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A Greener HPTLC Approach for the Determination of β-Carotene in Traditional and Ultrasound-Based Extracts of Different Fractions of *Daucus carota* (L.), *Ipomea batatas* (L.), and Commercial Formulation

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Abstract: Various analytical approaches for determining β-carotene in vegetable crops and commercial dosage forms have been documented. However, neither the qualitative nor quantitative environmental safety and greener aspects of the literature analytical methodologies of β-carotene analysis have been assessed. As a result, the goal of this research is to develop and validate a reversed-phase "high-performance thin-layer chromatography (HPTLC)" approach for determining β-carotene in traditional (TE) and ultrasound-assisted (UBE) extracts of different fractions of *Daucus carota* (L.), *Ipomea batatas* (L.), and commercial formulation. The greener mobile phase for β-carotene analysis was a ternary mixture of ethanol, cyclohexane, and ammonia (95:2.5:2.5, $v\ v\ v^{-1}$). The detection of β-carotene was done at a wavelength of 459 nm. In the 25–1000 ng band^{-1} range, the greener reversed-phase HPTLC approach was linear. Other validation factors for β-carotene analysis, including as accuracy, precision, robustness, and sensitivity, were likewise dependable. The contents of β-carotene were found to be maximum in hexane: acetone (50:50%) fractions of TE and UBE of *D. carota* and *I. batatas* compared to their acetone and hexane fractions. The amount of β-carotene in hexane: acetone (50:50%) portions of TE of *D. carota*, *I. batatas* and commercial formulation A was estimated to be 10.32, 3.73, and 6.73 percent $w\ w^{-1}$, respectively. However, the amount of β-carotene in hexane: acetone (50:50%) portions of UBE of *D. carota*, *I. batatas* and commercial formulation A was estimated to be 11.03, 4.43, and 6.89 percent $w\ w^{-1}$, respectively. The greenness scale for the proposed HPTLC strategy was calculated as 0.81 using the "analytical GREEnness (AGREE)" method, indicating that the proposed HPTLC methodology has good greenness. The UBE approach for extracting β-carotene outperformed the TE procedure. These results indicated that the greener reversed-phase HPTLC approach can be utilized for the determination of β-carotene in different vegetable crops, plant-based phytopharmaceuticals, and commercial products. In addition, this approach is also safe and sustainable due to the utilization of a greener mobile phase compared to the toxic mobile phases utilized in literature analytical approaches of β-carotene estimation.

Keywords: analytical GREEnness (AGREE); β-carotene; greener high-performance thin-layer chromatography (HPTLC); *Daucus carota*; *Ipomea batatas*; ultrasound extraction; validation; vegetable crops

1. Introduction

Carotenes are pigments (yellow-orange, molecular formula: $C_{40}H_{56}$), abundantly found in the vegetable plants. The two principal isomers found in plants are α-carotene and β-carotene [1]. The β-carotene is the most prevalent form of carotene in plants and is an essential nutritional resource and a precursor of vitamin A in humans [1,2]. Carotenes have a wide spectrum of biological activity and animal health benefits, making them a promising substance for the pharmaceutical, food, and cosmetics sectors. Kim (2016) examined the most recent research on carotenes and their biological and pharmacological effects [3].

The root of *Daucus carota* L. (family: Apiaceae), commonly referred to as carrot, is an essential vegetable source of bioactive chemicals in human and animal diets, as well as having remarkable economic significance as the world's most plentiful food crop [4,5]. Dietary fiber, α- and β-carotene (vitamin A precursors), ascorbic acid, thiamine, riboflavin, niacin, carbohydrates, and a high potassium content are all found in the root of *D. carota* [6]. In addition to carotenoids and above-mentioned nutrients, carrots also contain anthocyanins, which enhance their nutritional value [5]. It is a very good source of antioxidant [7,8] along with anti-diabetic, anti-inflammatory, cardioprotective activities, hepatoprotective, nephroprotection, and anti-atherogenic activities [9,10].

The roots of Ipomoea batatas L. Lam. (family: Convolvulaceae), widely known as the sweet potato, have long been used as an energy source and a valuable source of sustenance for humans and animals. Sweet potato is the world's sixth most abundant food crop [11], and it continues to be of incredible economic worth. Dietary fiber, carbohydrates, vitamin A (as β-carotene), vitamin B6, vitamin C, copper, manganese, potassium, and iron are all found in the root of *I. batatas* [12]. Sweet potato also had some other carotenoids, including β-carotene-5,8,5′,8′-diepoxide, β-carotene-5,8-epoxide, and ipomoeaxanthin A, in addition to β-carotene [13]. Furthermore, it had great potential for bioenergy production [12,13]. Sweet potato antioxidant capacities have recently been studied due to the presence of phenolics, flavonoids, β-carotene, anthocyanins, and caffeoylquinic acid derivatives [14,15]. Its medical usage has been documented in several studies, particularly its antiviral and antidiabetic characteristics [16,17].

The β-carotene is the main biomarker compound of these crops and hence its qualitative and quantitative standardization is necessary. Several analytical procedures are used to determine β-carotene in a range of plant-based products for this purpose. Fewer ultraviolet (UV)-based spectrometry approaches are utilized for β-carotene estimation either alone or in combination with other phytopharmaceuticals in various vegetable crops [18,19]. Various "high-performance liquid chromatography (HPLC)" approaches are also used to determine β-carotene either alone or in combination with other phytopharmaceuticals in various vegetable crops, nutritional supplements, and commercial formulations [20–28]. Several "high-performance thin-layer chromatography (HPTLC)" approaches are also used to determine β-carotene either alone or in combination with other phytopharmaceuticals in various vegetable crops, nutritional supplements, and commercial formulations [29–34]. A gas-chromatography mass-spectrometry approach was also applied to identify various carotenoid contents including α-, and β-carotene of minimally processed carrots stored at different temperatures [35]. Some other analytical approaches such as fluorimetry, Fourier transforms-Raman spectroscopy, attenuated total reflectance-infrared, and near infra-red spectrometry approaches have also been applied in the determination of β-carotene either alone or in combination with other natural compounds in different food samples [36,37]. We noticed that the safety and greener characteristics of literature analytical approaches to β-carotene estimates were not appraised after reviewing literature analytical approaches. Greener HPTLC approaches present several merits over other liquid chromatograpy-based approaches [38–41]. As a result, in this investigation, the greener reversed-phase HPTLC technique for determining β-carotene was chosen. Various strategies for evaluating the greenness of various analytical procedures have been presented [40–45]; while only the "analytical GREEnness (AGREE)" technique applies all twelve principles/components of "green analytical chemistry (GAC)" for greenness evaluation [44]. Therefore, the "AGREE

metric approach" was utilized for the greenness assessment of the greener reversed-phase HPTLC approach [44]. The current study involves the development and validation of a rapid, sensitive, and greener reversed-phase HPTLC approach for determining β-carotene in its pure form, traditional extraction (TE) and ultrasound-based extraction (UBE) of different fractions of *D. carota* and *I. batatas*, as well as commercial formulation, based on these hypotheses. The "International Council for Harmonization (ICH)" Q2-(R1) guidelines [46] were used to validate the greener reversed-phase HPTLC approach for determining β-carotene.

2. Materials and Methods

2.1. Sampling

The fresh roots of *D. carota* (carrots) and *I. batatas* (sweets potato) were purchased in a local supermarket of Al-Kharj, Saudi Arabia, which were provided by the local cultivars of Al-kharj, Central region of Saudi Arabia. The commercial soft gelatin capsules of β-carotene i.e., formulation A were obtained from a pharmacy shop in "Al-Kharj, Saudi Arabia".

2.2. Chemicals and Reagents

The working standard of β-carotene (purity: 98.7%) was obtained from "Sigma Aldrich (St. Louis, MO, USA)". Chromatography-grade solvents such as ethanol (EtOH) and cyclohexane (CY) were procured from "E-Merck (Darmstadt, Germany)". Analytical grade solvents for the TE and UBE, including acetone, hexane, and ammonia (A) were procured from "E-Merck, Darmstadt, Germany".
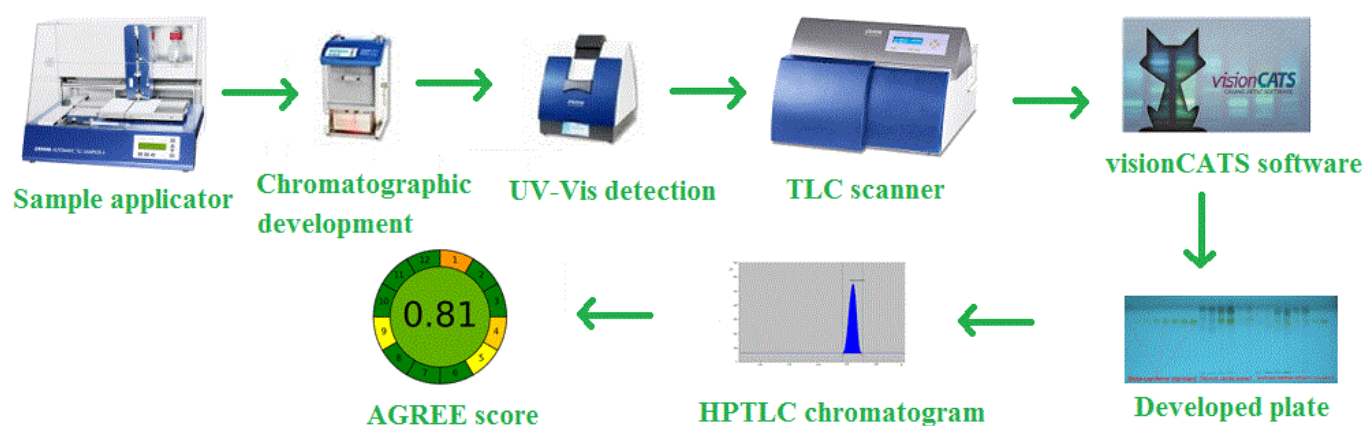
2.3. Chromatography and Instrumentation

The determination of β-carotene in its pure form, TE and UBE of various fractions of carrot, sweet potato, and marketed formulation A (soft gelatin capsule) was conducted using "HPTLC CAMAG TLC system (CAMAG, Muttenz, Switzerland)". The reversed-phase HPTLC analysis of β-carotene was performed via "10 × 20 cm glass plates pre-coated with RP silica gel 60 F254S plates (E-Merck, Darmstadt, Germany)". The samples were applied as the 6 mm bands using a "CAMAG Automatic TLC Sampler 4 (ATS4) Sample Applicator (CAMAG, Geneva, Switzerland)". The "CAMAG microliter Syringe (Hamilton, Bonaduz, Switzerland)" was connected to the sample applicator. The application rate for determining β-carotene was kept constant at 150 nL s^{-1}. Under linear ascending mode, the reversed-phase silica gel TLC plates were established in a "CAMAG automated developing chamber 2 (ADC2) (CAMAG, Muttenz, Switzerland)" with a distance of 80 mm. The ternary combination of EtOH-CY-A (95:2.5:2.5, $v\,v\,v^{-1}$) was used as the greener mobile phase. The development chamber was saturated previously with the vapors of EtOH-CY-A (95:2.5:2.5, $v\,v\,v^{-1}$) for 30 min at 22 °C. The detection of β-carotene was done at a wavelength of 459 nm. The slit dimensions (band length × width) and scanning rate were maintained constant at 4 × 0.45 mm and 20 mm s^{-1}, respectively. Each estimation was conducted in three or six replicates. The software applied was "WinCAT's (version 1.4.3.6336, CAMAG, Muttenz, Switzerland)". A schematic picture for the proposed HPTLC system with method development is presented in Figure 1.

2.4. β-Carotene Calibration Curve and Quality Control (QC) Samples

To obtain a stock solution of 100 μg mL^{-1}, the required amount of β-carotene (10 mg) was dissolved in 100 mL of EtOH-CY-A (95:2.5:2.5, $v\,v\,v^{-1}$). To acquire β-carotene concentrations in the 25–1000 ng band^{-1} range, several volumes of this stock solution were diluted further using the EtOH-CY-A (95:2.5:2.5, $v\,v\,v^{-1}$) greener mobile phase. The resulting β-carotene solutions were spotted onto reversed-phase silica gel TLC plates in various concentrations. Using the greener reversed-phase HPTLC approach, the HPTLC response for β-carotene was noted at each β-carotene concentration. Plotting the β-carotene concentrations against the recorded chromatographic response yielded the β-carotene calibration curve. In addition, three distinct QC samples were acquired separately for vali-

dation evaluation of the greener reversed-phase HPTLC approach, including low QC (LQC; 100 ng band^{-1}), middle QC (MQC; 400 ng band^{-1}), and high QC (HQC; 1000 ng band^{-1}).

Figure 1. A schematic diagram for the proposed "high-performance thin-layer chromatography (HPTLC)" instrument along with method development for the determination of β-carotene.

2.5. Sample Processing for the Determination of β-Carotene in TE of Carrots, Sweet Potato, and Commercial Formulation A

The uniform size of carrots and sweet potatoes were made using a knife and then washed with tap water several times. After 10 min of cutting, these samples were blended into small pieces using a blender, and put into a "Lyophilizer (Freezone® 2.5 model 76530, Labconco Corp., Kansas, MO, USA)", for 40 h and then stored at 20 °C. Approximately 10 g of each powder was separately extracted with 100 mL of hexane, hexane: acetone (50:50%), and acetone. After extraction, 10 mg/mL solution of each sample was separately prepared in the hexane and acetone (1:1) solvent for analysis of the presence of β-carotene utilizing the greener analytical approach.

A precisely weighed 4.0 mg of the contents from the soft gelatin capsules were dissolved in 10 mL of hexane: acetone (50:50 percent) for the measurement of β-carotene in commercial formulation A (soft gelatin capsules). Under lowered pressure, the solvent was evaporated. To reconstitute the remaining residue,.10 mL hexane: acetone (50:50 percent) was used. This solution was utilized for β-carotene estimation utilizing the greener reversed-phase HPTLC approach.

2.6. Sample Processing for the Determination of β-Carotene in UBE of Carrots, Sweet Potato, and Commercial Formulation A

The uniform size of carrots and sweet potatoes were made using A knife and then washed with tap water several times. After 10 min of cutting, these samples were blended into small pieces using a blender, and put into a "Lyophilizer (Freezone® 2.5 model 76530, Labconco Corp., Kansas, MO, USA)", for 40 h and then stored at 20 °C. The UBE was carried out utilizing ultrasonic vibrations with the help of the "Bransonic series (Model CPX5800H-E; Princeton, NJ, USA)". Approximately 10 g of each powder was separately extracted with 100 mL of hexane, hexane: acetone (50:50%), and acetone using the above apparatus. The organic solvents were evaporated separately utilizing a rotary vacuum evaporator. The residue obtained from each fraction was dissolved in 50 mL of hexane, hexane: acetone (50:50%), or acetone separately in a volumetric flask. Each UBE was ultrasonicated at 50 °C for about one hour. The obtained solutions were utilized for the determination of β-carotene in UBE of different fractions of carrots and sweet potato using the greener analytical approach.

A precisely weighed 4.0 mg of the contents from the soft gelatin capsules were dissolved in 10 mL of hexane: acetone (50:50 percent) for the measurement of β-carotene in commercial formulation A (soft gelatin capsules). Under lowered pressure, the solvent was

evaporated. To reconstitute the remaining residue, 10 mL hexane: acetone (50:50 percent) was used. This solution was ultrasonicated at 50 °C for about one hour. This solution was utilized for the determination of β-carotene, utilizing the greener reversed-phase HPTLC approach.

2.7. Validation Studies

Following the ICH-Q2 (R1) criteria, the greener reversed-phase HPTLC approach for determining β-carotene was verified for varied validation settings [46]. By graphing β-carotene concentrations against its measured chromatographic response, the linearity of β-carotene was determined. For the greener reversed-phase HPTLC approach, the β-carotene linearity was tested in the 25–1000 ng band^{-1} range. The "retardation factor (R_f), asymmetry factor (As), and theoretical plates number (N m^{-1})" were used to evaluate the system appropriateness characteristics for the greener reversed-phase HPTLC approach. The "R_f, As, and N m^{-1}" values were computed using the equations previously given [47].

The percent recovery, which was evaluated at LQC (100 ng band^{-1}), MQC (400 ng band^{-1}), and HQC (1000 ng band^{-1}) for the greener reversed-phase HPTLC approach, was used to assess the accuracy of the greener reversed-phase HPTLC approach.

The intra/interday precision of the greener reversed-phase HPTLC approach was examined. Analysis of β-carotene at LQC, MQC, and HQC on the same day was used to determine intraday fluctuation. The study of β-carotene at LQC, MQC, and HQC on three different days was used to assess interday variation [46].

The robustness of the suggested analytical approach was tested by including some minor changes in the greener mobile phase. The original EtOH-CY-A (95:2.5:2.5, $v\,v\,v^{-1}$) greener mobile phase was replaced with EtOH-CY-A (96:2:2 $v\,v\,v^{-1}$) and EtOH-CY-A (94:3:3 $v\,v\,v^{-1}$) greener mobile phases, with the necessary chromatographic adjustments reported [46].

Using the standard deviation technique of blank, the sensitivity of the greener reversed-phase HPTLC methodology was examined as "detection (LOD) and quantification (LOQ) limits". The "LOD and LOQ" for β-carotene were computed using conventional methods previously described [46,47].

By comparing the R_f values and superimposed UV absorption spectra of β-carotene in TE of different fractions of carrots and sweet potato, UBE of different fractions of carrots and sweet potato, and commercial formulation A with those of standard β-carotene, the peak purity/specificity for the greener reversed-phase HPTLC approach was assessed.

2.8. Determination of β-Carotene in TE and UBE of Carrots, Sweet Potato, and Marketed Formulation A

The chromatographic responses of the prepared TE and UBE solutions of carrots, sweet potato, and commercial formulation A were observed on reversed-phase silica gel TLC plates. The β-carotene content of all produced solutions was calculated using the β-carotene calibration curve for the greener reversed-phase HPTLC approach.

2.9. Greenness Assessment

The "AGREE metric technique" [44] was used to assess the greenness of the greener reversed-phase HPTLC technology. The "AGREE: The Analytical Greenness Calculator (version 0.5, Gdansk University of Technology, Gdansk, Poland, 2020)" was used to calculate the AGREE scale (0.0–1.0) of the greener reversed-phase HPTLC technique.

3. Results and Discussion

3.1. Method Development

In the literature, there is a scarcity of green analytical methodologies for determining β-carotene. As a result, the goal of this study was to develop and validate a rapid, sensitive, and environmentally friendly reversed-phase HPTLC approach for determining β-carotene in TE and UBE fractions of carrots, sweet potatoes, and commercial formulations.

For the β-carotene estimation utilizing the greener reversed-phase HPTLC approach, different proportions of EtOH, CY, and A, including EtOH-CY-A (50:25:25, $v\,v\,v^{-1}$), EtOH-CY-A (60:20:20, $v\,v\,v^{-1}$), EtOH-CY-A (70:15:15, $v\,v\,v^{-1}$), EtOH-CY-A (80:10:10, $v\,v\,v^{-1}$), EtOH-CY-A (85:7.5:7.5, $v\,v\,v^{-1}$), EtOH-CY-A (90:5:5, $v\,v\,v^{-1}$), and EtOH-CY-A (95:2.5:2.5, $v\,v\,v^{-1}$) were evaluated as the greener solvent mixtures for the establishment of a reliable band for the determination of β-carotene in TE and UBE of different fractions of carrots, sweet potato, and marketed formulation. Figure 2 shows a representative image of the greener mobile phase, which was created under chamber saturation conditions.
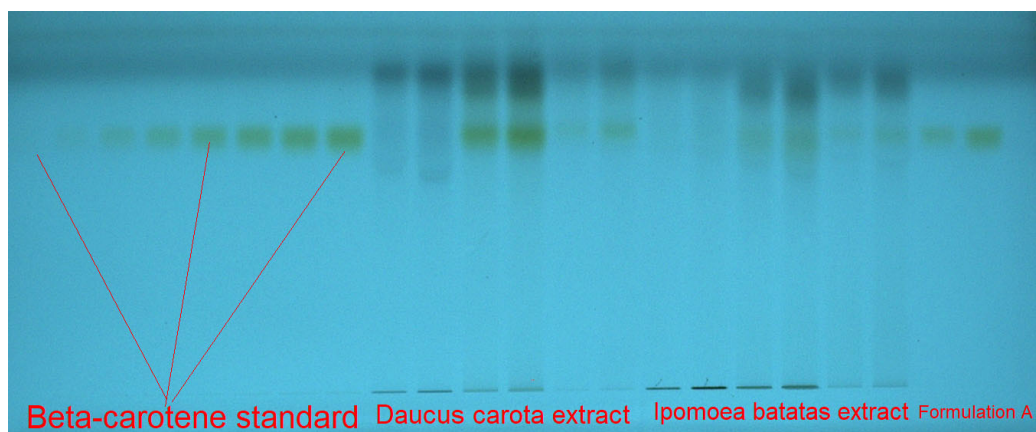
Figure 2. Developed thin-layer chromatography (TLC) plate for standard β-carotene, *Daucus carota* extract, *Ipomea batatas* extract, and commercial formulation A established using EtOH-CY-A (95:2.5:2.5, $v\,v\,v^{-1}$) as the greener mobile phase for the greener reversed-phase HPTLC approach.

The findings revealed that EtOH-CY-A (50:25:25, $v\,v\,v^{-1}$), EtOH-CY-A (60:20:20, $v\,v\,v^{-1}$), EtOH-CY-A (70:15:15, $v\,v\,v^{-1}$), EtOH-CY-A (80:10:10, $v\,v\,v^{-1}$), EtOH-CY-A (85:7.5:7.5, $v\,v\,v^{-1}$), and EtOH-CY-A (90:5:5, $v\,v\,v^{-1}$) greener solvent mixtures presented a poor chromatogram of β-carotene with a high value of As (As = 1.15–1.27). However, the EtOH-CY-A (95:2.5:2.5, $v\,v\,v^{-1}$) greener mobile phase presented a well-resolved peak of β-carotene at R_f = 0.64 ± 0.02 with a reliable value of As (As = 1.03) as indicated in Figure 3.
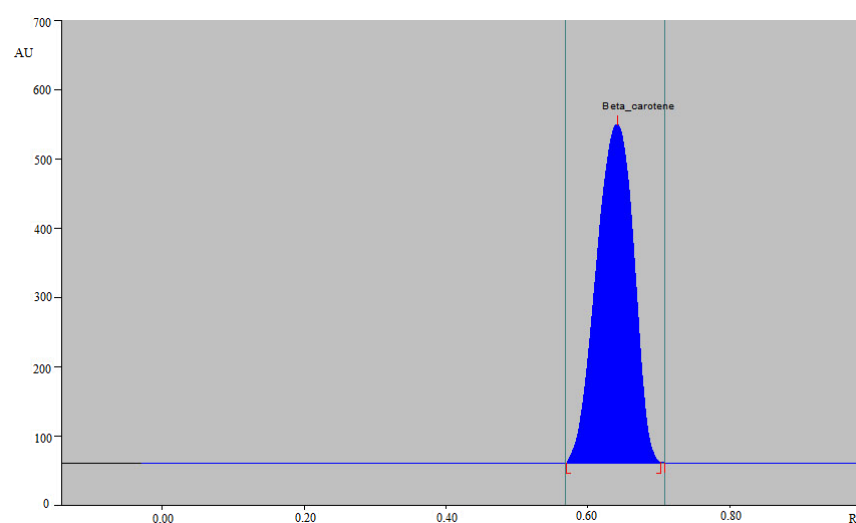
Figure 3. Representative chromatogram of pure β-carotene for the greener reversed-phase HPTLC approach.

As a result, for the determination of β-carotene in TE and UBE of various fractions of carrots, sweet potato, and commercial formulation, the EtOH-CY-A (90:2.5:2.5, $v\,v\,v^{-1}$)

greener mobile phase was chosen as the final mobile phase system. The chromatogram for the greener reversed-phase HPTLC approach was densitometrically examined, and the greatest chromatographic response for the greener reversed-phase HPTLC approach was determined at 459 nm. Hence, all the analyses of β-carotene were performed at 459 nm.

3.2. Validation Studies

The greener reversed-phase HPTLC approach for the determination of β-carotene was validated for various parameters by following the ICH-Q2-R1 recommendations [46]. The results for the regression analysis for linearity of the calibration plot of β-carotene for the proposed analytical approach are shown in Table 1. The β-carotene calibration curve was linear in the 25–1000 ng band^{-1} range for the greener analytical approach. The values of "determination coefficient (R^2)" and "regression coefficient (R)" for β-carotene were predicted as 0.9985 and 0.9992, respectively for the greener analytical approach. These observations offered good linearity between the β-carotene concentration and its chromatographic response.

Table 1. The regression analysis results for the determination of β-carotene utilizing a greener reversed-phase "high-performance thin-layer chromatography (HPTLC)" approach [a].

Parameters	Values
Linearity range (ng band^{-1})	25–1000
Regression equation	y = 47.696x + 297.73
R^2	0.9985
R	0.9992
Slope ± SD	47.696 ± 1.9400
Intercept ± SD	297.73 ± 3.4100
Standard error of slope	0.79216
Standard error of intercept	1.3924
95% confidence interval of slope	44.287–51.104
95% confidence interval of intercept	291.73–303.72
LOD ± SD (ng band^{-1})	8.84 ± 0.12
LOQ ± SD (ng band^{-1})	26.52 ± 0.36

[a] Mean ± SD; *n* = 6; LOD: limit of detection; LOQ: limit of quantification.

Table 2 shows the results of the system suitability parameters for the greener reversed-phase HPTLC approach. For the greener analytical approach, the "R_f, As, and N m^{-1}" were calculated as 0.64 ± 0.02, 1.03 ± 0.03, and 5741 ± 3.52, respectively. These findings demonstrated that the greener analytical approach was reliable in determining β-carotene in TE and UBE of various carrot fractions, sweet potato fractions, and marketed formulations.

Table 2. System suitability factors, such as retardation factor (R_f), asymmetry factor (As), and theoretical plates number (N m^{-1}) of β-carotene for a greener reversed-phase HPTLC approach [a].

Parameters	Value
R_f	0.64 ± 0.02
As	1.03 ± 0.03
N m^{-1}	5741 ± 3.52

[a] Mean ± SD; *n* = 3.

Table 3 contains the results of the accuracy estimation for the greener analytical approach. At LQC, MQC, and HQC, the percent recovery of β-carotene was calculated to be 101.23 percent, 99.41 percent, and 101.22 percent, respectively, for the greener analytical approach. The accuracy of the greener analytical approach for the determination of β-carotene in TE and UBE of different fractions of carrots, sweet potato, and marketed formulation was represented by these percent accuracy values.

Table 3. Evaluation of accuracy of β-carotene for a greener reversed-phase HPTLC approach [a].

Conc. (ng Band−1)	Conc. Found (ng Band−1) ± SD	Recovery (%)	CV (%)
100	101.23 ± 0.61	101.23	0.60
400	397.64 ± 2.17	99.41	0.54
1000	1010.24 ± 4.64	101.02	0.45

[a] Mean ± SD; $n = 6$.

The precision evaluation results for the greener analytical approach were predicted in terms of percent of the coefficient of variation (percent CV), as shown in Table 4. Using the intraday precision, the percent CVs of β-carotene for the greener analytical approach were assessed to be 0.50 percent, 0.47 percent, and 0.46 percent at LQC, MQC, and HQC, respectively. For the intermediate precision, the percent CVs of β-carotene for the greener analytical approach were 0.64 percent, 0.55 percent, and 0.47 percent at LQC, MQC, and HQC, respectively. These values of percent CV showed the precision of the greener analytical approach for the determination of β-carotene in TE and UBE of different fractions of carrots, sweet potato, and commercial formulation.

Table 4. Measurement of intra/inter-day precision of β-carotene for a greener reversed-phase HPTLC approach [a].

Conc. (ng Band−1)	Intraday Precision			Interday Precision		
	Conc. (ng Band−1) ± SD	Standard Error	CV (%)	Conc. (ng Band−1) ± SD	Standard Error	CV (%)
100	98.36 ± 0.50	0.20	0.50	98.74 ± 0.64	0.26	0.64
400	406.31 ± 1.94	0.79	0.47	396.21 ± 2.19	0.89	0.55
1000	988.23 ± 4.58	1.87	0.46	1008.54 ± 4.78	1.95	0.47

[a] Mean ± SD; $n = 6$.

Table 5 summarizes the findings of the robustness investigation for the greener reversed-phase analytical approach. For the greener analytical approach, the percent CVs for the robustness analysis were assessed to be 0.74–0.84 percent. For the greener analytical approach, the R_f values of β-carotene were anticipated to be 0.63–0.65. The durability of the greener analytical approach for the detection of β-carotene in TE and UBE of different fractions of carrots, sweet potato, and commercial formulation was demonstrated by modest fluctuations in R_f values of β-carotene and low percent CVs.

**Table 5.** Robustness analysis for β-carotene for a greener reversed-phase HPTLC approach [a].

Conc. (ng Band−1)	Mobile Phase Composition (EtOH-CY-A, $v\,v\,v^{-1}$)			Results		
	Original	Used	Level	Conc. (ng Band−1) ± SD	% CV	R_f
400	95:2.5:2.5	96:2:2	+1.0	387.42 ± 2.87	0.74	0.63
		95:2.5:2.5	0.0	398.21 ± 3.14	0.78	0.64
		94:3:3	−1.0	406.21 ± 3.45	0.84	0.65

[a] Mean ± SD; $n = 6$.

The "LOD and LOQ" values for the greener analytical approach were obtained, and these results are given in Table 1. The "LOD and LOQ" for the greener analytical approach were computed as 8.84 ± 0.12 and 26.52 ± 0.36 ng band^{-1}, respectively for the determination of β-carotene. The sensitivity of the greener analytical approach for the determination of β-carotene in TE and UBE of different fractions of carrots, sweet potato, and commercial formulation was demonstrated by these "LOD and LOQ" values for the greener analytical approach.

By comparing the superimposed UV spectra of β-carotene in different fractions of carrots, sweet potato, and commercial formulation with those of standard β-carotene, the peak purity/specificity for the greener analytical approach was evaluated. Figure 4 depicts

the superimposed UV spectra of standard β-carotene and β-carotene in various fractions of carrots, sweet potato, and commercial formulation. For the greener analytical approach, the maximum densitometric response for β-carotene in standard β-carotene and β-carotene in varied fractions of carrots, sweet potato, and commercial formulation was determined at 459 nm. The peak purity/specificity for the greener analytical approach was demonstrated by the same UV spectra, R_f values, and wavelength of β-carotene in standard β-carotene and β-carotene in varied fractions of carrots, sweet potato, and commercial formulation.
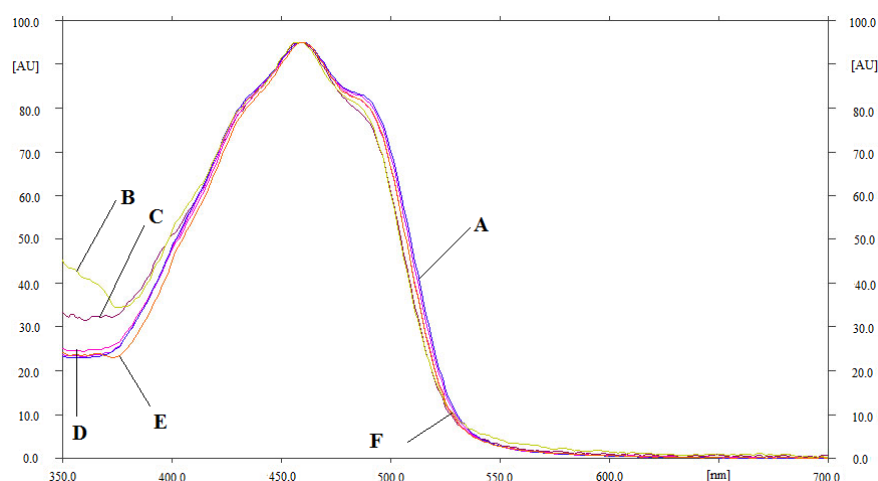
Figure 4. Superimposed UV absorption spectra of (**A**) pure β-carotene, (**B**) *D. carota* (hexane: acetone 50:50%), (**C**) *D. carota* (acetone 100%), (**D**) commercial formulation A, (**E**) *I. batatas* (hexane: acetone 50:50%), and (**F**) *I. batatas* (acetone 100%).

3.3. Determination of β-Carotene in TE and UBE of Carrots, Sweet Potato, and Marketed Formulation A

The greener reversed-phase HPTLC approach was applied in the determination of β-carotene in TE and UBE of varied fractions of carrots, sweet potato, and marketed formulation A. The chromatographic peaks of β-carotene from TE and UBE of varied fractions of carrots, sweet potato, and marketed formulation were verified by comparing their single TLC spot at $R_f = 0.64 \pm 0.02$ with those of a standard β-carotene for the greener analytical approach. The chromatographic peaks of β-carotene in all studied sample matrices were recorded at $R_f = 0.64 \pm 0.02$ without the presence of additional peaks.

The content ($\% \ w \ w^{-1}$) of β-carotene in all investigated sample matrices were determined by the calibration curve of β-carotene and results are summarized in Table 6. The content of β-carotene in TE and UBE of carrots (hexane 100%) was not detected. The content of β-carotene in TE of carrots (acetone 100%), carrots (hexane: acetone 50:50%), sweet potato (hexane 100%), sweet potato (acetone 100%), sweet potato (hexane: acetone 50:50%), and commercial formulation A was computed as 3.22 ± 0.08, 10.32 ± 0.14, 0.85 ± 0.02, $2.29 \pm 0.04\%$, $3.73 \pm 0.09\%$, and $6.73 \pm 0.134\% \ w \ w^{-1}$, respectively. However, the content of β-carotene in UBE of carrots (acetone 100%), carrots (hexane: acetone 50:50%), sweet potato (hexane 100%), sweet potato (acetone 100%), sweet potato (hexane: acetone 50:50%), and commercial formulation A was computed as 4.31 ± 0.11, 12.35 ± 0.20, 1.06 ± 0.03, $3.11 \pm 0.05\%$, $4.86 \pm 0.10\%$, and $8.52 \pm 0.16\% \ w \ w^{-1}$, respectively. The amount of β-carotene in ethanolic extracts of carrots has been reported as 30.30 µg L^{-1}, using the UV spectrometry approach [18]. The amount of β-carotene in acetone and oleyl alcohol fractions of carrots has been reported as 112.10 µg g^{-1} [24] and 41.06 µg g^{-1} [28], respectively using the HPLC approach. The amount of similar carotenoids, such as lycopene in various red-fleshed watermelon cultivars has been reported in the range of 3.91–6.30% $w \ w^{-1}$ using a spectrometry approach [48]. The amount of β-carotene recorded in different fractions of carrots and sweet potato in this study were considerably higher than those reported by UV and HPLC methods in literature [18,24,28]. However, the amount of β-carotene

recorded in different fractions of carrots and sweet potato in this study were comparable to those reported for lycopene using the spectrometry approach [48]. As a result, the studied fractions and proposed analytical methodology could be considered superior over reported UV and HPLC methods for the determination of β-carotene, and similar to the reported spectrometry approach for lycopene determination. The content of β-carotene was computed to be higher in TE and UBE of carrots (hexane: acetone 50:50%) fraction compared to other fractions of carrots and sweet potato studied. In addition, the content of β-carotene was significantly higher in UBE of all sample matrices in comparison to respective TE ($p < 0.05$). The UBE approach for extracting β-carotene in different fractions of carrots, sweet potato, and marketed formulation A is superior to the TE method of extraction based on these observations and results.

Table 6. Application of a greener reversed-phase HPTLC approach for the determination of β-carotene in TE and UBE of different fractions of *D. carota* and *I. batatas* and commercial formulation A [a].

Samples	TE	UBE
Amount of β-Carotene (% w w^{-1})		
D. carota (Hexane 100%)	0.00 ± 0.00	0.00 ± 0.00
D. carota (Acetone 100%)	3.22 ± 0.08	4.31 ± 0.11
D. carota (Hexane: acetone 50:50%)	10.32 ± 0.14	12.35 ± 0.20
I. batatas (Hexane 100%)	0.85 ± 0.02	1.06 ± 0.03
I. batatas (Acetone 100%)	2.29 ± 0.04	3.11 ± 0.05
I. batatas (Hexane: acetone 50:50%)	3.73 ± 0.09	4.86 ± 0.10
Formulation A	6.73 ± 0.13	8.52 ± 0.16

[a] Mean ± SD; *n* = 3.

Overall, these findings and results showed that the greener analytical approach can be successfully used to determine β-carotene in a variety of food and pharmaceutical samples containing β-carotene as an active medicinal ingredient.

3.4. Greenness Evaluation

For evaluating the greenness of analytical procedures, various qualitative and quantitative methodologies have been presented [40–45]. Only the AGREE quantitative technique, on the other hand, assesses GAC using all twelve components/principles [44]. As a result, the proposed analytical approach's greenness features were computed utilizing "AGREE: The Analytical Greenness Calculator (version 0.5, Gdansk University of Technology, Gdansk, Poland, 2020)". Figure 5 shows the computed AGREE scale for the greener analytical approach with respect to the twelve GAC principles. Figure 6 depicts the AGREE report sheet and AGREE scale for each particular GAC principle. The overall AGREE scale for the greener analytical technique was 0.81, indicating that the greener profile for determining β-carotene is excellent.

3.5. Comparison with Literature Analytical Approaches

For the determination of β-carotene, the greener reversed-phase HPTLC approach was compared to literature analytical approaches. The comparison results are summarized in Table 7. Three primary validation criteria of the greener reversed-phase HPTLC approach were compared to existing analytical approaches, including "linearity range, accuracy, and precision. A reported HPLC approach's linearity range and accuracy were 0.20–35 μg g^{-1} and 97.94–101.02%, respectively, which were somewhat less than the greener reversed-phase HPTLC approach (linearity range = 25–1000 ng band^{-1} and accuracy = 99.41–101.23%) [18]. Another HPLC approach's linearity range, accuracy, and precision were reported as 0.10–50 μg mL^{-1}, 97.50–102.10%, and 1.20–4.40% respectively, all of which were inferior to the greener reversed-phase HPTLC approach [23]. The reported HPTLC approach has a linearity range of 0.76–9.14 μg band^{-1}, which was significantly inferior than the greener reversed-phase HPTLC approach [31].
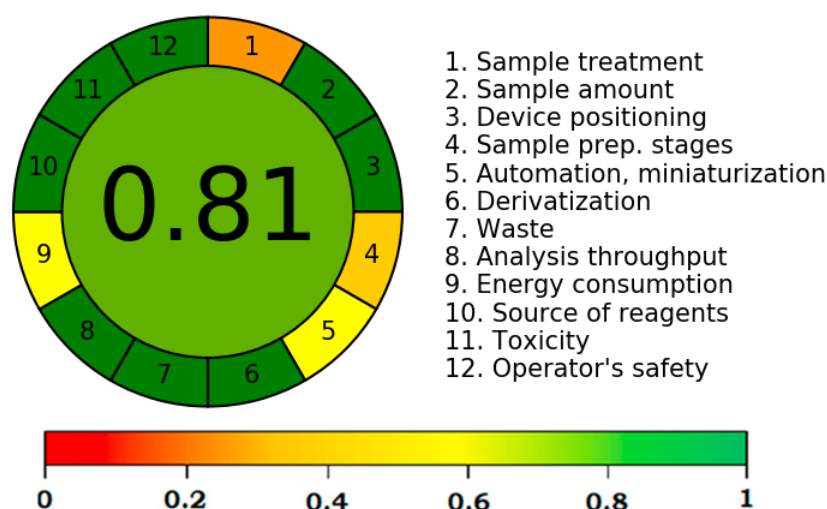
Figure 5. "Analytical GREEnness (AGREE)" scale for a greener reversed-phase HPTLC approach.

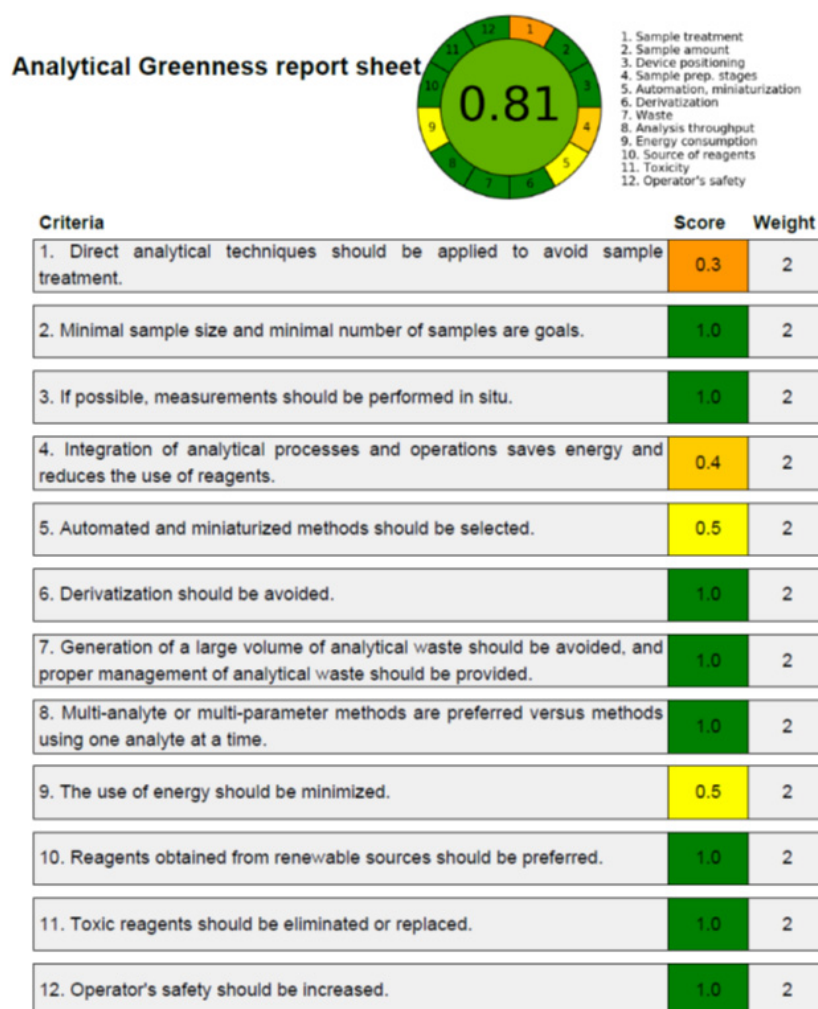| Criteria	Score	Weight
1. Direct analytical techniques should be applied to avoid sample treatment.	0.3	2
2. Minimal sample size and minimal number of samples are goals.	1.0	2
3. If possible, measurements should be performed in situ.	1.0	2
4. Integration of analytical processes and operations saves energy and reduces the use of reagents.	0.4	2
5. Automated and miniaturized methods should be selected.	0.5	2
6. Derivatization should be avoided.	1.0	2
7. Generation of a large volume of analytical waste should be avoided, and proper management of analytical waste should be provided.	1.0	2
8. Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time.	1.0	2
9. The use of energy should be minimized.	0.5	2
10. Reagents obtained from renewable sources should be preferred.	1.0	2
11. Toxic reagents should be eliminated or replaced.	1.0	2
12. Operator's safety should be increased.	1.0	2

Figure 6. AGREE scale sheet for a greener reversed-phase HPTLC approach of β-carotene, demonstrating the AGREE scale for 12 different components/principles of GAC.

Table 7. Comparative evaluation of the greener HPTLC approach with reported analytical approaches for the determination of β-carotene.

Analytical Method	Linearity Range	Accuracy (% Recovery)	Precision (% CV)	Ref.
HPLC	0.20–35 ($\mu g\ g^{-1}$)	97.94–101.02	-	[18]
HPLC	0.10–50 ($\mu g\ mL^{-1}$)	97.50–102.10	1.20–4.40	[23]
HPTLC	0.76–9.14 ($\mu g\ band^{-1}$)	99.59–101.04	0.68–0.87	[31]
HPTLC	25–1000 ($ng\ band^{-1}$)	99.41–101.23	0.46–0.64	Present work

However, the described HPTLC approach's accuracy and precision were comparable to the greener reversed-phase HPTLC approach [31]. All of these findings pointed to the superiority of the greener reversed-phase HPTLC approach for determining β-carotene over previously reported HPLC and HPTLC approaches in addition to the greener nature of the proposed analytical approach.

4. Conclusions

Due to the lack of a greener HPTLC approach for determining β-carotene in the literature, this study was conducted to develop and validate a rapid, sensitive, and greener reversed-phase HPTLC approach for determining β-carotene in TE and UBE of different fractions of carrots, sweet potato, and marketed formulation. For the determination of β-carotene, the greener analytical approach is sensitive, rapid, accurate, precise, robust, and greener. When compared to its TE, the UBE of carrots, sweet potato, and marketed formulation A had considerably more β-carotene. As a result, the UBE approach is suggested as the preferred method for extracting β-carotene from various fractions of carrots, sweet potato, and marketed formulations. The computed overall AGREE scale for the greener analytical approach suggested the excellent greener nature of the method for β-carotene estimation. The amount of β-carotene recorded in different fractions of carrots and sweet potato in this study were considerably higher than those reported by UV and HPLC methods in literature. Hence, the studied fractions and proposed analytical methodology could be considered superior over reported UV and HPLC methods for the determination of β-carotene. These findings suggest that the greener reversed-phase HPTLC approach can be used to determine β-carotene in a variety of food and pharmaceutical samples containing β-carotene as an active medicinal ingredient.

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References

1. Haskell, M.J. The challenge to reach nutritional adequacy for vitamin A: β-carotene bioavailability and conversion—Evidence in humans. *Am. J. Clin. Nutr.* **2012**, *96*, 1193S–1203S. [CrossRef] [PubMed]
2. Sommer, A.; Vyas, K.S. A global clinical view on vitamin A and carotenoids. *Am. J. Clin. Nutr.* **2012**, *96*, 1204S–1206S. [CrossRef]
3. Kim, J.K. An update on the potential health benefits of carotenes. *EXCLI J.* **2016**, *15*, 1–4. [CrossRef] [PubMed]
4. Prieto, J.M.; Chandrasekara, A.; Josheph, K.T. Roots and tuber crops as functional foods: A review on phytochemical constituents and their potential health benefits. *Int. J. Food Sci.* **2016**, *2016*, E3631647.
5. Blando, F.; Marchello, S.; Maiorano, G.; Durante, M.; Signore, A.; Laus, M.; Soccio, M.; Mita, G. Bioactive Compounds and Antioxidant Capacity in Anthocyanin-Rich Carrots: A Comparison between the Black Carrot and the Apulian Landrace "Polignano" Carrot. *Plants* **2021**, *10*, 564. [CrossRef]
6. Arscott, S.A.; Tanumihardjo, S.A. Carrots of Many Colors Provide Basic Nutrition and Bioavailable Phytochemicals Acting as a Functional Food. *Compr. Rev. Food Sci. Food Saf.* **2010**, *9*, 223–239. [CrossRef]
7. Andersen, O.M.; Jordheim, M. Basic anthocyanin chemistry and dietary Sources. In *Anthocyanins in Health and Disease*; Wallace, T.C., Giusti, M.M., Eds.; Taylor & Francis Inc.: Abingdon, UK; CRC Press: New York, NY, USA, 2013; pp. 13–90.
8. Blando, F.; Calabriso, N.; Berland, H.; Maiorano, G.; Gerardi, C.; Carluccio, M.A.; Andersen, Ø.M. Radical scavenging and biological activities of representative anthocyanin groupings from pigment-rich fruits and vegetables. *Int. J. Mol. Sci.* **2018**, *19*, 169. [CrossRef]
9. Esatbeyoglu, T.; Rodríguez-Werner, M.; Schlösser, A.; Liehr, M.; Ipharraguerre, I.; Winterhalter, P.; Rimbach, G. Fractionation of Plant Bioactives from Black Carrots (Daucus carota subspecies sativus varietas atrorubens Alef.) by Adsorptive Membrane Chromatography and Analysis of Their Potential Anti-Diabetic Activity. *J. Agric. Food Chem.* **2016**, *64*, 5901–5908. [CrossRef]
10. Bendokas, V.; Stanys, V.; Mažeikienė, I.; Trumbeckaite, S.; Baniene, R.; Liobikas, J. Anthocyanins: From the Field to the Antioxidants in the Body. *Antioxidants* **2020**, *9*, 819. [CrossRef]
11. Groppo, F.C.; Pochapski, M.T.; Fosquiera, E.C.; Esmerino, L.A.; Dos Santos, E.B.; Farago, P.V.; Santos, F.A. Phytochemical screening, antioxidant, and antimicrobial activities of the crude leaves′ extract from Ipomoea batatas (L.) Lam. *Pharmacogn. Mag.* **2011**, *7*, 165–170. [CrossRef]
12. Dini, I.; Tenore, G.C.; Dini, A. Saponins in Ipomoea batatas tubers: Isolation, characterization, quantification and antioxidant properties. *Food Chem.* **2009**, *113*, 411–419. [CrossRef]
13. Khan, M.Z.; Takemura, M.; Maoka, T.; Otani, M.; Misawa, N. Carotenoid analysis of sweetpotato Ipomoea batatas and functional identification of its lycopene β- and ε-cyclase genes. *Zeitschrift für Naturforschung C* **2016**, *71*, 313–322. [CrossRef] [PubMed]
14. Zheng, W.; Clifford, M.N. Profiling the chlorogenic acids of sweet potato (Ipomoea batatas) from China. *Food Chem.* **2008**, *106*, 147–152. [CrossRef]
15. Rumbaoa, R.G.O.; Cornago, D.F.; Geronimo, I.M. Phenolic content and antioxidant capacity of Philippine sweet potato (Ipomoea batatas) varieties. *Food Chem.* **2009**, *113*, 1133–1138. [CrossRef]
16. Miyazaki, Y.; Kusano, S.; Doi, H.; Aki, O. Effects on immune response of antidiabetic ingredients from white-skinned sweet potato (Ipomoea batatas L.). *Nutrition* **2005**, *21*, 358–362. [CrossRef] [PubMed]
17. Kwon, H.C.; Jung, C.M.; Shin, C.G.; Lee, J.K.; Choi, S.U.; Kim, S.Y.; Lee, K.R. A New Caffeoyl Quinic Acid from Aster scaber and Its Inhibitory Activity against Human Immunodeficiency Virus-1(HIV-1) Integrase. *Chem. Pharm. Bull.* **2000**, *48*, 1796–1798. [CrossRef]
18. Mustapha, Y.; Babura, S.R. Determination of carbohydrate and β-carotene content of some vegetables consumed in Kana metropolis, Nigeria. *Bay. J. Pure Appl. Sci.* **2009**, *2*, 119–121.
19. Nagata, M. A simple spectrophotometric method for the estimation of beta-carotene content in spinach acetone extracts. *Bull. Natl. Inst. Veg. Tea Sci.* **2009**, *8*, 1–5.
20. Speek, A.; Temalilwa, C.; Schrijver, J. Determination of β-carotene content and vitamin A activity of vegetables by high-performance liquid chromatography and spectrophotometry. *Food Chem.* **1986**, *19*, 65–74. [CrossRef]
21. Takahata, Y.; Noda, T.; Nagata, T. HPLC determination of β-carotene content of sweet potato cultivars and its relationship with color values. *Jpn. J. Breed.* **1993**, *43*, 421–427. [CrossRef]
22. Schuep, W.; Schierle, J. Determination of β-Carotene in Commercial Foods: Interlaboratory Study. *J. AOAC Int.* **1997**, *80*, 1057–1064. [CrossRef]
23. Szpylka, J.; DeVries, J.W.; Bhandari, S.; Bui, M.H.; Ji, D.; Konings, E.; Lewis, R.; Mass, P.; Parish, H.; Post, B.; et al. Determination of β-carotene in supplements and raw materials by reversed-phase high pressure liquid chromatography. *J. AOAC Int.* **2005**, *88*, 1279–1291. [CrossRef] [PubMed]
24. Ahamad, M.N.; Saleemullah, M.; Shah, H.U.; Khalil, I.A.; Saljoqi, A.U.R. Determination of beta carotene content in fresh vegetables using high performance liquid chromatography. *Sarhad J. Agric.* **2007**, *23*, 767–770.
25. Gupta, P.; Sreelakshmi, Y.; Sharma, R. A rapid and sensitive method for determination of carotenoids in plant tissues by high performance liquid chromatography. *Plant Methods* **2015**, *11*, 5. [CrossRef] [PubMed]
26. Varzakas, T.; Kiokias, S. HPLC Analysis and Determination of Carotenoid Pigments in Commercially Available Plant Extracts. *Curr. Res. Nutr. Food Sci. J.* **2015**, *4*, 1–14. [CrossRef]
27. Dhankhar, J.; Sharma, R.; Mann, B. Optimization of various steps for RP-HPLC determination of β-carotene in milk fat. *Int. Food Res. J.* **2017**, *24*, 1393–1398.

28. Dai, Y.; Row, K.H. Isolation and Determination of Beta-Carotene in Carrots by Magnetic Chitosan Beta-Cyclodextrin Extraction and High-Performance Liquid Chromatography (HPLC). *Anal. Lett.* **2019**, *52*, 1828–1843. [CrossRef]

29. Shi, L.; Zhu, X.L.; Liu, B.Z.; Gao, Y. Determination of beta-carotene in tobacco by HPTLC. *Chin. J. Chromatogr.* **1999**, *17*, 606–607.

30. Marsit, C.J.; Fried, B.; Sherma, J. High-performance thin-layer chromatographic analysis of lutein and beta-carotene in Cerithidia californica (Gastropoda) infected with two species of larval trematodes. *J. Parasitol.* **2000**, *86*, 635–636. [CrossRef]

31. Starek, M.; Guja, A.; Dąbrowska, M.; Krzek, J. Assay of β-Carotene in Dietary Supplements and Fruit Juices by TLC-Densitometry. *Food Anal. Methods* **2015**, *8*, 1347–1355. [CrossRef]

32. Das, S.; Gupta, P.; De, B. Thin Layer Chromatographic Characterization of Carotenoid Isolates in Sugar Date Palm (Phoenix sylvestris) Fruit Epicarp and Inflorescence Axis. *Int. J. Pharmacogn. Phytochem. Res.* **2017**, *9*, 680–684. [CrossRef]

33. Hynstova, V.; Štěrbová, D.; Klejdus, B.; Hedbavny, J.; Huska, D.; Adam, V. Separation, identification and quantification of carotenoids and chlorophylls in dietary supplements containing Chlorella vulgaris and Spirulina platensis using High Performance Thin Layer Chromatography. *J. Pharm. Biomed. Anal.* **2018**, *148*, 108–118. [CrossRef]

34. Ghosh, S.; Chatterjee, J.K.; Chalkroborty, B.; Kundu, P. Estimation of beta carotene from fruit peel waste by high performance thin layer chromatography. *J. Pharmacog. Phytochem.* **2019**, *8*, 2598–2600.

35. Condurso, C.; Cincotta, F.; Tripodi, G.; Merlino, M.; Giarratana, F.; Verzera, A. A new approach for the shelf-life definition of minimally processed carrots. *Postharvest Biol. Technol.* **2020**, *163*, 111138. [CrossRef]

36. Cioates, C.N.; van Staden, J.F. Fluorimetric determination of β-carotene in food samples using a fluorescent dye. *Anal. Lett.* **2020**, *53*, 152–163. [CrossRef]

37. Baranska, M.; Schütze, A.W.; Schulz, H. Determination of Lycopene and β-Carotene Content in Tomato Fruits and Related Products: Comparison of FT-Raman, ATR-IR, and NIR Spectroscopy. *Anal. Chem.* **2006**, *78*, 8456–8461. [CrossRef]

38. Alam, P.; Ezzeldin, E.; Iqbal, M.; Anwer, K.; Mostafa, G.A.E.; Alqarni, M.H.; Foudah, A.I.; Shakeel, F. Ecofriendly densitometric RP-HPTLC method for determination of rivaroxaban in nanoparticle formulations using green solvents. *RSC Adv.* **2020**, *10*, 2133–2140. [CrossRef]

39. Alam, P.; Iqbal, M.; Ezzeldin, E.; Khalil, N.Y.; Foudah, A.I.; Alqarni, M.H.; Shakeel, F. Simple and accurate HPTLC-densitometry method for quantification of delafloxacin (a novel fluoroquinolone antibiotic) in plasma samples: Application to pharmacokinetic study in rats. *Antibiotics* **2020**, *9*, 134. [CrossRef]

40. Ibrahim, F.A.; Elmansi, H.; Fathy, M.E. Green RP-HPLC method for simultaneous determination of moxifloxacin combinations: Investigation of the greenness for the proposed method. *Microchem. J.* **2019**, *148*, 151–161. [CrossRef]

41. Abou-Taleb, N.H.; El-Enany, N.M.; El-Sherbiny, D.T.; El-Subbagh, H.I. Digitally enhanced thin layer chromatography for simultaneous determination of norfloxacin and tinidazole with the aid of Taguchi orthogonal array and desirability function approach: Greenness assessment by analytical Eco-Scale. *J. Sep. Sci.* **2019**, *43*, 1195–1202. [CrossRef]

42. Abdelrahman, M.M.; Abdelwahab, N.S.; Hegazy, M.A.; Fares, M.Y.; El-Sayed, G.M. Determination of the abused intravenously self-administered madness drops (Tropicamide) by liquid chromatography in rat plasma; an application to pharmacokinetic study and greenness profile assessment. *Microchem. J.* **2020**, *159*, 105582. [CrossRef]

43. Duan, X.; Liu, X.; Dong, Y.; Yang, J.; Zhang, J.; He, S.; Yang, F.; Wang, Z.; Dong, Y. A Green HPLC Method for Determination of Nine Sulfonamides in Milk and Beef, and Its Greenness Assessment with Analytical Eco-Scale and Greenness Profile. *J. AOAC Int.* **2020**, *103*, 1181–1189. [CrossRef]

44. Pena-Pereira, F.; Wojnowski, W.; Tobiszewski, M. AGREE—Analytical GREEnness Metric Approach and Software. *Anal. Chem.* **2020**, *92*, 10076–10082. [CrossRef] [PubMed]

45. Nowak, P.M.; Koscielniak, P. What Color Is Your Method? Adaptation of the RGB Additive Color Model to Analytical Method Evaluation. *Anal. Chem.* **2019**, *91*, 10343–10352. [CrossRef] [PubMed]

46. Guideline, I.H.T. Validation of analytical procedures: Text and methodology. *Q2(R1)* **2005**, *1*, 5.

47. Foudah, A.I.; Shakeel, F.; Alqarni, M.H.; Alam, P. A rapid and sensitive stability-indicating green RP-HPTLC method for the quantitation of flibanserin compared to green NP-HPTLC method: Validation studies and greenness assessment. *Microchem. J.* **2021**, *164*, 105960. [CrossRef]

48. Fish, W.W.; Perkins-Veazie, P.; Collins, J.K. A Quantitative Assay for Lycopene That Utilizes Reduced Volumes of Organic Solvents. *J. Food Compos. Anal.* **2002**, *15*, 309–317. [CrossRef]