DETERMINATION OF TOTAL VITAMIN C IN FOODSTUFFS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION
- INTERLABORATORY STUDY -

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INTRODUCTION

We report here the results obtained with a sensitive and highly selective alternative HPLC method with electrochemical detection using L-cystein as precolumn reagent for the reduction of DHAA to AA for determination of total vitamin C in a wide range of fortified food products. This method can also be recommended for checking the presence of isoascorbic acid.

A collaborative study was performed with 3 laboratories to evaluate the performance of the method.
The samples used for this study, ranged from 5 to 4500 mg/100g, included: a certified reference material (Infant/Adult Nutritional Formula , SRM 1849a), dietetic milk powder, cereal based infant formula, fortified orange juice, fortified vegetable and cheese soup, apple and banana compote, multivitamin tablets.

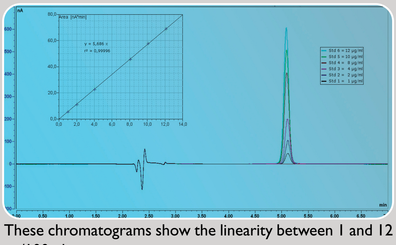
In addition, the SCL laboratory of Strasbourg (from French Ministry of Economy and Finances) in charge of the nutritional food compounds checking for many years and which organized in 1997 the interlaboratory validation study on a new vitamin C procedure become the EN14130 standard performed the analysis. Results reported in Fig.1 were similar to those found with the alternative method.

The European Standard EN 14130 for determination of vitamin C by HPLC with UV detection has been withdrawn. The method described in this standard was hardly used because of difficulties with the chromatographic analysis due to the use of a counter-ion in the mobile phase.
Another method should be selected to replace this standard and it is necessary to take in consideration the following points:
- A total vitamin C analysis involves the quantification of the two active forms, ascorbic acid (AA) and its oxidized form dehydroascorbic acid (DHAA). The quantification of DHAA is usually performed after its conversion into AA in the presence of reducing agents.
- The D-isomer of ascorbic acid, isoascorbic acid also called erythorbic acid which has a very limited antiscorbutic activity can be used as an antioxidant food additive. Therefore, in the field of nutritional control, a reliable vitamin C method should be able to differentiate between AA and isoAA.
Sample preparation described in the withdrawn EN 14130 (metaphosphoric acid extraction and reduction of DHAA to AA with L-cystein) is still of interest and the performance of analytical columns available nowadays allows a good resolution between AA and isoAA (there is no need to use counter-ion anymore).
Moreover, the use of electrochemical detection instead of UV detection is more specific and improves the chromatograms quality.

PROTOCOL & RESULTS

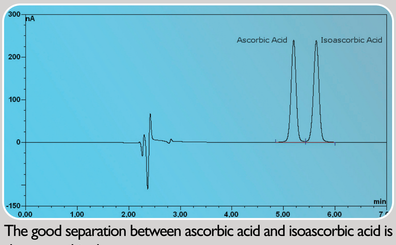
Vitamin C is extracted from samples with metaphosphoric acid 2%. After the conversion of DHAA into AA in the presence of reducing L-cystein solution, ascorbic acid is quantified by reverse phase HPLC with electrochemical detection. It is also to point out that no organic solvent is used in this method.

6 levels standard profile



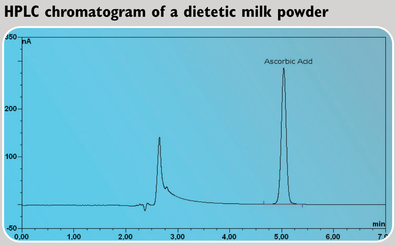
These chromatograms show the linearity between 1 and 12 µg/100ml.

HPLC chromatogram of AA and isoAA standards



The good separation between ascorbic acid and isoascorbic acid is shown on this chromatogram.

HPLC chromatogram of a dietetic milk powder



The chromatographic conditions offer a very short retention time (5 minutes) and there is no interference between the matrices (milk powder here) and ascorbic acid.

Method performance (Table 1):

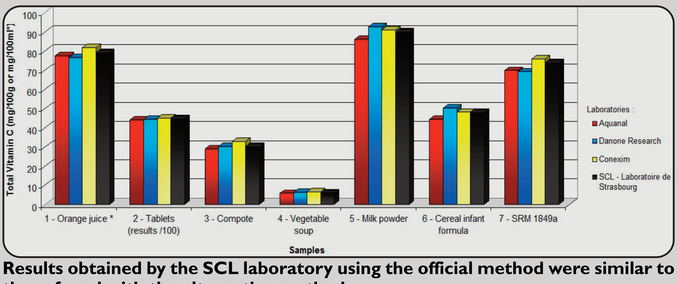
Parameters	Samples [a] 1	2	3	4	5	6	7
Number of participants	3	3	3	3	3	3	3
Number of tests *(all values - no invalid results)*	27	27	27	27	27	27	27
Labelled value *(mg/100g or mg/100ml*)*	42	4000	20	3,80	105	30	78,4
Average *(mg/100g or mg/100ml*)*	78,7	4448	30,7	6,24	90,0	47,6	71,5
s_r *(mg/100g or mg/100ml*)*	0,76	97,5	0,90	0,19	3,41	3,94	1,58
RSDr *(%)*	0,97	2,19	2,94	3,11	3,79	8,28	2,21
$r = 2{,}8s_r$ *(mg/100g or mg/100ml*)*	2,13	273	2,52	0,53	9,55	11,03	4,42
s_R *(mg/100g or mg/100ml*)*	3,37	231	2,30	0,47	5,19	4,82	4,33
RSD_R found *(%)*	4,28	5,20	7,48	7,60	5,77	10,11	6,05
$R = 2{,}8s_R$ *(mg/100g or mg/100ml*)*	9,44	647	6,44	1,32	14,53	13,50	12,12
RSD_R calculated *(%)*	5,84	3,19	6,73	8,55	5,73	6,30	5,93
HORRAT$_R$ *(RSD_R found / RSD_R calculated) (Horwitz ratio)*	0,73	1,63	1,11	0,89	1,01	1,60	1,02

[a] 1 = Orange juice; 2 = Tablets ; 3 = Compote ; 4 = Vegetable soup ; 5 = Milk powder ; 6 = Cereal infant formula ; 7 = SRM 1849a*

Whatever the matrices and vitamin C concentration (between 4 and 4000 mg/100g) this method yields good results and variability.

All the laboratories performed three independent triplicate analyses on the seven types of fortified samples. Comparable results were obtained between the 3 laboratories **(Fig.1).**

Collaborative results (Fig.1):
(Average of 9 results for each laboratory)



Results obtained by the SCL laboratory using the official method were similar to those found with the alternative method.

All the results were statistically analysed and the following parameters were calculated (Table 1): standard deviation of repeatability (S_r) and reproducibility (S_R), relative standard deviation of repeatability (RSD_r) and reproducibility (RSD_R), repeatability ($r = 2.8S_r$) and reproducibility ($R = 2.8S_R$), HORRAT value. The overall RSD_R values obtained on 7 samples analysed by 3 different laboratories ranged between 4.28 and 10.11%. The HORRAT parameter ranged between the limiting values of 0.5-2.0 demonstrates acceptable reproducibility of the method.

CONCLUSION

The proposed HPLC method for determining total vitamin C in foodstuffs is easily usable for routine analysis, has the advantage to be a "green method" and offers:

- accuracy,
- reliability,
- good repeatability and reproducibility,
- separation of AA and iso AA,
- a relative short analysis time.

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