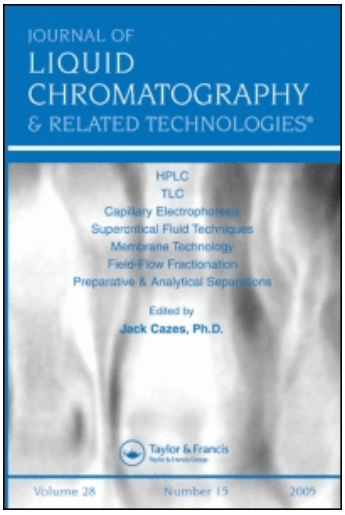

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HPLC ASSAY FOR THE DETERMINATION OF ASCORBIC ACID IN HONEY SAMPLES

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ABSTRACT

Determination of ascorbic acid in honey samples was performed by high performance liquid chromatography (HPLC) with UV detection at 254 nm. The separation was performed using a C-18-ODS column with a mobile phase consisting of a mixture of 15% methanol and 85% water, adjusted to pH 2.5 with metaphosphoric acid. The mobile phase was pumped at a flow rate of 0.9 mL/min. The retention time for ascorbic acid was found to be 5.1 minutes. The method is simple, fast, sensitive, and reproducible. The recovery of ascorbic acid is over 90% by the standard addition method.

INTRODUCTION

A number of reports on the determination of ascorbic acid (AA) have appeared in the last 10 years.[1-4] Numerous efforts have been directed towards the development of specific and sensitive detection systems in high performance liquid chromatography (HPLC).

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Ascorbic acid plays a very important role in human metabolism. Its functions include collagen synthesis, amino acid metabolism, synthesis of adrenalin, synthesis of anti-inflammatory steroids, and iron and copper metabolism.

Numerous HPLC methods have been developed for the determination of ascorbic acid in both food products and physiological samples.[5-10] However, limitations exist due to mobile phase complexity or incompatibility and column instability. An important factor in HPLC analysis is the mobile phase, because it interacts with solute species of the sample and has a significant influence on the separation. In the case of ionizable compounds, the mobile phase is even more important because controlling the pH of the mobile phase may determine if the compounds will dissociate or not.

In this paper, an ionizable compound was analyzed using a C-18 column, which requires that this compound can not be dissociated in order to have a useful retention time in the column. The method of ionic suppression was used to achieve a mobile phase that allows the separation of ascorbic acid in an aqueous sample. This paper reports a fast, reliable, and sensitive method, with a simple mobile phase, for the determination of ascorbic acid in honey samples. We have applied a reversed-phase HPLC method, developed for AA determination in honey samples, using a simple isocratic method. The method requires minimum sample preparation and no derivatization procedures.

EXPERIMENTAL

Materials

Ascorbic acid was purchased from VETEC (Rio de Janeiro, Brazil). The reagent solution was freshly prepared prior to use. Methanol (VETEC) was of chromatographic grade and metaphosphoric acid (VETEC) was of analytical grade. Water was purified with a Millipore Milli-Q Plus System. Membrane filters (0.45 μm) were obtained from PR COLA (Rio de Janeiro, Brazil).

Instrumentation and Methods

The high performance liquid chromatography system consisted of a solvent delivery pump model LC-10AS, equipped with a ultraviolet detector (Shimadzu Corp., Kyoto, Japan) model SPD-10A, and recorder integrator data module model CR-6A (Shimadzu). The samples were applied with a Rheodyne 7125i injector with a 20 μL loop. Chromatographic separation was carried out with a Shimadzu C-18-ODS column (250 mm x 4.6 mm i.d, 5 μm particles), together with an C-18-ODS pre-column (4 x 4 mm i.d.). Isocratic elution was made using

MeOH:H_2O (15:85), pH = 2.5, adjusted with metaphosphoric acid (MPA) at a flow-rate of 0.9 mL/min, and the detection was made at 254 nm. All measurements were carried out at room temperature.

For each honey sample, about 10 g were completely dissolved in distilled deionized water with 0.25% MPA, and the solution was transferred to an amber volumetric flask of 100.0 mL. The solution was filtered through a membrane filter (0.45 μm) and aliquots (20 μL) of the filtrate were injected into the chromatograph for the determination of AA. Ascorbic acid, in this sample solution, was stable for 2 days at 4°C. The AA peak/height ratio was constant for two days and, subsequently, decreased.

The stock standard was freshly prepared by dissolving 4 mg of AA in 100 mL of 0.25% metaphosphoric acid in an amber volumetric flask prior to use. This solution was filtered through a membrane filter (0.45 μm). Ascorbic acid present in this solution was stable at 4°C for 1 h, indicated by a slow decrease in peak/height ratio.

The determination of ascorbic acid in honey samples was performed at 254 nm after dilution with distilled deionized water adjusted with MPA to pH 2.5. The only other sample preparation step was filtration using a 0.45 μm membrane filter to remove any suspended material from honey.

RESULTS AND DISCUSSION

Retentivity and selectivity are influenced by mobile phase pH and ionic strength in reversed-phase chromatography of ionizable species.[11] Thus, as the net solute charge decreases, solute retentivity increases. A mobile phase with pH below the pKa of ascorbic acid (ca 4.17)[12] is necessary for ion suppression reversed-phase separation.

It has been reported, that ascorbic acid is rapidly oxidized in distilled water at pH 7.0, but the rate of oxidative degradation is dramatically reduced by the presence of metaphosphoric acid[13] or another reducing agent. With pH adjustment, this compound was present in the non-dissociated form. This procedure is called ionic suppression, which consists of reducing the ionization of solutes by alteration of the pH of the mobile phase. Compounds with a weak acidic character require the decrease of the pH of the mobile phase in order to shift the equilibrium towards the non-dissociated form, so it has useful retention on the C-18 column.

We have observed that, using 0.25% metaphosforic acid in the sample solution, no decrease in ascorbic acid peak height/area was observed over a period of 150 min. The rate of ascorbic acid oxidative degradation is also dramatically reduced if the sample is prepared in the mobile phase at low pH. This indicates that the chosen system is more appropriate for analysis, since no degradation of ascorbic acid occurs.

Ascorbic acid was completely separated by monitoring at 254 nm. However, several unknown peaks were detected after the retention time of ascorbic acid, and the time needed for the complete elution was approximately 15 minutes.

The chromatograms of honey samples using UV detection are shown in Figure 1. The total analysis time was 15.0 min per injection of sample and the AA was detected at 5.1 min.

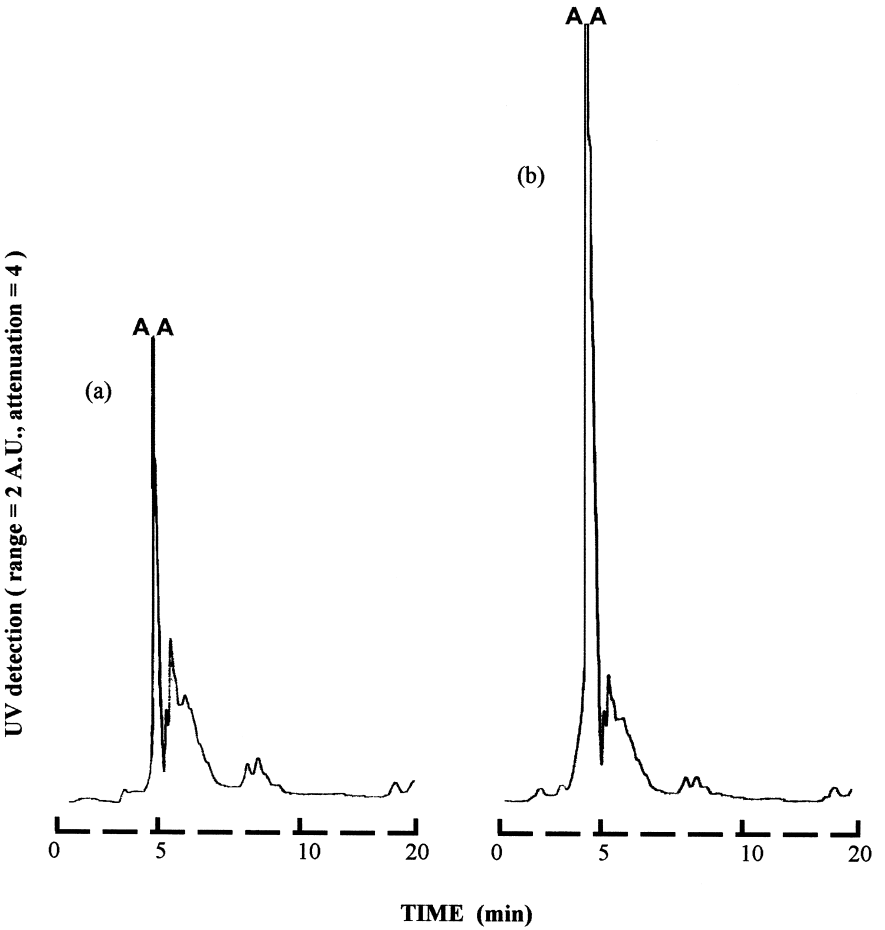

Figure 1. Chromatographic separation of ascorbic acid using methanol:water as mobile phase with pH adjustment, with metaphosphoric acid of a) honey sample, and b) honey sample spiked with 40 μg/mL of AA.

Table 1. Recovery of Ascorbic Acid Added to Honey

Ascorbic Acid (mg per 100 g)		
Added	Found	Recovery (%)
0	3.91	-
1.5	4.98	92.1
2.5	6.04	94.3
4.0	7.49	94.8

RSD = 1.6 % (n = 5)

A known amount of ascorbic acid was added to honey samples, and the overall recovery was estimated by the standard addition method. As shown in Table 1, the recovery of ascorbic acid is greater than 90% with a relative standard deviation of 1.6%. There is good agreement between the theoretical and experimental AA concentrations.

CONCLUSION

The use of ion suppression chromatography and isocratic elution with a simple mobile phase provides an excellent method for the analysis of ascorbic acid in honey samples.

This methodology has proven to be satisfactory with respect to selectivity, speed, and accuracy. It is simple and convenient, and therefore, applicable to the routine determination of AA in different honey samples. Unlike many methods which have been reported, this method has the advantages of requiring minimal sample preparation and no solute derivatization, apart from the filtration to remove suspended material. The method can be applied on a routine basis, with each analysis taking approximately 15 min to allow the elution of ascorbic acid.

REFERENCES

1. Iwase, H.; Ono, I. J. Chromatogr. A **1993**, *654*, 215–220.
2. Iwase, H. J. Chromatogr. A **1992**, *606*, 277–280.
3. Liau, L.S.; Lee, B.L.; New, A.L.; Ong, C.N. J. Chromatogr. A **1993**, *612*, 63–70.
4. Behrens, W.A.; Madère, R. J. Liq. Chromatogr. **1992**, *15*, 753–765.
5. Behrens, W.A.; Madère, R. Anal. Biochem. **1987**, *165*, 102–107.
6. Iwase, H.; Ono, I. J. Agric. Food Chem. **1997**, *45*, 4664–4667.

7. Marshall, P.A.; Trenerry, V.C.; Thompson, C.O. J. Chromatogr. Sci. **1995**, *33*, 426–432.
8. Denninson, D.B.; Brawley, T.G.; Hunter, G.L.K. J. Agric. Food Chem. **1981**, *29*, 927–929.
9. Acar, J.; Gokmen, V. Fruit Process. **1996**, *5*, 198–201.
10. Sawamura, M.; Ooishi, S.; Li, Z.F. J. Sci. Food Agric. **1990**, *53*, 279–281.
11. Lloyd, L.L.; Warner, F.P.; Kennedy, J.F.; White, C.A. J. Chromatogr. A **1988**, *437*, 447–452.
12. Crawford, T.C.; Crawford, S.A. Adv. Carbohydr. Chem. **1980**, *37*, 79–82.
13. van Niekert, P.J. *HPLC in Food Analysis*; Academic Press: London, 1982; 187.

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