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Assay of β-Carotene in Dietary Supplements and Fruit Juices by TLC-Densitometry

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Abstract β-carotene as a dietary supplement is a matter of interest to anyone who seeks to improve their skin condition or sight. Due to its antioxidative properties, carotenoids are involved in the prevention of severe conditions, such as cancer, heart disease, macular degeneration, or cataract, which makes them an object of scientific study. Thanks to its color, ß-carotene is used as a food colorant, improving the appearance of foodstuff. A presented method was established for the identification and quantification of β-carotene by thin-layer chromatography with densitometric detection. As a stationary phase, TLC Aluminiumoxid 60 F_{254} neutral was utilized. It were activated with methanol and dried at the temperature of 60 °C for 1 h. The development was carried out in a twin trough glass chamber saturated with a mobile phase consisting of chloroform/methanol/acetone/ammonium hydroxide (10:22:53:0.2, *v/v/v/v*). TLC scanner was used for densitometric scanning and analysis in the absorbance mode at 450 nm. The method was validated for specificity, linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ). Presented method illustrates simple, economical, and suitable manner for routine quantitative and qualitative determination of β-carotene in dietary supplements and carrot juice.

Keywords β-carotene · Dietary supplements · Fruit juices · TLC analysis · Densitometric detection

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Introduction

More than 600 carotenoids have been isolated in nature and the β-carotene being the best known (Faure et al. 1999; Goodman 1984). The structure β-carotene (Fig. 1) is conjugated hydrocarbon of eight repeating isoprene units. It is a provitamin A, being converted to vitamin A in a human body which is necessary for the proper functioning (Goodman 1984). Deficiency of vitamin A is the main cause of premature deaths in developing countries, especially among children (Burri 1997). Although most programs of supplementation utilize vitamin A, its high doses can induces a teratogenic effect. Therefore, it is recommended to use a rather β-carotene or eat fruits and vegetables, rich in this vitamin (De Pee et al. 1995).

β-carotene is an antioxidant, adopted in increasing quantities around the world to improve eyesight, the condition of the skin and nails, as well as to protect the body against free radicals (Burri 1997; Paiva and Russell 1999; Palozza 1998; Palozza et al. 2001). β-carotene affects the immune system, increasing the absolute number of T helper cells, as well as indicators of T cell function in animal and human models (Fryburg et al. 1995). Carotenes have also been reported to be associated with harmful events, such as at high doses for heavy smokers (Omenn et al. 1994). Therefore, its quantitative determination in food is important for the evaluation of the nutritional values, quality of fresh and processed products, and health benefits to humans.

Carotenoids are fat-soluble food components and practically insoluble in water and ethanol, but due to the high water content in the tissues from which they are harvested, an initial extraction of a water miscible solvent is usually needed (Cortes et al. 2004). The nature of the end groups of carotenoids affects their polarity, hence the different solubility in various solvents, for example, acetone, tetrahydrofuran, n-hexane, pentane, petroleum ether, methanol, and their

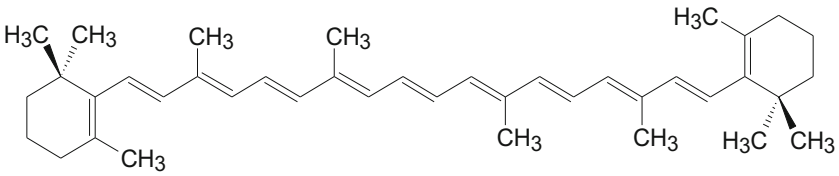

Fig. 1 Chemical structure of β–carotene

mixtures, and the need for specific methods of extraction (Taungbodhitham et al. 1998). Some of authors point to the fact that the tetrahydrofuran and diethyl ether may produce peroxides decomposing β-carotene, and therefore, it is advisable to add a butylhydroxytoluene as an antioxidant (Marsili and Callahan 1993; Quackenbush and Smallidge 1986). Different extraction procedures were used to isolate carotenoids, including simple solvent extraction, lipid phase distribution (Minguez-Mosquera and Garrido-Fernandez 1989), solid-phase extraction (Gutierrez et al. 1989), accelerated solvent extraction (Breithaupt 2004), and supercritical-fluid extraction (Gamlieli-Bonshtein et al. 2002). Extraction of carotenoids should be performed quickly, avoiding exposure to light, oxygen, high temperature, and prooxidant metals in order to minimize auto-oxidation and isomerization (Van den Berg et al. 2000).

As the reference method to the quantification of β-carotene, the spectrophotometry at 455 and 483 nm is recommended (European 2001). As the methods for the determination of β-carotene, spectrophotometric (Biswas et al. 2011; Schierle et al. 2002) and chromatographic methods, both in normal and reversed phase, were used (Brabcova et al. 2013; Burri et al. 1997; Guedes De Pinho et al. 2001; Hart and Scott 1995; Khachik et al. 1991; Oliver and Palou 2000; Rodriguez-Bernaldo De Quiros and Costa 2006). The chromatographic methods with the electrochemical detection were also described (Ferruzzi et al. 1998; Murata et al. 1992). Although the presence of double conjugated bonds in the structure of β-carotene determines its electrochemical activity, however, applicable in analytical oxidation and reduction processes is limited. Ziyatdinova et al. conducted the voltammetric analysis in an aprotic organic medium adding various surfactants (Ziyatdinova et al. 2012).

Thin-layer chromatography (TLC) was found to have the potential to be the first choice for analysis of carotenoids in vegetable and biological samples (Murkovic and Zeb 2010). TLC is still a widely used technique due to its reliability, simplicity, reproducibility, and speed in analysis of pharmaceuticals, botanicals, foodstuff, environmental, and clinical samples (Renger et al. 2011). Direct applications of suspensions, dirty, or turbid samples also are possible (Dorni et al. 2007). Additionally, composition of used mobile phase is not very important for detection as it is observed in HPLC. For the determination of β-carotene in fruits, musts, wines, snails, leeches, and fancy carp, the TLC analysis was referenced with the use of eluents in reversed phase with mobile phases composed of petroleum ether or diethyl ether (Arthur et al. 2006; Bundit et al. 2008; Evans et al., 2004; Ligor and Buszewski 2007; Martin et al. 2005). Sherma and Fried described the qualitative analysis of β-carotene by TLC from an extract of spinach (Sherma and Fried 2004). A mobile phase containing the petroleum ether and various solvents in a different ratio has been used for TLC analysis of β-carotene, oxidized carotenoids, vitamin A, and carotenoid mixtures (Martin et al. 2005; Minguez-Mosquera et al. 1992; Arthur and Sherma 2007). Although several techniques have been used for analysis of carotenoids form various sources, literature describing the analysis of carotenoids by TLC in detail is limited (Jarusiewicz et al. 2006; Sherma 2000). In available literature, no papers were found describing determination of β-carotene in dietary supplements and fruit juices by TLC with densitometric detection and using a mobile phase without ether which is consequently eliminated from routine analysis because of its toxicity.

The purpose of this research was to present a rapid, precision, and accurate analytical method for the determination of β-carotene in dietary supplements and fruit juices based on a simple extraction method, as well as a TLC-densitometry technique. The presented method employs a TLC-Aluminiumoxid layer, automated band-wise sample application, densitometric scanning of the yellow β-carotene standard, and sample bands.

Materials and Methods

Materials and Chemicals

The standard of β-carotene was purchased from SIGMA (cat no C9750). Dietary supplements and juices were used: BetaKaroten optimal, 6 mg/capsule (Alpepharma, Jelenia Góra, Poland); BetaSolar, 15 mg/capsule (Olimp Lab., Dębica, Poland); β-carotene plus, 6 mg/tablet (Aflofarm, Ksawerów, Polska); BetaSunBio, 6 mg/tablet (Sun-Farm, Kołbiel, Poland); BelissaSun, 10 mg/tablet (Aflofarm, Ksawerów, Polska); *Natus* carote juice (Natus, Poland); *Kubuś* juice, composition: purees and juice from Mazurska carrot, bananas, and apples, water, glucose-fructose syrup, citric acid, vitamin C (MWS, Poland); *Dizzy* juice, composition: carrot puree, water, bananas puree, apple puree, sugar, citric acid, vitamin C; juice containing no less than 2.5 mg of β-carotene (Lidl Stiftung&Co.KG). Methanol, glacial acetic

acid 99.5 %, n-hexane, cyclohexane, toluene, ammonium 35.04 g/mol were purchased from Chempur (Piekary Śląskie, Poland). Acetone, isopropanol, ethyl acetate, chloroform, butanol, dichloromethane were purchased from POCH, Gliwice. All used reagents were of analytical grade.

Preparation of Standard Solution

Ten milligrams of β-carotene standard were weighed and transferred to a 5-mL volumetric flask. Ethyl acetate was added. The flask was shaken, and volume was made up to the mark to give a 0.2 % (*w/v*) solution.

Sample Preparation

Five capsules or tablets were weighed and powder was collected from the shell. The powder equivalent to 5-mg β-carotene was accurately weighed and transferred to a volumetric flask. Ethyl acetate was added and shaken for 10 min. Next, the volume was made up to the mark with ethyl acetate. The above solution was filtered through Whatman filter paper (0.45 μm); this would give a 0.2 % (*w/v*) solution.

Forty milliliter of juice and 80 mL of chloroform were dispensed to the separatory funnel of capacity 200 mL. Contents were shaken for 15 min, left to the phase separation, and next, the chloroform layer was separated from the aqueous layer. The extraction process was repeated three times. The collected chloroform layer was evaporated at 30 °C to a volume of 50 mL and used for the study.

TLC Conditions

TLC analysis was performed on the precoated TLC Aluminiumoxid 60 F_{254} neutral (typ E) (Merck, Darmstadt, Germany) 10×10-cm plates, cut from 20×20 cm. Before using, the plates were activated with methanol. Next, they were dried in the incubator at temperature 60 °C by 1 h. The samples in 10-μL volumes of appropriate solution were applied on thus prepared stationary phase with Linomat V applicator (CAMAG, Muttenz, Switzerland), as 10 mm width bands, with distance of 10 mm from the plate bottom, 10 mm from the edge, and 8-mm distance between the bands. The mobile phase consisted of chloroform/methanol/acetone/ammonia 25 % (10:22:53:0.2, *v/v/v/v*). Chromatograms were developed in a room temperature on the distance of 9 cm within about 40 min in glass chromatographic chambers (17.5×16×8.2 cm in size; Sigma-Aldrich), saturated with vapor of mobile phase for 15 min. After drying at the air in a dark place, plates were scanned and obtained spots were recorded by using TLC Scanner 3 (CAMAG, Muttenz, Switzerland) with Cats 1.3.4 software at 450 nm.

Retardation factor (R_f) for the β-carotene (standard and samples) was designated. For the quantitative analysis of β-carotene, the values of the peak area obtained on the densitograms were recorded.

Method Validation

The developed TLC method was validated as per International Conference on Harmonisation (ICH) guidelines (ICH 2000) by specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, robustness, and ruggedness.

Specificity

The specificity of the method was confirmed by analyzing the standard substance and the extract. The band for β-carotene in the sample was confirmed by comparing the R_f values and absorption spectra of the band with that of the standard. The peak purity of the β-carotene was assessed by comparing the spectra at three different levels, viz., peak start (S), peak apex (M), and peak end (E) positions of the band.

Linearity

The linearity was checked for six solutions in a range from 0.5 to 9.5 μg per band. Results of calibration curve parameters were estimated by Statistica software.

Limit of Detection and Limit of Quantitation

The LOD and LOQ were establish based on standard deviation of intercept (S_b) and slope of a straight line (a), obtained for the calibration curve. Following formulations, LOD= 3.3 S_b/a and LOQ=10 S_b/a were applied for the estimation.

Precision

Repeatability of the sample application and measurement of peak area were carried out using nine determinants (3 concentrations/3 replicates) covering the specified range for the procedure (1, 5, and 9 μg per band of β-carotene) and was expressed in terms of relative standard deviation (% RSD). Acceptance criteria for a procedure's repeatability or intermediate precision are based on the intended use of the analytical method. A repeatability showing a random standard deviation of ≤1.0 % may be acceptable for assaying substance in the formulations with a given specification range. Indirect precision was estimated in another day and by different analyst in analogical way.

Accuracy

To investigate the accuracy in sample preparation (i.e., extraction efficiency), a spiked solution by adding known amounts

of related substances into a sample matrix was prepared. Thereafter, responses of the spike solutions and the standard solutions were taken to assess the recovery from the sample preparation. Recovery studies were carried out by addition of standard β-carotene to the sample at three different concentration levels (80, 100, and 120 %), taking into consideration percentage purity of added bulk preparations samples. The experiment was conducted three times.

Ruggedness

β-carotene in solution of concentration 2 μg per band was prepared and analyzed on day 0 and after 12 and 24 h. Data were treated for % RSD to assess the ruggedness of the method.

Robustness

By introducing small changes in the mobile phase volume, duration of mobile phase saturation, and activation of prewashed TLC plates with methanol, the effects on the obtained results were examined. Robustness of the method was done in triplicate at a concentration level of 2 μg per band for β-carotene, and the % RSD values were calculated.

Determination of β-Carotene in Dietary Supplements and Fruit Juices

Determination of β-carotene was carried out according to the procedure describing above in five dietary supplements and three fruit juices. For each preparation and juice, ten measurements were performed. Estimation of β-carotene content was done by comparing peak areas for standard solutions with peak areas for studied solutions. For each series of results, the statistical analysis was done.

Results and Discussion

Here in the paper, the conditions for the identification and determination of β-carotene in dietary supplements and fruit juices were determined and presented. The assay was provided using TLC technique with densitometric detection.

Optimization of TLC Conditions

In the first stage of studies, conditions for separation of β-carotene were established. For the selection of the stationary phase, various plates were tested, such as TLC Cellulose F, TLC Kieselgel 60$WF_{254}S$, Silica gel 60 F_{254}, TLC Polyamide $_{11}F_{254}$, TLC Aluminiumoxid 60 F_{254} neutral (typ E) (Merck, Darmstadt, Germany). On these plates, 10-μL 0.02 % standard solution and selected supplements were applied as a band of 1-cm wide by means of an automatic sampler. The mobile phase composition was established by checking the solvent mixtures of varying qualitatively and quantitatively compositions, based on the eluotropic series. The following testing mobile phases were tried: toluene/ethyl acetate/glacial acetic acid (15:4:1, *v/v/v*), cyclohexane/ethyl acetate (7:3, *v/v*), acetone/n-hexane (3:7, *v/v*), n-hexane/isopropanol/methanol (10:0.2:0.02, *v/v/v*), chloroform/acetone/toluene (12:5:2, *v/v/v*), ethanol/isopropanol/glacial acetic acid/water (4:4:1:3, *v/v/v/v*), n-hexane/acetone (4:1, *v/v*), chloroform/acetone/toluene (12:5:2, *v/v/v*), acetone/n-hexane/methanol (4:1:1, *v/v/v*), butanol/glacial acetic acid/water (12:3:5, *v/v/v*), ethanol/water/glacial acetic acid (10:2:0.1, *v/v/v*), chloroform/methanol/acetone/ammonia 25 % (10:22:53:0.05, *v/v/v/v*), chloroform/methanol/acetone/ammonia 25 % (10:22:53:0.2, *v/v/v/v*), chloroform/methanol/acetone/butylamine (10:22:53:1, *v/v/v/v*), chloroform/methanol/ammonia 25 % (10:22:0.2, *v/v/v*), chloroform/methanol/butylamine (10:22:10, *v/v/v*). The plates were developed on the way of 9 cm at room temperature and then air-dried. The obtained chromatograms were analyzed in terms of color of the spots and background. The acquired densitograms were rated in terms of separate and shape of the peaks. Finally, TLC Aluminiumoxid 60 F_{254} neutral (typ E) plates as the stationary phase were found, after initial purification by prewash with methanol and activation in the oven (BMT, Brno, Czech Republic) for 1 h at 60 °C. Furthermore, chloroform/methanol/acetone/ammonia 25 % (10:22:53:0.2, *v/v/v/v*) was found to be satisfactory and gave good separation for β-carotene without any interference from excipients (Fig. 2). The appointed R_f value for β-carotene was 0.90. The obtained chromatograms were subjected to densitometric analysis by recording absorption spectra within the wavelength range of 200–600 nm. The scanning spectra of β-carotene revealed that at 450 nm, it possesses significant absorbance (Fig. 3). So, it was selected as detection wavelength. Application of the described above conditions has allowed to obtain a compact spots and well-shaped peaks, allowing to perform the quantitative determination of β-carotene.

Extraction of β-Carotene

In order to optimize the extraction of β-carotene, the following solvents were tested: methanol, ethyl acetate, chloroform, dichloromethane, and hexane, in terms of the solubility of the β-carotene substances. Finally, ethyl acetate was chosen for the extraction of β-carotene from dietary supplements and chloroform for the extraction from the juices. In these conditions, the best recovery of the substance was achieved.

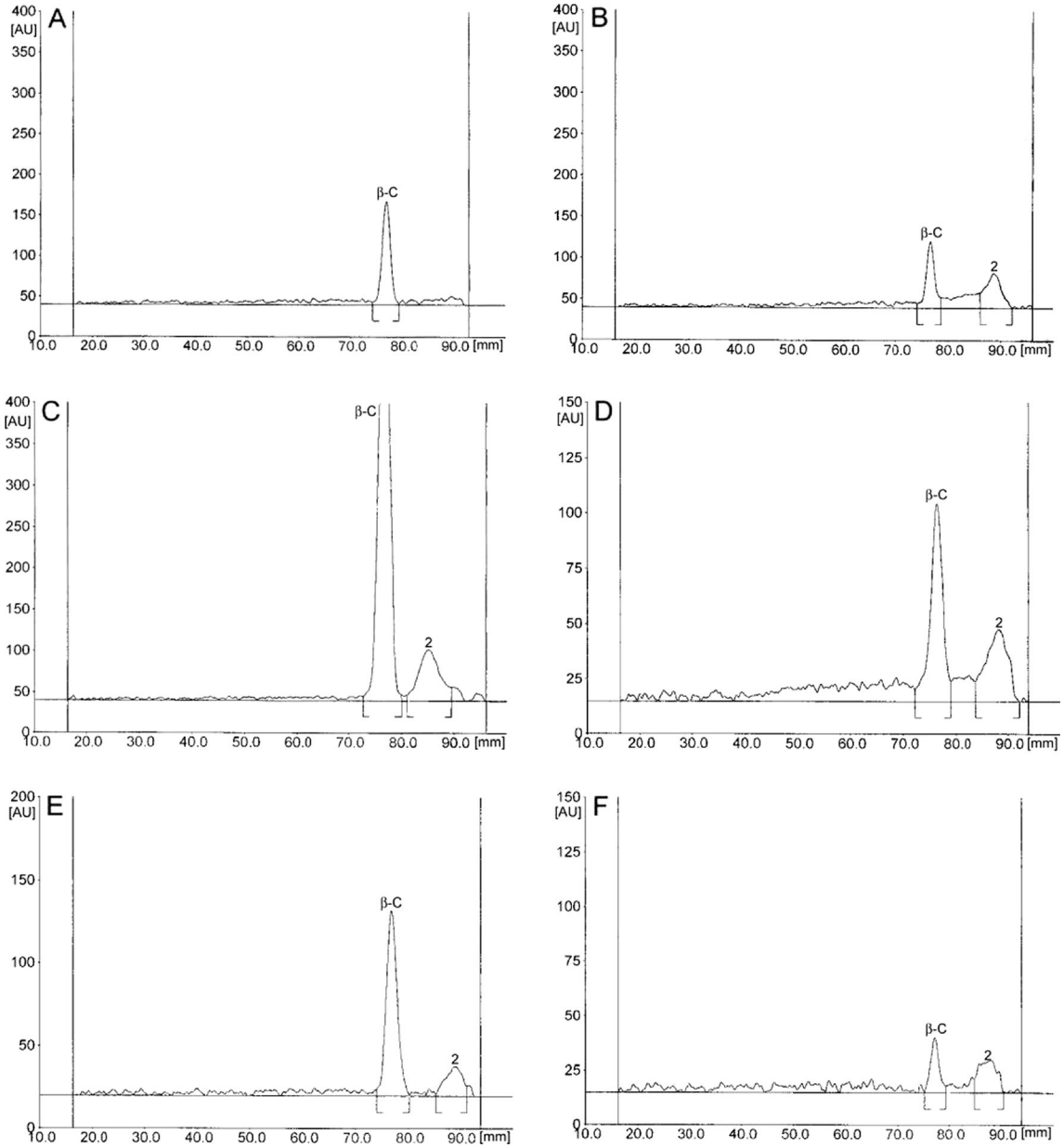

Fig. 2 An example of densitograms obtained for preparations: BetaSunBio (**a**), BetaSolar (**b**), Betacaroten plus (**c**), *Natus* juice (**d**), *Kubuś* juice (**e**), and *Dizzy* juice (**f**) under described conditions (β-C—β-carotene; 2—additional peaks)

Validation of the Method

The presented method was validated for assay of β-carotene in accordance with ICH guidelines. The specificity of the method was confirmed by analyzing the standard compound and the extracts. The band for β-carotene in the sample was confirmed by comparing the R_f values and spectra of the band with those of the standard. The peak purity of β-carotene was assessed by comparing the absorption spectra at three different levels, viz., peak start (S), peak apex (M), and peak end (E) positions of the band. The mobile phase used enabled good resolution for β-carotene at R_f 0.90 and wavelength 450 nm. Chromatograms obtained from standard and sample solutions are shown in Fig. 2.

Amounts of standard solutions equivalent to 0.76–9.14 μg of β-carotene per band were applied to the plate. The plate was developed and scanned. The data of peak areas plotted against the corresponding concentrations were treated by least square regression analysis. The slope, intercept, and correlation coefficient were also determined. Over the studied calibration range, the correlation coefficient for β-carotene was found to be r=0.9990. The regression parameters are shown in Table 1.

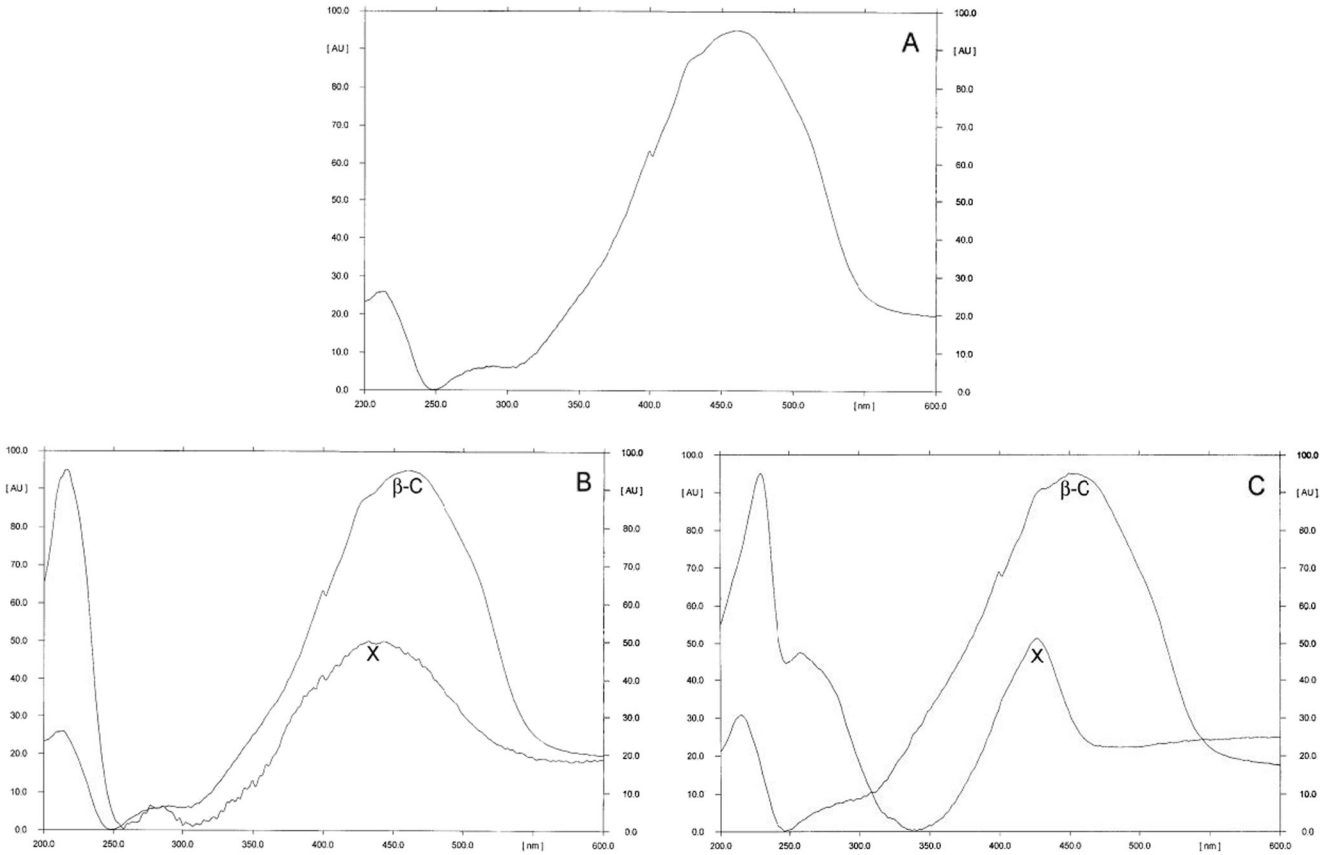

Fig. 3 An absorption spectrum for β-carotene standard (**a**). An absorption spectra for Betacaroten plus (**b**) and *Kubuś* juice (**c**) recorded for each two registered peaks (presented on Fig. 2; β-C–β-carotene; X–additional substance). All spectra were obtained directly from the chromatograms

The sensitivity of measurement of β-carotene using the proposed method was estimated as LOQ, and the lowest concentration detected under these chromatographic conditions as LOD. The obtained LOD and LOQ for β-carotene were 103 and 313 mg per L, respectively.

Table 1 The results of the method validation

Parameter	Result
Linearity (μg/band)	0.76–9.14 P=2214.5 c +650.2; r=0.9990
LOD (mg/L)	103
LOQ (mg/L)	313
Intra-day precision	10719.7; 10753.7; 10894.2; 10744.5; 10858.6; 10841.3; 10910.2; 10890.5; 10765.1 x_m=10819.76; S=73.93; S_{xm}=24.64; RSD=0.68 %
Inter-day precision	10763.8; 10936.5; 10712.6; 10716.9; 10957.1; 10857.5; 10857.5; 10912.9; 10916.4 x_m=10847.91; S=94.48; S_{xm}=31.49; RSD=0.87 %
Accuracy	Level 80 %: 100.79; 99.85; 101.04; 100.06; 99.88; 99.94 x_m=100.26; S=0.52; S_{xm}=0.21; RSD=0.52 % level 100 %: 100,11; 99,02; 101,03; 99,97; 100,77; 100,57; x_m=100.25; S=0.72; S_{xm}=0.29; RSD= 0.72 % level 120 %: 99.96; 99.51; 99.80; 100.17; 99.33; 98.74 x_m=99.59; S=0.51 S_{xm}=0.21; RSD= 0.51 %

P peak area, *c* concentration, *r* correlation coefficient, x_m arithmetic mean, *S* standard deviation, S_{xm} standard deviation of the mean, *RSD* relative standard deviation (%)

The precision of the method, as intra-day variation (% RSD), was determined by analysis of standard solutions in the range of 1–9 μg per band three times on the same day. Inter-day precision (% RSD) was assessed by analysis of the same solution on three different days over a period of 1 day. The relative standard deviation values for both intra- and inter-day precision values were ≤1.0 %. The results from study of precision are shown in Table 1.

The accuracy of the method was determined by analysis of standard additions at three concentration levels. The obtained results of accuracy of β-carotene in formulation are <1 % of RSD, and they are given in Table 1. Obtained results indicated that the proposed method is accurate.

By introducing small changes in the mobile phase volume, duration of mobile phase saturation, and activation of prewashed TLC plates with methanol, the effects on the results were examined.

A β-carotene solution was prepared and analyzed on day 0 and after 12 and 24 h. Data were treated for % RSD to assess ruggedness of the method. The relative standard deviation of the results obtained by different analysts was <1.0 %. Thus,

Table 2 Statistical evaluation of β-carotene content in tested dietary supplements and juices with the statistical evaluation

Preparation	Declared content (mg)	Determined content (mg)	Statistical data (n=10)
BetaKaroten optimal	3.6 mg/capsule	0.18, 0.18, 0.18, 0.18, 0.17, 0.18, 0.18, 0.18, 0.18, 0.18	x_m=0.1790 S=0.0032; S_{xm}=0.0010 RSD=1.77 %
Betasolar	15 mg/capsule	0.88, 0.89, 0.89, 0.90, 0.91, 0.89, 0.90, 0.91, 0.90, 0.88	x_m=0.8950 S=0.0108; S_{xm}=0.0034 RSD=1.21 %
BetaSunBio	6 mg/capsule	1.65, 1.69, 1.69, 1.65, 1.65, 1.68, 1.64, 1.64, 1.66, 1.64	x_m=1.6620 S=0.0210; S_{xm}=0.0066 RSD=1.26 %
BelissaSun	10 mg/tablet	10.42, 10.37, 10.26, 10.21, 10.36, 10.42, 10.24, 10.35, 10.28, 10.26	x_m=10.3170 S=0.0762; S_{xm}=0.0241 RSD=0.74 %
Betacaroten plus	10 mg/tablet	15.69, 15.84, 15.85, 15.78, 15.36, 15.86, 15.86, 15.56, 15.50, 15.94	x_m=15.7240 S=0.1906; S_{xm}=0.0603 RSD=1.21 %
Natus fresh carrot juice	–/100 mL	19.53, 19.75, 19.93, 20.21, 20.11, 20.20, 19.83, 19.92, 20.07, 19.78	x_m=19.9330 S=0.2181; S_{xm}=0.0690 RSD=1.09 %
Kubuś juice	–/100 mL	12.03, 12.16, 12.23, 11.72, 11.86, 12.10, 11.96, 11.77, 12.08, 11.95	x_m=11.9860 S=0.1663; S_{xm}=0.0526 RSD=1.39 %
Dizzy juice	>2.5 mg/100 mL	5.23, 5.13, 5.18, 5.13, 5.13, 5.14, 5.10, 5.11, 5.17, 5.06	x_m=5.1380 S=0.0469; S_{xm}=0.0148 RSD=0.91 %

"–" no data, x_m arithmetic mean, S standard deviation, S_{xm} standard deviation of the mean, *RSD* relative standard deviation (%)

statistical analysis showed no significant difference between results obtained by applying the analytical conditions established for the method and those obtained in experiments in which some of the conditions were varied slightly.

Determination of β-Carotene in Dietary Supplements and Fruit Juices

Usefulness of the developed method for routine studies was proved during determination of β-carotene in studied formulations. The five tested dietary supplements confirmed the presence of the active compound (Table 2) but not always in an amount declared by the manufacturer.

Relative standard deviation obtained from determination of β-carotene confirmed insignificant differences between results obtained from BelissaSun dietary supplement and declaration of contents. In some of the preparations, deficiency of β-carotene was observed (BetaSolar, BetaKaroten optimal, BetaSunBio), and in β-carotene plus the excess of this component was observed. Thus, this results indicate a possible decomposition of β-carotene during storage time in out-data supplements. β-carotene is commonly used in food processing as a coloring additive. A high β-carotene content in the formulation β-carotene plus, relative to the declared value, may derive from the presence of coloring additives of a similar chemical structure to the determined substance. Additionally, β-carotene content in the juices was determined in the range of 5.1380–19.9330 mg of the substance per 100 mL of juice with a good RSD value <1.5 %. In the case of three supplement formulations, on the recorded chromatograms, only one peak of β-carotene was obtained, and no matrix component was detected by densitometric detector. While analyzing two dietary supplements (BetaSolar, β-carotene plus) and all juices, there were additional spots in chromatograms. These spots differ in position (various R_f values), but their absorption spectra were related (Fig. 3), thus suggesting that additional peaks may have a similar chemical structure. β-carotene formed a symmetrical peak, clearly separated from the additional peaks, which confirmed parameters calculated for registered peaks, R_s (resolution factor) and α (separation factor) (Table 3).

As a result of presented study, the conditions were established to make determination of β-carotene in commonly available dietary supplements and fruit juices. The analyses of β-carotene were carried out by employing a simple and economical TLC-densitometry procedure that, with the advances in densitometric instrumentation, provides new opportunities for dietary supplements and juice quality control.

Conclusions

A sensitive, rugged, and reproducible TLC method was developed and validated according to the ICH guidelines, for the subsequent separation and detection of β-carotene. Validation experiments provided proof that TLC analytical method is linear in the proposed working range as well as accurate,

Table 3 Results presenting resolution of β-carotene and additional peaks on TLC Aluminiumoxid 60 F_{254} plates

Preparation	Substance	R_s	α
BetaSolar	β-carotene—add peak (R_f=0.87)	0.66	2.33
Betacaroten plus	β-carotene—add peak (R_f=0.83)	0.29	1.75
Natus juice	β-carotene—add peak (R_f=0.86)	0.40	2.19
Kubuś juice	β-carotene—add peak (R_f=0.88)	0.55	2.50
Dizzy juice	β-carotene—add peak (R_f=0.86)	0.60	2.19

R_s resolution factor, R_s=2 (distance between the centers of two adjacent spots)/(sum of the widths of the two spots in the direction of development), α separation factor, α=[(1/R_{f1})-1]/[(1/R_{f2})-1]

precise, and specific, being able to separate the main compound from the matrix. The proposed mobile phase of TLC effectively resolves β-carotene. The method can be used for qualitative as well as quantitative determination of β-carotene in dietary supplements and fruit juices and for purity estimation. TLC fingerprints were obtained for all the extracts giving reliable indication of the same identity. This method can be also used in routine analysis of food and pharmaceuticals containing β-carotene, especially due to the speed of analysis and reduction of the consumption of organic solvents.

Conflict of Interest Małgorzata Starek declared that she has no conflict of interest. Anna Guja declared that she has no conflict of interest. Monika Dąbrowska declared that she has no conflict of interest. Jan Krzek declared that he has no conflict of interest. This article does not contain any studies with human or animal subjects.

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