Food Sci. Biotechnol. 19(5): 1199-1204 (2010)
DOI 10.1007/s10068-010-0171-2

RESEARCH ARTICLE

Rapid Method for Determination of β-Carotene in Foods Using Ultra High Performance Liquid Chromatography

Jaeho Ha, You-Shin Shim, Hye-Young Seo, Hyun-Jin Nam, Masahito Ito, and Hiroaki Nakagawa

Received: 3 March 2010 / Revised: 20 April 2010 / Accepted: 28 April 2010 / Published Online: 31 October 2010

Abstract The ultra high performance liquid chromatography (u-HPLC) method for determination of β-carotene in foods was validated in terms of precision, accuracy, and linearity. The u-HPLC separation was performed on a reversed column C_{18} (particle size 2 μm, i.d. 2 mm, length 50 mm), followed by ultra violet (UV) detection at 450 nm. The recovery of β-carotene was more than 84.4% and the limit of detection and limit of quantitation of u-HPLC analysis were 0.28 and 0.85 μg/mL for β-carotene with butylated hydroxytoluene (BHT) and 0.62 and 1.89 μg/mL for β-carotene without BHT, respectively. The calibration graph for β-carotene was linear from 0.1 to 25.0 μg/mL for u-HPLC. The intra- and interday precisions (relative standard deviations) were <7.5 and <7.8%, respectively. Benefits of u-HPLC analysis of β-carotene in foods is reduction of the analysis time to approximately 1/4, saving the volume of solvent to approximately 1/15. It seems that u-HPLC can offer significant improvements in speed, sensitivity, and resolution compared with conventional HPLC, this bodes well for future applications.

Keywords: β-carotene, rapid determination, ultra high performance liquid chromatography

Introduction

β-Carotene is an organic compound - a terpenoid, a red-orange pigment abundant in plants and fruits. Isolation of β-carotene from fruits abundant in carotenoids is commonly done using column chromatography. The separation of β-carotene from the mixture of carotenoids is based on the polarity of a compound. β-Carotene is a non-polar compound, so it is separated with a non-polar solvent such as hexane. Being highly conjugated, it is deeply colored, and as a hydrocarbon lacking functional groups, it is very lipophilic (1).

There have been many reports that β-carotene has the wide variety of function on the human health (2). For examples, Jialai *et al.* (3) and Sies *et al.* (4) reported that β-carotene, in addition to being an efficient quencher of singlet oxygen, can also function as a radical-trapping antioxidant. Numerous observational studies have found that major public health benefits could be achieved by increasing consumption of carotenoids-rich fruits and vegetables still appears to stand; however, the pharmacological use of supplemental β-carotene for the prevention of cardiovascular diseases and lung cancer, particularly in smokers, can no longer be recommended (5). Therefore, the demand of exact determination of β-carotene amount in foods is increased due to its two faces of the effect on the human health (6,7).

A lot of the analytical methods of β-carotene have been studied to date. The most common method for identification and quantification of β-carotene has employed the high performance liquid chromatography (HPLC) system combined with a ultra violet (UV) detector. An isocratic liquid chromatographic method was reported for the simultaneous determination of vitamin C, E, and β-carotene in human plasma (8). Especially, in the clinical chemistry, several researches have been made to determine the β-carotene in biological samples, such as human serum and tissues using liquid chromatography combined with reversed phase column and UV detection (9,10). Others

Jaeho Ha (✉), You-Shin Shim, Hye-Young Seo, Hyun-Jin Nam
Food Analysis Center, Korea Food Research Institute, Seongnam, Gyeonggi 463-746, Korea
Tel: +82-31-780-9127; Fax: +82-31-780-9280
Email: jhkfri@kfri.re.kr

Masahito Ito, Hiroaki Nakagawa
Naka Division, Hitachi High-Technologies Corporation, Hitachinaka-shi, Ibaraki-ken, 312-8504, Japan

employed the normal phase HPLC in a silica gel column with *n*-hexane-2-propanol as the mobile phase (11).

Dietz *et al.* (12) reported the reversed phase HPLC analysis of β-carotene from raw and cooked vegetable. Weissenberg *et al.* (13) also reported that a simple and rapid HPLC method had been devised in order to separate and quantify β-carotene present in red pepper fruits and food preparation using HPLC. A reversed-phase isocratic non-aqueous system enables the separation of β-carotene within a few minutes, with detection at 450 nm. Quantitative analysis of carotenoids and carotenoid esters in fruits by HPLC was introduced and analyzed without saponification using octadecyl silica as stationary and methanol-ethyl acetate as mobile phase (14). Recently ultra HPLC (u-HPLC) method coupled with mass spectrometry has been adopted in many areas of food and biological analysis due to its rapid analysis and remarkably excellent separation (15,16). Because u-HPLC has adopted the higher pressure, shorter column than conventional HPLC, minimizing peak dispersion providing improved speed, resolution, and sensitivity, it is well known that u-HPLC delivers fast analysis, higher resolution, and increased sensitivity without losing separation quality. Moreover, u-HPLC method has been known to be economical and environmentally friendly due to extremely rapid analysis. In concomitant with the fast analysis, the consumption of solvent for mobile phase can be reduced up to 5 to 10 fold, comparing with the conventional HPLC method. Therefore, our study was focused to evaluate the rapid analytical method for the determination of β-carotene in foods, using u-HPLC.

Materials and Methods

Materials Red pepper powder samples were purchased in the local market in Seongnam, Korea, and samples were stored at room temperature in an airtight container prior to analysis. All other samples for the applicability were also purchased in the local market in Seongnam, Korea. Standard β-carotene (>95%, $C_{40}H_{56}$, Fw 536.87, CAS 404-86-4) was purchased from Sigma-Aldrich (St. Louis, MO, USA). To make a stock solution for quantitation, weight accurately 0.2630 g β-carotene and 0.02 g butylated hydroxyl toluene (BHT), and dissolve in ethyl acetate: acetonitrile:acetic acid (30:68:2, v/v/v) with 0.22 mM BHT in a volumetric flask to produce a solution of approximately 2,500 μg/g. Other reagents such as ethylacetate and petroleum ether (ACS reagent grade), acetonitrile (chromatographic grade), and acetic acid (ACS reagent grade) were used.

Sample preparation The sample preparation was based on the conventional method except saponification procedure. Approximate 0.5 g of samples was taken into 22-mL vial (solid caps with PTFE liner 20-mm, Supelco, Bellefonte, PA, USA) and then 10 mL anhydrous ethanolic 1 N KOH and 0.02 g BHT were added, followed by tightly capping the tube. The vial was placed in a heating block (TECHNE DB-3D; Barloworld Scientific Ltd., Stone, UK) at 100°C for 30 min to saponify. After saponification, the vial was placed in a dark place and was allowed about 30 min in a room temperature to cool down. The supernatant was transferred from the vial into a 250-mL brown separating funnel through a filter paper (Whatman No. 2). Twenty mL of saturated NaCl solution and 40 mL of petroleum ether containing 0.22 mM BHT were added into the separating funnel. The separating funnel was vigorously shaken for 5 min, followed by collecting the supernatant into the 250-mL of round-bottom flask. The procedure above was repeated again and all the extracts were combined. After removing the petroleum ether under 40°C using a rotary evaporator, the concentrate was reconstituted up to 200 mL with a mobile phase into a volumetric flask. Finally, the sample solution was filtered through a 0.2-μm syringe type filter into a small glass vial for u-HPLC analysis.

Effect of BHT on the stability of β-carotene β-Carotene has been known to be light sensitive compound and deterioration can be easily occurred during storage and pretreatment of the sample. To avoid the change of total amount in samples during storage and pretreatment, it is recommended that BHT be added in the standard stock solution as well as sample solution for β-carotene analysis. For the rapid analysis of β-carotene, we adopted the heating block method instead of a refluxing method as a new way of reducing saponification time, accordingly, it was expected that the concentration of β-carotene could be affected by the procedure of sample preparation. At this point of view, we have evaluated the effect of BHT on the stability of β-carotene during method validation.

Analysis of β-carotene The concentration of β-carotene was determined using u-HPLC. The u-HPLC system (LaChromUltra L-2000 U Series; Hitachi-High Technologies Corp., Hitachinaka, Japan) was equipped with an eluant reservoir, u-HPLC pump (Model L-2200U), an autoinjection system of 5 μL injection at a fixed volume. LaChromUltra C_{18} (2 μm, 2 mm i.d.×50 mm L, Hitachi-High Technologies Corp.) was used as an analytical column. Mobile phase was ethylacetate: acetonitrile: acetic acid (30:68:2, v/v/v) with 0.22 mM BHT and flow rate was 0.2 mL/min. Detector was UV detector (Model L-2400U; Hitachi-High Technologies Corp.) set at the wavelength of 450 nm. For comparison purpose, conventional HPLC (c-HPLC) analysis was performed according to Korea Food Code.

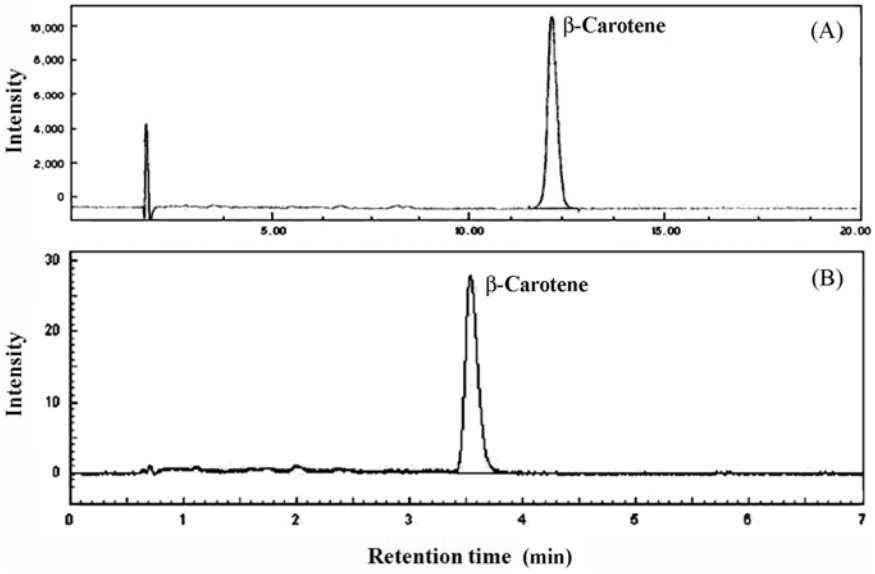

Fig. 1. Typical chromatograms of β-carotene. (A) Standard analyzed by c-HPLC, column: Atlantis column dC_{18} (particle size 5 μm, 4.6 mm i.d., length 150 mm; Waters, Ireland). Flow rate 1.0 mL/min, injection volume 20 μL; (B) standard analyzed by u-HPLC; column: LaChromUltra C_{18} (particle size 2 μm, 2 mm i.d. length 50 mm, Hitachi-High Technologies Corp.). Flow rate 0.2 mL/min, injection volume 5 μL.

Calibration graph Calibration graph for u-HPLC was based on peak area and prepared by injecting 5 μL of 0.5, 1.0, 5.0, and 25.0 μg/mL solutions prepared by the dilution of β-carotene stock solutions with a mobile phase.

Results and Discussion

Comparison of elution time by u-HPLC with conventional HPLC (c-HPLC) The chromatograms of β-carotene standard separated by c-HPLC and u-HPLC were illustrated in Fig. 1. The typical c-HPLC requires a considerable amount of analysis time (around 20 min) and considerable amount of solvent consumption for successful chromatographic analysis due to time of long-running. However, u-HPLC requires a short analysis time (around 5 min) for the achievement of the same chromatographic separation as c-HPLC, as shown in Fig. 1. It can be considered that u-HPLC method has been economically and environmentally friendly due to rapid analysis. Because of the fast analysis, the consumption of solvent for mobile phase can be reduced at least 4 fold.

Linearity To determine the linearity, 4 different concentrations of β-carotene standards were used in a working range from 0.5 to 25.0 μg/mL for u-HPLC method as illustrated in Fig. 2. Each solution was injected 3 times and the values represented the average of triplicate analysis. Regression analysis revealed a good relation (correlation coefficient R^2=0.9999 for β-carotene both without and with BHT). The graph showed a negligible

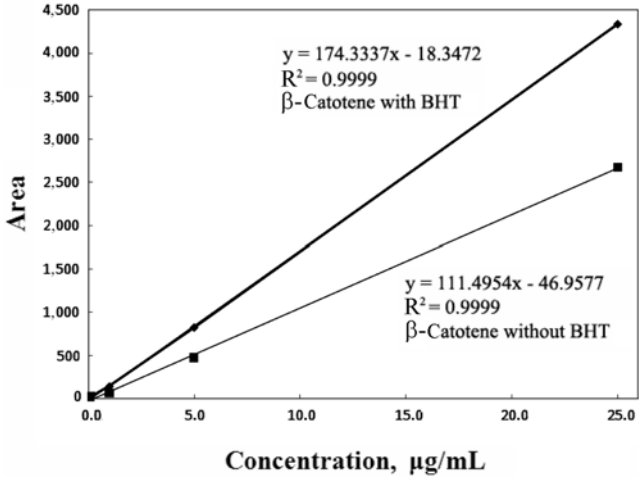

Fig. 2. Calibration graph of β-carotene with and without BHT by u-HPLC method.

intercept, which was calculated by the least-square method's regression equation.

Limit of detection (LOD) and limit of quantitation (LOQ) The calibration and sensitivity data of both β-carotene with BHT and without BHT were compared. The results are represented in Table 1. The LOD and the LOQ values of the method were estimated at an SD/b ratio of 3 and 10, where SD and b stand for the standard deviation of the intercept and slope of the regression line, respectively. LOD was 0.28 μg/mL for β-carotene with BHT and 0.62 μg/mL without BHT. LOQ was 0.84 μg/mL for β-carotene with BHT and 1.89 μg/mL without BHT. β-Carotene with

Table 1. Calibration and sensitivity data of β-carotene

Component	Linear range (μg/mL)	R^2	LOD (μg/mL)	LOQ (μg/mL)
β-Carotene (with BHT)	0.10-25.0	0.9999	0.28	0.84
β-Carotene (without BHT)	0.10-25.0	0.9999	0.62	1.89

Table 2. Precision and accuracy data of β-carotene in red pepper powder[1)]

Component	Spiked amount (μg/mL)	Intra-day (RSD%)	Inter-day (RSD%)	Recovery (%)
β-Carotene	1.4	2.4	4.3	84.4±2.6[2)]
	4.3	2.4	3.9	87.7±3.7
	12.8	7.5	7.8	85.8±5.3

[1)]Amount of red pepper powder taken=0.5 g; Values represent the mean of intra-day (n=3) and inter-day (n=12); Amount of β-carotene of red pepper powder in the initial sample was estimated to be 0.35±0.03 μg/mL.
[2)]Values represent the mean±SD (n=3).

BHT indicates more stable and less changeable than β-carotene without BHT at the same storage conditions. It has been revealed that the method with BHT had approximately 2 times more sensitivity than that without BHT. It can be elucidated that the differences of LOD and LOQ with and without BHT is caused by instability of standard solution of β-carotene in absence of BHT during analysis as well as sample preparation. Therefore, it is recommended that BHT should be added during preparation of standard solution as well as at the first stage of sample preparation.

Precision and accuracy Intra-day repeatability (n=3) and inter-day (n=12) test was performed on β-carotene in red pepper powder for u-HPLC methods. The relative standard deviations for intra-day repeatability at the spiked amounts at 1.4, 4.3, and 12.8 μg/g revealed 2.4, 2.4, and 7.5% and inter-day repeatability revealed 4.3, 3.9, and 7.6 %, respectively, as shown in Table 2. In order to study the accuracy of the methods, recovery experiments were carried out with the standard addition method. The recovery of the added standard to the assay samples was calculated from:

$$\text{Recovery } (\%)=[(C_t-C_u)/C_a]\times 100$$

where C_t is the total concentration of the analyte found, C_u is the concentration of the present analyte in the original red pepper powder, and C_a is the concentration of the pure analyte added to the original red pepper powder. The results are given in Table 2. The average recoveries obtained were quantitative 84.4-87.7%, indicating good accuracy of the u-HPLC method.

Table 3. Comparison results of the analysis of β-carotene in red pepper powders by u-HPLC and c-HPLC methods

(Unit: μg/g)

	u-HPLC	c-HPLC
Mean[1)]	147.26	146.13
SD	5.76	9.63
RSD	3.91	6.59
t-test[2)]	0.21 (2.02)	

[1)]n=6 for all the methods; SD=standard deviation; RSD=relative standard deviation
[2)]Tabulated values of t at p=0.05 are shown in parentheses.

Table 4. Applicability of the u-HPLC methods to various foods

Foods	β-Carotene (μg/mL)
Red-pepper powder	137.48±31.16
Strawberry	0.56±0.01
Tomato	0.82±0.02
Pomegranate	0.52±0.05
Red pimento-1	2.47±0.07
Red pimento-2	1.56+0.04

Applicability Application of u-HPLC method coupled with a heating block for the saponification of sample has been examined with the various foods. Because u-HPLC method has adopted the higher pressure and shorter column than conventional HPLC, peak dispersion is minimized, analysis speed is improved, and resolution and sensitivity is provided. In the meantime, the conventional HPLC can adopted as a short column as 5 cm in length, but, the resolution is sometimes not good enough to separate β-carotene in foods due to complex matrix of the food samples. We have found that the good separation can be achieved without losing resolution quality, using u-HPLC method coupled with a heating block for the saponification of food samples. Moreover, u-HPLC method has been known to be economical and environmentally friendly due to extremely rapid analysis because of the shorter column. In concomitant with the rapid analysis, the consumption of solvent for mobile phase can be reduced up to 5 to 10 fold, comparing with the conventional HPLC method.

Comparison results of the analysis β-carotene in red pepper powders are illustrated in Table 3. It is postulated that two methods have no significant difference in the results. To apply the analysis method to various foods, 6 samples, containing a different amount of β-carotene, were analyzed by the u-HPLC methods. The results showed that the u-HPLC method could be applicable to the wide variety of foods including strawberry, tomato, pomegranate, and red pimento and achieved the good separation without hindrance of interferences in foods as shown in Table 4 and Fig. 3.

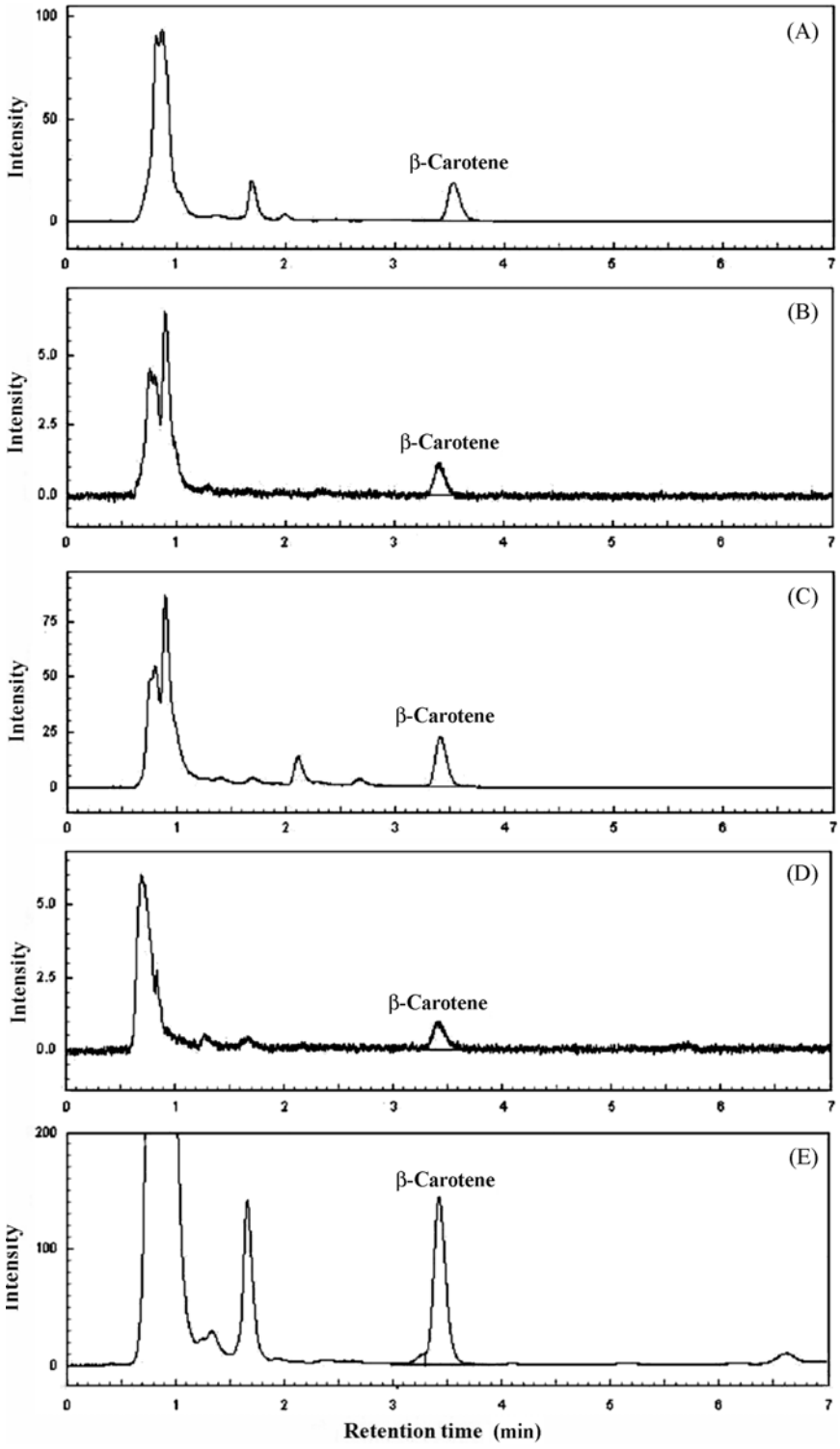

Fig. 3. Chromatograms of β-carotene in various foods using u-HPLC. Concentration of the β-carotene in the samples has been adjusted by the dilution of sample with a mobile phase to fit the peak height. A, red pepper powder; B, straw berry; C, tomato; D, pomegranate; E, red pimento

Acknowledgments We especially thank Hitachi-High Technologies Corporation for its financial support and also appreciate the technical advice received from Analytical Systems Design Department, Naka Division.

References

1. Wikipedia, The Free Encyclopedia. Beta-carotene. Available from http://en.wikipedia.org/wiki/beta-Carotene. Accessed Dec. 10, 2009.
2. Bertram JS. Carotenoids as cancer preventive agents. pp. 307-310.

In: Retinoids and Carotenoids in Dermatology. Vahlquist A, Duvic M (eds). Informa Healthcare USA, Inc., New York, NY, USA (2007)
3. Jialai I, Norkus EP, Cristol L, Grundy SM. Beta-carotene inhibits the oxidative modification of low-density lipoprotein. Biochim. Biophys. Acta 1086: 134-138 (1991)
4. Sies H, Stahl W, Sundquist AR. Antioxidant functions of vitamins. Vitamins E and C, beta-carotene, and other carotenoids. Ann. NY Acad. Sci. 669: 7-20 (1992)
5. Mayne ST. Beta-carotene, carotenoids, and disease prevention in humans. FASEB J. 10: 690-701 (1996)
6. Burton GW, Ingold KU. Beta-carotene: An unusual type of lipid oxidant. Science 224: 569-573 (1984)
7. Peto R, Doll R, Buckley JD, Sporn MB. Can dietary beta-carotene materially reduce human cancer rates? Nature 290: 201-208 (1981)
8. Zhao B, Tham SY, Lai MH, Lee LKH, Moochhala SM. Simultaneous determination of vitamin C, E, and β-carotene in human plasma by high-performance liquid chromatography with photodiode-array detection. J. Pharm. Pharm. Sci. 7: 200-204 (2004)
9. Driskell WJ, Bashor MM, Neese JW. Beta-carotene determined in serum by liquid chromatography with an internal standard. Clin. Chem. 29: 1042-1044 (1983)
10. Stahl W, Sundquist AR, Hanusch M, Schwarz W, Sies H. Separation of beta-carotene and lycopene geometrical isomers in biological samples. Clin. Chem. 39: 810-814 (1993)
11. Aaran RK, Nikkari TJ. HPLC method for the simultaneous determination of beta-carotene, retinol, and alpha-tocopherol in serum. J. Pharmaceut. Biomed. 6: 853-857 (1988)
12. Dietz JM, Kantha SS, Erdman Jr JW. Reversed phase HPLC analysis of α- and β-carotene from selected raw and cooked vegetables. Plant Food Hum. Nutr. 38: 333-341 (1988)
13. Weissenberg M, Levy A, Schaeffler I, Menagem E, Barzilai M. Rapid isocratic HPLC analysis of beta-carotene in red peppers (*Capsicum annuum* L.) and food preparations. Chromatographia 46: 399-403 (1997)
14. Gregory KG, Chen TS, Philip T. Quantitative analysis of carotenoids and carotenoids esters in fruits by HPLC: Red bell peppers. J. Food Sci. 52: 1071-1073 (2006)
15. Estella-Hermoso de Mendoza A, Campanero MA, Mollinedo F, Blanco-Prieto MJ. Comparative study of A HPLC-MS assay versus an UHPLC-MS/MS for anti-tumoral alkyl lysophospholipid edelfosine determination in both biological samples and in lipid nanoparticulate systems. J. Chromatogr. B 877: 4035-4041 (2009)
16. Mallett DN, Ramirez-Molina C. The use of partially porous particle columns for the routine, generic analysis of biological samples for pharmacokinetic studies in drug discovery by reversed-phase ultra-high performance liquid chromatography-tandem mass spectrometry. J. Pharmaceut. Biomed. 49: 100-107 (2009)