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Analytical Methods

Extraction and quantification of major carotenoids in processed foods and supplements by liquid chromatography ☆

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ABSTRACT

A simple, fast and robust method with minimum steps, small sample size and amounts of solvents was developed to determine major carotenoids contents in processed foods, tablets and gel capsules. The method involves dispersion of the sample in hot water (60 °C) with added butylated hydroxytoluene (BHT) in ethanol to minimize oxidation, followed by extraction with chloroform and analysis by liquid chromatography. Chromatographic parameters were: a C30 column protected with a C18 guard cartridge; gradient elution at the rate of 1.0 mL/min starting with 100% methanol (A) and ending with 40:60 (v/v) methanol/isopropanol (B); detection set at 450 nm for carotenoids, and 325 nm for retinol, retinyl acetate and retinol palmitate. The method exhibited: (i) high degree of repeatability (%rsd); (ii) linear calibration curves ($r^2 \geqslant 0.9998$); (iii) low detection; and quantification limits. The method was validated with standard reference material 2383 for *trans*-β-carotene; and tested for α-, and β-carotenes, lutein, zeaxanthin, cryptoxanthin, *trans*-retinol in processed foods, tablets and gel supplements.

1. Introduction

Numerous epidemiological and several clinical studies have shown an association between consumption of carotenoids and reduced risk of several chronic diseases. Canada and the US allow several health claims regarding specific nutrient or dietary supplement and its role in maintaining good health condition or reducing the risk of certain disease(s). The role of dietary carotenoids in enhancing immune function has been reviewed by Hughes (2001), as well as the role of lutein in eye health (Fullmer & Shao, 2001).

β-Carotene has enjoyed centre stage for a number of years because of its provitamin A activity. Vitamin A deficiency is most notable in developing countries, especially during winter months that have major impact on the growth of children (Scrimshaw, 2000). Lutein is associated with reduced incidence of cataract (Granado, Olmedilla, & Blanco, 2003; Olmedilla, Granado, Blanco, Vaquero, & Cajigal, 2001), age-related macular degeneration (AMD) (Bone, Landrum, Guera, & Ruiz, 2003; Bone et al., 2001; Sheddon, Ajani, & Sperduto, 1994), and cancer (Michaud et al., 2000); lycopene is linked with reduced risk of prostate cancer (Clinton, 1998; Giovannucci, 1999; Rao & Agarwal, 1999). As such carotenoids are promoted globally as value added products for maintaining good health. Incidently, a recent report predicts "the world carotenoid market is expected to reach Euro 0.77 billion ($1.06 billion) by 2010 as consumers continue to look for natural ingredients" (Nutraingredient, 2007). This has resulted in and will continue introduction and promotion of a myriad of foods and dietary supplements that are expected to provide daily requirements of carotenoids for a healthy lifestyle. The continued and projected demands for carotenoids, especially β-carotene, lutein, zeaxanthin are bound to increase misuse of the term carotenoids and adulteration with sources that may not contain bioavailable carotenes. Such abuses of carotenoids has necessitated the need for better analytical methods that are inexpensive, rapid, robust for the detection and quantification of a variety of heath promoting carotenoids in a variety of food matrices, vitamin tablets, supplements, and gels among others. Although, spectrometry is still the most often used technique for the analysis of β-carotene in commercial products, it has limitation because it cannot differentiate between isomers and other carotenes (Schierle, Pietsch, Ceresa, Fizet, & Waysek, 2004). The quantitation of *cis* and *trans* isomers of β-carotene is important because they have different physiological benefits (Szpylka & DeVries, 2005). A number of liquid chromatography (LC) methods for the analysis of carotene and vitamin A have been published (Sun, 1999; Sundaresan, 2002; Tee & Khor, 1995; Ye, Landen, & Eitenmiller, 2000). Only a few reports have focussed on the simultaneous detection of retinols and carotenoids in food products using dual wavelength techniques (Bueno, 1997; Sundaresan, 2002; Tee & Khor, 1995; Ye et al., 2000). High performance liquid chromatography (HPLC) is the method of

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choice for separation and quantification of carotenoids. Recent articles review the analysis of carotenoids in vegetable and plasma samples (de Quirós & Costa, 2006; Ladislav, Vera, Karel, & Karel, 2005). International Expert Review Panel (IERP) (AOAC, 2003) on β-carotene evaluated a number of methods including that reported by Sundaresan (2002) with a view of having a method that would simultaneously analyze both carotenoids and retinoids. This was followed by a single laboratory validation report (Schierle et al., 2004), and a collaborative study that involved twelve laboratories located in four countries for the determination of β-carotene in supplements and raw materials using reversed phase HPLC (Szpylka & DeVries, 2005). In recent years HPLC has been reported to detect and quantify other carotenoids including β-carotene in palm oil (Mortensen, 2005), pumpkin (Seo, Burri, Quan, & Neidlinger, 2005), and vegetables (Barba, Hurtado, Mata, Ruiz, & de Tejada, 2006).

The known and/or perceived health benefits of antioxidants have created an extraordinary demand for compounds with antioxidant activity. Demand for carotenoids and especially β-carotene has led to unprecedented growth in foods, drinks, supplements, ingredients with carotenoids. This has also introduced potential for adulteration and false claims. In recent years consumer weary of medicine are relying heavily on over the counter herbal and natural health products. In order to protect consumers, there is a strong need for a rapid, robust, inexpensive and accurate method for monitoring carotene content, especially α-, and β-carotenes, and lutein in various food products. Very recently, Nutraingredients USA (2005) reported the validation of β-carotene in supplements except in gel formulation.

Our research was aimed at identifying a robust method that detects, separates and quantitates a number of important carotenoids (α-, β-carotenes, lutein) in diets and supplements including gels rapidly and in minimum of steps. β-Carotene sold in different amounts in tablets, gels, juice are taken by people as an immune booster. A vast majority of human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AID) patients take herbal supplements including β-carotene concurrently with therapeutic protease inhibitor. The amount of β-carotene listed on supplements is not always accurate or guaranteed. Accuracy of the amount of β-carotene in supplement was essential for a planned study on the effect of β-carotene on the disposition of a protease inhibitor when taken concomitantly. The method reported here was employed to establish the amount of β-carotene in supplements at the start and stability during clinical trials. Further, we validated accuracy of the method with National Institute of Science and Technology (NIST) standard reference material 2383 (Sharpless, Arce-Osuna, Thomas, & Gill, 1999; Sharpless, Gill, Margolis, Wise, & Elkins, 1999), and extended the use of this method to determine β-carotene content in supplements, juice and baby food puree. However, in the current studies no special efforts were made to separate, identify and quantify other minor isomers (e.g. 13-*cis*-β-carotene) in any of the materials analyzed. The method reported here involves treating the sample with 2,6-di-*tert*-butyl-4-methylphenol (commonly referred to as BHT) in ethanol followed by dissolution/suspension/dispersion of sample in hot (60 °C) 0.1 N HCl and then by partition/extraction with chloroform. The chloroform extract, after drying and reconstituting in 50:50 (v/v) methanol/isopropanol 0.05% BHT, is then analyzed on a C30 column using a gradient mobile phase starting with 100% methanol to a 40:60 (v/v) methanol/isopropanol in 45 min. This method resolves lutein, α-, β-carotenes and 13-*cis*-β-carotene (standard was not available, tentative identification based on retention time and spectral profile reported (Marx, Schieber, & Carle, 2000), zeaxanthin and cryptoxanthin at 450 nm and retinol, retinol acetate and retinol palmitate at 325 nm.

2. Experimental

2.1. Chemicals and solvents

HPLC grade solvents methanol (MeOH), ethanol, hexane, isopropanol (IPA), and chloroform were purchased from Caledon Laboratories Ltd., Georgetown, ON, Canada. Standards from Sigma (St. Louis, MO, USA) include: β-carotene (synthetic minimum 95%, carotene), a mixture of α- and β-carotene from carrots (in 42:57 ratio); lutein (xanthophyll from Alfalfa 93% purity), retinol >95% retinol acetate (94% purity) and retinol palmitate (90.2% purity), 2,6-di-*tert*-butyl-4-methylphenol (BHT > 99.0%).

β-Cryptoxanthin (95% purity) and zeaxanthin (purity not listed) were purchased from Indofine Chemical Company, Hillsborough, NJ, USA.

2.2. Standard reference material

Standard reference material 2383 (Baby Food Composite with certified concentrations of several carotenoids; certificate valid until 31 August 2007) was purchased from National Institute of Standards and Technology, Washington, DC, USA (NOTE: The certificate does not provide a HPLC chromatogram).

2.3. Over the counter (OTC) products

Premixes, supplements, carrots puree, and juices were purchased over the counter from local grocery stores. Exact β-carotene capsule (25000 IU β-carotene); Exact essentra élite tablets (multivitamins and multiminerals formula for senior, Pharmetics Inc., 3000 IU β-carotene, 3000 IU retinol acetate, 0.25 mg lutein/zeaxanthin); Jamieson β-carotene tablets (25000 IU β-carotene); Spectrum multivitamin tablets (Life brand 3000 IU β-carotene, and 2000 IU retinol acetate); Icap multivitamin tablets (lists 3300 IU β-carotene, 2 mg lutein/zeaxanthin); Swiss natural sources of soft gel capsules (claims 10000 IU β-carotene); Bolthouse Farms 100% carrot juice (lists a 240 mL serving would provide 700% of vitamin A (α-, and β-carotene); Heinz beginner carrot puree (360% DV vitamin A per 64 mL serving).

2.4. High performance liquid chromatography (HPLC)

Liquid chromatography was performed on a Surveyor HPLC system equipped with quaternary pump with built-in degasser, an autosampler, compartment temperature control unit, and photodiode array detector (PDA) from Thermo-Finnigan, San Jose, CA, USA. Separation was carried out at 30 °C (Davey, Keulemans, & Swennen, 2006; Schierle et al., 2004) on a Develosil 5 μm RP-AQUEOUS C30 (4.6 × 250 mm) with guard system with a C18 cartridge (4 mm × 3.0 mm) from Phenomenex, Torrance, CA, USA. The binary gradient mobile phase with a flow rate 1.0 mL/min was setup as shown in Table 1.

Table 1
Mobile phase composition during a run

Time (min)	MeOH (%A)	MeOH:IPA 40:60 (%B)
0	100	0
5	98	2
10	20	80
44	0	100
45	100	0
50	100	0

2.5. Preparation of stock and analytical standard solutions

Stock solutions (0.1 mg/mL) of mixed α- and β-carotene and all *trans*-β-carotene were prepared by weighing and dissolving in hexane containing 0.05% BHT. Other carotenoids and retinols were dissolved in ethanol containing 0.05% BHT except for lutein which was dissolved in ethyl acetate. All preparations were subdivided and stored in amber autosampler vials at −20 °C before use. The concentration of the working standards were determined from the extinction coefficient using a dual beam Cary 3C spectrophotometer (Varian Analytical Instruments, Walnut Creek, CA, USA) to measure the stock solutions concentration. For β-carotene at 450 nm the concentration is calculated as follows:

$$C_{\text{all-E-}\beta\text{carotene}}\ (\text{mg/L}) = \text{Absorption} * 10,000/2592 \times (\text{dL/g cm, extinction coefficient unit}) \quad (1)$$

where 2592 (Sharpless, Arce-Ossuna et al., 1999) is the extinction coefficient in hexane and 10,000 is the factor to convert% to mg/L (Schuep & Schierle, 1997). Similarly, the extinction coefficient of 2550 (Sharpless, Gill et al., 1999) and 1560 (DeLeenheer, Lambert, & Van Bocxlaer, 2000) in ethanol were used for quantifying lutein and retinol acetate standards at 444 and 325 nm, respectively. Analytical standards were prepared from stock solutions with appropriate dilution in 50:50 (v/v) MeOH/IPA:0.05% BHT. The standards were corrected for chromatographic purity (CP) with the following formula (Szpylka & DeVries, 2005):

CP = (Area of standard)/(sum of the areas of all relevant peaks)

The peak area of the standard is the percentage of the total peak area when run at the standards detection maximum wavelength. Fifty microlitres of stock were added to 1.0 mL 50:50 (v/v) MeOH/IPA:0.05% BHT and run six times at the standard's detection wavelength. Having the software report the area% gives the CP result.

2.6. Recovery of fortified standards

For recovery studies, two multivitamin tablets (Life Spectrum Forte) were crushed with an all glass mortar and pestle. The outer coat was removed by sieving (mesh 600 μm). One-gram portion of each was placed in two separate 50 mL polyethylene tube (Falcon). One was spiked with known amounts of β-carotene, lutein, or retinyl acetate while the other was used as blank and was processed the same as the spiked powder. To each sample was added 0.5 mL of BHT solution in ethanol (30 mg BHT/mL of ethanol) followed by 10 mL of 0.1 N HCl kept at 60 °C. The sample was then vortexed for 15 s followed by the addition of 15.0 mL of chloroform. The entire mixture was rocked gently (no vigorous shaking) to avoid serious emulsion, and then centrifuged at 3220 g for 5 min. The organic layer was removed and the aqueous layer was re-partitioned with 15.0 mL of chloroform. A 1.0 mL aliquot of the combined organic layer was evaporated under a stream of nitrogen, and the residue was reconstituted with 1.0 mL of 50:50 (v/v) MeOH/IPA:0.05% BHT for HPLC analysis. Recoveries (n = 5–10) were calculated using AOAC method (1998).

2.7. Extraction of standard reference material and over the counter (OTC) products

The supplements were stored at room temperature in their containers prior to analysis. Food, reference material and drink samples were stored at 4 °C in the dark. All extraction and analyses were performed under subdued lighting.

2.7.1. Standard reference material 2383

The content of the standard was transferred to a 250 mL plastic container to expedite through mixing followed by sub-sampling. A 2.5 g aliquot was placed in a Potter-Elvehjem 45 mL tissue homogenizer (Thomas Scientific, Swedesboro, NJ, USA) tube, to which was added 10.0 mL chilled 0.1 N HCl containing BHT (calculated amount 15 mg), and 15 mL chloroform. The sample as agitated by a polytetrafluoroethylene (PTFE) pestle at 150 rpm for 12 strokes followed by spinning at 3220 g to separate layers. Organic layer was withdrawn, and the procedure was repeated twice with the aqueous phase. A 5 mL chloroform layer was withdrawn and evaporated to dryness under nitrogen, and the residue was redissolved in 50 μL of tetrahydrofuran (THF), which was diluted with 1.0 mL of 50:50 (v/v) MeOH/IPA with 0.05% BHT and was analyzed by HPLC.

2.7.2. Supplements (tablets and gel capsules, but not beads)

To avoid light induced reactions, weighed sample was placed in a 50 mL aluminum foil covered Falcon tube along with 0.5 mL of BHT (30 mg/mL in ethanol). To this was added 10 mL of hot (60 °C) 0.1 N HCl. After standing for 1 min, the gel capsule breaks up releasing its content. In general, water soluble tablets require vortexing to disperse them. Using a volumetric pipette, 15.0 mL of chloroform was added to the above mixture at room temperature. It was gently vortexed for 30 s and then centrifuged at 3220*g* for 5 min at room temperature. The organic phase was removed and stored in aluminum foil covered Falcon tube while the aqueous phase was re-extracted with another 15.0 mL of chloroform. Extracts were combined and a 10 μL aliquot was withdrawn and diluted with 1.0 mL 50:50 (v/v) MeOH/IPA:0.05% BHT for HPLC analysis. In cases where larger volume of extract was needed, the solvent was removed under nitrogen and the residue was re-constituted into 1 mL of 50:50 (v/v) MeOH/IPA:0.05% BHT for HPLC analysis.

2.7.3. Pureed food

Pureed baby food contents were mixed before sub-sampling. An aliquot (~2 g) was accurately weighed into a Potter–Elvehjem 45 mL tissue homogenizer (Thomas Scientific, Swedesboro, NJ, USA). The sample was vortexed for 30 s after addition of 0.5 mL of BHT in ethanol (30 mg/mL) and 10 mL of 0.1 N HCl (60°C). Upon adding 15.0 mL chloroform, the mixture was stirred with a motor-driven PTFE pestle at 150 rpm for 12 strokes. The mixture was then transferred into a 50 mL covered Falcon tube and centrifuged at 3220*g* for 5 min, extracted as reported in 2.7 (i) at room temperature. A 0.5 mL aliquot was withdrawn into an amber tube, dried under nitrogen, and the residue was re-constituted with 1 mL of 50:50 (v/v) MeOH/IPA:0.05% BHT for HPLC analysis.

2.7.4. Juice

The same procedure as for pureed food was followed, except that 2.0 mL of juice was used and there was no addition of 0.1 N HCl.

Samples and standards of 20 μL were detected in the photodiode array detector set to scan from 192 to 798 nm with discrete wavelengths of 325 and 450 nm.

2.8. Statistical analysis

The recoveries and quantitative analyses of carotenoids and retinols were conducted in triplicate and the percent coefficient of variation (%rsd) was determined using the formula (standard deviation of array X) × 100/(average of array X).

3. Results and discussion

Carotenoid supplements, in particular β-carotene and lutein, are used extensively for their antioxidant properties. Over the years a number of methods for the analysis of carotenoids mainly for

vitamin A and β-carotene have been published and used for monitoring purposes. However, collectively they involve steps including saponification followed by extraction, clean up, analysis by thin layer chromatography, spectrophotometry, and liquid chromatography. In view of the continued consumer demands for the quality of supplements, there is a stronger need to identify a method that is robust, less cumbersome and has the potential for universal adaptability including in third world countries where resources are limited. Under the WTO agreements developing countries are eying markets in developed countries to export raw and processed materials for use in tablets, capsules, food products, but need to meet standards set by individual importing country's quality control standards. At the same time, the importers must be assured of the quality of imported materials. Hence there is a compelling need for a robust method.

The use of both C18 and C30 columns for separation under various solvent systems and column operating temperatures was investigated. In all systems investigated a C30 column provided the best separation and resolution compared to a C18 column. A combination of other solvent mixtures consisting of methanol, acetonitrile, ethyl acetate was also investigated, but none provided a satisfactory separation and resolution (not reported). The choice of 30 °C column operating temperature was to satisfy efficiency criteria such as separation of major carotenoids, sharpness of peaks and an improved throughput without effecting the value of data. An additional consideration was the application of the HPLC conditions in the developing countries where it would be economically feasible to maintain a temperature at 30 °C than ambient (22–25 °C in western countries). We showed independently that carotenoids, especially lutein, did not degrade during the analysis. This may, in part, be due to short stay in column (retention time 11–12 min). In recent years several researchers have reported operating C18 and C30, columns at 30 °C and a slightly higher temperature for the analysis of carotenoids without any mention of degradation (Albert, 1998; Barba et al., 2006; Breithaupt & Schlatterer, 2005; Sander, Sharpless, & Pursch, 2000; Zanatta & Mercandante, 2007). Finally, a solvent system consisting of methanol and isopropanol in various ratios were tested. The ratios of MeOH and IPA listed in Table 1 provided the best system for separation of all important carotenoids except lycopene, which did not elute under these conditions. However, lycopene could be made to elute with the addition of 10% methyl *tert*-butyl ether (MTBE), but baseline separation of other carotenoids was compromised and run time was increased. For this reason there was no attempt to elute

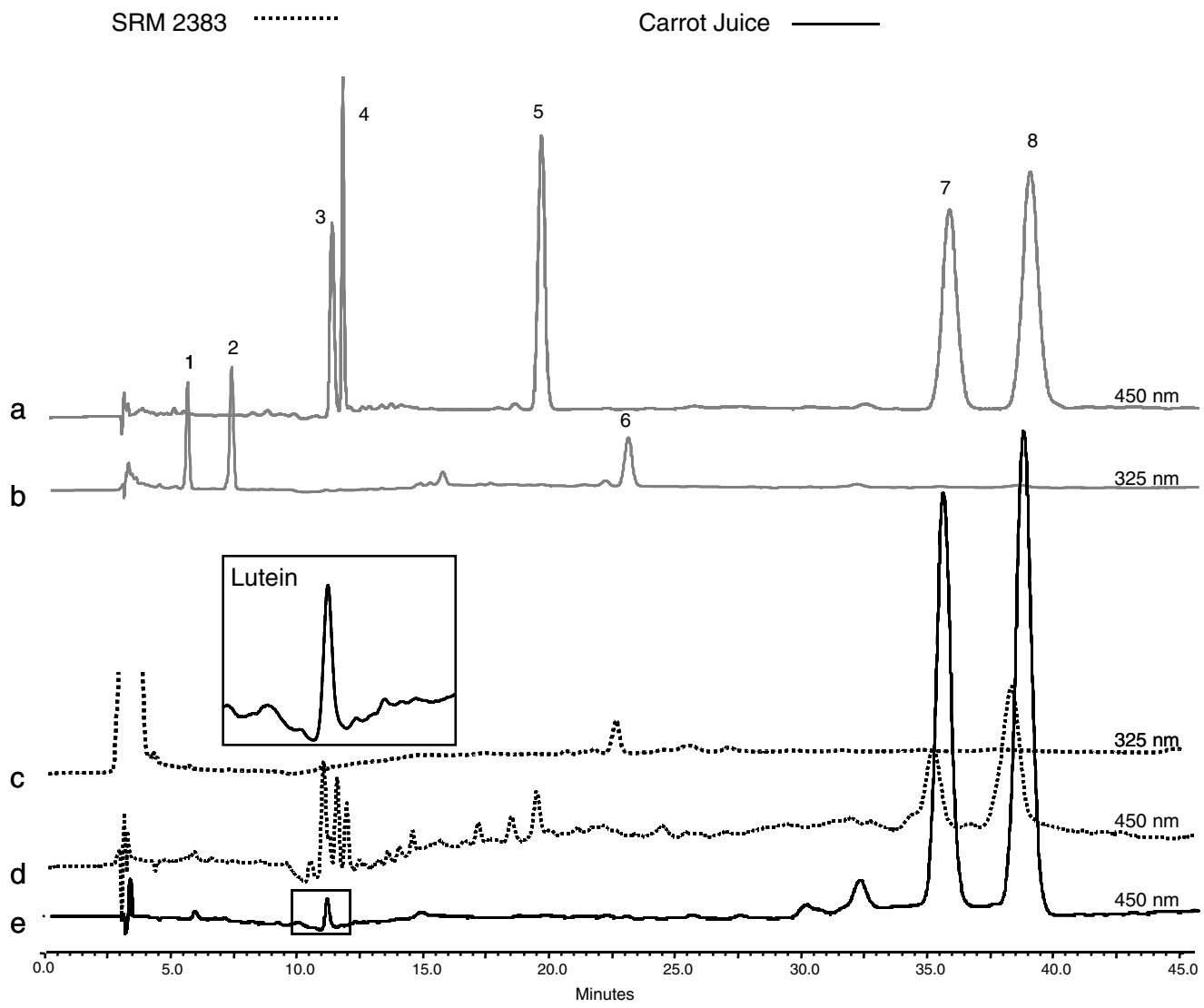

Fig. 1. Chromtograms of standards. Peaks in a and b: (1) all *trans* retinol; (2) retinol acetate; (3) all-*trans*-lutein; (4) zeaxanthin; (5) β-cryptoxanthin; (6) retinol palmitate; (7); all-*trans*-α-carotene and (8) all-*trans*-β-carotene, Standard Reference Material 2383 (c, d – NIST) and Carrot Juice (e – Bolthouse Farms, 100%).

lycopene with other carotenoids in the same run. Carotenoids were detected at 450 nm; whereas retinol and its acetate and palmitate derivatives exhibited signals at 325 nm. Fig. 1a and b are chromatograms of all carotenoids at 450 nm and the retinol and derivatives at 325 nm, respectively. It is seen that all carotenoids of interest are fully separated from each other, including α- and β-carotenes. There existed a linear relationship between (0.1–8.5 ng/μL) concentration on the column and the absorbance, and the relative coefficient (r^2) values for all compounds tested were greater than 0.999 (Table 2), which were calculated using linear regression equation:

$$y = mx + b;$$

where m is the slope and b is the intercept. These values for individual compound are reported in Table 2.

Our next effort was to develop a simplified extraction method that required minimum steps and also avoided use of large quantities of solvents including chlorinated solvents. A review of published methods showed that most sample preparation used large sample size and amount of solvents including chloroform and dichloromethane, and included steps that are cumbersome and/or unnecessary. Firstly, the sample size was reduced for spiked and test materials (supplements, tablets, puree, juice, etc.), but increased the number of replicates. Secondly, the saponification step was eliminated because we found it to be cumbersome and involved extra efforts without any benefits. It is worth mentioning here that previous workers (Marx et al., 2000; Szpylka & DeVries, 2005) also did not saponify samples when analyzing carrot juice and oily samples for carotenes. Further, we were also mindful of the Panel's consensus that saponification while advantageous for the analysis of vitamin A in the form of the acetate and palmitate ester would be detrimental to the analysis of carotenoids (International Expert Review Panel Community Assessment of Beta Carotene Methods, 2003). Thirdly, several smaller amounts of extracts were used for further work-up and analysis by HPLC. The efforts to replace halogenated solvents with non-halogenated system were not very fruitful. Also replacing chloroform with dichloromethane and other non-halogenated solvent was not very productive because recoveries were considerably low (<70%) and not reproducible. Hence, chloroform was used as the extraction solvent, but in reduced quantities. Craft and Soares (1992) had established that chloroform is the best compromised organic solvent for solubility, stability, and absorptivity.

Table 2
Validation parameters β-carotene, lutein, retinyl palmitate and retinol

Source	*trans* Retinol	Lutein	Retinyl palmitate	β-Carotene
Repeatability %rsd	0.60 (n = 10)	0.60 (n = 12)	0.61 (n = 11)	5.55 (n = 12)
Linear range μg/mL	0.03–5.97	0.05–8.78	0.04–8.21	0.059.09
r^2	0.9998	0.9996	0.9999	0.9998
slope (m) in [$y = mx + b$]	907,878	1,083,156	467236	436,453
intercept (b) in [$y = mx + b$]	4908	33361	−10630	−11828
LC purity %	99.268	91.615	93.959	87.921
intermediate precision %rsd	–	3.87 (1 month)	1.50 (1 week)	6.58 (4 months)
Detection limit 3× noise	0.003 μg/mL	0.008 μg/mL	0.013 μg/mL	0.012 μg/mL
Quantitation limit 10× noise	0.011 μg/mL	0.028 μg/mL	0.043 μg/mL	0.041 μg/mL

Table 3
Recovery of various carotenoids and retinyl acetate from fortified tablets

	Amount spiked mg	Recovery % (#samples)	% rsd	Intraday %rsd	Detectable level ng
β-Carotene	0.048–0.082	103.8 (8)	7.11	1.10	4.8
β-Carotene	0.172–0.192	100.6 (5)	3.29	–	–
Lutein	0.097–0.098	105.8 (10)	5.97	0.32	1.4
Retinyl acetate	0.041–0.085	97.7 (4)	6.78	1.15	1.4

Table 3 lists recoveries of β-carotene, lutein and retinyl acetate from fortified tablets. Recoveries of spiked substances were excellent and reproducible for all compounds studied. For example, recovery for all-*trans*-β-carotene ranged between 100.6% and 103.8% (n = 13; relative standard deviation (rsd%) ranged between 3.3 and 7.1); lutein 101.7% and 110.9% (n = 9; rsd 5.87%) and retinyl acetate 89.7% and 105.3% (n = 4; rsd 6.78%). These data are indicative of robustness and accuracy of the method for supplements.

In order to validate the precision, accuracy and values of our method including recoveries, we analyzed standards reference material 2383 (baby food composite). The concentration using our method was 89.2% (0.67 mg/kg vs. 0.75 mg/kg) and 105.4% (2.53 m/kg vs 2.4 mg/kg) of those listed in Table 2 of the NIST certificate of analysis for free lutein and free (unsaponified) β-carotene, respectively – an excellent validation (Table 4). The content of α-carotene in baby food composite was also calculated, but the values were considerably (almost double: 1.71 mg/kg vs. 0.86 mg/L) higher than those listed on the certificate, for which we have no explanation except that we used β-carotene calibration curve to calculate content of α-carotene. This approach was tested with a mixture of α-, and β-carotene (42:57) obtained from Indofine, and was found to be satisfactory. This technique was employed for the calculation of α-carotene in all other samples.

Having validated the method with NIST certified baby food composite, the validity of the current method was tested by analyzing several over the counter supplements (OTC), juice, baby food that claimed to contain specific amounts of β-carotene and lutein. A number of the OTC are sold mainly as multivitamins and contain substances other than β-carotene and lutein. Table 4 lists the claimed and observed values of β-carotene and lutein in various samples tested. The results clearly show the β-carotene and lutein amounts were in good agreement with what were claimed. The Exact essentra élite contained 4,049 IU of β-carotene, a 35% higher than the claimed 3000 IU per tablet.

The Bolthouse 100% carrot juice (made exclusively from fresh-pressed baby-cut carrots-information from web) lists a serving of 240 mL will provide 700% daily requirements of vitamin A in the form α and β-carotenes (no ratio provided). Fig. 1e is the chromatogram of Bolthouse carrot juice extract, which confirms that major carotenoids were indeed α-and β- carotenes, and was present in 44:56 ratio, along with small amounts of lutein (0.33 mg per serving). Furthermore, the chromatographic profile of Bolthouse carrot juice (Fig. 1e) was very similar to that reported for carrot juice (Marx et al., 2000). The small peak in Fig. 1e preceding major α-carotene peak tentatively may be assigned to 13-*cis*-β-carotene because of similar retention times (32.5 min vs. 34 min (estimated) were reported previously (Marx et al., 2000). However, its identity was not confirmed due to no-availability of authentic standard.

Until recently the 1968 National Academy of Sciences' recommended daily allowance (RDA) of 5000 IU for vitamin A was used as reference. However in 2001 the Food and Nutrition Board (FNB) of the Institute of Medicine (2000) revised the RDA for vitamin A as preformed vitamin A (retinol) for male adults at 3000 IU and females 2333 IU. The average RDA for vitamin was established at 2667 IU or 800 retinol equivalent (RE) of vitamin A. Similarly, in Canada, the RDA was 1000 RE for all persons of more than 2 years of age (Canadian Food Inspection Agency, 2003), but is now harmonized with the US (Health Canada, 2003).

To determine RE values, the conversion factors are as follows (Dietary Reference Intakes for Vitamin A et al., 2000):

Table 4
α and β-carotene, lutein and retinol content of selected dietary supplement and food products

Source	Retinol mg ± %rsd	Lutein mg ± %rsd	α-Carotene I.U. ± %rsd	β-Carotene I.U. ± %rsd
SRM 2383 Baby food composite	1.05 ± 5.42 (77.8%)[g] mg/kg	0.669 ± 3.94 (89.2%)[b] mg/kg	2.84 ± 8.7 (200.8%)[a] 1.71 mg/kg	4.22 ± 13.1 (105.4%)[c] 2.53 mg/kg
Exact beta carotene/capsule	n.d.	n.d.	n.d.	24513 ± 1.1 (98.1%)[a] 98272 mg/kg
Jamieson Beta Carotene/tablet	n.d.	n.d.	n.d.	23005 ± 2.4 (92%)[a] 22710 mg/kg
Exact essentra élite/tablet	3643 (121%)[d]	n.d.	n.d.	4049 ± 1.3 (135%)[a] 1443 mg/kg
Life Brand –Spectrum	2347 (117%)[c] mg/tablet	0.404 ± 1.52 (109%)[a] mg/tablet	n.d.	3317 ± 2.3 (110.5 %)[a] 1523 mg/kg
Icap tablets	n.d.	4.74 ± 0.48 (125%)[a] μg/tablet	n.d.	2866 ± 2.2 (86.8%)[a] 1803 mg/kg
Swiss Natural sources – soft gel capsule	n.d.	n.d.	n.d.	10194 ± 1.3 (101.9%)[a] 5257 mg/kg
Bolthouse farms – 100% carrot juice/serving (240 mL)	n.d.	0.8 ± 1.98([e])	20061 ± 0.9([f]) 50.2 mg/L	25612 ± 1.0 (94.3%)[f] 64.0 mg/L
Heinz beginner carrot/serving (64 mL)	n.d.	0.9 ± 2.07[e]	4449 ± 1.3([e]) 43.0 mg/kg	8232 ± 1.4 (86.0%)[a] 78.4 mg/kg

[a] Reported label values.
[b] Lutein (free).
[c] *trans*-β-Carotene.
[d] As retinol acetate.
[e] Not reported on label.
[f] Reported as alpha and beta carotene.
[g] As retinol palmitate.

1 RE = 1 μg retinol = 12 μg β-carotene = 24 μg α-carotene = 24 μg β-cryptoxanthin.

The IU values in one serving of 240 mL of Bolthouse juice were determined to be 20,060 and 25,612 for α- and β-carotene, respectively. Using the conversion factors given above a single serving of 240 mL juice would provide 5940 RE (1672 RE from α-carotene and 4268 from β-carotene), which is 66 times higher or 660% of the daily recommended allowance of 800 RE. This compares favourably well with claimed value of 700% RDA.

The analysis of Exact, Jamieson, Icap and Swiss brand β-carotene supplements, where the values of β-carotene are given, provide a very good confirmation of the β-carotene content in those samples, which were 98%, 92%, 87% and 102%, respectively (Table 4) of the listed values. Also, the analysis found both α-, and β-carotenes in Heinz beginner carrot, which was 86% of the β-carotene. Free retinol was not present in any material analyzed. A few samples had detectable and quantifiable amounts of retinyl acetate.

4. Conclusions

The method reported above is simple, fast and suitable for analysis of several free major carotenoids of high interest in several matrixes including juice, puree, gels and tablets. One of the major advantages is that the method is reproducible and eliminates saponification step, uses small amounts of sample and organic solvents and the analysis time is fairly reasonable (under an hour). The method has great potential for adoption by most labs globally including developing countries strapped for funds but engaged in routine monitoring analysis of supplements, food products for free α- and β-carotenes and lutein for domestic and international markets. The method has not been fully validated for other carotenoids and retinyl derivatives.

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