Simultaneous Determination of Vitamin A and β-Carotene in Dietary Supplements by Liquid Chromatography

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Several liquid chromatography (LC) methods for analysis of vitamin A in foods and feeds have been previously reported but only a few have been applied in non-food matrices. A validated LC method is needed for determination of vitamin A and β-carotene in the various matrixes presented by dietary supplements. The performance of a reversed-phase method with methanol–isopropanol gradient elution was evaluated with standard retinyl derivatives and β-carotene. The reversed-phase method is capable of separating retinol from other derivatives such as retinyl acetate, retinyl palmitate, and β-carotene. Two types of extraction were used to extract the analytes from the dietary supplements: a hexane–methylene chloride extraction for soft-gel capsules containing β-carotene, and a direct solvent extraction for dietary supplements in tablet form. The direct solvent extraction consisted of treatment with ethanol and methylene chloride following addition of hot water (55°C). Results with the reversed-phase method for vitamin A and β-carotene in the products examined (n = 8) indicated excellent method performance. The main form of vitamin A or β-carotene in dietary supplements was the all-trans isomer. The reversed-phase method avoids saponification and is rapid, accurate, precise, and suitable for simultaneous determination of retinyl derivatives and β-carotene in dietary supplements.

Conventional methods for determination of fat-soluble vitamins such as A and E and carotenoids have used colorimetry or fluorimetry together with thin-layer chromatography or open-column chromatography with alumina or silicic acid. These assays lack specificity, are time-consuming, and are not amenable to simultaneous determination of the vitamins from a single sample preparation (1–3). Analysis of each of these vitamins and carotenoids often requires saponification, solvent extraction, and sample cleanup by open-column chromatography. Quantitation is achieved by spectrophotometric or colorimetric analysis of column eluent. Results using such methods are dependent upon the skills of the individual analyst and generally lack precision when performed within and between laboratories. In the last 2 decades, liquid chromatography (LC) has become the predominant and best analytical technique for the determination of vitamins A and E in foods and vitamin-containing pharmaceutical preparations. A number of LC methods for analysis of β-carotene and vitamin A have been published (4–9). In addition, Eitenmiller and Landen (10) have comprehensively reviewed other LC methods for the analysis of retinol and its metabolites in foods, feeds, and pharmaceutical and biological materials (e.g., Tables 1.6 and 1.7 in ref. 10). This paper describes a validated LC method for the simultaneous determination of vitamin A and β-carotene in dietary supplements of different matrices.

Both β-carotene and retinyl derivatives of vitamin A (retinol, retinyl acetate, retinyl palmitate) are capable of forming geometric isomers. Biopotency studies in animals suggest that the cis-isomers of β-carotene and vitamin A have lower biologic activities than the respective all-trans forms (11, 12). It is important to be able to quantify these isomeric forms because cis-isomers of β-carotene and retinyl acetate may occur in dietary supplements.

The present study was designed to investigate methods of extraction of these nutrients with or without saponification from dietary supplements, and the applicability of the reversed-phase method currently used in our laboratory for analysis of vitamin A in serum and tissue (13) to extracts of dietary supplements (soft-gel capsules, tablets) containing vitamin A and/or β-carotene. The extraction method described here was developed from previous reports of analysis of vitamin A and β-carotene in infant formula and multivitamin-multimineral tablets (14–16). Direct extraction methods for multivitamin tablets and capsules have been reported (7, 17–19).

Experimental

Apparatus

(a) LC system.—The reversed-phase LC system consisted of Beckman Model 346 (Beckman Instruments, Inc., Fullerton, CA) equipped with a System Gold Model 168 photodiode array detector, Model 507 autosampler, Model 166 programmable UV-Vis detector and a PC Express-based data integration system.

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(b) Column for reversed-phase LC.—25 cm × 4.6 mm, 5 μm, C₁₈ Econosphere column (Alltech Associates, Deerfield, IL) and 5 μm precolumn packed with reversed-phase C₁₈ (Alltech Associates).

c) Normal-phase LC.—25 cm × 4.6 mm, 5 μm, Lichrosorb Si 60 column with Lichrosorb Si 60 precolumn (Alltech Associates).

(d) Apex silica column.—15 cm × 4.5 mm, 3 μm, with Apex silica precolumn for AOAC Method 992.04 (20; Jones Chromatography, Columbus, OH).


(f) Polytron homogenizer.—Kinematica Model PT10/35 (Brinkman Instruments, Westbury, NY).

(g) Refrigerated centrifuge.—Beckman Model TJ-6 (Beckman Instruments, Inc.).

**Chromatographic Conditions**

(a) Mobile phase solution for reversed-phase LC.—The mobile phase was 100% methanol (solvent A) and isopropanol–methanol (50 + 50, v/v; solvent B). The gradient procedure was, as follows: 100% solvent A at flow rate of 1.5 mL/min was used for 5 min, followed by linear gradient to 100% solvent B at flow rate of 1.0 mL/min, 9 min hold at 100% solvent B, and then 2 min gradient back to 100% solvent A.

(b) Mobile phase solution for normal-phase LC.—0.5% isopropanol in hexane. LC was performed isocratically.

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**Table 1. Precision of reversed-phase method versus normal-phase method for analysis of standards**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Reversed-phase method</th>
<th>Normal-phase method</th>
<th>RSD, %</th>
<th>RSD, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean, μg/mL</td>
<td>Interday</td>
<td>Intraday</td>
<td>Mean, μg/mL</td>
</tr>
<tr>
<td>Retinyl acetate</td>
<td>30.9</td>
<td>1.1 (31)</td>
<td>0.6 (8)</td>
<td>29.6</td>
</tr>
<tr>
<td>Retinyl palmitate</td>
<td>63.0</td>
<td>1.1 (25)</td>
<td>0.5 (6)</td>
<td>63.6</td>
</tr>
<tr>
<td>Retinol</td>
<td>26.9</td>
<td>1.0 (25)</td>
<td>0.3 (6)</td>
<td>ND</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>8.9</td>
<td>4.1 (25)</td>
<td>1.1 (8)</td>
<td>13.9</td>
</tr>
</tbody>
</table>

* Number of analyses shown in parentheses.

* ND = Not determined. Retinol does not elute from the column with the same mobile phase under the conditions described.
Mobile phase solution for AOAC Method 992.04.—4% Isopropanol in heptane.

Detection.—The UV wavelength detector was set at 325 nm and used at maximum sensitivity (0.001 absorbance units, full scale); the visible wavelength, set at 450 nm, was also used at maximum sensitivity (0.001 absorbance units, full scale).

Injection loop.—20 μL.

Temperature.—Ambient (24 ± 0.5°C).

Reagents

(a) Chemicals.—Methanol, ethanol, methylene chloride, hexane, heptane, cyclohexane, and petroleum ether were LC grade. Diethyl ether and hexadecane were analytical reagent grade.

(b) Butylated hydroxytoluene (BHT).—Sigma (St. Louis, MO).

(c) Standard compounds.—All-trans retinyl palmitate and all-trans retinol were purchased from Sigma. All-trans retinyl acetate was USP vitamin A reference standard obtained from the U.S. Pharmacopeia (USP; Rockville, MD).

(d) Dry β-carotene beadlets.—10%; Hoffmann-La Roche (Nutley, NJ).

(e) Ethanolic pyrogallol solution.—2% Pyrogallol (1,3,5-trihydroxy benzene, 98%; Sigma) in 95% ethanol.

(f) Ethanolic KOH.—10% (w/v) KOH in 90% ethanol (prepared from absolute ethanol).

(g) Iodine solution.—Iodine (10 mg) was dissolved in 100 mL volumetric flask and diluted to volume with cyclohexane; 10 mL of this solution was further diluted to 100 mL with cyclohexane. This solution was prepared daily.

Procedure

All procedures involving preparation of standards and samples as well as saponification, extraction, and evaporation under nitrogen were performed in yellow light.

Preparation of Retinol from USP Vitamin A Reference Standard

The method is the same as that described by Bueno (21), except that concentration of the working standard of retinol was determined from the extinction coefficient value of retinol as described below. Five USP Vitamin A reference standard capsules were placed in a round-bottom flask; 50 mL water, 150 mL ethanol, and 25 mL 50% KOH solution (12.546 g/25 mL water) were added to the flask, and the mixture was refluxed with a short air-condenser for 30 min at 70°C. The mixture was extracted twice with 100 mL petroleum ether and the combined extracts were washed with water and dried over 65 g anhydrous sodium sulfate. The mixture was then filtered through a sintered glass funnel with medium porosity, containing 50 g anhydrous sodium sulfate, and washed with 50 mL petroleum ether to elute retinol quantitatively. BHT (10 mg) was added, and the petroleum ether extract was evaporated to 50 mL with a nitrogen jet on a warm water bath (40°C). This extract was quantitatively transferred to a 100 mL volumetric flask and diluted to volume with petroleum ether (stock standard solution). BHT (10 mg) was added, and the petroleum ether extract was evaporated to 50 mL with a nitrogen jet on a warm water bath (40°C). This extract was quantitatively transferred to a 100 mL volumetric flask and diluted to volume with petroleum ether (stock standard solution). A 1 mL aliquot of the stock standard was diluted to 100 mL in a volumetric flask to provide the working standard solution. The concentration of the working standard solution was determined by taking another 1 mL aliquot of the stock standard solution, evaporating the solution to dryness under nitrogen, and dissolving the residue in 1 mL isopropanol. The extinction coefficient of the solution was determined in isopropanol in a UV-Vis spectrophotometer, and the concentration of the solution was

<table>
<thead>
<tr>
<th>Amount of β-carotene beadlets analyzed, mg</th>
<th>Amount of β-carotene determined by LC, mg</th>
<th>Mean</th>
<th>Amount of β-carotene determined spectrophotometrically, mg</th>
<th>Recovery of β-carotene, %a</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.3</td>
<td>0.94</td>
<td>0.93</td>
<td>0.88</td>
<td>106</td>
</tr>
<tr>
<td>15b</td>
<td>1.84</td>
<td>1.84</td>
<td>1.59</td>
<td>116</td>
</tr>
<tr>
<td>22.5</td>
<td>2.45</td>
<td>2.45</td>
<td>2.39</td>
<td>103</td>
</tr>
</tbody>
</table>

a Recovery = (amount measured by LC/amount determined spectrophotometrically) × 100.

b 15 mg β-carotene beadlets is equivalent to 99% of 3087 IU β-carotene analyzed in the tablets. The theoretical amount of 15 mg β-carotene beadlets is 2505 IU β-carotene equivalents; 1 g β-carotene beadlets has 167 000 IU provitamin A activity.
calculated by using the E1%, 1 cm value of 1820 for retinol in isopropanol at 325 nm (22).

Preparation of β-Carotene Standard Solution

About 5 mg β-carotene was weighed into a 50 mL volumetric flask and dissolved in hexane containing BHT (0.005%). A few drops of methylene chloride were added to completely dissolve the β-carotene, and the solution was diluted to volume with hexane containing BHT. This was the stock solution of β-carotene. A working standard was prepared by diluting 0.5 mL stock solution to 10 mL with hexane. The absorbance of the working standard was measured in a scanning spectrophotometer at 450 nm. The concentration of the β-carotene standard was measured by using the E1%, 1 cm value of 2592 in hexane at 450 nm (23).

Preparation of All-trans Retinyl Acetate and All-trans Retinyl Palmitate Standards

All-trans retinyl acetate.—One USP Vitamin A reference standard capsule was weighed accurately. The capsule was sliced open and the contents were transferred to a container with a Teflon-lined screw cap. The contents were dissolved in hexane in a 50 mL volumetric flask and diluted to volume. This was labeled “reference standard” stock solution. A 1 mL aliquot of this solution was diluted to 10 mL in another 10 mL volumetric flask, and this was labeled “working standard” solution for retinyl acetate. The concentration of the working standard was determined by taking 1 mL reference standard stock solution, placing it in a 10 mL volumetric flask, evaporating the solution with a nitrogen jet, dissolving the residue in isopropanol, and diluting the solution to volume with isopropanol. The absorbance of the working standard was measured by a scanning UV-Vis spectrophotometer at 325 nm in isopropanol. The concentration was calculated by using the extinction coefficient (E1%, 1 cm) of 1530 at 326 nm in isopropanol. The absorbance of the working standard was measured at 325 nm in ethanol. The concentration of the solution was calculated by using the extinction coefficient (E1%, 1 cm) of 975 at 325–328 nm in ethanol (24).

Determination of Retinol in Standard Reference Material 1846 (Infant Formula)

The method for extraction and determination of retinol in SRM 1846 is described in AOAC Official Method 992.04 (20). A 140 g amount of SRM 1846 infant formula powder was weighed into a 1 L volumetric flask containing a small magnetic stirrer. Water (previously boiled and cooled) was added almost to the mark. The mixture was flushed with nitrogen, and the flask was stoppered and stirred for 30 min. The solution was diluted to volume after the foam was allowed to collapse, and 40 mL of this dispersion was pipetted into a 100 mL digestion flask, followed by addition of 10 mL ethanolic pyrogallol and 40 mL ethanolic KOH. More ethanolic pyrogallol was added until the liquid was ca 1 cm below the graduation mark. The flask was then stoppered, wrapped in aluminum foil for protection against light, and stirred for 18 h. Ethanolic pyrogallol was added to dilute the contents to the mark, the flask was stoppered, and the contents were mixed. A 5 mL portion of the digest was transferred to a 15 mL centrifuge tube; 2 mL water and 7 mL extraction solvent (hexane–diethyl ether, 85 + 15) were added, and the mixture was shaken for 30 s. The mixture was centrifuged for 5 min at 2000 rpm in a Beckman centrifuge, and the hexane layer was transferred to a 25 mL volumetric flask, taking care not to carry over more than a trace of aqueous digest. The extraction step was repeated twice with 7 mL portions of extraction solvent, and the extracts were pooled in the 25 mL flask. A 1 mL aliquot of hexadecane–hexane mixture was then added, and the flask was diluted to volume with hexane. The flask was inverted to mix and allowed to stand 5 min to let any aqueous digest settle. A 15 mL volume of the clear solvent was pipetted from the center of the flask, avoiding contaminating the aqueous digest, into a 15 mL centrifuge tube, and evaporated under nitrogen. The residue was immediately dissolved in 1 mL heptane and transferred to a sealable vial for quantitation by LC on the Apex silica column. The same NIST 1846 saponified extract was also injected into the reversed-phase system with quantitation against the same retinol standard.

Sample Preparation

Dietary supplements used in this study were purchased locally. Two were soft-gel capsules containing β-carotene or vitamin A; 4 were multivitamin-multimineral tablets containing both vitamin A and β-carotene. One supplement contained lutein in addition to vitamin A and β-carotene. Retinyl acetate was the predominant form of vitamin A in the tablets. A composite of 20–30 tablets (20% of the total number of tablets in the bottle) was used for each analysis to obtain representative values for β-carotene and retinyl acetate.

### Table 4. Recovery of retinyl acetate from added standard retinyl acetate

<table>
<thead>
<tr>
<th>Analyte measured in unfortified sample, µg/g</th>
<th>Analyte added, µg/g</th>
<th>Analyte measured in fortified sample, µg/g</th>
<th>Rec., %&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>2135</td>
<td>1596</td>
<td>3758</td>
<td>102</td>
</tr>
<tr>
<td>2135</td>
<td>1596</td>
<td>3751</td>
<td>101</td>
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<td>2135</td>
<td>3141</td>
<td>5186</td>
<td>97</td>
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<td>3141</td>
<td>5107</td>
<td>95</td>
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<td>2135</td>
<td>5033</td>
<td>6767</td>
<td>92</td>
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<td>2135</td>
<td>5033</td>
<td>6670</td>
<td>90</td>
</tr>
<tr>
<td>2135</td>
<td>5033</td>
<td>6739</td>
<td>95</td>
</tr>
</tbody>
</table>

<sup>a</sup> Recovery, % = (analyte recovered/analyte added) × 100.
**Carotene/Vitamin A Soft-Gel Capsules**

Two soft-gel capsules were weighed and opened with a sharp blade. The contents were carefully transferred to a 50 mL volumetric flask and dissolved with 20 mL methylene chloride containing BHT (50 μg/mL). A 20 mL volume of hexane was added to this solution and diluted to 50 mL with hexane. A 1 mL aliquot of this solution was diluted to 10 mL with hexane in a 15 mL glass-stoppered centrifuge tube, and labeled “solution 1.” A 1 mL aliquot of solution 1 was further diluted to 10 mL with hexane and labeled “solution 2.” A 20 μL aliquot of this solution was injected into the liquid chromatograph to determine β-carotene and retinyl acetate simultaneously by reversed-phase column.

**Multivitamin-Multimineral Tablets**

A composite of 20 tablets was accurately weighed and ground to a uniform powder in small amounts with mortar and pestle. Duplicate portions of this powder (ca 5 g) were weighed into 125 mL glass-stoppered volumetric flasks, and 30 mL hot water (55–60°C) was added to each flask. The flasks were shaken for 2 min. The samples were allowed to stand for 30 min in the dark. The sample volumes were measured and duplicate aliquots (5 mL) were taken after mixing, and placed in separatory funnels. A 10 mL volume of absolute ethanol was added to each sample, followed by 20 mL methylene chloride. The mixtures were shaken for 2 min and allowed to stand for 1 min. They were then centrifuged at 1500 rpm for 5 min at 4°C. The supernatants were carefully transferred to a second separatory funnel and 15 mL methylene chloride was added. The funnels were shaken for 2 min. The mixtures were then centrifuged at 1500 rpm for 5 min in a Beckman centrifuge. This process was repeated a second time. The methylene chloride extracts were combined and dried over 5–10 g anhydrous sodium sulfate. They were filtered through a sintered glass funnel containing 0.45 mm filter paper, and diluted to 100 mL. A 4 mL portion of this solution was evaporated under nitrogen and taken up in 1 mL isopropanol. A 20 μL aliquot of this solution was injected into the liquid chromatograph for analysis by the reversed-phase system.

**Spectrophotometric Determination of β-Carotene in β-Carotene Beadlets**

β-Carotene beadlets (50 mg; containing 10% β-carotene) were accurately weighed into a 200 mL volumetric flask; 50 mL hot water (55–60°C) was added to the flask and mixed well to disperse the sample. The sample was cooled to room temperature and diluted to 200 mL with water (solution 1). A 5 mL portion of solution 1 was transferred to a 50 mL centrifuge tube to which 2–3 g sodium sulfate decahydrate, 2 mL 1N HCl, and 20 mL methylene chloride were added, in that order. The mixture was shaken for 10 min and then centrifuged at 1500 rpm for 5 min in a Beckman centrifuge. The aqueous
layer was removed and discarded. About 2 g sodium sulfate was added to the methylene chloride solution and shaken vigorously. The solution was allowed to settle (solution 2); a 5 mL portion of this was then transferred to a 50 mL volumetric flask, and 30 mL cyclohexane was added and mixed well. A 0.05 mL volume of iodine solution was then added, diluted to volume with cyclohexane, and mixed well (solution 3). This solution was kept in the dark for 3 h. The absorbance of solution 3 at the wavelength maximum of 452 nm was immediately measured against cyclohexane in the reference cell. The amount of $\beta$-carotene in the beadlets was calculated as percent $\beta$-carotene, as follows:

$$\frac{A \times 8000 \times 100}{223 \times \text{sample weight (mg)}} = \% \beta$$-carotene

where $A$ = absorbance of solution 3 at 452 ± 1 nm; 8000 = dilution factor; 100 = factor for conversion to percent; 223 = reference absorptivity of $\beta$-carotene at 452 ± 1 nm.

Quantitation

Retinol, retinyl acetate, retinyl palmitate, and $\beta$-carotene in samples were quantitated after LC from standard curves prepared of various concentrations of standards (retinol, retinyl acetate, retinyl palmitate, and $\beta$-carotene). Concentrations of standards were determined spectrophotometrically.

Results and Discussion

Linear responses from the reversed-phase method were observed from 1.7 to 27.5 $\mu$g/mL for retinol, 1.9 to 31 $\mu$g/mL for retinyl acetate, 5.3 to 84.9 $\mu$g/mL for retinyl palmitate, and 2.5 to 81.6 $\mu$g/mL for $\beta$-carotene. The correlation coefficients of the standard curves and the corresponding regression equations were retinol ($r$, 0.99932, $n$ = 12; $Y = 1.212409X + 2.16117$); retinyl acetate ($r$, 0.99924, $n$ = 10; $Y = 1.031764X + 0.875665$); retinyl palmitate ($r$, 0.99981, $n$ = 12; $Y = 0.882477X + 0.59445$); and $\beta$-carotene ($r$, 0.99861, $n$ = 12; $Y = 0.499768X + 2.876466$).

Several LC methods have been described for determination of vitamin A and $\beta$-carotene in foods, feed materials, and dietary supplements (4–10). However, few studies have addressed the occurrence and quantitation of isomers of retinol, retinyl acetate (25), and $\beta$-carotene (26) in dietary supplements.

In the present study, a reversed-phase method with methanol–isopropanol gradient elution was examined for simultaneous baseline separation of retinol, retinyl acetate, retinyl palmitate, and $\beta$-carotene. Figure 1 shows the dual chromatogram in 2 channels of all-trans retinol, all-trans retinyl acetate, all-trans retinyl palmitate, and all-trans $\beta$-carotene obtained with the reversed-phase method. The chromatogram obtained in channel B is superimposed on the chromatogram obtained in channel A to show that there is good baseline separation of the retinyl acetate peak from the retinol, retinyl palmitate, and $\beta$-carotene peaks, and there is no interference of the $\beta$-carotene peak with the retinyl acetate or the retinyl palmitate peaks. The chromatographic run was completed in 22 min. The gradient reversed-phase method was capable of separating retinol from other retinyl derivatives (retinyl acetate, retinyl palmitate) and $\beta$-carotene as well...
### Table 6. β-Carotene and vitamin A content of selected dietary supplements

<table>
<thead>
<tr>
<th>Dietary supplements</th>
<th>Analyzed Low</th>
<th>Analyzed High</th>
<th>Treatment</th>
<th>Vitamin A, IU</th>
<th>Total IU</th>
<th>% of label</th>
<th>% of label</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-trans β-carotene IU</td>
<td>30.96</td>
<td>31.38</td>
<td>Hot water treatment and direct extraction</td>
<td>1200</td>
<td>1300</td>
<td>100</td>
<td>120</td>
</tr>
<tr>
<td>cis β-carotene IU</td>
<td>7.14</td>
<td>8.07</td>
<td>Hot water treatment with pyrogallol and direct extraction</td>
<td>1201</td>
<td>1301</td>
<td>100</td>
<td>120</td>
</tr>
<tr>
<td>as 13-cis isomers from the all-trans forms of retinyl acetate and β-carotene, respectively.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results of analysis of the same extract of SRM 1846 infant formula powder by the reversed-phase method and by AOAC Method 992.04 for infant formula powder (20) are shown in Table 2. The concentration of retinol obtained by the reversed-phase method is comparable to the certified value provided by National Institute of Standards and Technology (NIST; 27), whereas results obtained by AOAC Method 992.04 are less than half the certified value. It is possible that the characteristics of the column packing prescribed for use in AOAC Method 992.04 (20) in the present study may have differed from those of columns used in previous studies.

The recoveries of β-carotene from β-carotene beadlets (β-carotene 10%) in amounts equivalent to 50, 99, and 132% of the amount measured in the tablets are shown in Table 3. The data show the excellent recovery using the reversed-phase method for determination of β-carotene in the beadlets as well as in dietary supplements.

The recoveries of retinyl acetate from varying amounts of crystalline retinyl acetate added to multivitamin-multimineral tablets are shown in Table 4. In preliminary experiments, we found that added retinyl acetate binds to some component in dietary supplements and is not easily extractable with methylene chloride even after hot water treatment. We found it necessary to use sonication of the water-soluble mixture for 10 min before methylene chloride extraction. This was followed by polytron homogenization of the water-soluble mixture + methylene chloride for 45–60 s and centrifugation at 1500 rpm for 5 min at 4°C. This process was repeated twice, and the combined methylene chloride extracts were dried over anhydrous sodium sulfate, and then filtered. An aliquot was analyzed by reversed-phase LC as in the Experimental section. Recoveries of 9.2, 18.1, and 29.2 mg pure retinyl acetate added to multivitamin-multimineral tablets were 102, 96, and 92%, respectively.

The results of analysis of dietary supplements containing β-carotene alone (soft-gel capsule) or vitamin A acetate and β-carotene in combination (multivitamin-multimineral tablets) are shown in Table 5. In the β-carotene soft-gel capsule, a cis isomer occurs to the extent of 8.4% of the total activity of β-carotene. The total analyzed β-carotene activity, in IU (all-trans + cis) agrees well with the label value of 25 000 IU vitamin A equivalents.
Figure 2 shows the chromatographic profile of all-trans β-carotene and a cis isomer of β-carotene. The absorption spectrum of the cis isomer was obtained with the photodiode array detector, and the cis isomer was tentatively identified as 13-cis β-carotene. This tentative identification was based on its absorption maximum (λmax = 446 nm) and on the basis of the Q-ratio (absorbance at the wavelength maximum/absorbance at the cis peak; 28, 29) and its comparison to published values (30–32). The Q-ratio obtained for the cis isomer in our study was 2.3 compared with 10 for the all-trans isomer. This agrees well with the Q-ratios of 2.2 and 11.3 reported by O’Neil et al. (29), and 2.3 and >12 found by Quackenbush (31) for 13-cis and all-trans β-carotene, respectively.

In multivitamin-multimineral tablets containing the carotenoid lutein, we found that lutein was present at a level of 244.4 μg/tablet (98% of the label value of 250 μg; results not shown). Recoveries of β-carotene and retinyl acetate from this supplement were 62 and 87%, respectively, of the label value. Recoveries of 95 and 78% for β-carotene and retinyl acetate, respectively, were found for a high potency multivitamin-multimineral tablet preparation (Table 5).

When a soft-gel capsule containing 25 000 IU vitamin A and 1000 IU vitamin D was analyzed for vitamin A, the chromatographic profile of the contents did not correspond to any of the known esters of retinol (retinyl acetate, retinyl palmitate, or retinyl stearate). The contents of the soft-gel capsule were saponified in the presence of pyrogallol, and vitamin A was estimated as retinol. The recovery was 71% of the label value. The source of vitamin A in this supplement was fish liver oil. No peak other than retinol was found by LC. Dehydroretinol with λ maximum at 350 nm (ethanol) was not found. Because we could not identify the retinyl ester present, we estimated vitamin A by LC and calculated vitamin A as retinyl palmitate equivalents. This calculation gave a recovery of 77% of the label value (Table 5).

Preliminary experiments with multivitamin-multimineral tablets containing 10 000 IU vitamin A with 25% as β-carotene indicated that almost 55% of the β-carotene was destroyed after saponification. However, the recovery of retinyl acetate as retinol was 112% of the label value. Therefore, we applied the direct extraction method with hot water at 55–60°C, as described earlier. The recoveries of β-carotene and retinyl acetate were 125 and 113% of the label values, respectively (Table 6).

A prenatal multivitamin-multimineral supplement labeled as containing 4000 IU of total vitamin A and β-carotene activity per tablet required additional treatment with 2% pyrogallol before hot water treatment. The recovery of total vitamin A and β-carotene activity in this case was 94% of the label value (Table 6).

Analysis of a prenatal vitamin supplement and a multivitamin-multimineral tablet supplement by AOAC Method 992.04 gave incorrect results because of overlapping of the β-carotene peak with the retinyl acetate peak. The peak areas for retinyl acetate are thus overestimated and the corresponding values for retinyl acetate are inaccurate. The recovery of total vitamin A and β-carotene as a percent of label is not provided (Table 6).

Conclusions

The LC gradient method developed in our laboratory for vitamin A and β-carotene in dietary supplements is fast, simple, and specific for vitamin A and its derivatives (retinyl acetate, retinyl palmitate) and β-carotene. It avoids alkaline hydrolysis of dietary supplements for vitamin A and β-carotene analysis and is capable of providing high resolution of closely related compounds (13-cis isomers from all-trans isomers of vitamin A and β-carotene) in a single run. Furthermore, the LC method is accurate, as indicated by the results of the analysis of SRM 1846 and the excellent recovery of standard retinyl acetate added to the tablets. The method also exhibits high precision, as shown by the inter- and intraday coefficients of variation for each standard assay.

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