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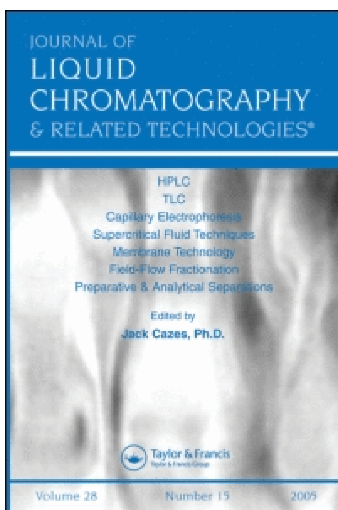

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L-ASCORBIC ACID DETERMINATION IN FRUITS AND MEDICAL FORMULATIONS BY ION INTERACTION REAGENT REVERSE PHASE HPLC TECHNIQUE

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ABSTRACT

The content of L-ascorbic acid has been evaluated in juices of fruits and in typical medical formulations. Grapefruits, orange, lemon and kiwi ,as well as tablets of vitamin C and antipyretic medicine added with vitamin C have been analyzed.

The ion interaction reagent reverse phase HPLC chromatographic technique has been employed, making use of octylaminium salicylate as interaction reagent and of a C-18 spherical 5 μm column as stationary phase.

Sensitivity levels of the order of 100 pmoles can be obtained. The dependence on time and temperature of the oxidation processes which L-ascorbic acid undergoes during its preservation has also been studied.

INTRODUCTION

It would seem that the widespread opinion that considers L-ascorbic acid as a sort of health-giving panacea has to be reconsidered, at least from the point of view of the ingested

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amount. Recent studies reported by mass media are suggesting that assumption of ascorbic acid could cause diseases if a certain daily amount is exceeded. In particular assuefaction, dependence and even mental diseases are indicated as possible consequences. The good properties of L-ascorbic acid in contrasting and preventing a series of pathologies are however still recognized. Therefore , in order to correctly plane its dosage, the working out of reliable and fast analytical methods for the quantitation of ascorbic acid in foods and in medical formulations are receiving always increasing interest.

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Literature reports different methods for the analysis and the quantitation of L-ascorbic acid, mainly performed by means of spectrophotometric [1-3] and HPLC techniques, with [4,5] or without derivatization [6-13] .

In this paper a new methodology is presented which has been shown to be rapid, selective and very sensitive. It makes use of the ion interaction reagent reverse phase HPLC chromatographic technique with spectrophotometric detection at λ=254 nm.

The method was applied in the analysis and quantitation of L-ascorbic acid in juice of some fresh fruits, in preserved fruit juice and in two medical formulations.

EXPERIMENTAL

Apparatus

Analyses were performed by using a Varian LC 5000 chromatograph, equipped with a Vista 401 Data System and UV-100 spectrophotometric detector. For pH measurements a Metrohm 654 pH-meter was employed.

Chromatography

The stationary phase was a Merck Lichrospher RP-18,250 x 4, 5 μm column, equipped with a guard column Merck Hibar Lichrocart Lichrosorb RP-18, 25 x 4 .

The solution of octylamine salicylate to be used as ion interaction reagent was prepared, as described in previous papers (14-17) by dissolving the weighed amount of the amine in ultra-pure water and bringing the solution to a pH 6.3 $\pm$ 0.4 by additions of salicylic acid. The solution was freshly prepared every three days. The chromatographic system was conditioned by flowing the eluent through the column for times of at least an hour. Between uses, columns were regenerated by the passage of water-methanol (1:1). By adopting this treatment, no particular deterioration of the column was observed with respect to its use in other chromatographic techniques.

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Chemicals

Ultra-pure water from a Millipore Milli-Q was used for the preparation of solutions. Octylamine, L-ascorbic and dehydroascorbic acids were Fluka analytical grade reagents. Salicylic acid and all other reagents were Carlo Erba analytical grade chemicals.

Samples preparation.

For the analysis concerning the content of ascorbic acid in kiwi, the fruit was homogeneized, ultracentrifugated at 3000 rpm for 10 min and then filtered through Nucleopore Syrfil 25 mm 0.45 μm filters. Juices were obtained for squeezing the fruits , ultracentrifugation and filtration as above described.

The samples of medical formulations to be injected , namely vitamin C tablets and antipyretic tablets containing acetylsalicylic acid added with vitamic C , were prepared by dissolving the tablets in 1000.0 ml of ultra pure water and filtering.

Standard solution of L-ascorbic acid were freshly prepared each three hours.

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RESULTS AND DISCUSSION

The ion interaction reagent reverse phase HPLC technique has been shown very efficaceous for the separation and analysis of anions, both organic and inorganic [14,15,17].

In a previous work [17] a methodology has been developed for the separation of some organic anions in wines and drinks. The use of different reverse phase columns as well as of different aminium salicylates as ion interaction reagents was tested and compared.

The good properties of sensitivity and resolution shown by L-ascorbic acid when using spectrophotometric detection suggested to search for the best chromatographic conditions for its evaluation and quantitation.

Preliminar experiments showed that a reverse phase C-18 5 μm spherical packing column as stationary phase and octylaminum salicylate 0.0050 M flowing at 1.0 ml/min as ion interaction reagent are the most suitable chromatographic conditions,from the point of view both of sensitivity and resolution. Salicylate ion shows at 254 nm a relatively high adsorptivity ,($\varepsilon=(3.08 \pm 0.02).10^2\ l\ m^{-1}cm^2$, which permits both direct and indirect spectrophotometric detections. Due to its high adsorptivity (ε =

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($8.72 \pm 0.04).10^3 lm^{-1} cm^2$) at this wavelength, L- ascorbic acid comes out as positive peak and can be easily identified among other anions which are generally also present in fruits, like chlorides, nitrates, o-phosphates, tartrates and citrates. These anions are in fact transparent at 254 nm and come out as negative peaks.

In addition, sensitivity shown by L-ascorbic acid in these chromatographic conditions can be evaluated of the order of 113 pmoles injected. Figure 1 shows the chromatogram obtained for injection of 100 µl of a standard solution containing 1.00 mg/l.

Figure 2 shows the chromatograms recorded within an hour from sample preparation, on a kiwi juice undergone to different successive dilutions. Figure 2a refers to the not diluted juice, figure 2b to a dilution 1/10 v/v and 2c to a dilution 1/75. It can be evidenced the high sensitivity of detection of L-ascorbic acid, which makes its evaluation free from interference by the other anionic components present.

In turn, figure 3 shows the chromatogram of a sample of commercial aspirin added with vitamin C. A tablet of the antipyretic,mainly containing acetylsalicylic acid ,was dissolved in 1000.0 ml of ultrapure water and the resulting solution was

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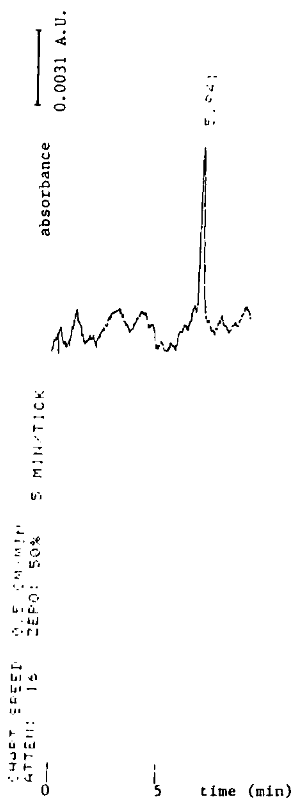

Figure 1 . Standard L-ascorbic acid 1.00 ppm.
Injection : 100 μl .
Ion interaction reagent : Octylaminium salicylate 0.005 M.
Flow rate : 1.0 ml/min.
Stationary phase : Merck Hibar Lichrospher C 18, 5 μm.
Spectrophotometric detection , λ =254 nm.

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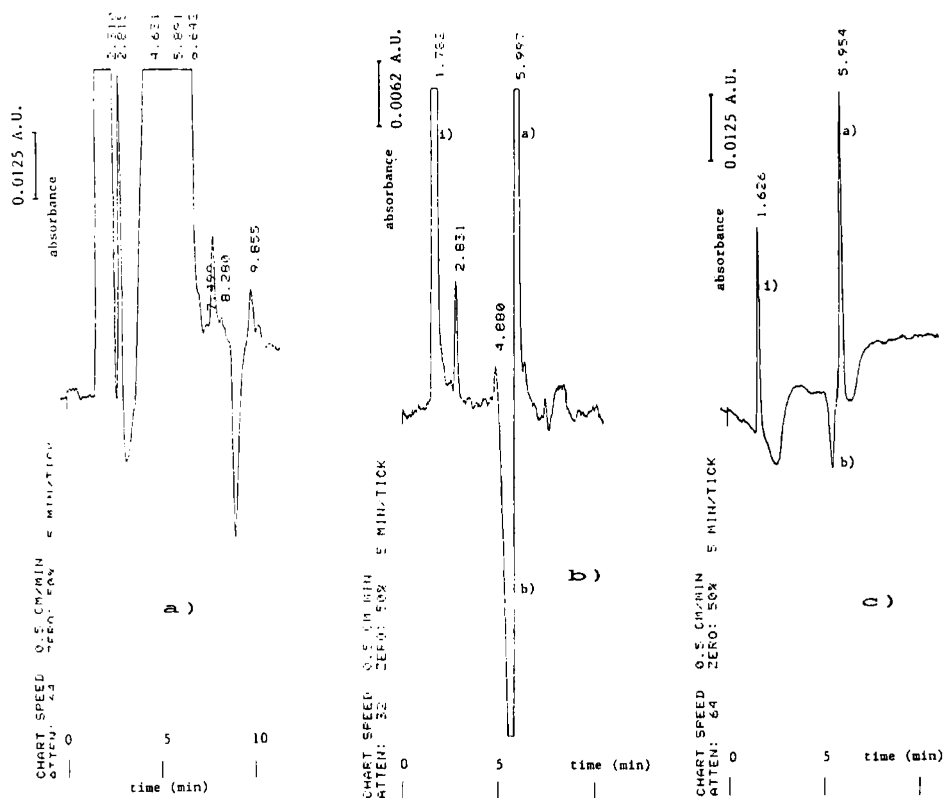

Figure 2 . Grapefruit juice.
a) non diluted b) diluted 1/10 v/v c) diluted 1/75 v/v
Chromatographic conditions as in figure 1.

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furtherly diluted 1/50 v/v before the chromatographic analysis. Figure 3 shows that the analysis is free from interference,even in the presence of acetylsalicylic acid .

As it concerns the quantitative evaluation, standard additions method was employed.Measurements performed with known

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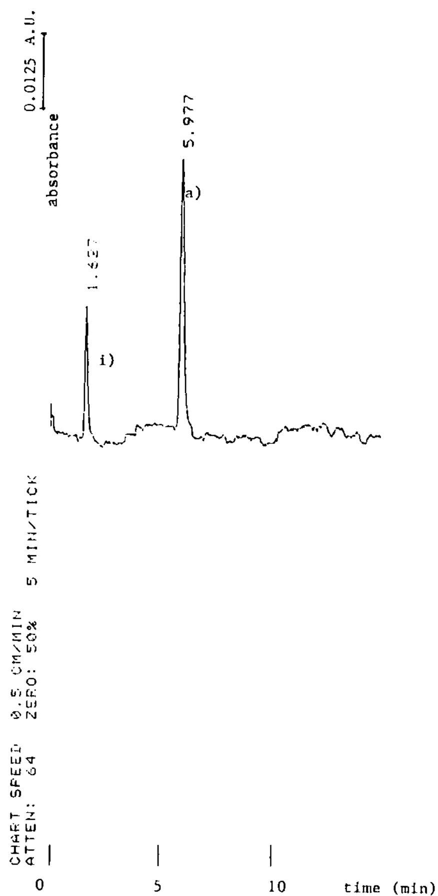

Figure 3 . Aspirine added of vitamin C. One tablet was dissolved in 1000.0 ml. This solution was furtherly diluted 1/50 v/v. Chromatographic conditions as in figure 1.

added amount of the analyte permitted to exclude any matrix effect on the determination. The calibration plots relating the peak areas with standard L-ascorbic concentrations showed an exceptionally good linear relationship (average correlation

coefficient equal to 0.996) over the range 0.2 - 10.0 mg/l. Reproducibility (standard deviation estimate) is within 1% between repeated injections. Anyway, in order to take into account also average variations in the vitamin C content coming from oxidation processes , the average quantitation data reported in table I have been calculated from the results collected within an hour from sample preparation.

Table I lists the average amount of L-ascorbic acid evaluated for grapefruit, lemon, orange, kiwi, preserved grapefruit juice, C vitamin tablets and aspirin .For medical formulation tablets also the expected values are reported.

As it concerns stability of L-ascorbic acid, literature (18) agrees in indicating a certain decay in the time and the addition of 0.5 % metaphosphoric acid is suggested to prevent this effect. Being furthermore vitamin C indicated as a thermolabile one, it is a general opinion that it can be longer preserved in refrigerators at 4°C, with respect to room temperature .

Taking into account that additions of metaphosphoric acid to the sample does not interfere in our chromatographic conditions with ascorbate determination, a series of analyses was

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TABLE 1

Quantitation of L-ascorbic acid (mg/l) in Fruit juices and Medical formulations. Each analysis was performed within one hour from sample preparation.
Estimates of Standard Deviation were calculated on the basis of at least four measurements.

Fruit	found (g/l)	expected (g/l)
grapefruit	0.47 ± 0.05	
lemon	0.51 ± 0.04	
orange	0.67 ± 0.03	
kiwi	0.95 ± 0.04	
preserved grapefruit juice	0.29 ± 0.02	
C vitamin tablets *	0.98 ± 0.05	1.000
aspirin added with C vitamin *	2.99 ± 0.05	2.400

* 1 tablet was dissolved in 1000.0 ml of ultrapure water.

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performed on the lemon juice, added or not of metaphosphoric acid. The L-ascorbate content was followed as a function of time and temperature.

The results , listed in table 2, confirm that a natural loss of L-ascorbate in the time surely verifies. Natural decay is as relevant as about 17 % during the first 3 hours, becoming

TABLE 2

Lemon juice Analysis. Dependence of L-ascorbic acid content on Temperature and Addition of 0.5 % Metaphosphoric acid.

temperature	25°C		4°C	
	added of m-phosphoric	without addition	added of m-phosphoric	without addition
time after preparation				
1 hour		0.51 ± 0.04		
3 hours	0.51 ± 0.06	0.43 ± 0.04	0.51 ± 0.06	0.41 ± 0.04
1 day	0.33 ± 0.04	0.34 ± 0.03	0.34 ± 0.03	0.36 ± 0.03
2 days	0.26 ± 0.03	0.30 ± 0.02	0.29 ± 0.02	0.28 ± 0.02

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afterwards practically negligible. Additions of m-phosphoric acid were shown to prevent the decay in the first hours, loosing its efficacy after about 12 hours.

No significant differences, on the other hand , neither in the first hours, could be observed between samples preserved at room temperature and at 4°C.

The decay of vitamin C is due to oxidation processes which mainly lead to the formation of a dimeric form, the dehydroascorbic acid. Being this last characterized at wavelength of 254 nm by a practically null value of absorptivity

its evaluation would be possible in the here adopted experimental conditions through indirect spectrophotometric detection. The detection sensitivity that can be achieved in these conditions is however about 200 folds lower than that of L-ascorbic acid, so that a direct decomposition kinetic investigation is precluded.A previous reduction of dehydroascorbate to ascorbate is suggested (6,10), by using for example DL-homocysteine as reducing agent : two runs, performed on the same sample added or not of reducing agent allow to evaluate the concentration ratio of oxidized to reducted form.

It can be concluded that the proposed methodology offers undeniable advantages of reproducibility, accuracy and very good sensitivity.Times not greater than 7 minutes are necessary and interference by other reducing species and monosaccharides are excluded. Furthermore, the sample does not require any pretreatment or derivatization other than squeezing, filtering and dilution.

This feature, which has in any case the undeniable advantage of time-saving, assumes a particular relevance in analysis in which the analyte is unstable and can undergo degradations depending both on time and preliminar treatments.

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