Determination of Carotenoids in Human Serum and Breast Milk Using High Performance Liquid Chromatography Coupled with a Diode Array Detector (HPLC-DAD)

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Abstract: High performance liquid chromatography (HPLC) coupled with a diode array detector (HPLC-DAD) for the identification and quantification of carotenoids, namely all-trans lutein, zeaxanthin, β-cryptoxanthin, α-carotene, and β-carotene, in biological samples such as human serum and breast milk, has been developed and validated. Good chromatography separation was achieved using a binary mobile phase system on a YMC C30 column (150 × 2.1 mm, 3 µm) at 30 °C. Owing to the smaller column particle size and diameter of the column, the separation was achieved in 18 min, which is significantly reduced from the typical 30–40 min of other methods. The diode array detector (DAD) acquisition was set at a wavelength of 445 nm; 3D spectra ranging from wavelengths of 240–600 nm were also recorded. Peaks were identified by matching their retention time and spectra with those of standards. Quantification was achieved by internal standard calibration using echinenone as the internal standard. Good linearity was obtained for each compound (R2 > 0.9999). The method quantification limits (MQLs) for serum and breast milk were 10 ng/mL and 5 ng/mL, in matrix, respectively. A spike recovery study and standard reference material (SRM) from the National Institute of Standards and Technology (NIST) 968e analysis has proven that the method has a high degree of accuracy, precision, and robustness. The stability study showed that the carotenoid standard and sample extracts could be stored in a chilled autosampler at 8 °C up to 48 h without being comprised, which provides guidance on re-test time frames. The freeze/thaw process was found to be detrimental to carotenoids, and should always be avoided. Most importantly, UV standardization of the stock standard is to be performed prior to each assay, and simply taking the values on Certificate of Analysis (CoA) for calculation of the standard concentration is not recommended.

Keywords: carotenoids; high performance liquid chromatography; human breast milk; human serum; internal standard

1. Introduction

Carotenoids are fat-soluble micronutrients that play an important role in human health. Studies have shown that carotenoids can provide a multitude of health benefits, including provitamin A activities, a role as antioxidants, preventing age-related macular degeneration (AMD), and reducing the risk of cancer [1]. In addition, evidence has associated the presence of carotenoids and the
cognitive development of fetuses and infants [2–5]. In order to support clinical and pre-clinical studies, a bioanalysis method has been developed and validated for the accurate determination of key carotenoids and isomers in human breast milk and serum. The target analytes are all-trans lutein, zeaxanthin, β-cryptoxanthin, α-carotene, and β-carotene.

High performance liquid chromatography (HPLC), using C18 or C30 columns, is widely adopted for analyzing carotenoids in biological samples. The problems with this approach are long run times, insufficient resolution of peaks, or tedious sample-preparation steps [6–11]. Recently, a liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based bioanalysis method has been developed [12,13]. However, due to the large sample sizes from clinical or pre-studies, where great sample variation is expected, isotopic-labeled standards are desired for the MS/MS method to minimize potential matrix effects. To the best of our knowledge, not all isotopic standards for the target carotenoids are available, and, thus, an HPLC-based method was finally chosen due to its high reliability.

It is well known that carotenoids are susceptible to degradation [14–17], which poses great challenges for their accurate determination. Therefore, we have conducted stability studies that assessed factors affecting the quantitation of carotenoids, such as freeze/thaw cycles, storage conditions, and stability of the reference standard solution and sample extracts. The findings complement current knowledge and provide guidelines on the proper handling of carotenoids samples and standards.

2. Results and Discussion

2.1. Optimization of Sample Preparation for Breast Milk Samples

Sample extraction of breast milk was carefully optimized due to the complexity of the sample matrices. Water was added to the samples to adjust the aqueous/organic ratio to prevent the formation of an emulsion [18]. Five percent potassium hydroxide was added for saponification to remove significant matrix interference from the high level of lipids in the samples [18–20]. To minimize the loss of lutein and zeaxanthin, we chose a low-concentration alkali saponification and shortened the saponification duration. Tetrahydrofuran (THF) was added as a modifier to enhance the solubility and improve the resolution [18,20–22]. A small amount of methyl tert-butyl ether (MTBE) was added to the final diluent (MTBE:Ethanol with butylated hydroxytoluene (BHT), 2:8, v/v) to improve the solubility of carotenes. Overall, the saponification and modifier step aimed at improving the method’s performance.

2.2. HPLC Separation and Specificity

By using a column with a smaller particle size and column diameter, YMC C30, 150 × 2.1 mm, 3 μm (YMC, Kyoto, Japan), the run time per sample was significantly reduced, from the typical 30–40 min to 18 min. The signal was recorded at a wavelength of 445 nm, which provided better specificity compared to low-range UV wavelengths. Retention time and spectra were compared with those of standards to confirm the identity of the compounds. The spectra found in sample were also matched with those in standards using the library matching function in Chemstation. Typical chromatograms are shown in Figure 1. An example of spectrum-matching of all-trans-lutein in standards and samples is demonstrated in Figure 2, where a specific peak is successfully identified as all-trans-lutein with a high matching score of 993.402. The peak purities were checked in Chemstation. The purity factors for the five carotenoids in samples were all above 950, which are considered acceptable for such a bioanalysis with high complexity.
Figure 1. Typical chromatograms of standard (red trace) and sample (blue trace) shown in overlaid pattern. Peaks: (1) all-trans-lutein; (2) zeaxanthin; (3) β-cryptoxanthin; (4) Internal standard; (5) α-carotene; (6) β-carotene.

Figure 2. The spectrum of a peak found in a serum sample at retention time of 5.649 is matched against the library, which contains the spectra obtained from carotenoid standards. The peak is identified as all-trans-lutein with a matching score of 993.402 and a purity factor of 998.601.
2.3. Linearity

The $R^2$ obtained were $>0.9999$ for all compounds, indicating excellent linearity.

2.4. MDL/MQL

The method detection limit (MDL) was determined as the concentration at which the signal to noise ratio (S/N) of the analyte peak equals 3. The method quantification limit (MQL) equals 3 times that of MDL. The MQL obtained in this study for each carotenoid was 10 ng/mL in serum and 5 ng/mL in breast milk.

2.5. Accuracy

For serum samples, Standard Reference Material (SRM) 968e from the National Institute of Standards and Technology (NIST) was analyzed using six determinants at three certified concentrations on different days. The concentrations obtained in the study were then compared with the certified values which can be found on NIST website. It was shown that all results were within the ranges except one sample with lutein concentration (88 ng/mL) lower than the assigned range (90–104 ng/mL) (Table 1). This is expected, as the assigned value for the NIST 968e is for total lutein while this method merely quantifies all-trans-lutein.

As there is no reference material available for human breast milk, an over-spike study was carried out: milk samples were spiked with known amounts of carotenoids at two concentrations prior to extraction. The obtained values were later subtracted from the sample blank (without standard addition) and then divided by the known amount and expressed in percentage. The spike was performed at two levels, representing low level (5 ng/mL; at the MQL), which is most likely only found in mature breast milk, and high level (50 ng/mL), which can be observed in both colostrum and mature milk. The recoveries varied from 85% to 105% (Table 2), which meet the requirement of the bioanalysis validation guideline from the US. Food and Drug Administration [23], and demonstrates a high degree of accuracy.

### Table 1. National Institute of Standards and Technology (NIST) Standard Reference Material 968e analysis results for serum (unit: ng/mL in serum).

<table>
<thead>
<tr>
<th>Level I</th>
<th>NIST 968e Certified Value</th>
<th>Day 1 ($n = 3$)</th>
<th>Mean a</th>
<th>RSD b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>1-1</td>
<td>1-2</td>
</tr>
<tr>
<td>Lutein c</td>
<td>67</td>
<td>59–75</td>
<td>74</td>
<td>75</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>31</td>
<td>26–36</td>
<td>36</td>
<td>33</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>41</td>
<td>35–47</td>
<td>43</td>
<td>42</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>11</td>
<td>6–16</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>β-carotene</td>
<td>88</td>
<td>78–98</td>
<td>89</td>
<td>87</td>
</tr>
<tr>
<td>Level II</td>
<td>2-1</td>
<td>2-2</td>
<td>2-3</td>
<td>2-4</td>
</tr>
<tr>
<td>Lutein</td>
<td>97</td>
<td>90–104</td>
<td>89</td>
<td>93</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>29</td>
<td>23–35</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>40</td>
<td>34–46</td>
<td>40</td>
<td>42</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>31</td>
<td>27–35</td>
<td>29</td>
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</tr>
<tr>
<td>β-carotene</td>
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<td>183–223</td>
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<tr>
<td>Level III</td>
<td>3-1</td>
<td>3-2</td>
<td>3-3</td>
<td>3-4</td>
</tr>
<tr>
<td>Lutein</td>
<td>124</td>
<td>114–134</td>
<td>131</td>
<td>121</td>
</tr>
<tr>
<td>Zeaxanthin</td>
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<td>24–34</td>
<td>34</td>
<td>26</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>21</td>
<td>17–25</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>15</td>
<td>13–17</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>β-carotene</td>
<td>363</td>
<td>325–401</td>
<td>377</td>
<td>364</td>
</tr>
</tbody>
</table>

a: Mean of six determinants in the study; b: RSD; relative standard deviation of the six determinants in the study; c: Reported value in Certificate of Analysis (CoA) is for total lutein. This method quantifies all-trans-lutein.
Table 2. Mature milk (MM) and colostrum (CL) over-spike recoveries.

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Mean</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>5 ng/mL in Mature Milk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lutein</td>
<td>89%</td>
<td>101%</td>
<td>89%</td>
<td>96%</td>
<td>106%</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>93%</td>
<td>100%</td>
<td>95%</td>
<td>88%</td>
<td>87%</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>107%</td>
<td>93%</td>
<td>81%</td>
<td>89%</td>
<td>93%</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>92%</td>
<td>106%</td>
<td>91%</td>
<td>85%</td>
<td>104%</td>
</tr>
<tr>
<td>β-carotene</td>
<td>92%</td>
<td>109%</td>
<td>96%</td>
<td>86%</td>
<td>86%</td>
</tr>
<tr>
<td><strong>50 ng/mL in Mature Milk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lutein</td>
<td>91%</td>
<td>90%</td>
<td>92%</td>
<td>93%</td>
<td>88%</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>89%</td>
<td>90%</td>
<td>91%</td>
<td>92%</td>
<td>91%</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>89%</td>
<td>92%</td>
<td>91%</td>
<td>92%</td>
<td>85%</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>85%</td>
<td>87%</td>
<td>86%</td>
<td>86%</td>
<td>87%</td>
</tr>
<tr>
<td>β-carotene</td>
<td>89%</td>
<td>94%</td>
<td>89%</td>
<td>90%</td>
<td>97%</td>
</tr>
<tr>
<td><strong>50 ng/mL in Colostrum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lutein</td>
<td>91%</td>
<td>92%</td>
<td>100%</td>
<td>104%</td>
<td>102%</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>93%</td>
<td>95%</td>
<td>98%</td>
<td>98%</td>
<td>99%</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>95%</td>
<td>96%</td>
<td>97%</td>
<td>97%</td>
<td>105%</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>91%</td>
<td>93%</td>
<td>98%</td>
<td>94%</td>
<td>103%</td>
</tr>
<tr>
<td>β-carotene</td>
<td>92%</td>
<td>90%</td>
<td>95%</td>
<td>97%</td>
<td>103%</td>
</tr>
</tbody>
</table>

*: Mature milk is pooled milk collected from multiple donors, four-weeks postpartum, and colostrum is pooled milk collected from multiple donors, one-week postpartum; b: MML: Mature milk low spike; MMH: Mature milk high spike; CLH: Colostrum high spike; c: Mean of six determinants in the study; d: RSD: relative standard deviation of the six determinants in the study.

2.6. Repeatability and Intermediate Precision

Injection repeatability was demonstrated by four consecutive injections of WS3. The relative standard deviations (RSDs) of the peak ratios, obtained over the course of method validation, were <3%. It was shown that the RSDs of the SRM results were lower than 15% for all compounds (Table 1), and the RSDs of over-spike recoveries in milk were all within 10% for colostrum and mature milk (Table 2). Therefore, the method is proven to be highly repeatable and precise.

2.7. Storage and Stability

2.7.1. Sample Storage

It has been previously indicated that carotenoids are stable in plasma stored at −70 °C for at least 28 months, or at −20 °C for five months [16]. However, there is an absence of data on long-term storage of breast milk. Assuming a similar behavior of breast milk at ultra-low temperatures, the samples are recommended to be stored at −70 °C for optimal preservation [17].

2.7.2. Stability of Standard Solution and Sample Extracts in HPLC Autosampler

To determine whether overnight automated analysis or re-injection was feasible, the standard solution (ethanol:MTBE, 80:20, v/v) and sample extracts were kept in an autosampler (chilled to 8 °C), and repeated injections were conducted at time points of 0, 24, and 48 h. The concentrations of the re-injections were calculated using the original calibration curve derived from time 0.

It was found that only mild fluctuations in concentration occurred at 48 h at 8 °C in the dark (Figure 3). The small changes in the concentrations of carotenoids in solution, and extracts, may be due to the protection rendered by BHT, as well as compensation from the internal standard. Thus,
if troubleshooting is needed, standard solutions and sample extracts are allowed to be re-injected within 48 h.

Figure 3. Stability of carotenoids in (a) solution (MTBE:Ethanol with BHT, 80:20, v/v) and in different sample extracts (b–d) in an autosampler at 8 °C. Testing points at 0, 24, and 48 h. Y axis: Concentrations of carotenoids (normalized to percentage). X axis: Testing points.

2.7.3. Freeze/Thaw Cycle

The impact of the freeze/thaw cycle on sample integrity was also evaluated. The samples were thawed to room temperature, and an aliquot was taken for analyses. The remaining part was re-frozen at −70 °C. After 24 h, the remaining sample was thawed and then re-tested; this process was repeated three times. It was found that freezing/thawing was detrimental to carotenoids, as all compounds
started to degrade after one cycle (Figure 4). Therefore, if the samples have ever been subjected to a freeze/thaw cycle before, the concentrations of these carotenoids may likely be underestimated.

![Graph showing the effect of freeze/thaw on carotenoids in mature milk, colostrum, and serum samples stored at −70 °C.](image)

**Figure 4.** Effect of freeze/thaw on carotenoids in (a) mature milk; (b) colostrum; and (c) serum samples stored at −70 °C. Y axis: Concentrations of carotenoids (normalized to percentage). X axis: Freeze/thaw cycles.

### 2.7.4. Stock Solution Stability

Carotenoids are highly susceptible to degradation in solutions; thus, UV standardization of the stock solution, prior to use, is highly recommended to ensure a high accuracy (preparation procedures are detailed in Section 3.2.2). In order to understand the stability of stock solutions at −70 °C (see Section 3.2.1) and to save on effort for checking the concentration every time, UV standardization was performed on aliquot stock solutions, periodically, over a span of 24 days. The trends are shown in Figure 5. It was shown that zeaxanthin degrades rapidly in solutions (up to 40%) during the first week, and α- and β-carotene exhibited high fluctuation patterns, which could be due to precipitation in ultra-low temperatures, which affect the homogeneity of the solution. Therefore, after three readings, the experiment was discontinued for the three compounds. Lutein exhibited 11% degradation and β-cryptoxanthin was stable up to 24 days. Thus, it can be concluded that, except for β-cryptoxanthin, the standard solutions all need to be UV standardized, each time, prior to use for best accuracy.
10 mL with ethanol/BHT solution. Stock solutions were then distributed in aliquots into amber vials wrapped in aluminum foil when being transported through, or into, an area without shielded lighting.

β-carotene (00003211) was purchased from Chromadex or United States Pharmacopeia (USP, Rockville, MD, USA) ((ASB-00012453) and (00026504) respectively). β-cryptoxanthin (0007), and echinenone (0283) were purchased from Carotenature (Ostermundigen, Switzerland). β-carotene (00003211) was purchased from Chromadex or United States Pharmacopeia (USP, Rockville, MD, USA) (1065480). Standard Reference Material (SRM) NIST 968e was purchased from the National Institute of Standards and Technology (Gaithersburg, MD, USA).

Human serum and breast milk samples, which were used for spike-recovery studies, were purchased from LeeBio (Maryland Heights, MO, USA).

Carotenoids are light sensitive. All samples and standards were prepared and handled under shielded lighting with a minimum UV cutoff of 385 nm. The standards and samples were tightly wrapped in aluminum foil when being transported through, or into, an area without shielded lighting.

3. Materials and Methods

3.1. Chemicals and Reagents

Absolute ethanol was purchased from Merck Millipore (Billerica, MA, USA). Methanol, dichloromethane, methyl tert-butyl ether (MTBE), butylated hydroxytoluene (BHT), ammonium acetate, petroleum ether, tetrahydrofuran (THF) with BHT as inhibitor (250 ppb), and potassium hydroxide (ACS grade, ≥85%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). N-hexane was purchased from ThermoFisher Scientific (Waltham, MA, USA). Unless otherwise specified, all reagents were of HPLC grade or higher. Ethanol/BHT solution was prepared by adding 30 mg of BHT into 1 L of absolute ethanol.

All-trans-utein (X6250-1MG) and zeaxanthin (14681-1MG-F) were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Chromadex (Irvine, CA, USA) ((ASB-00012453) and (00026504) respectively). β-cryptoxanthin (0055), α-carotene (0007), and echinenone (0283) were purchased from Carotenature (Ostermundigen, Switzerland). β-carotene (00003211) was purchased from Chromadex or United States Pharmacopeia (USP, Rockville, MD, USA) (1065480). Standard Reference Material (SRM) NIST 968e was purchased from the National Institute of Standards and Technology (Gaithersburg, MD, USA).

3.2. Standard Preparation

3.2.1. Stock Solution Preparation

Stock solutions (about 100 µg/mL) of lutein, zeaxanthin, and β-cryptoxanthin were individually prepared by dissolving 1 mg of the standard in 1 mL of THF and then diluting to 10 mL with ethanol/BHT solution. Stock solutions (about 100 µg/mL) of α-carotene, β-carotene, and echinenone were individually prepared by dissolving 1 mg of the standard in 7 mL of THF, and then diluting to 10 mL with ethanol/BHT solution. Stock solutions were then distributed in aliquots into amber vials (e.g., 200 µL each), sufficient for one-time use, and then stored at −70 °C.
3.2.2. UV Standardization of Stock Solutions

Unlike the preparation of stable compounds, for carotenoids analyses, concentrations of the standard should not follow the certified values on the Certificate of Analysis (CoA). This is because the standards may be subjected to significant degradation during transportation and/or prolonged storage. Therefore, UV standardization of the stock solution (Section 3.2.1) must be performed to calculate the true working standard concentrations.

The standards in Section 3.2.1 were diluted 100 times to 1 µg/mL. The diluent for lutein, zeaxanthin, and β-cryptoxanthin was the ethanol/BHT solution, while α-carotene and β-carotene were dissolved in hexane for better solubility. The cuvette for UV standardization of α-carotene and β-carotene must be made of quartz, while, for the rest of the compounds, disposable cuvettes made of polystyrene (PS) are acceptable. A $\lambda_{\text{max}}$ of 445, 452, 452, 444, and 452 nm and an absorptivity of 2550, 2540, 2356, 2800, and 2592 dL/(g·cm), for all-trans-lutein, zeaxanthin, β-cryptoxanthin, α-carotene, and β-carotene were used, respectively [24]. Spectrum scans needed to be performed to ensure the experimental $\lambda_{\text{max}}$ was within ±2 nm of the reference values.

3.2.3. Working Standard Solution Preparation

The internal standard solution was prepared by dissolving echinenone at a concentration of 0.2 µg/mL in ethanol/BHT solution.

Five-point working standard solutions were freshly prepared at concentrations of 10, 20, 100, 400, and 600 ng/mL with an internal standard of 500 ng/mL in ethanol/BHT solution.

3.3. Sample Preparation

Briefly, samples (100 µL of serum or 200 µL of breast milk) were first spiked with internal standard and then vortexed to mix. Serum samples were then extracted with hexane (0.8 mL) twice. Extracts were dried under a gentle stream of nitrogen at 35 °C, reconstituted with 100 µL reconstitution solution (MTBE:Ethanol/BHT, 80:20, v/v), centrifuged, and the clear supernatants were transferred into amber autosampler vials with a 250 µL micro-insert. Reagents were added sequentially in breast milk samples: ultrapure water (200 µL), 5% potassium hydroxide (0.4 mL), and THF (0.2 mL), followed by extraction with the extraction solvent (0.4 mL; petroleum ether:hexane:dichloromethane containing BHT 50 mg/L, 4:4:2, v/v/v) twice. The solutions were vortexed for 2 min between additions. The upper organic layer from the extraction was then transferred to a new vial and treated the same way as the extracts from the serum samples.

For quality control, each batch included at least one standard reference material (NIST 968e) for serum and at least one over-spike for breast milk or colostrum. The results of NIST 968e analyses should be within the assigned reference values, and the recovery for the over-spike should be 80–120% of the target value.

3.4. HPLC-DAD Conditions and Methods

The instrument used was an Agilent 1290 Infinity, equipped with a photodiode array detector (DAD) able to scan between 190 and 640 nm (60 mm Max-Light Cartridge Flow Cell). Separation of the carotenoids was achieved on a YMC C30 column (150 × 2.1 mm, 3 µm) at 30 °C, with a binary mobile phase system. The autosampler temperature was kept at 8 °C, and the injection volume was 10 µL. Mobile phase A (MPA) was methanol with 1.5% ammonium acetate solution at a ratio of 98:2, v/v, and mobile phase B (MPB) was a mixture of MTBE, methanol and 1.5% ammonium acetate solution at a ratio of 90:8:2, v/v/v. The gradient started at 100% MPA, which was held for 1.5 min, then ramped to 55% at 9.4 min, then to 5% over 2.0 min, held for 2.0 min, and finally returned to 100% MPA over 0.1 min, and held for 3.0 min. The flow rate was constant at 0.5 mL/min. Carotenoids were detected at 445 nm, with a band width of 2.0 nm (reference wavelength: 560 ± 80 nm). A three-dimensional
spectrum, ranging from 240–600 nm, was also recorded. Identification was conducted by matching the peak retention times ($R_t$) and spectra with those of the pure standards.

Internal standard calibration was used by plotting the peak area ratio of analytes to internal standard (IS) against the concentration ratio of analytes to IS.

As all-trans-lutein reference standards may contain significant amounts of impurities (e.g., zeaxanthin or other lutein isomers), such contributions need to be carefully examined.

4. Conclusions

We have successfully developed and validated a method for simultaneously quantifying five major carotenoids in human serum and breast milk. Good chromatography separation was achieved on a YMC C$_30$ column with a significantly shorter run time. The method had a high degree of accuracy and precision, and the MQLs were sufficiently low for studies on carotenoids levels in human biological samples. The stability study showed that the carotenoid standard and the sample extracts could be stored in a chilled autosampler at 8 °C without being significantly comprised for 48 h, which provides guidance on re-test time frames. Freeze/thaw was found to be detrimental to carotenoids, and should always be avoided. Most importantly, although the standard/standard solution was stored at ultra-low temperatures, UV standardization of the stock standard should be performed prior to each assay to obtain the true concentrations, and the use of CoA values is not recommended.

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Author Contributions: Jing Tan and Chunyan Zhang conceived and designed the experiments; Jing Tan, Jason Gek Leong Neo and Tania Setiawati performed the experiments, analyzed the data and wrote the paper.

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References


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