Quantitative Analysis of Vitamin C by Ion Suppression Chromatography

Application Note

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Introduction

This note describes the optimization of an ion suppression reversed phase HPLC method to detect L-ascorbic acid, and use of the method in the quantitative determination of the acid in fruit juice. PLRP-S columns were used in this investigation. PLRP-S is a rigid macroporous styrene/divinylbenzene HPLC phase with outstanding chemical and physical stability, and high surface area. This chemical stability permits the use of low pH eluents and ion suppression reversed phase HPLC.

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Materials and Reagents

Reference samples: L-ascorbic acid and D-erythorbic acid (D-*iso* ascorbic acid)

Conditions

Column: PLRP-S 100Å 5 µm, 250 x 4.6 mm (p/n PL1512-5500)
Eluent: 0.2 M NaH_2PO_4, pH 2.14
Flow rate: 0.5 mL/min
Detector: UV, 244 and 220 nm

Results and Discussion

Mobile phase optimization was achieved with 0.2 M NaH_2PO_4 at pH 2.14 (Figure 1) with reference to the separation of L-ascorbic acid and D-erythorbic acid. Reducing the pH to 2.14 at 0.2 M produced an increase in the capacity factor for L-ascorbic and D-erythorbic acids; further reductions in pH had minimal effect. There was no effect of ionic strength (from 0.2 M to 0.4 M) on the capacity factor of L-ascorbic acid. However, there was more variation in the capacity factor of D-erythorbic acid, giving a maximum in the calculated selectivity between 0.2 M and 0.3 M. Therefore, it was concluded that the optimum mobile phase to resolve L-ascorbic acid from the solvent front and D-erythorbic acid was 0.2 M sodium dihydrogen phosphate adjusted to pH 2.14 with HCl.

Addition of acetonitrile as an organic modifier reduced the solute capacity factors of both acids such that baseline resolution was lost at 1% ACN.

For quantification of L-ascorbic acid in fruit juice a calibration graph was first developed (Figure 2). Two wavelengths were used; 244 nm, the wavelength for the maximum absorption of the acid, and 220 nm. The variable wavelength UV detectors were employed in series. Using the same experimental conditions, 20 µL of samples of diluted fruit juice (1:50 with eluent) were injected and produced the chromatograms shown in Figures 3 and 4. With no pretreatment other than filtration, the resolution obtained was sufficient to allow quantitation of vitamin C in the juices, as shown in Table 1.

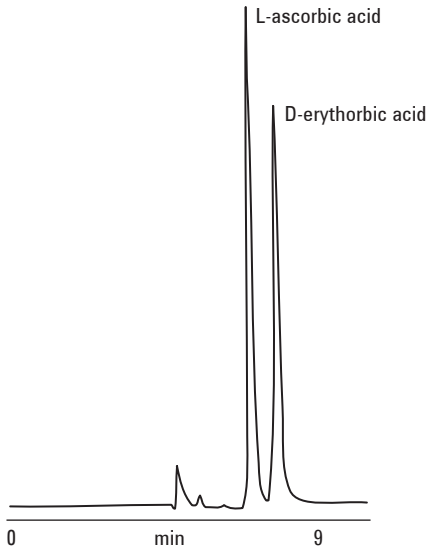

Figure 1. Separation of L-ascorbic acid and D-erythorbic acid using optimized mobile phase.

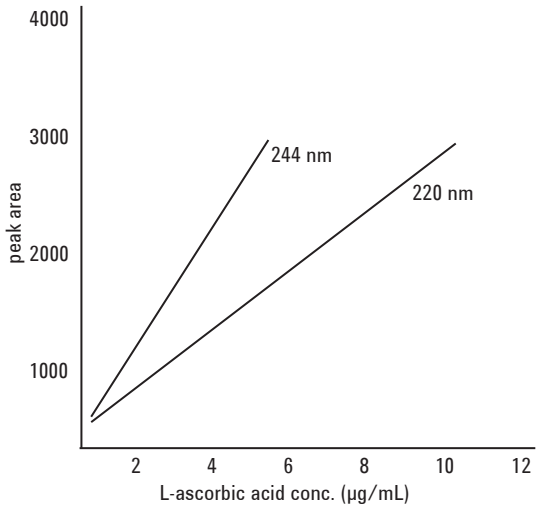

Figure 2. Calibration curve of L-ascorbic acid at 244 and 220 nm.

Table 1. Vitamin C content of some fruit juices.

Juice	Vitamin C concentration in undiluted juice (mg/L)
Grapefruit	440
Lemon	594
Orange	727

The complete data set and analysis is available in Lloyd *et al*. (1988).

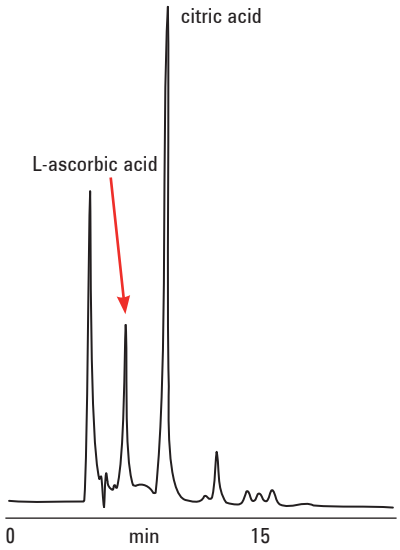

Figure 3. Elution profile of orange juice on PLRP-S columns.

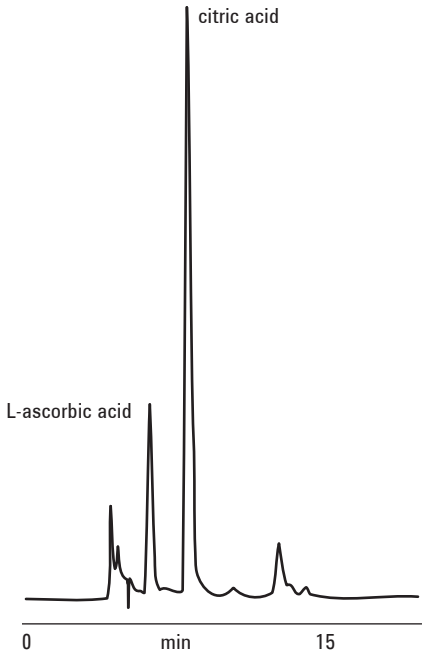

Figure 4. Elution profile of grapefruit juice on PLRP-S columns.

Conclusion

Ion suppression HPLC using PLRP-S columns successfully quantified L-ascorbic acid in fruit juice. Using these polymeric reversed phase materials, the optimized system was able to resolve L-ascorbic acid from D-erythorbic acid in nine minutes.

The method reported here is suitable for the qualitative and quantitative analysis of vitamin C in fruit juices. A simple isocratic eluent is used with no derivitization of the solute prior to analysis.

Reference

Lloyd, LL, Warner, FP, Kennedy, JF, White, CA (1988) Quantitative analysis of Vitamin C (L-ascorbic acid) by ion suppression reversed phase chromatography. *Food Chem.*, 28, 257-268.

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