Application of an HPLC method for the quality control of vitamin C content in foods for infants

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Vitamin C is an important water-soluble vitamin that humans are not able to synthesize, so it needs to be provided by the diet.

Babies less than 1 year usually do not eat foods naturally rich in vitamin C, so the main sources are breast milk and/or infant formulas.

Adequate intake of vitamin C is very important to assure children’s good health and development, so it is crucial to evaluate vitamin C content in this type of foods.

**Aim**

The aim of this study was to validate a high-performance liquid chromatography (HPLC) method for the quality control of vitamin C content in infant foods (2 infant formulae (IF), 2 follow-on formulae (FF), 2 processed cereal-based foods (PCF) and 2 baby foods (BF)).

**Materials and Methods**

In 2016, the infant foods were collected from major supermarket chains and parapharmacies in the region of Lisbon (Portugal).

Two of the acquired samples were already ready-to-use, while the remaining samples were prepared according to manufacturer’s instructions.

Sample extraction and chromatographic analysis were performed according to Valente et al. (2014) and Silva et al. (2017)\textsuperscript{4,5}.

The HPLC-DAD analytical method was validated for selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy, using an infant formula, according to international guidelines \[6,7\].

Figure 1 shows HPLC chromatograms for L-ascorbic acid standard (A) and for sample BF2 from baby foods’ category.

Calibration curves were linear over the range 1-100 µg/mL. The achieved LOD and LOQ were 0.026 and 0.086 µg/mL, respectively (Table 1).

The repeatability RSD (n=6) for vitamin C ranged from 0.54% to 1.85%. The intermediate precision RSD (n=18) was 4.63%, 7.64 ± 0.35 µg/mL (Table 2).

Vitamin C content in the analysed samples ranged from 1.5 ± 0.01 to 178 ± 1.01 mg/100 g for BF1 and PCF2, respectively (Figure 2).

**Results and Discussion**

**Conclusions**

The developed method is rapid, specific, precise and accurate, for the quantification of vitamin C in different categories of foods for infants and young children, showing satisfactory data for all the tested parameters. Since these type of food products are very important for such young and vulnerable consumers, regulation and assurance of an adequate intake of essential nutrients, like vitamin C, is extremely important for a healthy development.

**References**


**Acknowledgements**

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Table 1. Linearity, LOD and LOQ for vitamin C determination in infant foods.

<table>
<thead>
<tr>
<th>Concentration range (µg/mL)</th>
<th>Mean ± SD</th>
<th>LOD (µg/mL)</th>
<th>LOQ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope (n=6)</td>
<td>1 - 100</td>
<td>9.58 ± 10^{-1} × 1.59 ± 10^{2}</td>
<td>1.66</td>
</tr>
<tr>
<td>Intercept (n=6)</td>
<td></td>
<td>7.28 ± 10^{-4} × 1.98 ± 10^{4}</td>
<td>27.23</td>
</tr>
<tr>
<td>Determination coefficient (r²)</td>
<td></td>
<td>Mean ± SD</td>
<td>0.9996 ± 0.00</td>
</tr>
<tr>
<td>Equipment repeatability LOD (µg/mL)</td>
<td></td>
<td>RSD (%)</td>
<td>0.03</td>
</tr>
<tr>
<td>LOQ (µg/mL)</td>
<td></td>
<td>RSD (%)</td>
<td>0.07</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.026</td>
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<td></td>
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<td>0.086</td>
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Table 2. Validation data for method precision.

<table>
<thead>
<tr>
<th>Precision</th>
<th>Vitamin C</th>
<th>Mean (µg/mL) ± SD</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1 (n=6)</td>
<td></td>
<td>7.48 ± 0.04</td>
<td>0.54</td>
</tr>
<tr>
<td>Repeatability</td>
<td></td>
<td>7.34 ± 0.14</td>
<td>1.85</td>
</tr>
<tr>
<td>Day 3 (n=6)</td>
<td></td>
<td>8.10 ± 0.09</td>
<td>1.13</td>
</tr>
</tbody>
</table>

Intermediate precision (n=18): 7.64 ± 0.35 µg/mL (4.63)

**Figure 1.** HPLC chromatogram of: (A) standard of L-ascorbic acid and (B) baby food, sample BF2.

**Figure 2.** Vitamin C content (mg/100 g of sample) of the analysed samples. IF – Infant formula, FF – Follow-on formulae, PCF – Processed cereal-based foods, BF – Baby foods.