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validation for quantification of β-carotene in the stained release supplement formulation containing solid gel *in situ*

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A B S T R A C T

Despite the health benefits of β-carotene, its activity has been hampered by poor aqueous solubility and low oral bioavailability. Therefore, it is crucial to develop a new approach to overcome these problems. In this study, we developed a dry powder supplement comprising a combination approach of solid dispersion and floating gel *in situ* of β-carotene to enhance the solubility and achieve sustained release behavior. Here, we validated an HPLC method to quantify β-carotene as per the guidelines from ICH. The analytical method was validated in methanol and Fasted-State Simulated Gastric Fluid (FaSSGF) to determine β-carotene in recovery and *in vitro* release studies, respectively. A simple HPLC method using Xselect CSH™ C18 column (Waters, 3.0 × 150 mm) with the particle size of 3.5 μm was validated with 100% acetonitrile as the mobile phase. The calibration curves were found to be linear with LLOQ values < 3 ng/mL. Importantly, the method was accurate and precise without a carry over effect and successfully applied to determine the β-carotene concentration in the content analysis of the compound and *in vitro* drug release from floating gel *in situ* laden with solid dispersion formulations. The sensitivity of the method obtained here offers a wide potential use in various applications in drug delivery systems.

A R T I C L E I N F O

Keywords:
β-carotene
Solid dispersion
Floating gel *in situ*
HPLC-UV
Validation

tal biological agents that is extensively and many plants. This bioactive compd has fascinated significant consideration n pharmaceutical and food science, due th benefits [1,2]. Several investigations ional consumption of β-carotene may eases [3]. This compound is able to proging effects of radiation of UV [4]. Acting ene is able to remove excessive reactive body. This action makes the β-carotene generative illnesses, including cardiovasses and numerous kinds of cancer. In its an active pharmaceutical compound, in

enefits mentioned previously, the poor bioavailability limit its application [6].

Additionally, this compound has been found to be unstable with oxygen, light and heat [5]. Accordingly, it is necessary to find an approach that can improve the water solubility and bioavailability of this compound.

Currently, different approaches have been tried to solve the solubility issues of lipophilic bioactive compounds [7,8]. Amongst all the approaches, solid dispersion method is a promising approach for the improvement of water solubility of lipophilic compounds [9]. To prepare this approach, several hydrophilic polymers, namely polyvinylpyrrolidone (PVP), hydroxypropylmethyl cellulose (HPMC), hydroxypropyl cellulose (HPC), and cyclodextrin derivates are commonly used as vehicles [3,10]. Furthermore, to control the release of β-carotene, the selection of the final dosage form is crucial.

Gastroretentive drug delivery system (GRDDS) is a method that has been developed to overcome the bioavailability problem occurring from hydrophobic compounds. These approaches are intended to retain the active compounds in the gastrointestinal and sustain the release of the drug. This system would be beneficial to be used as a food supplement for the daily use of β-carotene. One of GRDDSs is the floating raft system

1. Introduction

β-carotene is one of the vi contained in fruits, vegetables pound is lipophilic in nature an from researchers, particularly i to its benefits for human heal have proven that the nutriti decrease the risk of several dis tect human cells from the dama as a strong antioxidant, β-carot oxygen species produced in the show the ability to prevent deg cular diseases, infectious disea application, it can be utilized a cosmetics and in foods [5].

Nevertheless, despite the b water solubility and poor oral

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(GFRS), well-known as *in situ* floating gel, which would be well preserved in the stomach because of the ability of this system to float in the acid environment [11]. Several biopolymers such as sodium alginate, gum and many others have been utilized to formulate this approach due to several benefits, including low price, accessibility, biodegradation, low toxicity and sustainability. Sodium alginate is a biodegradable polymer that swells in a normal pH aqueous environment and forms a gel in the aqueous acid environment [12,13]. Therefore, this polymer could potentially be used as a pH sensitive polymer in *in situ* floating gel formulation. Importantly, this system has been developed into dry form, which could be potentially further formulated into a dry powder for food supplements reconstituted with water prior to its consumption.

In the formulation process, an analytical method is one of the important parts. This part would allow the formulation scientists to detect and quantify the analytes of interest in the *in vitro* release study, especially for the sustained release dosage form. With respect to β-carotene analysis, generally, the analytical method was carried out using high performance liquid chromatography (HPLC) with a reversed phase C-18 columns and detection at the visible part of spectra [4,14]. Several studies have developed analytical methods to quantity β-carotene in different conditions. However, the limit of quantification values obtained were all above 50 ng/mL [4,15]. Considering that the oral dose of β-carotene is considerably low (around 14–45 mg daily) [16], accordingly, in controlled and sustained formulation development, which would release the compound in low concentration, it is extremely important to develop a new analytical method which allows us to detect β-carotene in very low concentration.

In the present work, we report a simple and sensitive HPLC method to quantify and analyze β-carotene. The analytical method was validated as per the guidelines from International Council for Harmonization (ICH). The validated method was successfully applied to determine the β-carotene. Furthermore, in this study, the β-carotene of palm oil extract was formulated into solid dispersion, which was further incorporated into *in situ* floating gel system. Finally, the validated method was again applied to quantify the content analysis of the compound and the β-carotene released in the *in vitro* release study.

2. Material and methods

2.1. Chemicals and reagents

β-carotene, HPLC grade acetonitrile, poly(vinylpyrrolidone) PVP (30 kDa) and polyethylene glycol (PEG) 6000 were obtained from Sigma-Aldrich Pte Ltd (Singapore). Xselect CSH™ C18 column with 3.0 × 150 mm and particle size of 3.5 μm was purchased from Waters (Dublin, Ireland). All other chemicals used in this study were of analytical grade.

2.2. Instrumentation and chromatographic conditions

Chromatographic analyses were performed in an HPLC (Shimadzu Prominence, Shimadzu, Kyoto, Japan) coupled with a photodiode array detector. The separation of the analyte of interest was achieved using Xselect CSH™ C18 column (Waters, 3.0 × 150 mm) particle size of 3.5 μm at 35 °C. Acetonitrile 100% was used as a mobile phase which was degassed for 15 min prior to use in the bath sonicator. The analysis run time was 15 min, and β-carotene was detected at 452 nm. The flow rate and the injection volume were 0.5 mL/min and 50 μL, respectively. Peaks of β-carotene in the extract, formulation and drug release medium were recognized by observing the retention time in each sample and were matched to the retention time of β-carotene authentic standards.

2.3. Preparation of stock solutions, calibration standards and quality control samples

The preparation of β-carotene stock solutions was carried out by dissolving 5 mg of standard β-carotene in 100 mL of methanol. The stock solution was further diluted in suitable solvents to achieve calibration standard solutions with concentrations between 1 ng/mL and 1000 ng/mL. The standard solutions were prepared in two solvents, namely methanol and Fasted-State Simulated Gastric Fluid (FaSSGF) neutralized with NaOH 1 M pH 7 (solvent B). Briefly, FaSSGF was made by solubilizing 1.999 g of NaCl in 1 L purified water, altered to pH 1.6 with HCl 1 M [17]. Additionally, three quality control solutions were prepared by diluting the stock solution solutions (β-carotene in methanol and FaSSGF to achieve low quality control (3 ng/mL), medium quality control (350 ng/mL) and high-quality control (750 ng/mL). All calibration standards and quality control solutions were made in three replications.

2.4. Method validation

The HPLC method developed previously was validated according to International Conference on Harmonization (ICH) guidelines [18,19]. Several parameters, namely selectivity, linearity, limit of detection (LOD), lower limits of quantification (LLOQ), carry-over, dilution integrity, precision and accuracy, were all evaluated.

2.4.1. Selectivity

The selectivity test was carried out by analyzing the standard solutions of β-carotene in methanol and FaSSGF [20]. The chromatogram of the standard solutions was compared to the blank methanol and FaSSGF, as well as the blank formulation of solid dispersion and floating gel *in situ* prepared in methanol.

2.4.2. Linearity, LOD and LLOQ

The linearity of the analytical method was assessed by constructing the calibration curve consisting of the analytes concentration (x axis) and the peak area (y axis). The calibration was considered to be linear if the correlation coefficient (r^2) were above 0.998 [21]. Furthermore, the LOD and LLOQ values were calculated using the following Equations:

$$LOD = 3.3\sigma/S \quad (1)$$

$$LLOQ = 10\sigma/S \quad (2)$$

Where σ = the SD of the response of the data and S = the slope of the calibration curve.

2.4.3. Carry-over and dilution integrity

To investigate the presence of carry-over during the analysis, two blank samples were injected directly following the injecting of HQC solutions. The carry over was considered when the area of the blank samples was more than 20% of the area of the LLOQ solutions [22].

To evaluate the dilution integrity, β-carotene solutions were prepared at 2500 ng/mL in methanol and FaSSGF. Furthermore, the solutions were diluted 5 and 10 times with appropriate solvents and the area of analytes was observed.

2.4.4. Accuracy and precision

Accuracy and precision were evaluated for the intra-day and the inter-day. The intra-day evaluation was carried out on the same day, while the inter-day evaluation was performed on three consecutive days. Following this evaluation, the relative standard deviation (RSD, %) and relative errors (RE, %) were calculated to represent the values of accuracy and precision, respectively. In these evaluations, samples at LLOQ, LQC, MQC and HQC were used.

2.5. Formulation of solid dispersion

Solid dispersions of β-carotene were prepared using a solvent evaporation method. Briefly, 500 mg β-carotene, PEG (500 mg) and PVP (500 mg) were accurately weighed and dissolved in 25 mL chloroform. The solution was then evaporated at 50 °C to obtain solid dispersion

powder. The process was carried out in the dark condition to protect against the degradation of β-carotene. Subsequently, the solid dispersion was crushed in a mortar and pestle before passing through a 45 μm sieve. The obtained powder was packed in an airtight container, stored in a desiccator prior to further investigations [23].

2.6. Formulation of solid dispersion loaded dry floating gel in-situ

The dry floating gel *in situ* formulations were prepared by mixing solid dispersion (equal to 100 mg of β-carotene), sodium alginate (2 g), HPMC K100 (2 g), sodium bicarbonate (1.5 g), calcium carbonate (1.5 g) in the mortar. The mixture was stored in airtight container before evaluation. As a control, floating gel *in situ* containing pure β-carotene and solid dispersion containing pure β-carotene were also prepared.

2.7. Application of the method

2.7.1. Determination of β-carotene recovery in solid dispersion and floating gel in situ formulation

The recoveries of β-carotene in solid dispersion and floating gel *in situ* formulations were determined by dissolving each formulation which was equal to 10 mg of β-carotene in 100 mL methanol in a bath sonicator for 1 h. All samples were centrifuged at 12,000 rpm for 15 min. The supernatant was collected and diluted appropriately prior to analysis using the validated HPLC method.

2.7.2. Determination of β-carotene saturation solubility in solid dispersion formulation

Four different media were used to determine the saturation solubility of β-carotene following the formulation of solid dispersion, namely purified water, FaSSGF, phosphate buffer saline (PBS) solution pH 6.8, and PBS solution pH 7.4. In this study, an excessive amount of solid dispersion was added into each medium. The mixture was stirred at 100 rpm 37 °C for 24 h. Finally, the mixture was centrifuged at 12,000 rpm for 15 min. The supernatant was collected and diluted appropriately prior to analysis using the validated HPLC method.

2.7.3. Determination of β-carotene in the in vitro release studies

The dry floating gel *in situ* formulation was reconstituted in water prior to the *in vitro* release study. The release profile of β-carotene from solid dispersion and floating gel *in situ* formulations were evaluated using USP Dissolution Apparatus II at 37 °C at 50 rpm. An aliquot of each formulation which was equal to 15 mg β-carotene was placed in a dissolution vessel filled containing 900 mL of FaSSGF. Samples (5 mL) were taken at a predetermined time (15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h and 24 h) from dissolution media and were replaced with 5 mL fresh media. The concentration of β-carotene was determined using the validated HPLC method. The kinetics of dissolution profile were assessed using various mathematic models, as follows:

$$Zero\ \ Order\ \ Kinetics: \quad C_t = C_0 + k_0 t \tag{3}$$

$$First\ \ Order\ \ Kinetics: \quad \ln C_t = \ln C_0 + k_1 t \tag{4}$$

$$Higuchi\ \ Model: \quad C_t = k_H \sqrt{t} \tag{5}$$

$$Korsmeyer - Peppas\ \ Model: \quad C_t = k_{KP} t^n \tag{6}$$

$$Hixson - Crowell\ \ Model: \quad C_t^{1/3} = C_0^{1/3} k_{HC} t \tag{7}$$

Where C_t is the concentration of β-carotene released at time t, C_0 is the initial amount of β-carotene in FaSSGF (at t = 0), k_0 is the constant of zero-order kinetics, k_1 is the constant of first-order kinetics, k_H is the constant of Higuchi model, k_{KP} is the constant of Korsmeyer-Peppas model, and k_{HC} is the constant of Hixson-Crowell model. All values were calculated using DD-solver software.

2.8. Statistical analysis

The mean, SD RSD and RE were calculated using Microsoft® Excel® 2016 (Microsoft Corporation, Redmond, USA). Furthermore, all data were displayed as mean ± SD. GraphPad Prism® version 6 (GraphPad Software, San Diego, California, USA) was utilized to statistically analyze all the data. p values < 0.05 were considered as significant differences.

3Results and discussion

3.1. Selectivity of HPLC method

The primary aim of this study was to develop and validate the analytical method of β-carotene in sustained release formulation. RP-HPLC was preferred for the analysis of the substance. This method is an analytical method which principally separates chemical substances depending on their polarity. In addition, this method is also able to identify substances rapidly and accurately and can be used in the purification of compounds.

Taking into consideration regarding the forthcoming utilizations of the methodologies developed, some other aspects that may affect separation were assessed in a selectivity evaluation, as illustrated in Fig. 1. In this step, blank methanol, blank FaSSGF, blank solid dispersion and blank floating gel *in situ* formulations were injected and analyzed using a similar method of β-carotene analysis. Specifically, two solvents were used in this study, namely methanol and FaSSGF neutralized with NaOH (1 M to pH 7). Methanol was used in the content analysis in solid dispersion and floating gel *in situ* formulations. Furthermore, FaSSGF was used to analyze β-carotene in the *in vitro* release studies. It was observed that the retention time of β-carotene was 9.74 min. The results showed that no interfering peaks were found in the chromatograms of β-carotene with blank methanol, blank FaSSGF, blank solid dispersion and blank floating gel *in situ* formulations. This showed that the method presented selectivity, with an excellent resolution of the peaks and the analytes of interest were not influenced by the appearance of expected endogenous or impurities compounds in *in vitro* studies.

3.2. Linearity, LOD, and LLOQ

In this step, the calibration curves in methanol and FaSSGF neutralized with NaOH 1 M to pH 7 were constructed. It was obtained that r^2 values of the calibration curves in methanol and FaSSGF were 0.999 and 0.997, respectively. Therefore, it could be considered that the curves were linear. Furthermore, the LOD values were 0.7 ng/mL for solvent A and 0.9 ng/mL for solvent B. The LLOQ values for methanol and FaSSGF were 2.5 ng/mL and 2.7 ng/mL. Importantly, LLOQ values in our study were considerably lower compared to those values reported in the previous study [4,15]. Accordingly, our method was found to be more sensitive to the current methods available. This could be beneficial for the quantification of sustained release behavior of small-dose compounds, like β-carotene.

3.3. Carry-over and dilution integrity

In the analytical process, especially when using HPLC, the carry-over effect should be avoided in order to ensure that the quantification of high-concentration samples did not affect the response of other samples. In this investigation, after the injection of β-carotene in HQC concentrations, there was no peak observed in the blank solvents. Thus, the method developed did not show any carry-over effect.

Additionally, it is important to bear in mind that the concentration of β-carotene released from sustained release formulation in the dissolution media could not be forecasted. Therefore, the dilution integrity was critical to be performed. After the dilution of a high concentration of β-carotene in both solvents with the dilution factors of 5 and 10, the

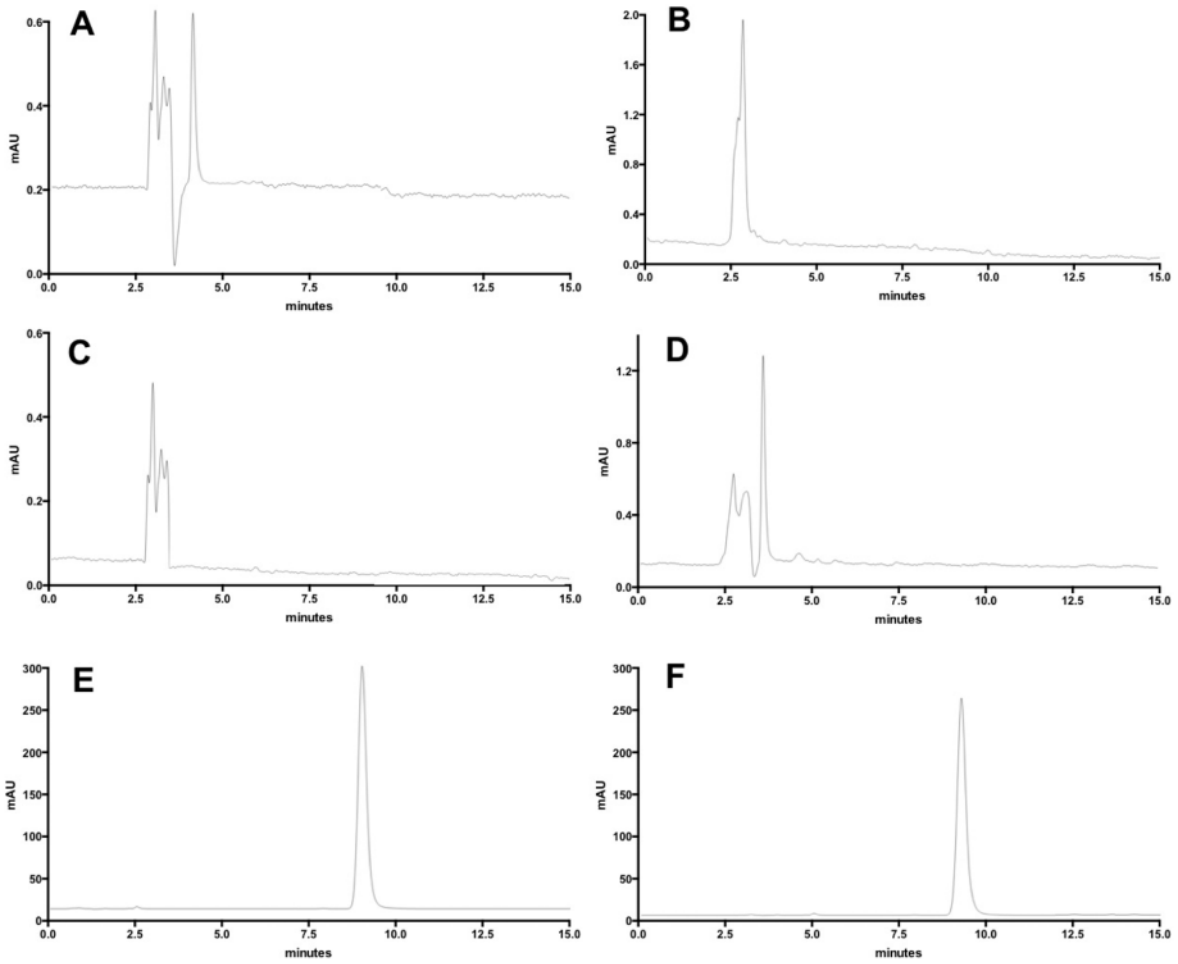

Fig. 1. Representative HPLC-UV of chromatograms of blank methanol (A), FaSSGF (B), blank solid dispersion (C), blank floating gel *in situ* (D), β-carotene in solvent A (E) and β-carotene in solvent B (F).

percentages of β-carotene recovery were between 97.89 ± 8.04% and 99.18 ± 9.32%. Furthermore, the precisions were found to be from 9.32% to 11.28%. Considering that the precision should be ± 15%, the dilution integrity assessment of this study fulfilled the prerequisite from ICH. Consequently, these findings implied that the concentration of β-carotene which were above the calibration standard solutions concentration can be determined by appropriate dilution.

3.4. Accuracy and precision

Inter-day and intra-day accuracy and precision of the developed HPLC method were assessed using β-carotene solutions with LLOQ and QC concentrations in methanol and FaSSGF. Table S1 and Table S2 show %RSD and %RE presenting the precision and accuracy of the method. It was found that inter-day and intra-day precision evaluation resulted in %RDS values which were in the range of 6.41–12.18% and 6.15–13.01%, respectively. Moreover, the accuracy evaluations exhibited %RE of − 6.33 to 7.78 for inter-day evaluation and − 7.20 to 9.20 for inter-day evaluation, respectively. Considerably, according to the limits established by ICH (15%), the developed HPLC to determine β-carotene was found to be accurate and precise.

As previously discussed, solid dispersion is a favorable approach to the solubility issue of β-carotene. Compared to other approaches, this method is a comparatively simple technique formed by the interaction between hydrophilic vehicles and hydrophobic compounds. Among numerous polymers used in solid dispersion preparation, PVP and PEG have been found to be the most utilized polymeric compounds. The application of these polymers in solid dispersion preparation has been widely discussed in numerous studies [9]. Several methods have been used to prepare solid dispersion from the polymeric matrix, namely solvent evaporation, kneading, melting, lyophilization and spray-drying. Solvent evaporation was chosen due to its simplicity because it does not need a specific machine to prepare the solid dispersion.

PEG is a semi-crystalline polymer of ethylene oxide with specific characteristics, namely its ability to produce solid drug solutions, rapid solidification level, low melting point, non-hazardous, and reasonably low price [24]. Furthermore, PVP is an amorphous polymer possessing excellent biocompatibility and high solubility in water. This polymer has been widely utilized in solid dispersion preparation due to its capability to stabilize amorphized drug crystals [25]. In this study, β-carotene solid dispersion was prepared using the combination of PVP K-30 and PEG 6000.

Following the formulation of solid dispersion, the saturation

solubilities of β-carotene were examined in four media with various pH values. Specifically, the validated HPLC method developed in this study was used to assess the saturation solubility values. The results showed that our solid dispersion formulation was able to improve the saturation solubility of β-carotene in the extract and standard forms, when compared to pure extract and standard β-carotene. Table 1 shows the saturation solubility of β-carotene in various systems evaluated in this study. In the solid dispersion system, the hydrophilic carrier plays a role as a solubilizer agent. The presence of these carriers could enhance the wettability of hydrophobic compounds, leading to improved solubility in an aqueous environment.

To sustain the release of β-carotene from solid dispersion formulation, it was further incorporated into a floating gel *in situ* system. In this study, the formation of gel was due to the crosslink reaction between alginate and calcium ions. *In situ* delivery system using ion crosslinking-based to physically retain the formulation in the upper part of the gastric provides a distinctive approach in the formulation of a controlled release dosage form in the liquid state. As a calcium source, calcium carbonate was used in the formulation. It is non-soluble compound which did not react with the alginate in the formulation. When reaching the acid environment, calcium carbonate released the calcium ion and reacted with alginate, forming ion crosslinking-based gel [26]. Furthermore, the addition of $NaHCO_3$ would help the formulation to float on the surface of gastric solution. Finally, HPMC is a hydrophilic polymer used to control the release of β-carotene from the final formulation product. Fig. 2 exhibits the representative floating gel *in situ* formulations in the FaSSGF media, showing that the formulation swelled in floated in the media.

The further application of the HPLC method validated in this study was to determine the β-carotene content in the solid dispersion containing β-carotene, floating gel *in situ* formulation containing β-carotene, as well as the floating gel *in situ* formulation containing solid dispersion of β-carotene. Fig. 2 presents the percentage of β-carotene contents in all formulations tested. The results showed that all recovery values were between 96.54% and 100.34%. This indicates that the excipients and method applied in the formulation process did not affect the β-carotene concentration in the formulations. Additionally, this also implied that the method applied produced the homogeneous formulation. ICH has recommended the acceptable recovery percentage was 95–105%, indicating that all formulations tested fulfilled the requirement.

Furthermore, the validated HPLC method was applied to determine the cumulative *in vitro* release of β-carotene from solid dispersion formulation, as compared to only β-carotene extract. Fig. 3 exhibited the comparison of the β-carotene release profile from solid dispersion and its free form. After 24 h, the solid dispersion formulation released 74.65 ± 9.56% β-carotene. Instead, only 16.75 ± 2.45% of β-carotene was released from pure β-carotene. This indicated that the solid dispersion formulation could improve the release percentage of β-carotene by three times higher than pure β-carotene. There was considerably higher of the release of β-carotene following solid dispersion formulation ($p < 0.003$) compared to pure β-carotene. As previously discussed, the enhanced release profile of β-carotene in the solid dispersion system was due to the presence of the hydrophilic carrier, which improved the wettability, resulting in an enhanced *in vitro* release profile [27]. It was also crucial to investigate the release mechanism of β-carotene from solid dispersion formulation. This was performed by fitting the release profile to

Table 1
Saturation solubility of β-carotene in solid dispersion formulation compared to pure β-carotene (means ± S.D., $n = 3$).

Media	Saturation solubility values	
	Pure β-carotene	Solid dispersion
Purified water	3.29 ± 0.23 ng/mL	28.11 ± 2.52 μg/mL
FaSSGF	2.93 ± 0.22 ng/mL	23.87 ± 2.32 μg/mL
PBS pH 6.8	3.75 ± 0.19 ng/mL	29.98 ± 2.09 μg/mL
PBS pH 7.4	3.79 ± 0.34 ng/mL	34.32 ± 3.17 μg/mL

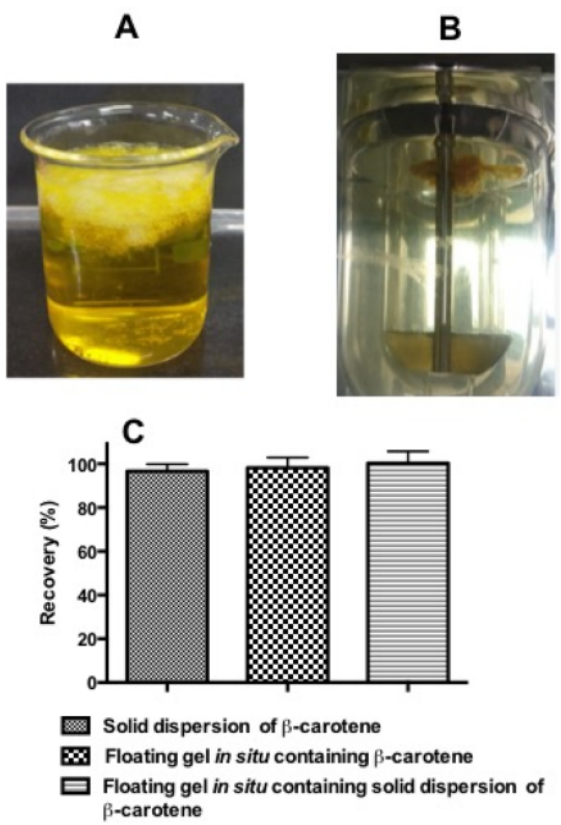

Fig. 2. Representative images of floating ability of floating gel *in situ* formulation containing solid dispersion of β-carotene (A and B). The percentage of recovery of β-carotene in various formulations (n = 3, mean ± SD).

different mathematic kinetic models. Following the calculation, the release profile of the formulation resulted in correlation coefficient of 0.59, 0.90, 0.49, 0.87 and 0.82 for Zero-order, First-order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell, respectively. With these values in mind, the most suitable model for β-carotene release from solid dispersion was First-order model, indicating that the release profile of β-carotene depended on the concentration of β-carotene in the polymeric matrixes [7,28].

Finally, the *in vitro* release behavior of β-carotene following the incorporation of solid dispersion was evaluated using the validated HPLC method, as shown in Fig. 3. According to the results obtained, the formulation of solid dispersion β-carotene into the alginate-based floating gel *in situ* formulation was able to sustain the release over 24 h, resulting in 98.54 ± 10.32% of the drug released percentage. On the other hand, the formulation of pure β-carotene into the floating gel *in situ* significantly decreased ($p < 0.05$) the release of β-carotene with only 9.24 ± 2.32% released after 24 h. Moreover, following being fitted to mathematic kinetic models, it was found that the correlation coefficient values were observed to be 0.85, 0.89, 0.43, 0.97 and 0.88 for Zero-order, First-order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell, respectively. The incorporation of solid dispersion into the floating gel *in situ* formulation resulted in the release mechanism following the Korsmeyer-Peppas model. This model has been generally found in a polymeric drug delivery system. Furthermore, it was found that the *n* value was 0.56, showing the mechanism of release following anomalous diffusion (non-Fickian). As the main compound of this approach was polymeric material, the mechanism of β-carotene from this system was

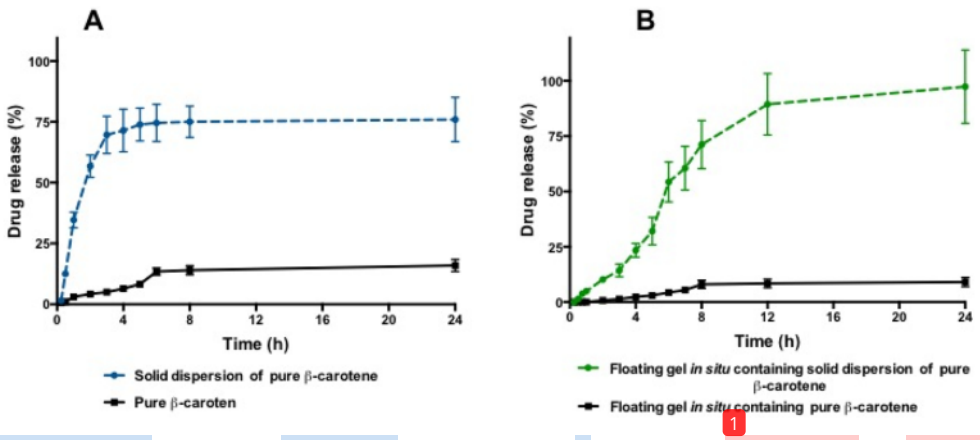

Fig. 3. *In vitro* dissolution study of β-carotene from solid dispersion formulation compared to pure β-carotene (n = 3, mean ± SD) (A). *In vitro* release profile of β-carotene from floating gel *in situ* formulations (n = 3, mean ± SD) (B).

hypothetically based on the diffusion of β-carotene from the polymeric material [29], as well as the erosion and the degradation of the materials [30].

Based on the results found in this study, the combination of solid dispersion and floating gel *in situ* was able to improve both the solubility and sustain the release of β-carotene for 24 h. Essentially, the validated HPLC method developed in this study was successfully applied to quantity β-carotene in the drug loading and *in vitro* release study of our combination approach. However, in forthcoming steps, the release profile of β-carotene following this approach should be carried out in a suitable *in vivo* animal model. To achieve this, the analytical method for *in vivo* studies must also be developed and validated.

4. Conclusion

The current investigation reports a sensitive an analytical method to quantify β-carotene from a sustained release formulation. The analysis process was carried out using HPLC which was validated according to ICH guidelines. The analysis was performed using 100% acetonitrile in Xselect CSH™ C18 column with 3.0 × 150 mm internal diameter and particle size of 3.5 μm at 35 °C. Specifically, the HPLC method was validated in 2 different solvents, namely methanol and FaSSGF neutralized with NaOH 1 M to pH 7 (solvent B). Methanol was used in the content analysis of the compound and FaSSGF was used in the *in vitro* release study. The method was found to be linear, selective, sensitive, accurate and precise. In this study, we also successfully developed an approach to combine solid dispersion and floating gel *in situ* to improve the solubility and sustain the release of β-carotene. Finally, the validated method was applied to quantify β-carotene in the content analysis of the compound and *in vitro* drug release, showing that the formulation possessed uniform content and excellent recovery with sustained release behavior following 24 h. Lastly, the development of *in vivo* analytical method should be carried out and applied in *in vivo* pharmacokinetic studies.

Authors contribution

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

CRediT authorship contribution statement

Cindy Kristina Enggi: Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Funding acquisition, Validation. **Fitrah Mahardika**: Writing – review & editing, Project administration. **Delly Mayari Devara**: Methodology, Investigation, Data curation, Formal analysis. **Mesakh Diki Saputra**: Methodology, Investigation, Data curation. **Nurfadilla Wafiah**: Methodology, Investigation, Data curation. **Muhammad Raihan**: Supervision, Investigation, Data curation, Writing – review & editing, Validation. **Andi Dian Permana:** Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing, Validation, Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jpba.2022.115041.

References

[1] B. Wang, T.O. Akanbi, D. Agyei, B.J. Holland, C.J. Barrow, Encapsulation and Delivery Tool for Hydrophobic Biofunctional Compounds, Elsevier Inc, 2018, https://doi.org/10.1016/B978-0-12-811448-3/00007-3.

[2] Q. Lin, D. Wu, H. Singh, A. Ye, Improving solubility and stability of β-carotene by microencapsulation in soluble complexes formed with whey protein and OSA-modified starch, Food Chem. 352 (2021), 129267, https://doi.org/10.1016/j.foodchem.2021.129267.

[3] K. Ishimoto, S. Miki, A. Ohno, Y. Nakamura, S. Otani, M. Nakamura, S. Nakagawa, β-Carotene solid dispersion prepared by hot-melt technology improves its solubility in water, J. Food Sci. Technol. 56 (2019) 3540–3546, https://doi.org/10.1007/s13197-019-03793-8.

[4] I. Brabcová, M. Hlaváčková, D. Šatínský, P. Solich, A rapid HPLC column switching method for sample preparation and determination of β-carotene in food supplements, Food Chem. 141 (2013) 1433–1437, https://doi.org/10.1016/j.foodchem.2013.04.063.

[5] J. Fiedor, K. Burda, Potential role of carotenoids as antioxidants in human health and disease, Nutrients 6 (2014) 466–488, https://doi.org/10.3390/nu6020466.

[6] D.T. Piorkowski, D.J. McClements, Beverage emulsions: recent developments in formulation, production, and applications, Food Hydrocoll. 42 (2014) 5–41, https://doi.org/10.1016/j.foodhyd.2013.07.009.
[7] A.D. Permana, M.T.C. McCrudden, R.F. Donnelly, Enhanced intradermal delivery of nanosuspensions of antifilariasis drugs using dissolving microneedles: a proof of concept study, Pharmaceutics 11 (2019) 346.
[8] A.D. Permana, R. Nurul, P. Layadi, A. Himawan, N. Juniarti, Q. Kurnia, E. Utomo, S. Aulia, A. Arjuna, R.F. Donnelly, Thermosensitive and mucoadhesive in situ ocular gel for effective local delivery and antifungal activity of itraconazole nanocrystal in the treatment of fungal keratitis, Int. J. Pharm. 602 (2021), 120623.
[9] A.D. Permana, E. Utomo, M.R. Pratama, M.N. Amir, Q.K. Anjani, S.A. Mardikasari, S. Sumarheni, A. Himawan, A. Arjuna, U. Usmanengsi, R.F. Donnelly, Bioadhesive-thermosensitive in situ vaginal gel of the gel flake-solid dispersion of itraconazole for enhanced antifungal activity in the treatment of Vaginal Candidiasis, ACS Appl. Mater. Interfaces 13 (2021) 18128–18141, https://doi.org/10.1021/acsami.1c03422.
[10] U.H. Gala, D.A. Miller, Y. Su, A. Spangenberg, R.O. (Bill) Williams, The effect of drug loading on the properties of abiraterone–hydroxypropyl beta cyclodextrin solid dispersions processed by solvent free KinetiSol® technology, Eur. J. Pharm. Biopharm. 165 (2021) 52–65, https://doi.org/10.1016/j.ejpb.2021.05.001.
[11] M. Moganti, H.N. Shivakumar, Oral raft forming in situ gelling system for site specific delivery of calcium, J. Drug Deliv. Sci. Technol. 61 (2021), 102113, https://doi.org/10.1016/j.jddst.2020.102113.
[12] G. Falcone, M. Saviano, R.P. Aquino, P. Del Gaudio, P. Russo, Coaxial semi-solid extrusion and ionotropic alginate gelation: a successful duo for personalized floating formulations via 3D printing, Carbohydr. Polym. 260 (2021), 117791, https://doi.org/10.1016/j.carbpol.2021.117791.
[13] G.B. Celli, M.S.L. Brooks, A. Ghanem, Development and evaluation of a novel alginate-based in situ gelling system to modulate the release of anthocyanins, Food Hydrocoll. 60 (2016) 500–508, https://doi.org/10.1016/j.foodhyd.2016.04.022.
[14] J. Karppi, T. Nurmi, B. Olmedilla-Alonso, F. Granado-Lorencio, K. Nyyssönen, Simultaneous measurement of retinol, α-tocopherol and six carotenoids in human plasma by using an isocratic reversed-phase HPLC method, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 867 (2008) 226–232, https://doi.org/10.1016/j.jchromb.2008.04.007.
[15] A.I. Olives Barba, M. Cámara Hurtado, M.C. Sánchez Mata, V. Fernández Ruiz, M. López Sáenz De Tejada, Application of a UV–vis detection-HPLC method for a rapid determination of lycopene and β-carotene in vegetables, Food Chem. 95 (2006) 328–336, https://doi.org/10.1016/j.foodchem.2005.02.028.
[16] N.V. Dimitrov, C. Meyer, D.E. Ullreyy, W. Chenoweth, A. Michelakis, W. Malone, B. Charles, G. Fink, Bioavailability of β-carotene in humans, Am. J. Clin. Nutr. (1988) 298–304.
[17] A.D. Permana, R.N. Utami, A.J. Courtenay, M.A. Manggau, R.F. Donnelly, L. Rahman, Phytosomal nanocarriers as platforms for improved delivery of natural antioxidant and photoprotective compounds in propolis: an approach for enhanced both dissolution behaviour in biorelevant media and skin retention profiles, J. Photochem. Photobiol. B Biol. (2020), https://doi.org/10.1016/j.jphotobiol.2020.111846.
[18] ICH, Validation of analytical procedures: text and methodology Q2 (R1). ICH harmonised tripartite guideline, 2005.
[19] FDA, Guidance for Industry: Bioanalytical Methods Validation, US Department of Health and Human Services, 2018, pp. 1–37.
[20] D. Ramadon, A.J. Courtenay, A.D. Permana, I.A. Tekko, E. McAlister, M.T. C. McCrudden, H.O. McCarthy, R.F. Donnelly, A sensitive HPLC-UV method for quantifying vancomycin in biological matrices: application to pharmacokinetic and biodistribution studies in rat plasma, skin and lymph nodes, J. Pharm. Biomed. Anal. 189 (2020), 113429.
[21] A.D. Permana, I.A. Tekko, H.O. McCarthy, R.F. Donnelly, New HPLC–MS method for rapid and simultaneous quantification of doxycycline, diethylcarbamazine and albendazole metabolites in rat plasma and organs after concomitant oral administration, J. Pharm. Biomed. Anal. 170 (2019) 243–253.
[22] A.D. Permana, E. Wahyudin, Ismail, M.N. Amir, M. Raihan, Q.K. Anjani, E. Utomo, P. Layadi, R.F. Donnelly, New and sensitive HPLC-UV method for concomitant quantification of a combination of antifilariasis drugs in rat plasma and organs after simultaneous oral administration, Anal. Methods 13 (2021) 933–945.
[23] A.L. Raj, Y.S. Kumar, Preparation and evaluation of solid dispersion of nebivolol using solvent evaporation method, Int. J. Pharm. Sci. Drug Res. 10 (2018) 322–328, https://doi.org/10.25004/ijpsdr.2018.100418.
[24] J.O. Eloy, J.M. Marchetti, Solid dispersions containing ursolic acid in Poloxamer 407 and PEG 6000: a comparative study of fusion and solvent methods, Powder Technol. 253 (2014) 98–106, https://doi.org/10.1016/j.powtec.2013.11.017.
[25] A.M. Yousaf, U.R. Malik, Y. Shahzad, T. Mahmood, T. Hussain, Silymarin-laden PVP-PEG polymeric composite for enhanced aqueous solubility and dissolution rate: preparation and in vitro characterization, J. Pharm. Anal. 9 (2019) 34–39, https://doi.org/10.1016/j.jpha.2018.09.003.
[26] P.S. Rajinikanth, B. Mishra, Floating in situ gelling system of acetohydroxamic acid for clearance of *H. pylori*, Drug Dev. Ind. Pharm. 34 (2008) 577–587, https://doi.org/10.1080/03639040701831819.
[27] C.L. Ng, S.E. Lee, J.K. Lee, T.H. Kim, W.S. Jang, J.S. Choi, Y.H. Kim, J.K. Kim, J. S. Park, Solubilization and formulation of chrysosplenol C in solid dispersion with hydrophilic carriers, Int. J. Pharm. 512 (2016) 314–321, https://doi.org/10.1016/j.ijpharm.2016.08.062.
[28] S. Dash, P.N. Murthy, L. Nath, P. Chowdhury, Kinetic modelling on drug release from controlled drug delivery systems, Acta Pol. Pharm. – Drug Res. 67 (2010) 217–223.
[29] A.D. Permana, I.A. Tekko, M.T.C. McCrudden, Q.K. Anjani, D. Ramadon, H. O. McCarthy, R.F. Donnelly, Solid lipid nanoparticle-based dissolving microneedles: a promising intradermal lymph targeting drug delivery system with potential for enhanced treatment of lymphatic filariasis, J. Control. Release 316 (2019) 34–52.
[30] E. Larrañeta, C. Martínez-Ohárriz, I. Vélaz, A. Zornoza, R. Machín, J.R. Isasi, In vitro release from reverse poloxamine/α-Cyclodextrin matrices: modelling and comparison of dissolution profiles, J. Pharm. Sci. 103 (2014) 197–206, https://doi.org/10.1002/jps.23774.

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