample was extracted with a mixture of n-hexane and ethanol at a ratio of 2: 5 (v/v). The method was evaluated for system suitability, specificity, calibration curve, precision and accuracy with limit of detection and limit of quantification were 15.0 µg/g and 50.0 µg/g, respectively. The analysis results of 10 real samples including 03 pellet samples and 07 powder samples showed that the method is suitable for analyzing samples with different concentration ranges.

***Keywords:*** *Gamma oryzanol, dietary supplement, HPLC.*

## 1. INTRODUCTION

Gamma oryzanol (γ-oryzanol, GO) refers to a group of phytosteryl ferulate compounds typically found in rice bran oil at concentrations of 1-2%. In recent years, GO has garnered significant attention due to its demonstrated biological effects, including the reduction of

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blood cholesterol levels [1, 2], hypoglycaemic properties [3], anti-inflammatory and antioxidant effects [4], as well as benefits related to the nervous system and premenopausal symptoms [5, 6]. This has led to increased interest in GO's presence in rice products (such as rice oil and rice bran film) and health supplements.

At least ten distinct compounds of GO have been identified, categorized into two groups: (1) ferulic forms of sterols (including campesterol, sitosterol, and stigmasterol) and (2) triterpenoids (such as cycloartenol and 24-methylenecycloartenol). Among these, campesteryl, cycloartenyl, 24-methylenecycloartanyl, and sitosteryl ferulate constitute 80-95% of the total GO content and are commonly used as markers for its quantification [7, 8] (see Figure 1).

GO appears as a white or yellowish crystalline powder that is odorless and tasteless, with a melting point ranging from 137.5°C to 138.5°C. It is insoluble in water, slightly soluble in diethyl ether, ethanol, and n-heptane, and readily soluble in chloroform and acetone [5]. GO is typically isolated from rice bran oil through hydrolysis in an alkaline environment at 80°C, yielding ferulic acid salts [9], or by precipitation with calcium ions following alkaline hydrolysis and subsequent dilution with water [10].

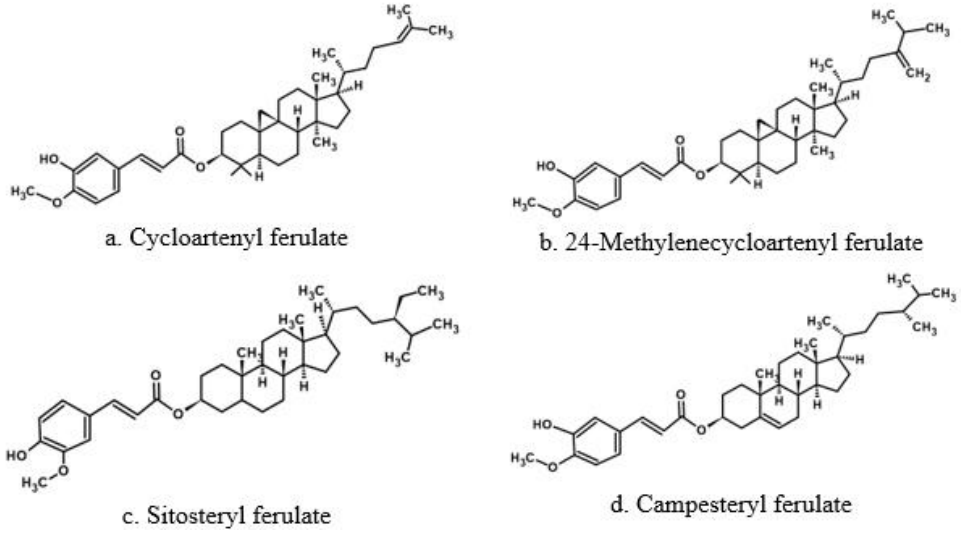


***Figure 1.*** *Structure of 4 main compounds of gamma oryzanol*

The publications in the world and Vietnam mainly use UV-Vis spectrophotometry, high-performance liquid chromatography (HPLC), LC-MS mass spectrometry liquid chromatography [16, 17], and GC-MS mass spectrometry gas [18] for the determination of GO in rice oil, rice, and rice bran film samples. However, the number of research published about the quantitation of GO in supplements is still limited [19].

In Vietnam, the UV-Vis method is applied in TCVN 7597:2013, and TCVN 12107:2017 which have been issued to control the quality of vegetable oil and rice oil, respectively. Nonetheless, it is hard to use the same technic on the supplement matrix to investigate GO because of the influence of background such as curcumin. In this case, HPLC method, a good specificity, simplicity, efficiency, and wide applicability technic, becomes a promising method to analyze GO in supplements.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals and equipments

*2.1.1. Standards and chemicals*

Gamma oryzanol (TRC, code O675500, lot number: 3-OBI-37-1, purity 95.0%). The stock solution of 400 µg/mL GO was used to prepare the intermediate and working standard solution. According to the information on the *Certificate of Analysis*, GO standard consists of two main components: Cycloartenyl ferulate, 24-Methylene Cycloartanylferulate and four other substances including Campesteryl ferulate, Campestanyl ferulate, β-Sitosteryl ferulate and Cycloartanyl ferulate. In this study, the peak area of GO was calculated as the total peak area present on the chromatogram, which is also consistent with previously published studies [13, 14, 16, 19].

The chemicals used in the study included: acetonitrile (ACN), ethanol (EtOH), methanol (MeOH), isopropanol (IPA), n-hexane provided by Merck, and pure water used for chromatography in the laboratory.

The sample named Kieu Xuan Skin Whitening Tablets produced by Sao Kim Pharmaceutical Joint Stock Company, batch number 220621 have been chosen to be test sample in this study to develop and validate method. GO content announced on the label is 20 mg/tablet and other ingredients claimed on the product label include: EstroG-100 (Herbal Estrogen) 257 mg, glutathione 20 mg, dehydroepiandrosterone (DHEA) 5 mg, pregnenolone acetate 5 mg, collagen 100 mg, cassava root 50 mg, curcumin 20 mg and some other excipients.

A placebo sample was self-prepared by mixing ingredients that are similar to the composition of the Kieu Xuan Skin Whitening Tablets but without GO.

*2.1.2. Equipments*

Alliance Waters e2695 high-performance liquid chromatography system connected to a PDA detector from Waters, USA; Waters XBrigde BEH C18 chromatographic column (250 mm × 4.6 mm, 5 µm). Other instruments include: Entris, Sartorius analytical balances (with a reading of 0.0001 g); Elmasonic ultrasonic vibrator; Hermle centrifuges, other auxiliary instruments, and equipment in the laboratory.

### 2.2. Methods

*2.2.1. Analytical method*

This study involved an investigation of various chromatographic conditions, including the selection of detection wavelength, composition of the mobile phase, and sample processing parameters (extraction solvent and extraction time). The evaluation of extraction solvents included six options: isopropanol (IPA), n-hexane, methanol (MeOH), ethanol (EtOH), n-hexane (5:2, v/v), and n-hexane (2:5, v/v). Additionally, the extraction time was assessed over three intervals: 15 minutes, 30 minutes, and 45 minutes.

*2.2.2. Validation method*

- System suitability: conduct repeated analysis of a GO standard solution 06 times, record chromatograph, area and retention time ($t_R$). System suitability is expressed by the relative standard deviation (RSD%) of $t_R$ ($\leq$ 1.0%), peak area ($\leq$ 2.0%), resolution of peaks ($>$ 1.5), theoretical plate number ($N \geq 2000$) and symmetry factor ($A_s \leq 2.0$) [20].

- Specificity: analysis of standard sample, placebo sample and spiked samples. Record $t_R$ and peak area. Requirements: the placebo sample does not appear the peak at the same time of the peak of GO in standard sample; the RSD% of $t_R$ of GO in standard sample and spiked sample is not more than 0.1 minutes [21]. The peak of GO in the test sample reached peak purity with purity angle (PA) < Purity threshold (TH) [22].

- Standard curve: prepare and analyze 5 standard solutions with a concentration of 50% - 150% of the concentration of GO in the test sample. Determine the regression equation y = ax + b. Requirements: $R^2 \geq 0.995$; the bias of each point ≤ 15% [21].

- Precision: evaluated through repeatability and reproducibility. Repeat the analysis of the test sample with two different testers, each tester analyses 06 times. The expected result in test sample is 20 mg/tablet (equivalent to 29.0 mg/g). Requirements: $RSD_r$ repeatability ≤ 1.9%, and $RSD_R$ reproducibility ≤ 3.0% [21].

- Accuracy: determined by recovery (R%) by adding a standard to the placebo at three levels of 50%, 100%, 1450% quantitative concentration (40 µg/mL), each level is repeated three times. Requirements: R% is about 97.0% – 103.0% [21].

## 3. RESULTS AND DISCUSSIONS

### 3.1. Optimization of chromatographic conditions

*3.1.1. Selection of detection wavelength*

To select the detection wavelength, spectrum of GO was collected by scan 40 µg/mL GO standard solution from 210 nm to 400 nm, the results are shown in Figure 2.

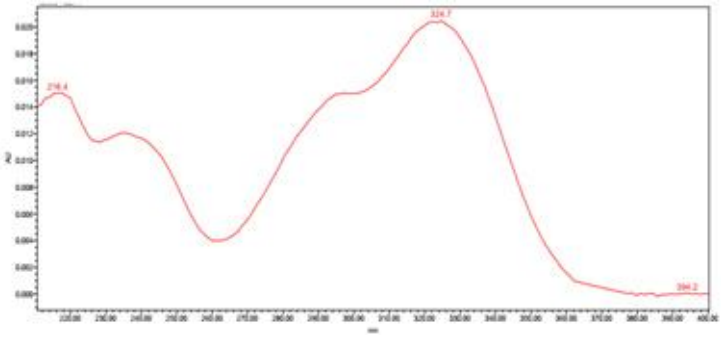


***Figure 2.*** *Absorption spectrum of GO standard*

The results of the standard solution spectrum show that GO has the maximum absorption at a wavelength of 324.7 nm. Therefore, the 325.0 nm wavelength was chosen for GO detection and quantification.

*3.1.2. Selection of mobile phase*

The XBrigde BEH C18 chromatographic column (250 mm × 4.6 mm, 5 µm) was selected for analysis as station phase according to publication and laboratory conditions. After reviewing the literature and considering the available laboratory conditions, the XBridge BEH C18 chromatographic column (250 mm × 4.6 mm, 5 µm) was used as station phage for the analysis. There are 3 different ratios of MeOH and ACN including 35:65 (v/v), 50:50 (v/v), and 70:30 (v/v), were evaluated. The column temperature was set to 40°C, and the flow rate was maintained at 1.2 mL/min. The results of the analysis are summarized in Table 1 and presented in Figure 3.

***Table 1.*** *Results of investigation composition of extraction solvent*

| Ratio of MeOH: ACN (v/v) | Retention ($t_R$) (min) | | | | Resolution (Rs) | | |
|---|---|---|---|---|---|---|---|
| | $t_{R1}$ | $t_{R2}$ | $t_{R3}$ | $t_{R4}$ | $R_{s12}$ | $R_{s23}$ | $R_{s34}$ |
| 35: 65 | 10.967 | 11.789 | 12.888 | 14.293 | 1.58 | 2.00 | 2.04 |
| 50: 50 | 11.836 | 12.836 | 12.151 | 15.677 | 1.10 | 1.39 | 2.15 |
| 70: 30 | 9.987 | 10.602 | 11.446 | 12.531 | 0.93 | 1.14 | 1.72 |

The analysis results from Table 1 showed that the retention time of the GOs decreases depend on the increase of methanol. On the other hand, the Rs among 4 GOs went to under 1.5 when the ratio of methanol went up 50%. Therefore, the isocratic mobile phase consisting of MeOH: ACN (35:65) was set up in this study to get the good resolution and optimize the analysis time.

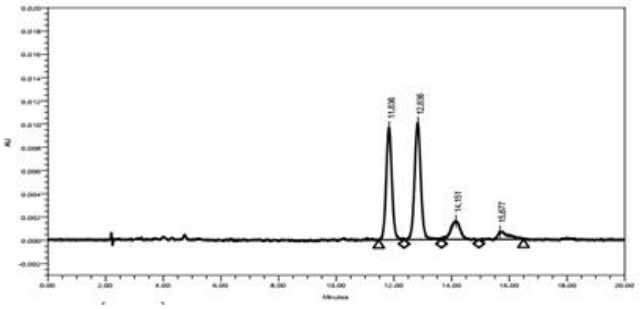

***Figure 3.*** *Chromatogram of standard GO*

### 3.2. Sample preparation optimization

*3.2.1. Extraction solvent*

The investigation of extraction solvents aims to determine the optimal solvent for dissolving GO while minimizing the influence of the sample background. In brief, the extraction solvent was applied to extraction GOs from a spiked sample by sonication twice for 15 minutes each time. Each solvent was analysed the recovery three times, and the average result was recorded. The comparison of recovery rates and chromatographic parameters is shown in Figure 4 and Figure 5.

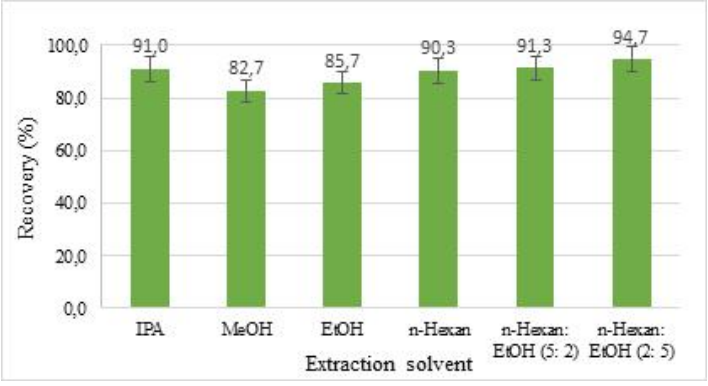


***Figure 4.*** *Result of investigation extraction solvent*

Based on the comparison of recovery (Figure 4) and chromatography (Figure 5), alcohol-based solvents such as IPA, EtOH, and MeOH performed better separation but worse recovery efficiency compared with n-hexane-containing solvents. Gradually reducing the proportion of hexane in the extraction solvent improved both extraction efficiency and resolution of the peak. Therefore, a n-hexane: EtOH (2:5, v/v) was selected as the extraction solvent for subsequent studies.

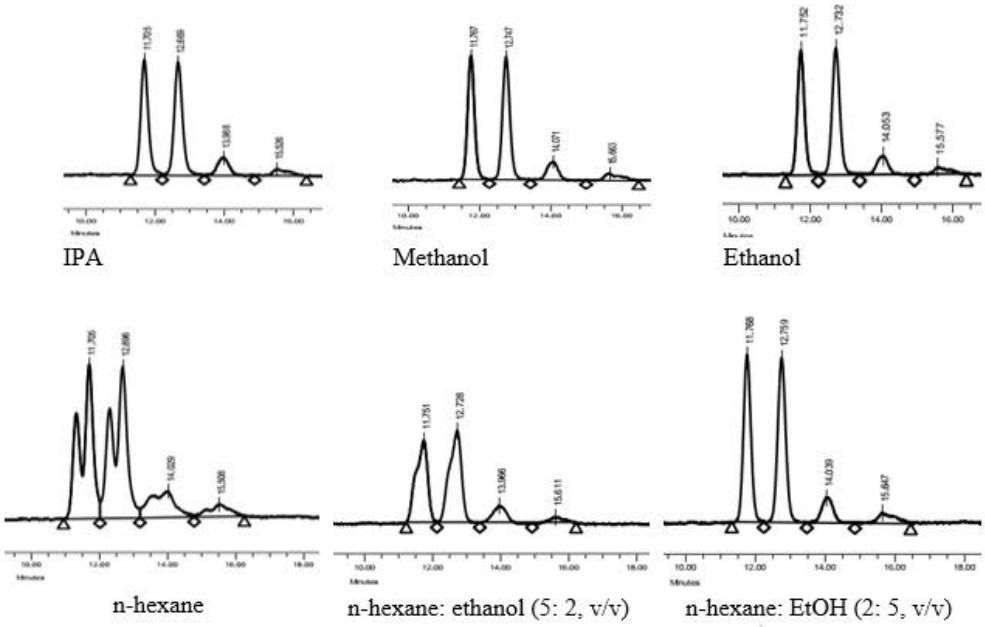


***Figure 5.*** *Chromatogram of GO extracted by 6 solvents*

*3.2.2. Extraction time*

An expected procedure for sample extraction was established using sonication method and n-hexane: EtOH (2:5, v/v) as extraction solvent. Extraction times of 15, 30, and 45 minutes were evaluated, with each condition tested in triplicate to obtain average values. The results of the GO analysis are presented in Figure 6.

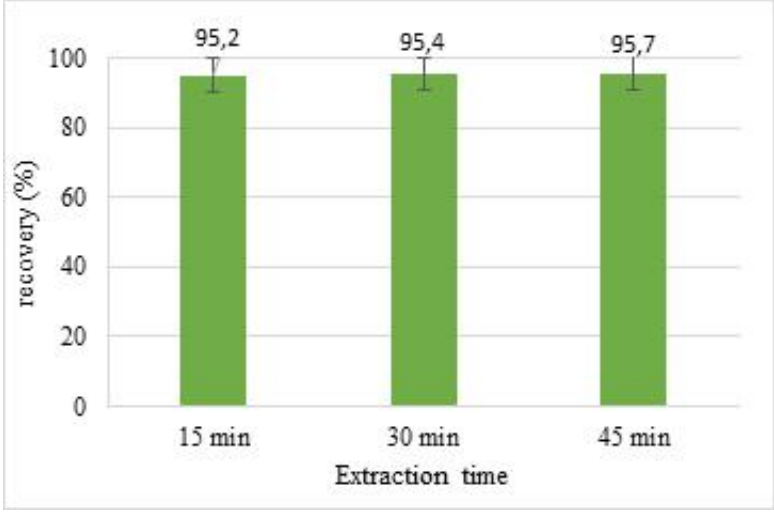


***Figure 6.*** *Result of investigation extraction time*

The results indicated that varying the extraction time from 15 to 45 minutes had minimal impact on extraction efficiency. This outcome is consistent with the fact that GO in health food samples is typically added in its refined form. Consequently, an extraction time of 15 minutes is deemed optimal, as it minimizes analysis time without influence on extraction efficiency.

Based on the evaluation findings, the GO analysis method was determined as follows:

*Sample preparation:* weigh an accurate amount of 0.5 – 2.0 g of the sample (or 20 mg GO equivalent) into a 50 mL centrifuge tube. Add 35 mL of n-hexane: EtOH (2:5, v/v), sonication for 15 minutes, and centrifugation at 6000 rpm for 5 minutes. Transfer the supernatant into a 50 mL volumetric flask. Next, the second extraction was done by adding 15 mL of extraction solvent and sonicate for 15 minutes. Repeat the centrifugation to collect supernatant into a 50 mL volumetric flask. Fulfill the volumetric flask by extraction solvent. Gently mix the extraction solution and filter using filter paper. Dilute the filtered solution 10 times before analysis by HPLC.

*Chromatographic conditions:* station phase of XBrigde BEH C18 (250 mm × 4.6 mm, 5 μm); mobile phase: isocratic MeOH: ACN, (35:65, v/v); flow rate: 1.2 mL/min; column temperature: 40°C; injection volume: 10 μL; detection and quantification wavelength: 325 nm.

### 3.3. Validation method

*3.3.1. System suitability*

Perform six replicate injections of GO at a concentration of 40 μg/mL under the optimized chromatographic conditions. Evaluate the total peak area, retention time, resolution, theoretical plate number, and symmetry factor. The results are summarized in Table 2 and Table 3.

The analysis results show that the repeatability of the total peak area is less than 2.0%; the retention time is less than 1.0%; the Rs > 1.5; AF from 0.90 to 1.87 and the N > 2000. Thus, the HPLC method is suitable for GO analysis.

***Table 2.*** *Results of validation system suitability – retention time and resolution*

| STT | Total peak area | Retention time (min) | | | | Resolution ($R_s$) | | |
|---|---|---|---|---|---|---|---|---|
| | | $t_{R1}$ | $t_{R2}$ | $t_{R3}$ | $t_{R4}$ | $R_{S12}$ | $R_{S23}$ | $R_{S34}$ |
| 1 | 1090776 | 11.127 | 12.044 | 13.251 | 14.716 | 1.50 | 2.02 | 2.14 |
| 2 | 1095395 | 11.120 | 12.033 | 13.253 | 14.717 | 1.50 | 1.95 | 2.07 |
| 3 | 1087194 | 11.083 | 12.039 | 13.228 | 14.698 | 1.51 | 1.97 | 2.08 |
| 4 | 1078206 | 11.080 | 11.988 | 13.178 | 14.644 | 1.50 | 1.96 | 2.10 |
| 5 | 1087853 | 11.074 | 11.989 | 13.210 | 14.635 | 1.51 | 2.05 | 1.93 |
| 6 | 1076275 | 11.122 | 11.985 | 13.176 | 14.638 | 1.57 | 2.03 | 2.08 |
| **Average** | **1085950** | **11.101** | **12.013** | **13.216** | **14.675** | **1.52** | **2.00** | **2.07** |
| RSD% | 0.68 | 0.22 | 0.24 | 0.26 | 0.27 | 1.81 | 2.09 | 3.46 |

***Table 3.*** *Results of validation system suitability theoretical plate number and symmetry factor*

| No. | Theoretical plate number (N) | | | | Symmetry factor ($A_s$) | | | |
|---|---|---|---|---|---|---|---|---|
| | $N_1$ | $N_2$ | $N_3$ | $N_4$ | $A_{s1}$ | $A_{s2}$ | $A_{s3}$ | $A_{s4}$ |
| 1 | 18019 | 18430 | 11562 | 8897 | 1.08 | 1.06 | 0.94 | 1.46 |
| 2 | 18373 | 18519 | 11177 | 8166 | 1.07 | 1.07 | 0.98 | 1.35 |
| 3 | 18133 | 18270 | 10849 | 8804 | 1.06 | 1.06 | 0.93 | 1.44 |
| 4 | 17892 | 18290 | 10652 | 7287 | 1.07 | 1.10 | 0.94 | 1.57 |
| 5 | 18486 | 18242 | 11673 | 7019 | 1.08 | 1.07 | 0.90 | 1.87 |
| 6 | 17833 | 18097 | 10086 | 8036 | 1.09 | 1.08 | 0.93 | 1.55 |
| **Average** | **18123** | **18308** | **11000** | **8035** | | | | |

*3.3.2. Specificity*

Analyse blank samples, placebo sample, standard sample, and real sample. Inject each of these solutions into the HPLC following the optimized chromatographic conditions.

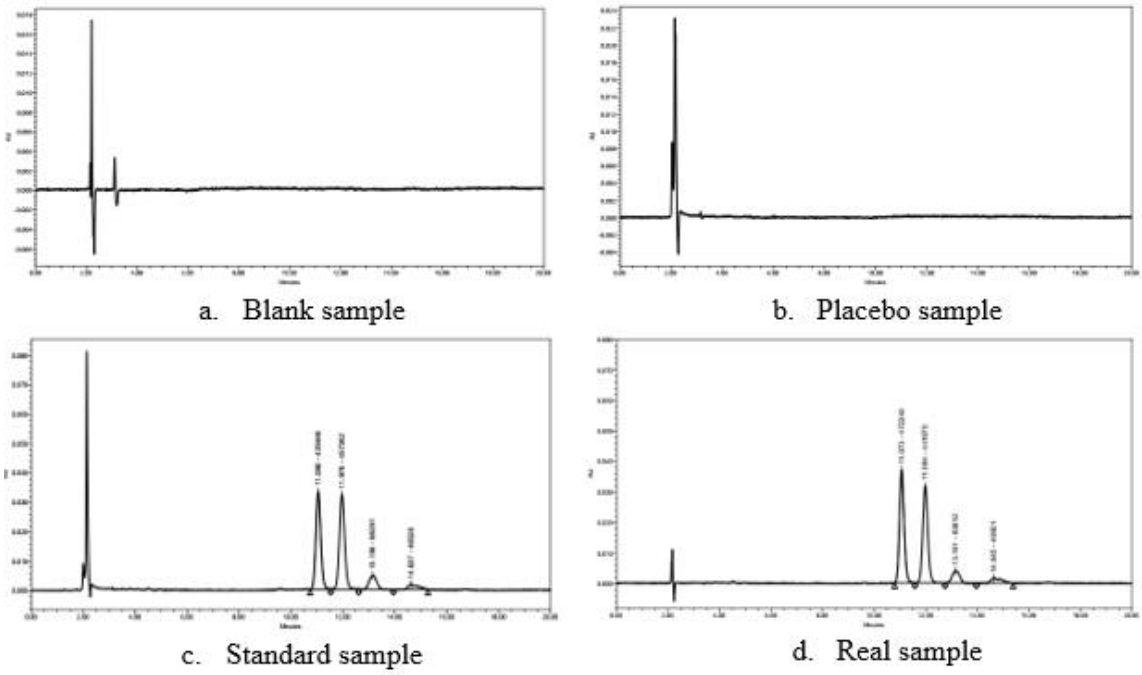


***Figure 7.*** *Chromatogram of specificity – Retention time and Resolution*

As demonstrated in Figure 7, chromatogram of the blank and placebo samples revealed no peak at the retention time of the GO in standard sample. The retention times for the real samples were $t_{R1}$ 11.066 min, $t_{R2}$ 11.976 min, $t_{R3}$ 13.156 min, and $t_{R4}$ 14.637 min, while the corresponding retention times for the standard samples were $t_{R1}$ 11.073 min, $t_{R2}$ 11.984 min, $t_{R3}$ 13.161 min, and $t_{R4}$ 14.645 min. These results indicate a good similarity in retention times between the real and standard samples. A peak purity assessment was subsequently performed on the real sample using the built-in software of the chromatographic system.

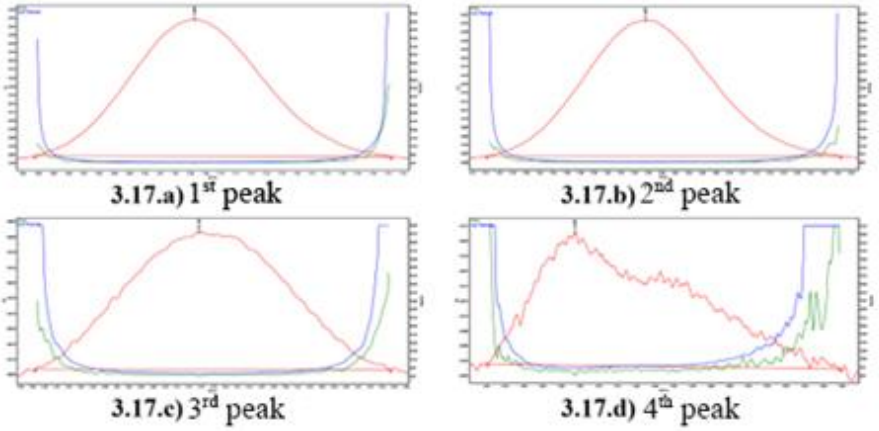


***Figure 8.*** *Chromatogram of specificity – Purity Angle and Purity threshold*

The PAs of the 1st and 2nd peak were less than 1 while the PAs of the 3rd and 4th peak were greater than 1 (Figure 8). However, the PA of all peak remained below the TH, which satisfy the peak purity criteria [22]. Consequently, both the real and standard samples exhibited similar retention times for each peak, and all peaks met the purity requirements, confirming their specificity for GO analysis.

*3.3.3. Linearity*

Prepare a six-point calibration solution for GO with concentrations of 2.5, 5.0, 10, 25, 50, and 100 µg/mL, and analyse the samples using HPLC. The standard curve is based on the peak area corresponding to the GO concentrations. The correlation between the standard curve and the area of the GO peak is illustrated in Figure 9.

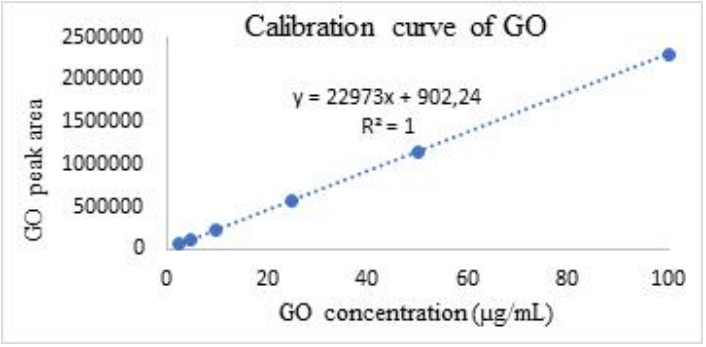


***Figure 9.*** *Standard curve of gamma oryzanol*

In the concentration range of 2.5 to 100 µg/mL, the standard curve for GO was linearity, with a coefficient of determination ($R^2$) equal to 1. The deviation of each data point from the calibration line is less than 15%, confirming the method's suitability for analyse samples containing varying content of GO.

*3.3.4. Precision*

Precision is evaluated through repeatability and reproducibility. Repeatability was assessed based on the standard deviation (RSD%) of 06 repeat tests of the same 01 tester on the same day of analysis. The reproducibility is performed similarly but on different days and different testers. The results of the relative standard deviation assessment are presented in Table 4.

***Table 4.*** *Results of validation precision*

| Tester 1 | | | | Tester 2 | | | |
|---|---|---|---|---|---|---|---|
| ***No.*** | ***m (g)*** | ***Peak area*** | ***Content (mg/g)*** | ***No.*** | ***m (g)*** | ***Peak area*** | ***Content (mg/g)*** |
| 1 | 0.7977 | 1047374 | 28.6 | 7 | 0.7879 | 1033624 | 28.5 |
| 2 | 0.7945 | 1022692 | 28.0 | 8 | 0.7582 | 1042070 | 29.9 |
| 3 | 0.8079 | 1030375 | 27.7 | 9 | 0.7883 | 1044252 | 28.8 |
| 4 | 0.7901 | 1033577 | 28.4 | 10 | 0.8014 | 1083275 | 29.4 |
| 5 | 0.7895 | 1054314 | 29.0 | 11 | 0.7904 | 1061524 | 29.2 |
| 6 | 0.7989 | 1056102 | 28.7 | 12 | 0.8029 | 1052612 | 28.5 |
| **Average** | | | **28.4** | **Average** | | | **29.1** |
| **$RSD_r$ (%)** | | | **1.70** | **$RSD_r$ (%)** | | | **1.87** |
| | | | **Average = 28.7** | | | | |
| | | | **$RSD_R$ (%) = 2.06** | | | | |

The results of the repeatability and reproducibility analysis on the real sample were at a reference content of <10% for $RSD_r$ < 1.9% and $RSD_R$ < 3.0%. Thus, the method meets the requirements of the AOAC of precision.

*3.3.5. Accuracy*

Accuracy was assessed using the standard addition method based on a placebo sample to prepare spiked sample and recovery calculations. Spiked samples were prepared at concentrations of 20, 40, and 60 µg/mL, corresponding to 50%, 100%, and 150% of the expected content level analysed in the real sample. Each concentration level was analysed in quadruplicate. The results are summarized in Table 5.

***Table 5.*** *Results of validation accuracy*

| No. | Spike level (mg/g) | $C_{spike}$ (µg/mL) | C (µg/mL) | R% | RSD% |
|---|---|---|---|---|---|
| 1 | 10.0 | 20.0 | 19.85 | 99.3 | 1.14 |
| 2 | 10.0 | 20.0 | 19.76 | 98.8 | |
| 3 | 10.0 | 20.0 | 19.45 | 97.0 | |
| 4 | 10.0 | 20.0 | 19.45 | 97.3 | |
| 5 | 20.0 | 40.0 | 39.84 | 99.6 | 0.93 |
| 6 | 20.0 | 40.0 | 39.22 | 98.1 | |
| 7 | 20.0 | 40.0 | 40.01 | 100.0 | |
| 8 | 20.0 | 40.0 | 39.41 | 98.5 | |
| 9 | 30.0 | 60.0 | 60.07 | 100.1 | 0.90 |
| 10 | 30.0 | 60.0 | 59.33 | 98.9 | |
| 11 | 30.0 | 60.0 | 59.68 | 99.5 | |
| 12 | 30.0 | 60.0 | 58.81 | 98.0 | |

The analysis results showed the recovery meet the requirement of 97.0% to 103%, with a repeatability of less than 2.7% at a concentration of 10 mg/g. At spike level of 20 and 30 mg/g, the recovery rates ranged from 98.0% to 102%, with a repeatability of less than 1.9% [21]. Thus, the method meets the accuracy requirements.

*3.3.6. Limit of detection and limit of quantitation*

10 spiked samples at spike level of 5 µg/mL were prepared and analysed by HPLC. The mean and standard deviation of the GO content were calculated. The LOD and LOQ were determined using the formulas: LOD = 3 × SD and LOQ = 10 × SD, with the requirement that $4 \leq R \leq 10$ (where $R = \bar{x}/LOD$). The results for the determination of LOD and LOQ are summarized in Table 6.

***Table 6**. Results of validation LOD-LOQ*

| No. | m (g) | Peak area | C (µg/mL) | R% | Content (µg/g) | Average (µg/g) | SD |
|---|---|---|---|---|---|---|---|
| 1 | 2.0963 | 111753 | 4.83 | 96.6 | 115.1 | 121.4 | 5.00 |
| 2 | 1.9818 | 116249 | 5.02 | 100.4 | 126.7 | | |
| 3 | 2.0114 | 115800 | 5.00 | 100.0 | 124.3 | | |
| 4 | 2.0811 | 111895 | 4.83 | 96.6 | 116.1 | | |
| 5 | 2.0335 | 111847 | 4.83 | 96.6 | 118.7 | | |
| 6 | 2.0016 | 119866 | 5.18 | 103.6 | 129.4 | | |
| 7 | 2.0035 | 117845 | 5.09 | 101.8 | 127.0 | | |
| 8 | 2.0565 | 112876 | 4.87 | 97.4 | 118.5 | | |
| 9 | 2.0746 | 114176 | 4.93 | 98.6 | 118.8 | | |
| 10 | 2.0439 | 113174 | 4.89 | 97.8 | 119.6 | | |

LOD value = 3×SD; LOQ= 10×SD is estimated to be 15.0 µg/g and 50 µg/g with R= 8.09. The accuracy is 96.6 – 103.6% and meets the requirements of recovery (90 – 107%).

The validation results of the quantitative method for GO indicate that the method exhibits specificity, repeatability, reproductivity, and accuracy that satisfied with AOAC requirement. This method can apply to analyse GO in real sample at various content level because of low LOD-LOQ, broad linearity.

**3.4. Application on product sample**

The method applied for the analysis of product which send to the National Institute for Food Control, including 3 capsule and 10 powder samples. The results of these samples analysis are presented in Figure 10.

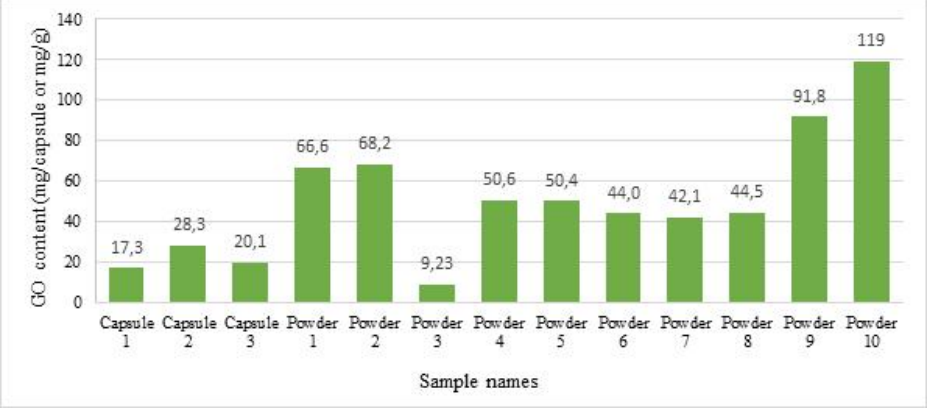


***Figure 10.** Results of GO content in real sample*

Capsules samples have a declared content of 20-30 mg/tablet, with results in the range of 80-120% according to the product's declaration dossier. Semi-finished powder samples without ingredient information have different levels ranging from 9.23 to 119 mg/g.

## 4. CONCLUSION

This study focused on the development and validation of a quantitative method for determining GO in dietary supplements. The validation results demonstrated that the method meets AOAC requirement for system suitability, specificity, accuracy (96.6% to 103.6%), and reproducibility (2.8%). A linear calibration curve was established over the concentration range of 2.5 to 100 µg/mL, with LOD of 15.0 µg/g and LOQ of 50 µg/g, indicating the method suitable for determining GO at various level. At the laboratory, the method was validated on the 2 backgrounds including soft capsule and liquid. However, on the market, GO was usually supplemented into hard capsules, tablets, and powders. Therefore, this publication only showed the results on powder as a representative background. The method has now been implemented to analyse the content of gamma oryzanol at the National Institute for Food Control.

## REFERENCES

[1]. A. Berger, D. Rein, A. Schäfer *et al.*, "Similar cholesterol-lowering properties of rice bran oil, with varied γ -oryzanol, in mildly hypercholesterolemic men," *European Journal of Nutrition*, vol. 44, no. 3, pp. 163-173, 2004.

[2]. C. Juliano, M. Cossu, M. C. Alamanni, L. Piu, "Antioxidant activity of gamma-oryzanol: Mechanism of action and its effect on oxidative stability of pharmaceutical oils," *International Journal of Pharmaceutics*, vol. 299, no. 1-2, pp. 146-154, 2005.

[3]. C. Kozuka, K. Yabiku, C. Takayama *et al.*, "Natural food science based novel approach toward prevention and treatment of obesity and type 2 diabetes: Recent studies on brown rice and γ-oryzanol," *Obesity Research & Clinical Practice*, vol. 7, no. 3, pp. 165-172, 2013.

[4]. W. Rungratanawanich, G. Abate, D. Uberti, "Pharmacological profile of γ-oryzanol: Its antioxidant mechanisms and its effects in age-related diseases," *Aging*, second edition, pp. 201-208, 2020.

[5]. K. Srikaeo, "Chapter 35 - Organic Rice Bran Oils in Health," *Wheat and Rice in Disease Prevention and Health*, pp. 453-465, 2014.

[6]. C. Lemus, A. Angelis, M. Halabalaki, A. L. Skaltsounis, "Chapter 32-γ-oryzanol: An attractive bioactive component from rice bran," *Wheat and Rice in Disease Prevention and Health*, pp. 409-430, 2014.

[7]. Y. H. Cho, S. Y. Lim, A. Rehman *et al.*, "Characterization and quantification of γ-oryzanol in Korean rice landraces," *Journal of Cereal Science*, vol. 88, pp. 150-156, 2019.

[8]. C. Saenjum, C. Chaiyasut, S. Chansakaow *et al.*, "Antioxidant and antiinflammatory activities of gamma oryzanol rich extracts from Thai purple rice bran," *Journal of Medicinal Plants Research*, vol. 6, pp. 1070-1077, 2011.

[9]. Z. Xu, J. S. Godber, "Purification and identification of components of γ-oryzanol in rice bran oil," *Journal of Agricultural and Food Chemistry*, vol. 47, no. 7, pp. 2724-2728, 1999.

[10]. P. K. Das, A. Chaudhuri, T. N. B. Kaimal *et al.*, "Isolation of γ-oryzanol through calcium ion induced precipitation of anionic micellar aggregates," *Journal of Agricultural and Food Chemistry*, vol. 46, no. 8, pp. 3073-3080, 1998.

[11]. Vietnam National Standard, TCVN 7597:2013, Vegetable oils (In Vietnameses).

[12]. Vietnam National Standard, TCVN 12107:2017, Rice bran oils (In Vietnameses).

[13]. Pham Canh Em, Nguyen Thi Kim Mo, Le Thi Tuong Vi, Nguyen Trong Tuan, "Process optimization for extracting tocopherol and γ -oryzanol contents in rice bran using response surface methodology," *Journal of Analytical Sciences,* vol. 21, no. 1, pp. 101-111, 2016 (In Vietnameses).

[14]. J. Y. Cho, H. J. Lee, G. A. Kim *et al.*, "Quantitative analyses of individual γ-oryzanol (Steryl Ferulates) in conventional and organic brown rice (*Oryza sativa* L.)," *Journal of Cereal Science*, vol. 55, no. 3, pp. 337-343, 2012.

[15]. Le Pham Van Anh, Le Nguyen Doan Duy, Nguyen Cong Ha, "Study on extraction and preservation of g-oryzanol and ferulic acid from rice bran," *Journal of Sciences Can Tho University*, 55, pp. 292-300, 2019.

[16]. Aladedunye F., Przybylski R., Rudzinska M. *et al.*, "γ-oryzanols of North American Wild Rice (Zizania palustris)," *Journal of the American Oil Chemists' Society,* vol. 90, no. 8, pp. 1101-1109, 2013.

[17]. Kim H.W., Kim J.B., Cho S.M. *et al.*, "Characterization and quantification of γ-oryzanol in grains of 16 Korean rice varieties," *International Journal of Food Science Nutrition*, vol. 66, no. 2, pp. 166-174, 2015.

[18]. A. Miller, T. Frenzel, H.-G. Schmarr *et al.*, "Coupled liquid chromatography–gas chromatography for the rapid analysis of γ-oryzanol in rice lipids," *Journal of Chromatography A*, vol. 985, no. 1-2, pp. 40-410, 2003.

[19]. E. Waraksa, K. Kowalski, E. Kłodzińska *et al.*, "A rapid and eco-friendly method for determination of the main components of gamma oryzanol in equestrian dietary and nutritional supplements by liquid chromatography - tandem mass spectrometry," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 172, pp. 339-348, 2019.

[20]. Ministry of Health, *Vietnam Pharmacopoeia V*, Medical Publishing House, pp. 138-141, 2017.

[21]. Jr. George W. Latimer, "Guidelines for Standard Method Performance Requirements," *Official Methods of Analysis of AOAC International*, Oxford University Press, 2023.

[22]. Waters, "Empower 3 PDA: What is Purity 1 Angle, Purity 1 Threshold (How is the peak purity determined)?", Waters Knowledge Base, 2021. Online: https://support.waters.com/KB_Inf/Empower_Breeze/WKB85832_Empower_3_PDA_What_is_Purity_1_Angle_Purity_1_Threshold_How_is_the_peak_purity_determined. [Accessed: Jul 06, 2024].