

# 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.

Regulation (EU) No. 609/2013, Commission Delegated Regulation (EU) No. 2016/127 and *Ministério da Agricultura, do Desenvolvimento Rural e das Pescas* (2008) establishes compositional and information requirements for foodstuffs for infants and young children [1-3].

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

### Sampling

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.



6 scoops of an infant formula powder



180 mL of boiled water



Infant formula

Sample extraction and chromatographic analysis were performed according to Valente *et al.* (2014) and Silva *et al.* (2017) [4,5].

## Results and Discussion

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).

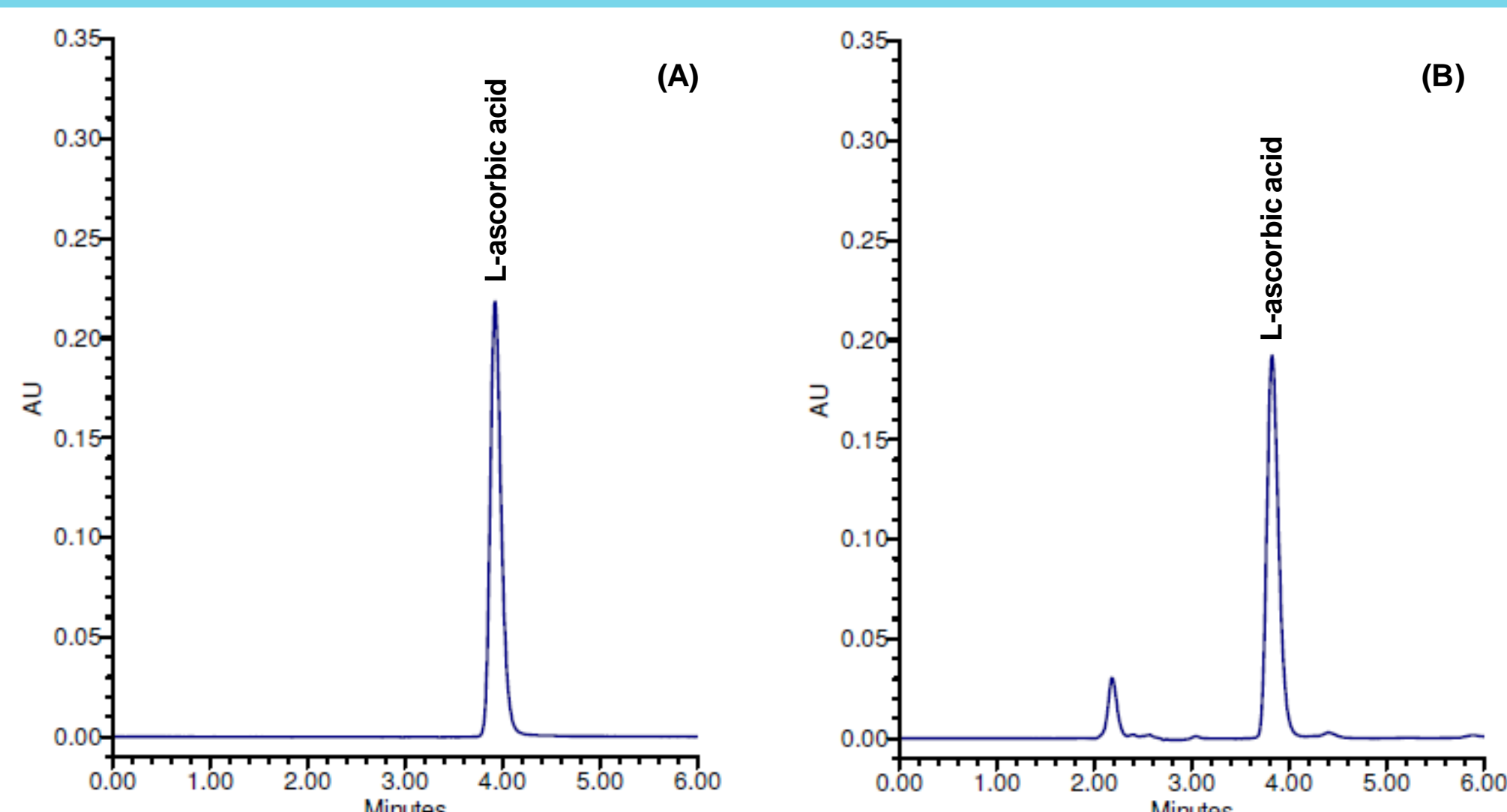


Figure 1. HPLC chromatogram of: (A) standard of L-ascorbic acid and (B) baby food, sample BF2.

## 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

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

Concentration range (µg/mL)		1 - 100
Slope (n=6)	Mean ± SD	9.58 x 10 <sup>4</sup> ± 1.59 x 10 <sup>3</sup>
	RSD (%)	1.66
Intercept (n=6)	Mean ± SD	7.28 x 10 <sup>4</sup> ± 1.98 x 10 <sup>4</sup>
	RSD (%)	27.23
Determination coefficient (r <sup>2</sup> )	Mean ± SD	0.9996 ± 0.00
	RSD (%)	0.03
Equipment repeatability	RSD (%)	0.07
	LOD (µg/mL)	0.026
LOQ (µg/mL)		0.086

Table 2. Validation data for method precision.

Precision		Vitamin C	
		Mean (µg/mL) ± SD	RSD (%)
Repeatability	Day 1 (n=6)	7.48 ± 0.04	0.54
	Day 2 (n=6)	7.34 ± 0.14	1.85
	Day 3 (n=6)	8.10 ± 0.09	1.13
Intermediate precision (n=18)		7.64 ± 0.35	4.63

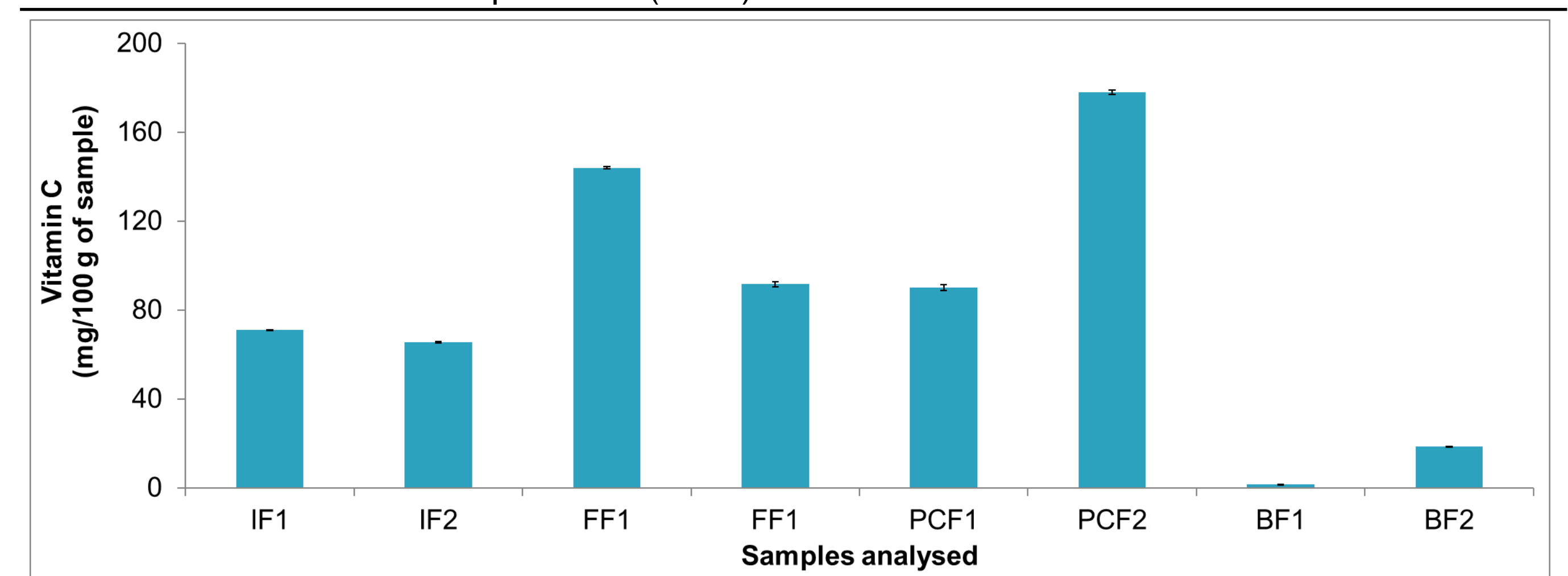


Figure 2. Vitamin C content (mg/100 g of sample) of the analysed samples. IF – Infant formula; FF – Follow-on formulae; PCF – Processed cereal-based foods; BF – Baby foods