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Development of a robust HPTLC method for estimation of β-carotene in nano-formulated apricot extract

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ABSTRACT

Fruits play a crucial role in our fight against diseases and disorders. Apricots are temperate fruits classified under the Rosaceae family. Fruits are rich in carotenoids, phenolic compounds, and flavonoids which contribute to color, taste, and nutritional value. Currently, no standardized high-performance thin-layer chromatography (HPTLC) method exists for analyzing apricots. This study aimed to develop and validate a sensitive, specific, economical, reliable, and precise HPTLC method for quantifying β-carotene in apricot extract and its nano-formulation. Apricot nano-formulation was prepared, characterized, and quantified. In this method, a silica gel aluminum TLC plate was used as the stationary phase, and toluene: acetone in a ratio of 7:3v/v was used as the mobile phase. Analysis was performed at wavelength 448 nm. To ensure method reliability, validation was performed according to ICH guidelines. Prepared nano-formulation showed particle size 101.7 ± 0.12 nm, polydispersity index of 0.125, entrapment efficiency % of 78.6%, zeta potential −25mV, and transmission electron microscopy analysis revealed vesicles are smooth spherical in shape. Regression analysis demonstrated a good linear relationship (R^2 = 0.9935) within a 100–500 ng/band concentration range. The LOD and LOQ were found to be 22.05 ng/band and 66.82 ng/band, respectively. The validated HPTLC method was successfully applied for quantification of β-carotene in apricot nano-formulation. Hence, the developed HPTLC method would be an important tool in utilizing the quality control methods for herbal formulations.

INTRODUCTION

Wound healing is an intricate network of overlapping biological processes to resolve tissue injuries. A ubiquitous challenge in clinical practice is the subject of continuous and in-depth research [1]. This research aims to develop novel techniques that prevent impaired or delayed healing and to discover methods to expedite the healing process [2]. Beyond their delightful taste, apricots are a nutritional powerhouse, packed with health-promoting ingredients, solidifying their place as a treasured fruit around the world [3]. Apricot, a nutritious stone fruit botanically known as *Prunus armeniaca Linn.* belongs to the family Rosaceae. It is commonly known as Khubani, Jhardalu, the egg of the sun (ancient Persians), and the moon of the faithful (eastern countries) [4]. Apricot fruit is rich in source of vitamins, carbohydrates, and minerals [5]. It contains varying levels of phytoconstituents such as carotenoids, phenolic compounds, and flavonoids which contribute to color, taste, and nutritional value [6]. Fruit has immense pharmacological activity such as antioxidants, anti-inflammatory, antimicrobial, antiallergic, anticarcinogenic, and hepatoprotective activity [7,8]. β-carotene reigns supreme among plant carotenes. This essential nutrient acts as a precursor to vitamin A in humans, but its benefits extend far beyond. Carotenes boast a broad spectrum of biological activities and demonstrably improve animal health. These attributes position them as a promising ingredient for the food, pharmaceutical, and cosmetics sectors [9]. Among the many nutritional carotenoids, β-carotene stands out as one of the most extensively studied, well-understood carotenoids and highly rich in apricots. β-Carotene functions as a provitamin A, offering a crucial benefit for human health [10]. It acts as a biological antioxidant, shielding cells and tissues

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from the harmful effects of free radicals and singlet oxygen. Additionally, it shows potential as an anti-carcinogen. This versatile nutrient is found abundantly in various yellow-orange fruits and vegetables, including apricots, mangoes, carrots, palm fruit, tomatoes, and a multitude of other plants [11]. High-performance thin-layer chromatography (HPTLC) is now an ideal, essential, valuable tool for the analysis of Herbal drugs with respect to different aspects of their quality. It is the widely accepted standard technique in analytical methods due to its many advantages such as low operating costs, short analysis time, easy sample preparation, numerous samples can be easily compared side by side on the same plate, high accuracy, precision, and reproducibility of results, extremely critical for screening of a selection of raw material selection [12]. Several established methods exist for β-carotene analysis, including UV spectrophotometry [13], HPLC [14], and HPTLC [15] methods. In a study, Zeb and Murkovic [16] described an HPTLC method for β-carotene analysis using a ternary mobile phase petroleum ether: hexane: acetone (2:3:1 v/v/v). Hynstova *et al.* [17] developed an HPTLC method for quantifying carotenoids and chlorophylls in dietary supplements containing Chlorella vulgaris and Spirulina platensis. Their method employed a complex five-component mobile phase (petroleum ether: cyclohexane: ethyl acetate: acetone: ethanol, 60:16:10:10:6 v/v/v/v/v). However, these techniques often suffer from limitations such as complex mobile phase compositions requiring expensive solvents. To the best of our knowledge, to date, the proposed study has not been reported for the estimation of β-carotene in apricots by the HPTLC method. The objective of the current study is to develop and validate new sensitive, specific, economical, reliable, accurate, and precise HPTLC method development for the quantification of β-carotene from the apricot extract and apricot nano formulation.

MATERIALS AND METHODS

Materials

Apricots (*Prunus armeniaca)* were procured from Leh, Ladakh, India. β-Carotene was purchased from Sigma Aldrich, Mumbai, India. Pre-coated aluminum silica gel 60 F_{254} HPTLC plates were purchased from Merck KGaA, Darmstadt, Germany. Toluene (purity ≥ 99.8%) and methanol (purity ≥ 99.8 %) were obtained from Sigma-Aldrich, Bangalore, India. Acetone (purity ≥ 99.8%) and chloroform (purity≥99.8 %) were procured from Fisher Scientific, Mumbai, India. Phospholipid was received as a gift sample from Lipoid GmBH, Ludwigshafen, Germany. Carbopol 934, Methylparaben, and triethanolamine were obtained from Hi-media Pvt. Ltd, Mumbai, India. All the solvents or reagents used were of analytical/pharmaceutical grade.

Preparation of apricot nanoformulation

Apricot nanoformulation was prepared using the thin film hydration method. Apricot extract and phospholipid were dissolved in chloroform: methanol (2:1 v/v) and evaporated via a rotary evaporator, forming a thin film in a round-bottom flask. After overnight storage in a desiccator, the film was hydrated, yielding a milky suspension with multilamellar vesicles. The suspension was sonicated for 10 minutes using a probe-sonicator to reduce vesicle size, producing small unilamellar vesicles. It was then filtered through 0.45 µ and 0.25 µ filters for uniformity and stored at 4°C [18]. A gel base was prepared by hydrating 1% Carbopol 934 in water. The nano-suspension was incorporated into the gel with continuous stirring. Methylparaben and triethanolamine were used as preservatives and neutralizers, respectively. Finally, the mixture was agitated thoroughly to obtain a homogenous gel [19].

Characterization of apricot nano-formulation

Vesicle size, polydispersity index (PDI), and zeta potential

The vesicle size, polydispersity index, and zeta potential of apricot nano formulation were determined by Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK). The specimens were diluted with milli-pore water [20].

Entrapment efficiency (EE%)

The entrapment efficiency of apricot nano formulation was determined by ultracentrifugation technique which determines the quantity of extract/drug trapped inside the formulation carrier. The Eppendorf tube containing the nano-formulation was centrifuged at 4°C for 2 hours at 15,000 rpm. After centrifugation, the free drug present in the supernatant was separated and diluted. The supernatant was determined by using the U.V spectrophotometer method at 448 nm [21]. The EE % was calculated by using the following equation:

$$EE\ \% = \frac{A - B}{A} \times 100$$

where A is the total amount of extract in the nanodispersion, B is the amount of extract present in the supernatant, and A-B is the amount of extract inside the vesicles.

Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM Tecnai, G20, Philips Scientific, and the Netherlands) was employed to examine the vesicular morphology of nanoformulation. To do this, a carbon-coated grid was used to hold a dried formulation sample, which was then dyed with phosphor-tungstic acid, then examined under a microscope at a magnification of 10–100 k times at a voltage of 200 kV [22].

Evaluation of apricot nanogel

Physical appearance

The prepared apricot nanogel was visually inspected for its color, consistency, and homogeneity. Homogeneity was tested for its appearance and the presence of aggregates.

pH determination

The pH of apricot nanogel was determined by using a digital pH meter (CyberScan pH 510 Eutech instruments, Mumbai, India). 1 g of gel was distributed in 10 ml of distilled water, then the pH was checked. To ensure accuracy, using

standard buffer solutions of pH 4 and 7, the pH meter was calibrated beforehand.

Viscosity

The viscosity of apricot nanogel was determined by cone and plate Brookfield viscometer technique (CAP 2000+, Brookfield Engineering Laboratories, MA, USA) at 25°C using disc spindle no.1 with an optimum speed at 50 rpm [23].

Spreadability

The spreadability of apricot nanogel was determined by the parallel plate method. 1 g of gel was placed on the glass plate within 2 cm of its diameter, a second glass plate was placed over it with a 100 g weight load, and we allowed it to rest for 5 minutes. The increase in the diameter of the gel was recorded. The time taken to separate the slides was also measured. Less time taken for the separation of two slides relates to better spreadability [24]. Values were calculated by using the following equation:

$$S = \frac{A \times B}{C}$$

where A = weight tied to upper slide, B = length of glass slide, and C = time taken to separate the slides.

HPTLC—Instrumentation and chromatographic condition

The HP-TLC system CAMAG® (Switzerland) consisted Linomat 5 auto sprayer connected to a nitrogen cylinder to precisely apply samples using100 µl TLC-Hamilton® glass syringe. Pre-coated silica gel 60 F_{254} HP-TLC plates (10 × 10 and 20 × 10 cm, Merck KGaA, Darmstadt, Germany) were used as stationary phase. The development was conducted in a twin trough chamber. The optimized duration of chamber saturation with the mobile phase was 10 minutes. A CAMAG TLC Scanner 4 was employed for scanning and capturing the digital images for further analysis using Software Vision CATS version 3. Samples were applied 10 mm from the bottom and side edges of the pre-coated TLC plates in the form of bands, maintaining a 10 mm inter-track spacing. Each band measured 7 mm in length.

Preparation of standard and sample solution

Ten milligrams β-carotene were accurately weighed and dissolved in 10 ml of Toluene for the standard preparation. The sample extract was prepared by weighing 1 g of apricot extract and dissolving it in 10 ml of toluene. The solution was stirred and filtered using Whatman Grade 1 filter paper. For the apricot nanogel, 1 g was dissolved in 10 ml of toluene and then placed in an ultrasonic bath with a frequency of 25kHz for 10 minutes. Furthermore, it was filtered through the Whatman Grade 1 filter paper before the analysis [25].

Validation parameters

The developed method was validated as per ICH Q2 (R1) guidelines [26].

Linearity and Range

The ability of an analytical method to yield results that are proportional to the analyte concentration within the specified concentration range is known as linearity. Several concentrations of standard β-carotene were applied to get a linear dynamic range. Based on the linearity, the slope, correlation coefficient (R^2), and y-intercept were determined [27].

Precision

The intra-day precision parameter is performed at three distinct periods on the same day, whereas the inter-day precision parameter is completed over 3 days. The method demonstrated excellent precision, with a relative standard deviation (%RSD) of less than 2%. This value falls well within the acceptable range established by ICH guidelines, signifying high confidence in the method's reproducibility [28].

Sensitivity

The sensitivity of the method was determined by LOD and LOQ parameters [29]

LOD: In analytical methods, the term limit of detection refers to the lowest concentration of analyte that may be detected, but this concentration may not be precisely measurable. It was determined by the following equation:

$$LOD = \frac{3.3 \text{ x Standard Deviation (SD) of response}}{\text{calibration curve slope}}$$

LOQ: The term limit of quantification refers to the lowest amount of a sample that can be accurately and precisely measured. It can be estimated using the following equation:

$$LOQ = \frac{10 \text{ x Standard Deviation (SD) of response}}{\text{calibration curve slope}}$$

Robustness

It was determined to cheque methods reliability based on chromatographic conditions. Changing the mobile phase composition, mobile phase volume/ratio and duration of chamber saturation are the chromatographic conditions that can be chosen. The effect of these changes on the retardation factor was determined [30].

Specificity

The specificity of the proposed HPTLC method was determined by the complete separation of bands within the formulation, β-carotene band in the formulation was identified by matching its Rf value. The peak purity of the β-carotene band was confirmed by comparing its spectrum to the standard, ensuring the absence of interfering peaks from other compounds [31].

Accuracy

Samples were spiked with β-carotene standard in triplicate at three levels: 80%(v/v), 100%(v/v), and 120%(v/v)

of the standard concentration. The developed analytical method was then used to analyze β-carotene in apricot extract and its nano-formulation. Standard deviation (SD) and percent relative standard deviation (%RSD) were calculated [32].

Forced degradation studies

Forced degradation studies stress drug samples with extreme conditions such as acid, base, and light exposure (photolytic). This simulates potential breakdown pathways during storage or use. By analyzing the degraded products, scientists can assess the inherent stability of the drug's active ingredients and identify potential breakdown products that arise [33].

Quantification

The quantification of β-carotene in the apricot extract and prepared nano-formulation was carried out by employing the proposed method [34].

RESULTS AND DISCUSSION

About 70%–80% worldwide population, especially in developing countries, relies on herbal medicines as their primary healthcare system because of their higher cultural acceptability, better compatibility with the human body, and fewer side effects. To cure injuries, illnesses, and diseases, as well as to promote health and healing, people often turn to herbal remedies, which are composed of plants or their parts. Traditional herbal remedies have been used for medical purposes since prehistoric times.

Characterization of apricot nano-formulation

Vesicle size and PDI of apricot nanoformulation were found to be 101.7 ± 0.12 nm and 0.125, respectively, Figure 1. Drug absorption is heavily influenced by vesicle size. Smaller vesicles offer a greater surface area, which significantly enhances the rate and extent of drug absorption. PDI was obtained within the range (below 0.8), which indicates that particles are uniform in size and homogenously distributed.

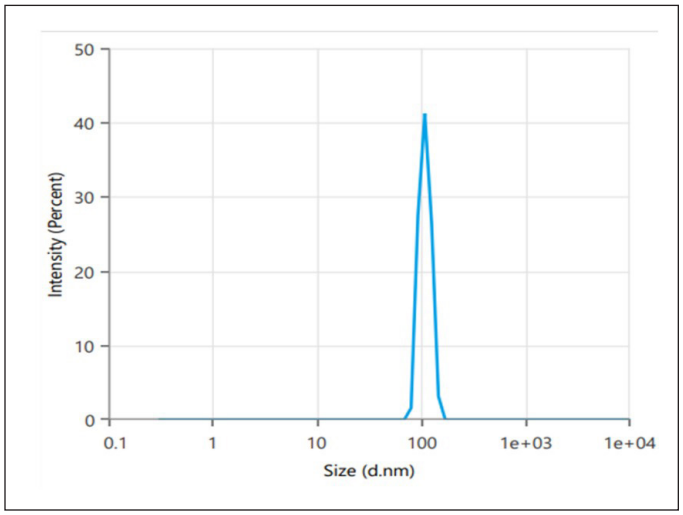

Figure 1. Vesicle size of Apricot nanoformulation.

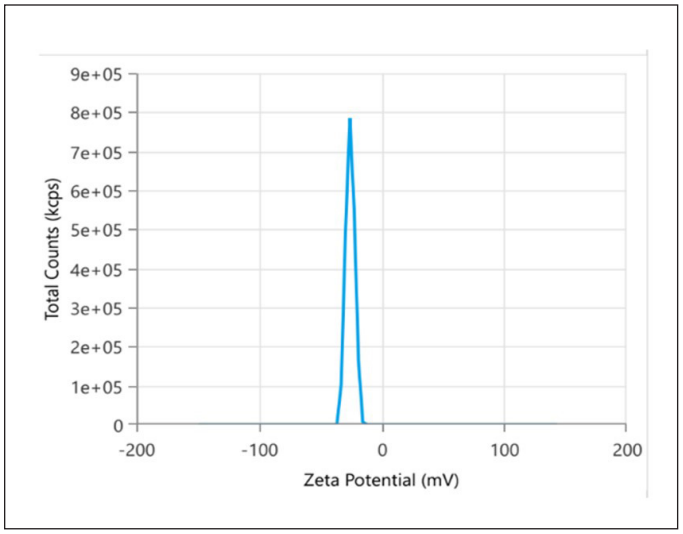

Figure 2. Zeta potential of Apricot nanoformulation.

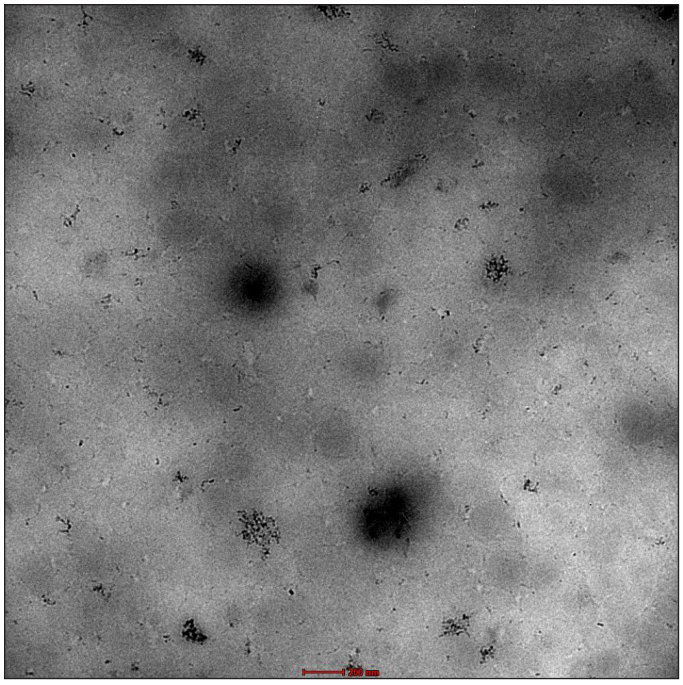

Figure 3. TEM image of apricot nanoformualtion.

Table 1. Summary of characterisation of Apricot Nano formulation.

Parameters	Results
Vesicle size	101.7 ± 0.12 nm
PDI	0.125
Zeta potential	−25mV
Entrapment efficiency	78.6%
TEM vesicle characteristics	Smooth, spherical in shape unilamellar vesicles
pH	6.2 ± 0.5
Viscosity	1524 ± 142 cPs
Spreadability	8.24 ± 18 g.cm/second

Figure 4. HPTLC plate of the developed method.

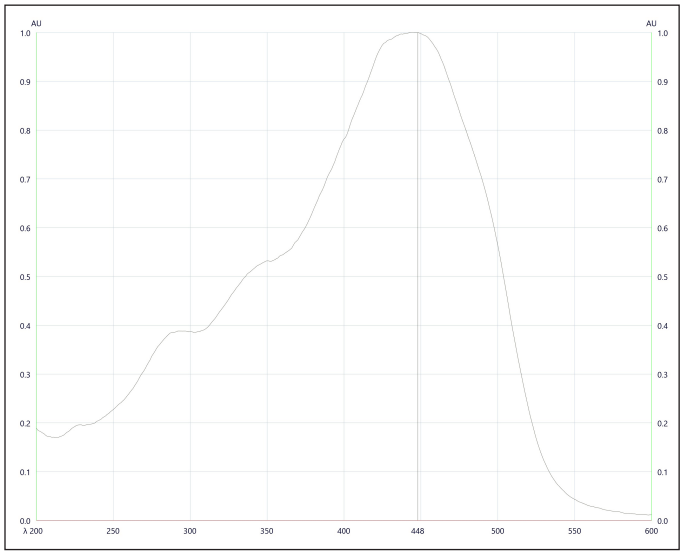

Figure 5. Spectra of β-carotene.

PDI less than 0.3 shows a homogeneous distribution of the particles. The formulation is said to be stable its zeta potential is within the range of -30 to + 30 mV. Zeta potential was found to be −25mV Figure 2. The entrapment efficiency was found to be 78.6% estimating that the EE% is critical since it determines the drug retaining capacity as well as the system's drug delivery capability. The TEM image in Figure 3 reveals that the apricot nano formulation consists of spherical particles. The vesicles are smooth, unilamellar vesicles, spherical in shape with sharp boundaries and with an aqueous internal phase. A summary of these characterizations is depicted in Table 1.

Evaluation of apricot nanogel

Apricot nanogel showed an orangish color, and good homogeneity with no lumps. The viscosity of gel was found to be 1524 ± 142 cPs as it is an important parameter to evaluate because a decrease in viscosity can impact the spreadability and stability of the formulation. The gel showed a good spreadability of 8.24 ± 18 g. cm/second and pH 6.2 ± 0.5 which is within the skin pH range. The evaluation of apricot nanogel is depicted in Table 1. It is ideally used for topical application because, they have favorable properties such as non-staining, easy to use, non-greasy, patient compliance, good adherence at the site of application, and non-toxic in nature.

Optimisation of mobile phase

The HP-TLC method was developed after multiple trials of various solvent systems. Several mobile phase compositions were evaluated based on the quality of the bands they produced and their ability to achieve consistent separation (Rf values) for the standard, extract, and formulation samples. Initial Trials with hexane: acetone 5:5 was initially tested, but it resulted in an unsatisfactory band appearance. A slightly higher ratio of hexane: acetone (6:4) was then examined, but it led to differing Rf values for the standard and extract, indicating insufficient separation. The solvent was changed, Toluene: acetone (5:5) was subsequently investigated, yielding lighter bands compared to the hexane mixtures. Further trials with varying toluene: acetone ratios (6:4, 4:6, and 3:7) continued to

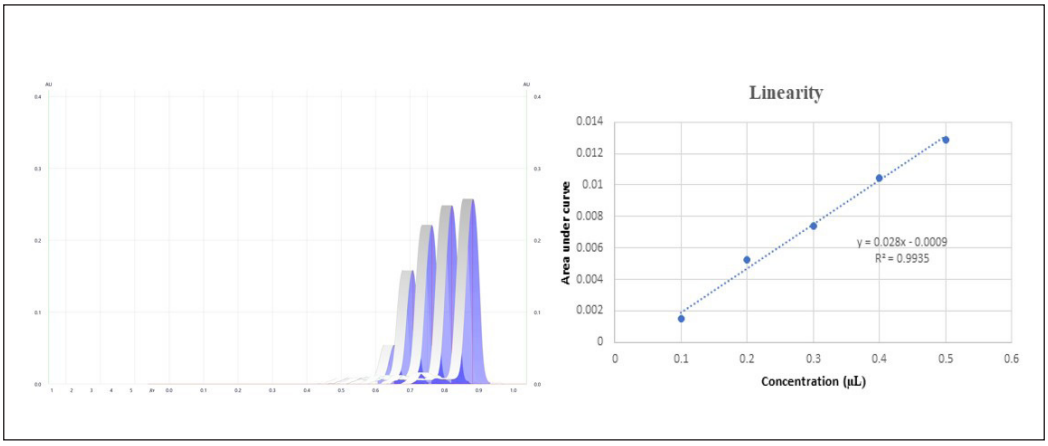

Figure 6. Linearity.

produce inconsistent band appearance and Rf values. Finally, a toluene: acetone ratio of 7:3 was assessed. This composition resulted in well-defined bands for all samples (standard, extract, and formulation) and yielded consistent Rf values. Consequently, this 7:3 toluene: acetone mixture was chosen as the optimal mobile phase for this specific TLC application also it was found to be the most suitable component/ratio of the mobile phase. This proves that the stationary phase transition went well (Fig. 4).

Method validation

The lambda max of β-carotene was found to be 448 nm, shown in Figure 5. A good linear regression was obtained over a concentration range from 100 to 500 ng/band. The linearity equation obtained was y = 0.028x – 0.0009 with a correlation coefficient R^2 of 0.9935. The mean value of slope for β-carotene was 0.0279 ± 0.1867 (Fig. 6). The precision data was subjected to %RSD calculations of the peak areas and determined for intra-day and inter-day variation which were within the acceptable criteria of less than 2% as depicted in Table 2 and Figure 7. LOD and LOQ of the developed method were calculated using a statistical formula, it were found to be 22.05 ng/band and 66.82 ng/band, respectively. The deliberate change

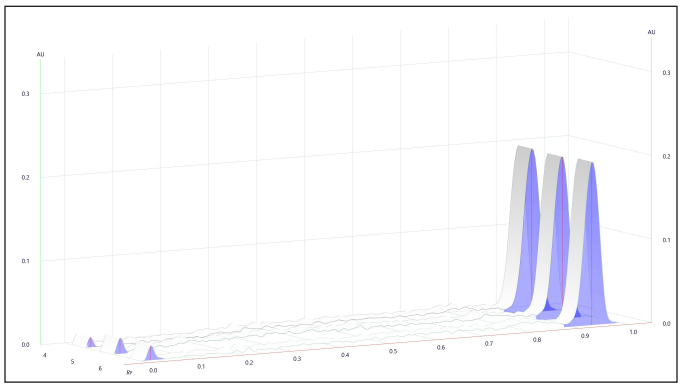

Figure 7. Precision.

Table 2. Precision.

Precision	Intra-day	Inter-day
Concentration (ng/band)	Peak area first hour	**Peak area first day**
100	0.0073,900	0.007450,000
300	0.0074,400	0.007410,000
500	0.0074,120	0.007550,000
Mean	0.007442,000	0.007446,667
%RSD	0.768629,382	0.713962,472
	Fourth hour	Second day
100	0.0074,152	0.0074,256
300	0.0073,148	0.0074,268
500	0.0074,658	0.0074,725
Mean	0.007,399	0.007,442
%RSD	1.04	0.359
	Eighth hour	Third day
100	0.0073,782	0.0073,729
300	0.0074,386	0.0072,845
500	0.0073,457	0.0073,472
Mean	0.007,388	0.007,335
%RSD	0.638	0.62

Table 3. Robustness.

Parameters	Altered conditions (levels)	Effect on Rf value
	Mobile phase composition ± 0.1 ml	
	Toluene: acetone (7:3 v/v)	
	+0.1	0.921
	0	0.927
	−0.1	0.931
%RSD		0.543349,006
	Mobile phase volume ± 1ml	
11 ml	+1	0.937
10 ml	0	0.931
9 ml	−1	0.931
%RSD		0.371286,347
	Saturation time ± 2 minutes	
12 minutes	+2	0.921
10 minutes	0	0.921
8 minutes	−2	0.931
%RSD		0.624612,624

Table 4. Accuracy/Recovery studies.

Samples	Amount added %	Amount recovered (mg) ±SD (n = 3)	% Recovery	% RSD
Sample extracted from apricot extract	80	78.12 ± 0.02	97.65	0.3,051
	100	99.4 ± 0.02	99.4	0.2,163
	120	118.5 ± 0.03	98.75	0.0,076
Sample extracted from apricot nano-formulation	80	77.2 ± 0.04	96.5	0.4,207
	100	98.6 ± 0.03	98.6	0.3,414
	120	117.3 ± 0.032	97.75	0.0,066

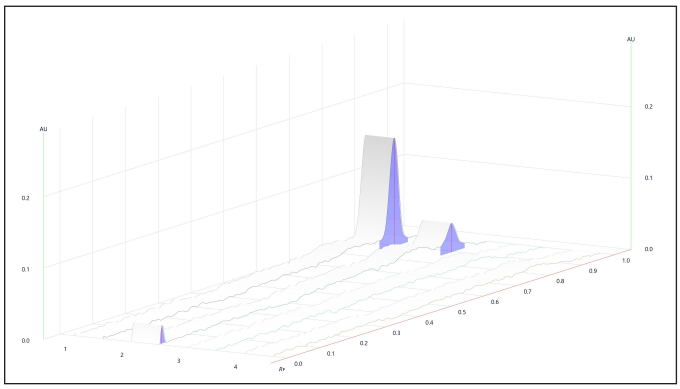

Figure 8. Specificity.

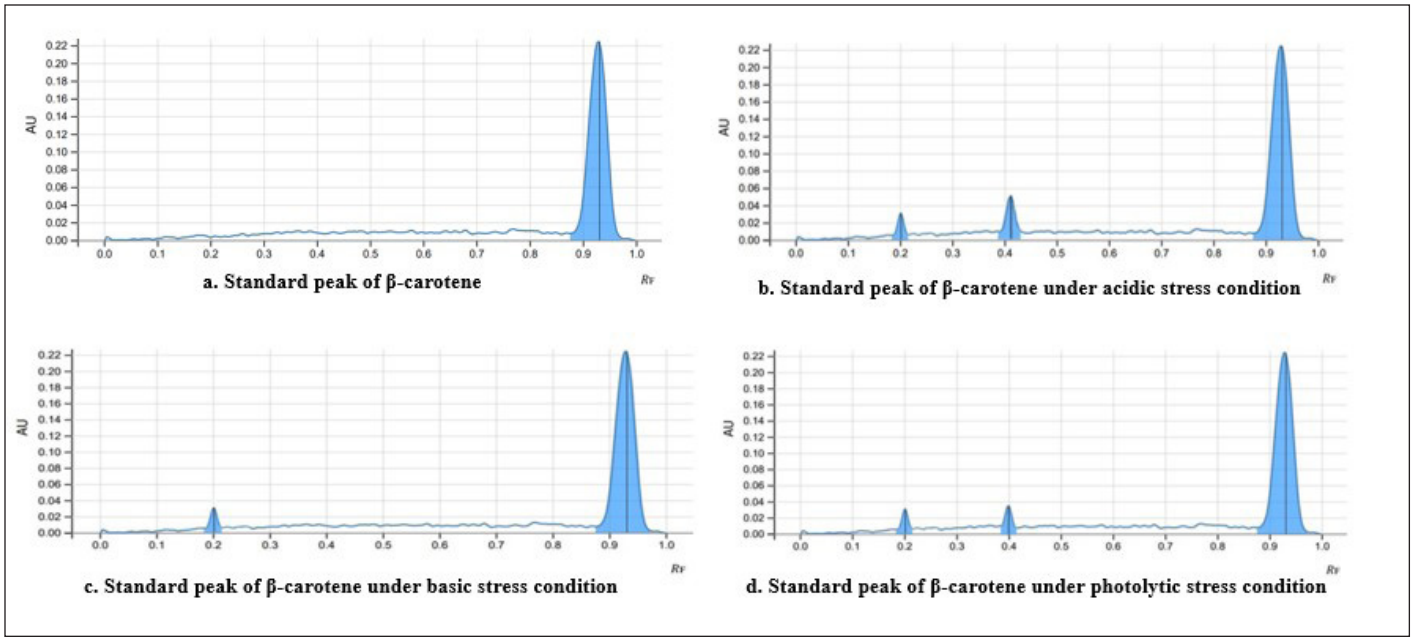

Figure 9. Force degradation studies. a Standard peak of β-carotene. b Standard peak of β-carotene under acidic stress condition. c Standard peak of β-carotene under basic stress condition. d Standard peak of β-carotene under photolytic stress condition.

Table 5. Forced degradation study.

Stress condition	Temperature °C	Time (hours)	% Degradation of β-carotene
Acidic	80	2	22
Basic	80	2	18
Photolytic	Sunlight	6	12

in composition, volume of mobile phase, and saturation chamber time did not deviate beyond the acceptance criteria and hence can be considered a robust method. The method demonstrated robustness with %RSD values below 2% for critical parameters: mobile phase composition (0.5433%), mobile phase volume (0.3712%), and saturation time (0.6246%). These results (Table 3) confirm the method's reliability and reproducibility. The separation of the sample and standards was not affected by the solvent system both showed the same Rf value at 0.9 which shows the specificity of the method (Fig. 8). To assess accuracy, samples were spiked with 80%(v/v), 100%(v/v), and 120%(v/v) of β-carotene. This technique compares the measured values to the known amounts added (true value), revealing any discrepancies between the method's results and the actual concentration. As presented in Table 4, the method exhibited high accuracy, with recovery rates ranging from 97.65% to 99.4% for apricot extract samples and 96.5% to 98.6% for apricot nano formulation samples. These results demonstrate the method's capacity to produce accurate and reliable analytical results with high confidence. This study demonstrates the method's suitability by achieving good separation between the drug peak and any degradation product peaks observed. This separation is crucial for accurate quantification of the drug and ensures the method's relevance for stability testing.

Table 6. Summary of validation parameters.

Sr. No.	Validation parameters	β-Carotene		
1	Linearity			
	Linearity range (µg/ml)	100–500 ng/band		
	Slope	0.028x		
	Intercept	0.0009		
	Correlation coefficient	0.9935		
2	Precision	100 ng/ band	300 ng/ band	500 ng/ band
	Intra-day (%RSD)			
	First hour	0.0,048	0.0,032	0.0,028
	Fourth hour	0.0,044	0.0,033	0.0,029
	Eighth hour	0.0,046	0.0,034	0.0,030
	Inter-day (%RSD)	0.0,054	0.0,038	0.0,032
	First day	0.0,052	0.0,036	0.0,030
	Second day	0.0,050	0.0,034	0.0,029
	Third day			
3	LOD	22.05 ng/band		
	LOQ	66.82 ng/band		
4	Robustness			
	Mobile phase composition (%RSD)	0.54,334		
	Mobile phase volume (%RSD)	0.37,128		
	Saturation time (%RSD)	0.62,461		
5	Recovery studies	80%	100%	120%
	Apricot extract (%RSD)	0.3051	0.2163	0.0076
	Apricot nano formulation (%RSD)	0.4207	0.3414	0.0066

Table 7. Comparison between previously published HPTLC paper.

Sr. No.	Mobile phase	Wavelength (nm)	Rf value	Limitations	Applications	Ref
1.	Toluene: Acetone (7:3 v/v)	448	0.90	-	Quantification in apricot nanoformulation	Present work
2.	Hexane: acetone (5:5v/v)	459	0.64	Comparative wavelength was more	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	35
3.	n-hexane: ethyl acetate (6:4 v/v)	254	0.27	Comparative wavelength was very less	Determination of β-carotene from Gymnosporia senegalensis (Lam.) Loes	36
4.	Chloroform: methanol: acetone: ammonium hydroxide (10:22:53:0.2 v/v/v/v)	450	0.90	Used complex solvent as mobile phase	Estimation of β-carotene in dietary supplements and fruit juices	37
5.	Ethanol: hexane (4:3 v/v)	450	0.87	The method was not applied in the quantification of β-carotene in any formulation.	Analysis of β-carotene from fruit peel.	38

As illustrated in Figures 9a–9d and Table 5, β-carotene exhibited varying degrees of degradation under different stress conditions. Acidic stress led to the most significant degradation, with a 30% loss. Base stress resulted in an 18% reduction, while photolytic stress caused a 12% decrease in β-carotene content. The standard β-carotene was quantified in the extract and the formulation which was found to be 2.8 ± 0.222 µg/ml and 0.47 ± 0.043 µg/ml, respectively. According to the ICH Q2 (R1) guidelines, the recently suggested method was validated for various parameters, including linearity, range, LOD, LOQ, precision, specificity, and robustness. This method offers several advantages compared to traditional HPLC techniques. Fast and simple (the HPTLC method provides a rapid and straightforward approach for β-carotene analysis), focused and specific (the method efficiently separates and targets β-carotene, minimizing interference from other compounds), precise and repeatable (it delivers highly accurate and reproducible results, ensuring consistency between analyses), robust and sensitive (the method is reliable under varying conditions and can detect β-carotene even at low concentrations). These combined features make the HPTLC method a valuable alternative for β-carotene quantification in apricot samples, offering a potentially more efficient and cost-effective approach compared to HPLC. The summary of validation parameters is depicted in Table 6.

Comparison with previously published HPTLC methods

A comprehensive comparison of the newly developed HPTLC method with existing methodologies was undertaken. Key parameters including mobile phase composition, Rf value, and practical applications were meticulously evaluated, which is depicted in Table 7. Notably, no single HPTLC method was previously available for β-carotene determination in apricot extract and apricot nanoformulation. The proposed method employs a toluene: acetone (7:3 v/v) mobile phase, yielding an Rf value of 0.9 for β-carotene and optimal detection at 448 nm. Adhering to ICH guidelines, the method underwent rigorous validation to ensure accuracy and reliability. The novel high-performance thin-layer chromatography (HPTLC) method effectively addresses a critical gap in current analytical techniques by providing a targeted, reproducible approach for the quantification of β-carotene in both apricot extracts and nano-formulations. This method demonstrates high sensitivity, precision, and efficiency, facilitating routine analysis in food products and biological matrices containing β-carotene. Its robustness and adaptability make it a valuable tool for applications in quality control and food science, underscoring its practical relevance and contribution to improving analytical standards for β-carotene levels in diverse samples. This enhanced analytical capability supports product development, regulatory compliance, and ongoing research into the nutritional and health benefits of β-carotene-containing foods and formulations [35–38].

CONCLUSION

We successfully developed and validated a novel HPTLC method for quantifying β-carotene in apricot extract and nano-formulation. The method was found to be quick, simple, focused, precise, specific, sensitive, repeatable, and robust and can be used for quantification of β-carotene in apricot extract and nanoformulation. This method is a good alternative to HPLC methods. It can also be utilized for the regular examination of foods and biological substances that contain β-carotene. The validation method was successfully followed according to ICH guidelines.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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