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TECHNICAL NOTE

Comparison of voltammetric and high performance liquid chromatographic methods for ascorbic acid determination in infant formulas

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Two methods — voltammetric and high performance liquid chromatographic (HPLC) — useful for determining ascorbic acid in foods, were compared to ascertain which of them could be used for routine determination of ascorbic acid in infant formulas. Both methods were used to assay 10 identical samples of an adapted cow's milk infant formula and 10 samples of soya protein formula. Precision was determined, the variance of each method was measured, and the methods were compared with each other. The variances of the two methods were not statistically different nor was there any significant difference between the results from the two methods. Therefore, it was concluded that both methods can be used for determination of ascorbic acid in infant formulas. The voltammetric method, because of its simplicity, cheapness and less time-consuming nature, could be used for routine determination.

INTRODUCTION

A number of physiological and biochemical functions have been described for vitamin C in the human being, although evidence for the precise mechanism of action is only available for collagen biosynthesis. Infants fed exclusively on diets based on cow's milk and the elderly having a monotonous diet are within the main risk groups susceptible to suffer from vitamin C deficiencies (NRC, 1990).

Cow's milk has a relatively low vitamin C content; furthermore, it is very unstable especially in the presence of air, light, and high temperatures used for hygienisation. For this reason, and to meet the recommended dietary allowance of 7 mg per 100 kcal (ESPGAN, 1987) (FDA, 1985) or 8 mg per 100 kcal (CODEX, 1987), ascorbic acid must be added to infant formulas during their manufacture (Hurrell *et al.*, 1989).

It is important to develop simple accurate analytical methods for routine determination of ascorbic acid in infant milk formulas. Those methods should be applicable to both industry and government laboratories.

Vitamin C has been analysed in infant formulas by the 2,6-dichloroindophenol titrimetric method (Martin *et al.*, 1985; Tanner & Barnett, 1985), which is useful but time consuming. In addition, it suffers from many interferences and only ascorbic acid (AA) but not dehydroascorbic acid (DHAA) can be estimated. One of the most specific methods for the determination of 'total vitamin C' (AA + DHAA) is the fluorometric procedure. This method is based on the coupling reaction of *o*-phenylenediamine across the *cis*-hydroxyl groups of DHAA to form a fluorescent quinoxaline derivative. Both titrimetric and fluorometric methods are AOAC (1990) final action procedures although they have certain limitations due to the presence of interfering substances.

High performance liquid chromatography (HPLC), equipped either with a UV or an electrochemical detector, is currently the most commonly used technique for the analysis of ascorbic acid in food (Rose & Nahrwold, 1980; Tsao & Salimi, 1982; Ashoor *et al.*, 1984; Kneifel & Sommer, 1985; Behrens & Madére, 1987; Margolis & Black, 1987; Bognar, 1988). There is

also interest in the simultaneous determination of both AA and its direct oxidation product (DHAA), since DHAA has a different antioxidant activity.

Voltammetric techniques are not as common as the HPLC methods but the advantage of low cost and very good sensitivity in the determination of ascorbic acid (Branca, 1980; Erb *et al.*, 1981; Gerhardt & Windermüller, 1981; Lechien *et al.*, 1982; Cherdkiatgumchai & Grant, 1987; Koza *et al.*, 1988; Sahbaz & Somer, 1992). The simplicity of the procedure and the equipment make these techniques an attractive alternative for routine control of the ascorbic acid in dietetic foods.

The aim of this study was to compare a voltammetric method with a HPLC one for the determination of ascorbic acid in infant formulas, to ascertain whether the first one could be a good alternative to the chromatographic method.

MATERIALS AND METHODS

Instrumentation

The current voltammetric measurements were obtained by a Polarographic System 663 VA STAND and a control unit 626 Polarecord (Methrom, Switzerland). The HPLC experiments were performed with a Waters Chromatograph (Milford, USA) comprising a 717 autosampler, 519 pump, 996 photodiode array detector and Millennium 2011 chromatography manager.

All reagents used were of analytical reagent grade. Deionised water (Milli-Q Water system, Millipore Inc., USA) with a metered resistance of 19 MΩ, was used to prepare samples and standards.

Metaphosphoric acid, sodium acetate, potassium phosphate, oxalic acid dihydrate and trichloroacetic acid (TCA) were supplied by Panreac (Montplet & Esteban S.A., Barcelona, Spain). Ascorbic acid was obtained from Merck (Darmstadt, Germany).

Samples

Twenty cans (400 g) of powdered infant formula in a nitrogen atmosphere from identical homogeneous batches; 10 cow's milk-based and 10 soya-based, were supplied by the manufacturer Puleva®.

Procedure

Determination of ascorbic acid by HPLC method
Ascorbic acid was determined according to Behrens & Madére (1987, 1989) with the following modifications: ascorbic acid content of infant formula (5 g) was brought to a final volume of 100 ml with 1% (w/v) metaphosphoric acid solution. Homogenates were submitted to 2000 *g* in a refrigerated centrifuge for 10 min. The clear supernatant was filtered through Whatman 501 paper. A portion of 1 ml was diluted to 5 ml with 1% (w/v) metaphosphoric acid and a 10 μl aliquot of this solution was injected into the HPLC system (column: stainless steel 5 μm Superspher 100RP-18 cartridge; mobile phase: potassium buffer solution 0·1 M pH 3·5, 1 ml/min; UV detection 245nm) to determine the concentration of ascorbic acid in the sample. Aliquots of 10 μl of ascorbic acid standards solutions (1·25– 10·00 μg/ml), prepared by appropiate dilution of working standard solution (250 μg/ml) in 1% (w/v) metaphosphoric acid solution, were used to do a standard curve by plotting peak area versus ascorbic acid amount after HPLC analysis.

Determination of ascorbic acid by voltammetric method
Ascorbic acid was determined according to Lau *et al.* (1985). A sample (0·5 g) was dissolved in 25 ml of extraction liquid, 1% (w/v) trichloroacetic acid / 1% (w/v) sodium sulphate (v/v), and filtered through Whatman 501 paper. An aliquot of 1 ml was diluted to 10 ml with 1% (w/v) oxalic acid solution, transferred quantitatively to the voltammetric cell and then 2 ml of 2 M acetic-acetate buffer solution (pH 4·5) was added. Nitrogen was bubbled for 2 min to eliminate air and the voltammogram was recorded by applying the following instrumental conditions: mode DP50-DME; potential initial: –0·15 V, final: + 0·20 V; scan rate: 5 mV/s; current sensitivity: 5 nA/mm; and drop time: 1 s.

The amount of ascorbic acid present in the sample solution was determined by standard-addition calibration. Standard ascorbic acid solution (100 μg/ml) in 1% (w/v) oxalic acid solution was freshly prepared.

Statistical analysis

Precision — the variances of the two methods were compared by using *F*-test comparison of variances.

A paired Student's *t*-test for mean comparison was applied to the ascorbic acid content values of 10 aliquots of the same infant formula coming from different packs.

RESULTS AND DISCUSSION

Official methods for the determination of vitamin C in food have been substituted for HPLC methods, which are more reliable and sensitive. Only a few polarographic methods have been described in the literature for the analysis of vitamin C.

In this study, we have compared a voltammetric method with an HPLC method to determine precision and to detect possible differences in contents determined by applying each of the methods. The data obtained for vitamin C analysis of 10 replicates are presented in Table 1. The ratio between variances of both methods did not exceed the tabulated *F* values even at 0·05 level of probability.

To detect differences in mean values, samples of 10 different batches of an adapted cow's milk formula and soya protein formula were analysed by HPLC and voltammetric methods. The ascorbic acid values obtained are shown in Table 2.

Table 1. *F*-test for comparison precision of voltammetric method and HPLC method for determination of ascorbic acid

	Voltammetric method		HPLC method		F^c
	N^a	S^{2b}	N^a	S^{2b}	
Milk-based infant formula	10	4·3	10	2·9	1·7
Soya-based infant formula	10	2·2	10	1·3	1·6

[a] N, number of replicates.
[b] S^2, corrected sample variance.
[c] $F = S_1^2/S_2^2$. Tabulated F values: $F_{0\cdot05(9,9)} = 3\cdot18$; $F_{0\cdot01(9,9)} = 5\cdot35$.

Table 2. Comparison of ascorbic acid contents (mg per 100 g) obtained by voltammetric method and HPLC method on milk-based infant formula and soya-based infant formula

Sample number[a]	Milk-based infant formula		Soya-based infant formula	
	Voltammetric method	HPLC method	Voltammetric method	HPLC method
1	50·93	45·97	43·09	39·72
2	50·98	46·86	44·38	42·61
3	51·22	45·01	44·32	41·71
4	49·56	48·03	42·20	43·36
5	48·55	46·45	50·00	43·43
6	49·05	45·35	45·37	41·99
7	43·56	49·61	45·00	43·09
8	52·76	48·03	48·84	42·40
9	47·73	47·82	49·89	42·61
10	48·52	44·94	39·61	44·94
$X \pm$ SD	49·28 ± 2·53	46·81 ± 1·55	45·27 ± 3·41	45·59 ± 1·35
d	2·48		2·68	
s	3·51		3·83	
$d/(s/n^{1/2})$	2·23		2·22	
t_{n-1}	2·26		2·26	

[a] d, mean of the differences; s, standard deviation of the differences; n, number of replicates.

Absolute mean values for the voltammetric methods were higher than those obtained by HPLC, but this difference was not significant at $P = 0\cdot05$.

These results suggest that both methods are equivalent in precision since their variances are not significantly different. Means of ascorbic acid in the infant formulas analysed were slightly lower than those obtained by the voltammetric method, but this difference was not significant at $P = 0\cdot05$, according to the results.

CONCLUSION

The voltammetric method is simpler and less time consuming than the HPLC. That means that it could be used for routine purposes, taking into consideration it will result in slightly higher values than those obtained by HPLC which apparently is highly sensitive. The latter can also be used when DHAA and AA must be analysed.

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