Development of a reference material for L-ascorbic acid in fruits and vegetables

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Introduction

Ascorbic acid (AA) is a water soluble vitamin that plays a determinant role in the defense against cellular damage through its antioxidant activity. Fresh fruits and vegetables are the main natural sources of AA [1]. Economic, rapid, accurate and sensitive methods for laboratory analysis are needed to improve the quality of analytical data. Regular use of a reference material and participation in proficiency testing schemes are helpful to evaluate the performance of laboratories as well as are useful for the quality control of analytical procedures [2, 3].

Aim

The aim of the study was to develop and validate a high-performance liquid chromatography method and to produce a reference material to be used as a quality control material in routine laboratory assays.

Methods

The analysis procedure has three major steps: a simultaneous deproteinization and stabilization with an extraction solution of perchloric acid and metaphosphoric acid; dilution with mobile phase and finally two consecutive filtrations. Chromatographic separation was performed on an Alliance 2695 equipment with UV detection at 246 nm, using a Phenomenex, SynergiTM Hydro-RP (150 x 4.6 mm, 4.0 µm) column with a SecurityGuard Cartridge AQ C18 (4.0 x 2.0 mm). Validation procedure was performed according to Food and Drug Administration or International Conference on Harmonization guidelines. Homogeneity study was performed by the analysis of 20 random samples selected from two different batches. Samples stability was tested in different periods of time, for one year, at two temperatures (+4 ºC and -80 ºC).

Results

Figure 1 shows a chromatogram of an orange juice sample. The method was linear over the range of 1-100 µg/ml (Table 1) with a LOD of 0.035 µg/ml and a LOQ of 0.090 µg/ml. The within-day and between-day precision were 0.58% and 3.67%, respectively (Table 2). The overall recovery values at three spiking levels (20, 60 and 100 µg/ml) were 96.7%, 96.6% and 97.3%, respectively (Table 3). In the homogeneity study, no significant differences were obtained between samples from the two batches. The short- and long-term stability studies showed that orange juice samples can be kept at -80 ºC for at least 12 months after initial treatment with a stabilization solution.

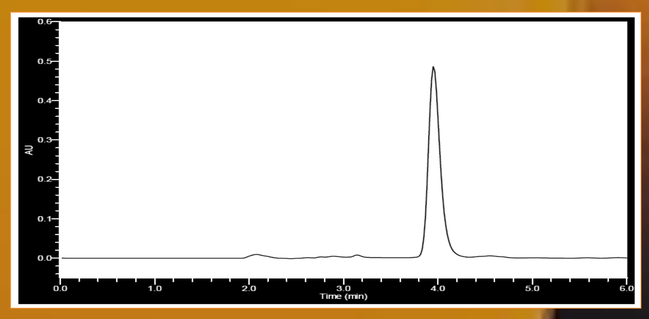

Fig. 1 - Chromatogram of an orange juice sample with 51.4 µg/ml of ascorbic acid.

Table 1 – Linearity of HPLC method for ascorbic acid analysis.

Parameter	Mean (µM) ± SD	CV%	n
Slope	$9.96 \times 10^4 \pm 1.41 \times 10^3$	1.41	6
Intercept	$-2.65 \times 10^4 \pm 1.52 \times 10^4$	57.36	6
r^2: determination coefficient	0.9990 ± 0.0023	0.24	6

Table 2 – Validation data for method precision.

Precision		Mean (µg/ml) ± SD	CV%
Repeatability [a]	day 1	63.8 ± 0.28	0.43
	day 2	69.0 ± 0.41	0.60
	day 3	68.7 ± 0.48	0.70
Intermediate precision [b]	between-day	67.2 ± 2.47	3.67

[a] Analysis of six samples in triplicate on the same day
[b] Analysis of six samples in triplicate for 3 days

Table 3 – Validation data for method accuracy.

Ascorbic acid added (µg/ml)	Mean (µg/ml) ± SD	Recovery[a] (%)	CV%
20	19.3 ± 0.26	96.7	1.32
60	58.0 ± 1.34	96.6	2.31
100	97.3 ± 1.53	97.3	1.57

[a] Overall mean recovery of ascorbic acid added to orange juice samples (96.9%).

Conclusion

A full validation of a highly sensitive, rapid, precise and accurate HPLC method was performed for laboratory routine use. This method is an excellent choice for quantification of AA in foods and to generate high quality analytical data. The homogeneity and stability studies of the produced in-house reference material showed that this product is suitable for use in quality control of AA laboratory assays.

Acknowledgments

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