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HPTLC SCANNER METHOD FOR THE SIMULTANEOUS DETERMINATION OF LYCOPENE AND *β*-CAROTENE IN GAC SEED ARIL

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Summary

This study successfully established and validated a High-Performance Thin-Layer Chromatography (HPTLC) method, HPTLC Scanner Method for the simultaneous quantification of two marker carotenoid compounds, lycopene and *β*-carotene, in the aril of Gac (*Momordica cochinchinensis* Spreng.). This method provides a rapid, cost-effective, and reliable analysis. The validation results, conducted according to international standards (ICH and AOAC), indicated that the new method possesses high reliability, with intra-day and inter-day precision both being below 5%, and the accuracy falling within the acceptable range of 96.50% to 102.12%. The content of the two marker carotenoid compounds in ten samples of Gac seed aril collected in Vietnam revealed significant variability: lycopene ranged from 0.03% to 1.01%, and *β*-carotene ranged from 0.09 to 1.43 mg/g. The quantification results obtained by the HPTLC-scanner method showed a very high correlation with the previously developed High-Performance Liquid Chromatography (HPLC-DAD) method ($R^2 > 0.95$).

Keywords: *Momordica cochinchinensis* (Lour.) Spreng.; *HPTLC scanner; Validation method; Lycopene, β-carotene.*

1. Introduction

Gac (*Momordica cochinchinensis* (Lour.) Spreng., which belongs to the Cucurbitaceae family, originates from the South and Southeast Asian regions, including Vietnam and China [1],[2]. In traditional Vietnamese medicine, Gac is widely used, particularly for the deep red natural coloring in Gac sticky rice, and traditional Gac oil is applied in the supportive treatment of visual disorders (dry eyes, night blindness) [3],[4]. The Gac seed aril, which is the dark red membrane enveloping the seed, has been identified as the component with the highest nutritional and pharmacological value in the fruit. This seed aril has an exceptionally high carotenoid content, notably lycopene and *β*-carotene, with concentrations exceeding those found in common plant sources, such as tomatoes and carrots [3],[5]. Regarding lipid composition, oil accounts for approximately 22% of the aril's dry weight, with the major fatty acids including oleic acid (32%), palmitic acid (29%), and linoleic acid (28%) [3]. Furthermore, the presence of phenolic and flavonoid compounds contributes to the strong antioxidant capacity [6]. Studies have demonstrated that extracts from Gac seed aril possess potent anticancer activities, including the ability to exhibit potential anticancer activity against breast cancer and melanoma cells through the activation of programmed cell death [2],[6]. Moreover, Gac is also recognized for other biological effects such as improving fatty liver conditions, controlling obesity, and regulating the gut microbiota [7].

Currently, Gac products (Gac oil, Gac powder, and functional foods) are widely available in the market; therefore, quality control and quantifying active ingredients has become crucial [8]. The research group prioritized the establishment and development of an HPLC-DAD method for the simultaneous quantification of lycopene and *β*-carotene in Gac seed aril [9]. However, because carotenoids are a group of easily degradable compounds, analysis must be carried out quickly. To optimize the procedure and minimize the risk of sample degradation, the research group incorporated an HPTLC scanner. The HPTLC method is applied for preliminary and rapid screening of the main components in Gac seed aril, serving as a quick check before proceeding with precise quantification via HPLC. The combination of HPTLC and HPLC provides a flexible and cost-effective solution, particularly suitable for research and quality control of Gac seed aril.

2. Experimental

2.1. Samples

A selection of ten samples of Gac seed aril was collected randomly from different regions in Vietnam. The identity of these samples was formally authenticated by the Center for Medicinal Plant Resources at the National Institute of Medicinal Materials using morphological comparison methods [10]. All plant materials were dried at 50 ºC and stored in vacuum-sealed bags shielded from light using an external black bag until needed for analysis. Moisture content was quantified via the solvent distillation method as prescribed in Appendix 12.13 of the Vietnamese Pharmacopoeia V (VP V).

2.2. Chemicals

β-Carotene standard was purchased from Chemfaces, China (CAS: 7235-40-7; Lot: CFS202202; purity 98%), and lycopene (CAS: 502-65-8, Lot: 000220023, purity 98%) from Sigma-Aldrich.

Solvents and chemicals used for analysis by HPLC (acetonitrile, methanol) from Merck. Solvents and chemicals used to extract and prepare samples for quantitative analysis were all of analytical grade (P.A.).

2.3. Instruments

The HPLC system (Shimadzu, Japan) used for the analysis consisted of a binary pump LC-20AD, a SIL-20A HT autosampler, a CTO-10AS column oven, a diode-array detector (SPD-M20A), and an Agilent Eclipse Plus C_{18} column (250 x 4.6 mm, 5 μm).

The HPTLC system (Camag, Japan) used for the analysis consisted of Linomat 5 sample applicator, automatic developing chamber 2, TLC scanner 4, TLC visualizer 3, dosage syringe 100 µL for Linomat, vision CATS software, and HPTLC *Silica gel* 60 F_{254}S 25 glass plates 20 x 20 cm Merck.

2.4. Methods

2.4.1. Preparation of standard solutions:

Stock standard solutions of the carotenoid were initially prepared as 1000 µg/mL in *n*-hexane – acetone – ethanol (2:1:1, *v/v/v*) solvent, and then diluted with *n*-hexane – acetone – ethanol (2:1:1, *v/v/v*) solvent. Carotenoids utilized in the analysis were β-carotene and lycopene. Working concentrations were 6.25, 12.50, 25.00, 50.00, and 100.00 µg/mL for lycopene, 3.91, 7.81, 15.63, 31.25, 62.50, and 125.00 µg/mL for β-carotene.

2.4.2. Preparation of sample solution:

All crude drug samples were powdered to a homogeneous size using a mill. Each Gac seed aril sample was accurately weighed (1 g) and extracted twice with 25 mL of *n*-hexane – acetone – ethanol (2:1:1, *v/v/v*) solvent in a shaker for 30 min each time. The pooled extracts were adjusted to volume with the solvent mixture *n*-hexane – acetone – ethanol (2:1:1, *v/v/v*), shaken well to obtain the extract. The extract was filteredthrough a 0.45 μm filter to obtain the sample solution for HPLC analysis. The working solutions were kept protected from light at −20°C until use [9].

2.4.3. Chromatographic conditions for HPLC analysis:

The samples were analyzed using acetonitrile (solvent A) and methanol (solvent B) as a mobile phase. The isocratic program was set at 30% B for 40 minutes at a flow rate of 1.5 mL/min. The mobile phase was filtered through 0.45 μm PTFE membrane filters and degassed using an ultrasonic bath before analysis. The injection volume of standards and sample solutions was 10 μL. The wavelength was set at 475 nm for monitoring the chromatographic profile. All measurements were done in triplicate [9].

2.4.4. Developing a quantitative method for lycopene and β-carotene in Gac seed aril using TLC-Scanner:

2.4.4.1. Chromatographic Optimization Study:

- The wavelength was set at 475 nm.
- Mobile phase investigation: various mobile phase solvent systems with different constituent components were investigated.

Chromatographic procedure: Separately spot 10 µL each of the sample solution and the standard solution. After developing the chromatogram and observing under visible light, the chromatogram of the sample solution mobile phase 4 provided compact peaks the chromatogram of the reference substance solution. The selected solvent system is one that yields spots with retention factor (R_f) values between 0.2 and 0.8, and where the spots on the TLC chromatogram are clearly separated.

- The sample loading volume: spotting volumes were investigated at different volumes: 5 μL, 10 μL, 20 μL, and 30 μL.

2.4.4.2. Method Validation:

Method validation was conducted following the guidelines established by AOAC and ICH, assessing parameters including specificity, suitability, linearity (calibration curve), repeatability, recovery, and limit of detection (LOD) [11],[12].

2.5. Calculation

The content of analyzed compounds in Gac seed aril samples was calculated as follows:

$$X(\%) = \frac{C_t \times V \times 100}{M \times 10^6 \times (100-a)} \times 100 \qquad \textbf{(1)}$$

$$X(mg/g) = X(\%) \times 10 \qquad \textbf{(2)}$$

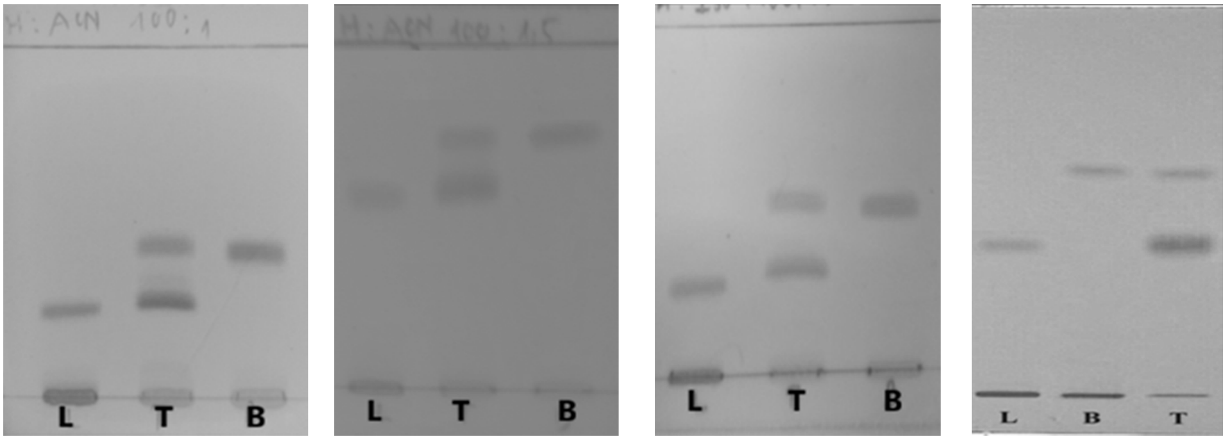
Where C_t: the concentration of analyzed compounds in the sample solution from the calibration curve equation (µg/band); V: volume of the sample solution (mL); M: weight of sample taken to prepare the sample solution (g); a: the moisture of the sample (%).

3. Results and discussion

3.1. Survey of chromatographic conditions

3.1.1. Mobile phase:

Testing various mobile phase solvents, the separation efficiency was evaluated based on the retention factor (R_f). The results obtained are shown in Fig. 1.



Mobile phase 1: *n*-hexane – acetonitrile (100:1, v/v)

Mobile phase 2: *n*-hexane – acetonitrile (100:1.5, v/v)

Mobile phase 3: *n*-hexane – isopropanol (100:1, v/v)

Mobile phase 4: *n*-hexane – acetone (100:1, v/v)

Fig. 1. TLC chromatograms of testing some different mobile phases
(L: lycopene; B: β-carotene; T: Gac seed aril sample)

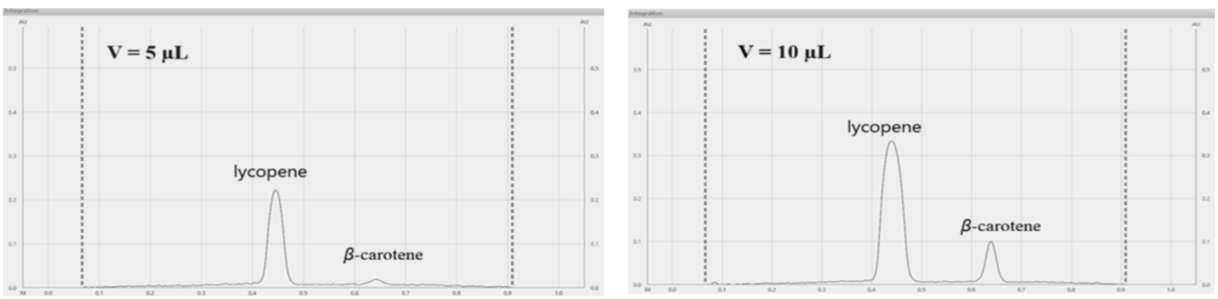
The results showed that the R_f values of lycopene and *β*-carotene in four TLC chromatograms were all within the range 0.2-0.8. However, mobile phase 4 gives compact peaks and spots in the middle of the thin plate. Therefore, mobile phase 4 was selected for TLC chromatography.

3.1.2. Sample loading volume:

Survey different sample loading volumes in the range of 5 – 30 *µ*L. The sample used for the survey was the extract of the Gac seed aril. The results obtained are shown in Table 1 and Fig. 2.

Table 1. Peak parameters of lycopene and β-carotene corresponding to different sample loading volumes

Sample loading volume (*µ*L)	**Lycopene**		***β*-Carotene**	
	Peak area	The spot width (ΔR_f)	Peak area	The spot width (ΔRf)
5	0.0026	0.083	*not detected*	*not detected*
10	0.0095	0.081	0.0010	0.065
20	0.0200	0.113	0.0028	0.082
30	0.0326	0.184	0.0055	0.090



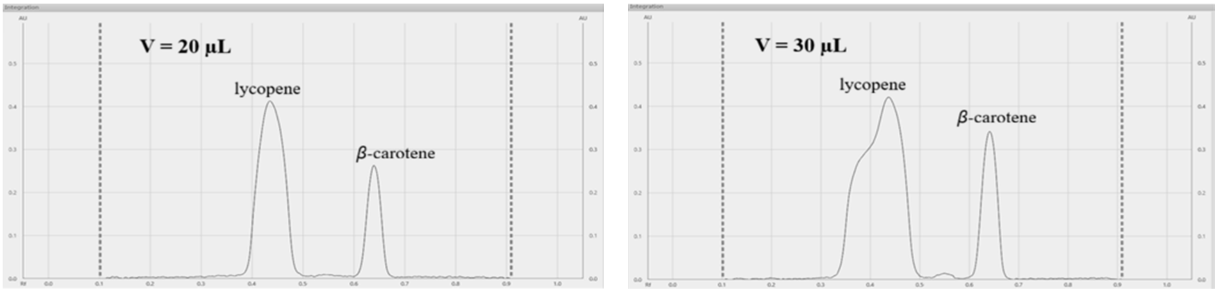


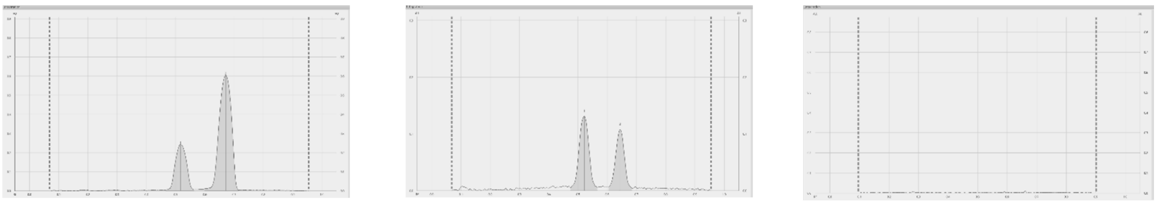
Fig. 2. Results of testing sample loading volumes

The sample injection volume is proportional to the received peak signal. The peaks are asymmetrical, and the spot width (ΔR_f) is very large, affecting the analysis process when sample loading volumes are above 20 μL. Therefore, the sample loading volume is about 10 μL, which is suitable for the method. In this study, a sample loading volume of 10 μL was selected for the next steps.

3.2. Method validation

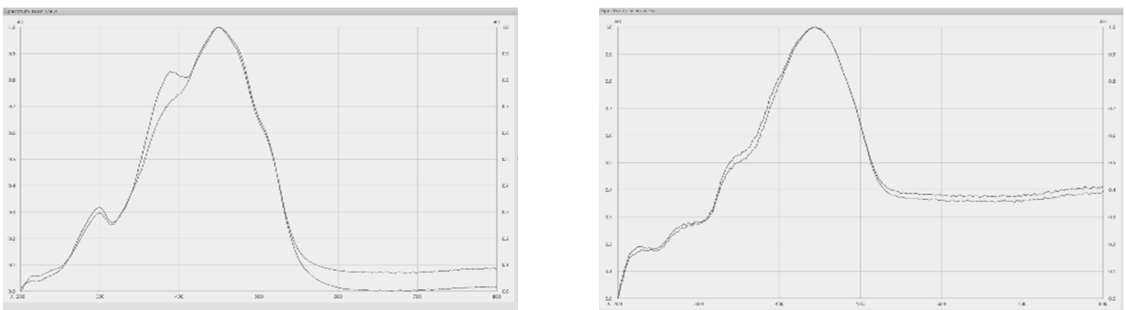
3.2.1. Specificity:

To evaluate the specificity of the method, conduct chromatography of blank sample, lycopene and β-carotene standard, and Gac seed aril sample solutions. The results are presented in Figures 3 and 4.



Mixed sample of lycopene and β-carotene standard | Gac seed aril sample solution | Blank sample

Fig. 3. TLC chromatogram to evaluate the specificity



Lycopene | *β-Carotene*

Fig. 4. UV spectra of the standard solution and test solution by HPTLC

The results obtained showed that clear and compact spots appeared in the test sample, with R_f values coinciding with the lycopene and β-carotene standard solutions. The blank sample did not show any spots with R_f values similar to the standard sample and the test sample. Furthermore, the match ratios for both lycopene and β-carotene in the sample solution compared to the standard solutions were greater than 0.99. These results collectively demonstrate that the constructed qualitative method meets the requirements for specificity.

3.2.2. System suitability:

Use a mixed sample of lycopene and β-carotene standards and repeat HPTLC analysis six times under the selected chromatographic conditions, record the R_f values and peak areas. The results obtained are presented in Table 2.

Table 2. Results of system suitability assessment

No.	Lycopene		β-Carotene	
	R_f	Peak area	R_f	Peak area
1	0.532	0.0104	0.682	0.0298
2	0.526	0.0105	0.678	0.0294
3	0.527	0.0109	0.678	0.0287
4	0.521	0.0102	0.674	0.0288
5	0.517	0.0108	0.671	0.0297
6	0.520	0.0107	0.672	0.0296
RSD (%)	**0.957**	**2.5551**	**0.569**	**1.4373**

The relative standard deviation (RSD, %) of the peak area and R_f value was less than 3%, showing that the chromatographic conditions selected and the HPTLC system used are appropriate and ensure the stability of quantitative analysis of lycopene and β-carotene.

3.2.3. Linearity:

Prepare standard solutions with different concentrations in the range 0.06 – 1.00 μg/band for lycopene and 0.04 – 1.25 μg/band for β-carotene. Carry out HPTLC chromatography of the standard solutions after dilution according to the selected conditions, record the peak area value corresponding to each standard, thereby building a standard curve representing the relationship between the concentration of each standard in the solution and the peak area value obtained on the chromatogram. The results are shown in Fig. 5 and Table 3.

Table 3. Linearity study data

Lycopene				β-Carotene			
Concentration (µg/mL)	**Applied quantity (µg/band)**	**Peak area**	**Bias (%)**	**Concentration (µg/mL)**	**Applied quantity (µg/band)**	**Peak area**	**Bias (%)**
100.00	1.00	0.0100	0.00	125.00	1.25	0.0690	0.26
50.00	0.50	0.0053	2.27	62.50	0.63	0.0357	-0.19
25.00	0.25	0.0025	-7.63	31.25	0.31	0.0189	-2.00
12.50	0.13	0.0016	7.22	15.63	0.16	0.0110	0.42
6.25	0.06	0.0009	-1.03	7.81	0.08	0.0070	4.05
				3.91	0.04	0.0050	11.30

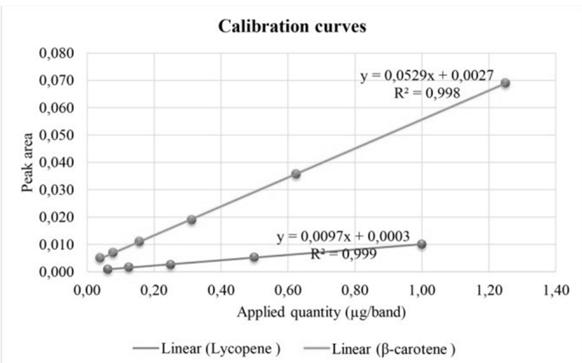


Fig. 5. Calibration curves of lycopene and β-carotene

The calibration curves had $P_{value\ (intercept)} > 0.05$, correlation coefficient $R^2 > 0.99$, indicating good linear correlation between β-carotene (lycopene) concentration and peak area value, the deviation is less than 15%, and no systematic error. Therefore, the developed method can be applied to the quantitative analysis of β-carotene and lycopene.

3.2.4 Repeatability:

The results of the intra-day and inter-day repeatability assessment were presented in Table 4.

Table 4. Results of evaluating intra-day and inter-day repeatability

No.	Mass (g)	Content lycopene (%)	Content β-carotene (%)
M1	1.0023	0.775	0.067
M2	1.0091	0.726	0.067
M3	1.0187	0.688	0.061
M4	1.0270	0.723	0.062
M5	1.0130	0.752	0.067
M6	1.0007	0.779	0.066

No.	Mass (g)	Content lycopene (%)	Content *β*-carotene (%)
Mean (n = 6)		0.741	0.065
RSD (%, n=6)		**3.982**	**3.686**
M7	1.0056	0.756	0.065
M8	1.0070	0.719	0.069
M9	1.0026	0.734	0.064
M10	1.0038	0.727	0.067
M11	1.0002	0.760	0.063
M12	1.0094	0.769	0.062
Mean (n = 12)		**0.742**	**0.065**
RSDr (%, n=12)		**4.594**	**4.931**

The obtained results showed that the RSD values (%) for intra-day and inter-day repeatability assessment were less than 4.0%, proving that the developed method had high repeatability, stability, and was suitable for analyzing and quantifying *β*-carotene and lycopene in the Gac seed aril sample.

3.2.5. Limit of detection and limit of quantification:

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated by diluting sample solutions until signal-to-noise ratios of 3 and 10, respectively. LOD and LOQ of two substances were also calculated, as shown in Table 5.

Table 5. LOD and LOQ of lycopene and *β*-carotene

		Lycopene	***β*-Carotene**
LOD	Concentration (μg/mL)	1.55	0.985
	Content (%)	0.008	0.005
LOQ	Concentration (μg/mL)	5.13	3.25
	Content (%)	0.026	0.016

The results obtained showed that LOD and LOQ values of lycopene and *β*-carotene by the HPTLC scanner method were 1.55 μg/mL and 5.13 μg/mL for lycopene, 0.985 μg/mL and 3.25 μg/mL for *β*-carotene, respectively.

3.2.6. Accuracy:

The accuracy was evaluated by spiking the previously quantified sample with known amounts of *β*-carotene standard solution and lycopene standard in solid form at three different levels (50%, 100%, and 150%) relative to the inherent concentration found in the unspiked sample. Each experiment was repeated three times. Conduct HPTLC scanner analysis of spiked and unspiked samples, determine the amount of standard added based on the calibration curve equations, and calculate the recovery efficiency compared to the actual amount of standard added.

Table 6. The results of evaluating the accuracy (n=3)

Compound	**Level**	**Recovery rate (%)**	**Mean (%)**
Lycopene	50% spiked (3.71 mg)	101.53	98.57 ± 2.14
	100% spiked (7.41 mg)	96.54	
	150% spiked (11.12 mg)	97.65	
β-Carotene	50% spiked (0.33 mg)	98.73	100.60 ± 1.41
	100% spiked (0.65 mg)	100.95	
	150% spiked (0.98 mg)	102.12	

The results in Table 5 showed that the recovery rates of the two compounds were in the range of 96.54% – 102.12%. The results indicated that the accuracy of the developed method was acceptable.

3.3. Applying the method to quantify β-carotene and lycopene in some Gac seed arils

Applying the HPTLC method to evaluate the content of *β*-carotene and lycopene in ten samples of Gac seed aril collected in Vietnam. The results obtained are presented in Table 7 and Fig. 6.

The results of evaluating the active ingredient content in ten samples of Gac seed aril showed that the lycopene content in Gac seed aril samples fluctuated greatly from 0.03 to 1.01%, and the *β*-carotene content in the test sample was in the range of 0.09 to 1.43 mg/g.

Table 7. Results of *β*-carotene and lycopene content in some samples of Gac seed aril collected in Vietnam by HPTLC scanner and HPLC-DAD methods

No.	Sample	HPTLC scanner method (n = 3)						HPLC-DAD method	
		Mass (g)	Lycopene		*β*-Carotene			Lycopene	*β*-Carotene
			Content (%)	Mean (%)	Content (%)	Content (mg/g)	Mean (mg/g)	Content (%)	Content (mg/g)
1	G1	1.0087	0.43	0.41	0.07	0.73	0.72	0.44	0.78
		1.0184	0.39		0.07	0.69		0.35	0.68
		1.0054	0.41		0.08	0.75		0.46	0.72
2	G2	1.0776	0.23	0.22	0.12	1.19	1.11	0.17	1.07
		1.0363	0.22		0.10	1.04		0.22	1.26
		1.0915	0.21		0.11	1.09		0.18	1.13
3	G10	1.0255	0.51	0.50	0.06	0.63	0.60	0.54	0.74
		1.0726	0.48		0.06	0.58		0.42	0.65
		1.0457	0.50		0.06	0.59		0.49	0.50
4	G12	1.0398	0.09	0.09	0.01	0.10	0.09	0.09	0.11
		1.0234	0.09		0.01	0.10		0.09	0.09
		1.0125	0.09		0.01	0.09		0.08	0.08
5	G13	1.0158	0.03	0.03	0.02	0.23	0.21	0.04	0.27
		1.0833	0.03		0.02	0.20		0.03	0.24
		1.0395	0.03		0.02	0.20		0.03	0.18
6	G19	1.0177	0.60	0.61	0.14	1.43	1.40	0.70	1.40
		1.0070	0.60		0.14	1.35		0.55	1.28
		1.0240	0.62		0.14	1.41		0.60	1.29
7	G29	1.0135	0.10	0.09	0.08	0.76	0.82	0.09	0.60
		1.0856	0.09		0.08	0.83		0.07	0.80
		1.0199	0.08		0.09	0.86		0.11	0.84
8	G30	1.0180	0.15	0.15	0.09	0.86	0.91	0.11	0.75
		1.0005	0.15		0.09	0.92		0.11	0.99
		1.0267	0.15		0.09	0.94		0.10	1.04
9	G33	1.0171	0.84	0.82	0.11	1.07	1.18	0.83	0.98
		1.0207	0.80		0.12	1.19		0.71	1.04
		1.0450	0.82		0.13	1.28		0.81	1.11
10	G34	1.0634	0.10	0.99	0.06	0.56	0.57	1.04	0.51
		1.0264	1.01		0.06	0.60		1.28	0.65
		1.0799	0.95		0.05	0.54		0.91	0.47

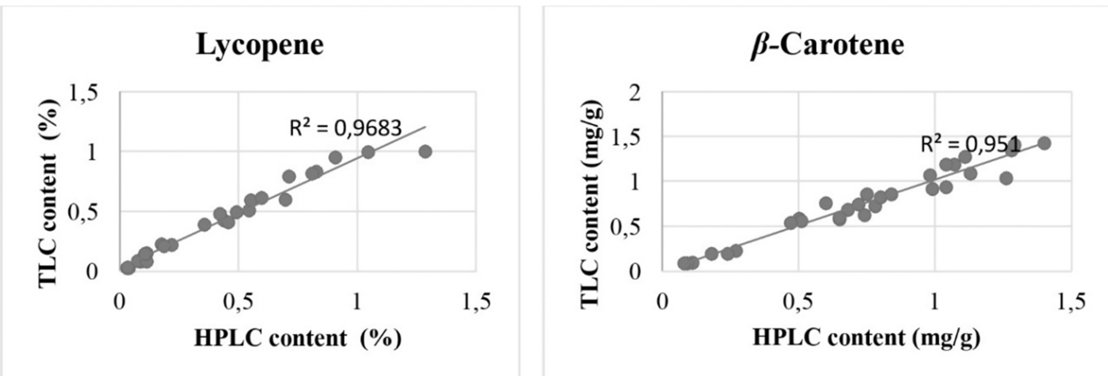


Fig. 6. Linear regression line comparing lycopene and *β*-carotene content determined by two methods

The R^2 between the HPTLC-scanner method and the reference HPLC-DAD method for lycopene and *β*-carotene were 96.83% and 95.10%, respectively. A paired $T_{\text{-test}}$ indicated no statistically significant difference between the two methods; the p-values for lycopene and *β*-carotene were above 0.05 (0.908 for lycopene and 0.260 for *β*-carotene). Furthermore, the results of the method's accuracy assessment showed high accuracy. These results showed that the HPTLC scanner method was highly accurate and can be used to semi-quantify lycopene and *β*-carotene in Gac seed aril samples.

3.4. Discussion

Gac is a rather complicated object due to the complex nature of Gac oil and the diversity/variation of ingredients. *β*-Carotene and lycopene vary depending on cultivar, cultivation, and harvesting conditions. In particular, carotenoid compounds such as β-carotene and lycopene are easily decomposed by heat, light, and oxygen, causing errors during processing and analysis. Therefore, for the analysis of β-carotene and lycopene in Gac, sample processing techniques and fast and accurate analysis methods are required for quantification to ensure accurate results. In the previous stage, the HPLC-DAD method was developed to simultaneously quantify β-carotene and lycopene in Gac seed arils to meet the above requirements. However, to contribute to expanding the application of modern methods in testing medicinal materials, research into other analytical techniques is necessary and plays an important role.

TLC is only used as a qualitative method in testing the quality of medicinal materials. However, with the development of science and technology, the HPTLC scanner system can be applied in both qualitative and quantitative analysis. For medicinal material samples, the HPTLC scanner method has the outstanding advantage of simultaneously analyzing many samples, saving costs and time, allowing quick quantification while still ensuring high specificity and reliability. The HPTLC scanner method to simultaneously quantify β-carotene and lycopene in Gac seed arils was established and validated according to ICH guidelines on the criteria of specificity, linearity, repeatability, and recovery efficiency. The results of quantitative analysis of β-carotene and lycopene in ten samples of Gac seed arils using HPTLC scanner and HPLC-DAD were compared and showed no statistically significant difference between the two data sets. The obtained results demonstrate that this method is suitable for applying in analyzing and controlling the quality of Gac seed aril medicinal herbs.

4. Conclusions

This study successfully developedand evaluated a method to simultaneously quantify lycopene and β-carotene in Gac seed arils using the HPTLC scanner system. The method uses the mobile phase solvent: *n*-hexane - acetone (100:1, v/v), a sample loading volume of 10 μL, observed at 475 nm, providing good separation of the two compounds to be analyzed. The method has good repeatability (RSD% < 5%), the limit of detection was 1.55 μg/mL (0.008%) for lycopene and 0.985 μg/mL (0.005%) for β-carotene, respectively, meeting qualitative and semi-quantitative requirements with the advantages of being fast and effective.

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References

1. Rahman M. N. A., Ismail A., Azlan A., Aziz A. A., Hassim M. D., Muhammad N. H. (2024), Physicochemical properties, proximate composition, and carotenoid content of *Momordica cochinchinensis* L. Spreng (Gac) fruit, *Pertanika Journal of Tropical Agricultural Science*, 47(2), 461-479.

2. Wimalasiri D. (2024), *Genetic diversity, nutritional and biological activity of Momordica cochinchinensis (*Cucurbitaceae*)*, Doctoral dissertation - RMIT University.

3. Chuyen H. V., Nguyen M. H., Roach P. D., Golding J. B., Parks S. E. (2015), Gac fruit (*Momordica cochinchinensis* Spreng.): a rich source of bioactive compounds and its potential health benefits, *International Journal of Food Science and Technology*, 50(3), 567-577.

4. Phan H. T., Waché Y. (2019), Behind the myth of the fruit of heaven, a critical review on Gac (*Momordica cochinchinensis* Spreng) contribution to nutrition, *Current Medicinal Chemistry*, 26(24), 4585-4605.

5. Abdulqader A., Ali F., Ismail A., Esa N. M. (2019), Antioxidant compounds and capacities of Gac (*Momordica cochinchinensis* Spreng) fruits, *Asian Pacific Journal of Tropical Biomedicine*, 9(4), 158-167.

6. Wimalasiri D., Dekiwadia C., Fong S. Y., Piva T. J., Huynh T. (2020), Anticancer activity of *Momordica cochinchinensis* (red Gac) aril and the impact of varietal diversity, *BMC Complementary Medicine and Therapies*, 20(1), 365.

7. Huang H. C., Chen C. J., Lai Y. H., Lin Y. C., Chiou W. C., Lu H. F., Chen Y. F., Chen Y. H., Huang C. (2021), *Momordica cochinchinensis* aril ameliorates diet-induced metabolic dysfunction and non-alcoholic fatty liver by modulating gut microbiota", *International Journal of Molecular Sciences*, 22(5), 2640.

8. Kha T. C., Nguyen M. H., Roach P. D., Parks S. E., Stathopoulos C. (2013), Gac fruit: nutrient and phytochemical composition, and options for processing, *Food Reviews International*, 29(1), 92-106.

9. Hien P. T., Hoa N. T. L., Thao T. T., Hang L. T. T., Thuong H. H., Khuyen P. T., Ly N. T. H. L. (2025), Developing HPLC-DAD methods for simultaneously determining content of lycopene and β-carotene in Gac seed aril using external standards and QAMS, *Journal of Medicinal Materials*, 30(3), 192-199.

10. Nguyen Nghia Thin (2007), Plant research methods, *Hanoi National University Publishing House*, 165.

11. Guideline ICH (2023), *Validation of analytical procedures: text and methodology*, *Q2 (R1)*, 1(20), 05.

12. AOAC International (2019), *Appendix K: Guidelines for Dietary Supplements and Botanicals.*