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Ascorbic Acid in Orange Juice

Ascorbic acid, commonly known as Vitamin C, is a dietary essential for humans, other primates and the guinea pig, but can be synthesised by all species of animals which have been investigated. The most prominent chemical property of the vitamin is its ability to oxidise to dehydroascorbic acid, which is catalysed by metal ions (Reference 1). This feature also renders Vitamin C suitable for analysis by HPLC using electrochemical detection, which is highly selective for ascorbic acid even in complex samples, and is highly sensitive, with detection limits in the picogram range. However, due consideration must be given to sample preparation procedures in order to avoid the loss of ascorbic acid by oxidative degradation prior to the chromatographic analysis. The HPLC instrumentation and sample preparation required for the analysis of ascorbic acid in orange juice are detailed below. The perchloric acid extraction technique is applicable to other sample matrices, *e.g.*, milk products, blood and tissue, with minor modification.

Figures 1 and 2 show ascorbic acid standard (ca. 10 ng on column) and a commercial orange juice sample, respectively.

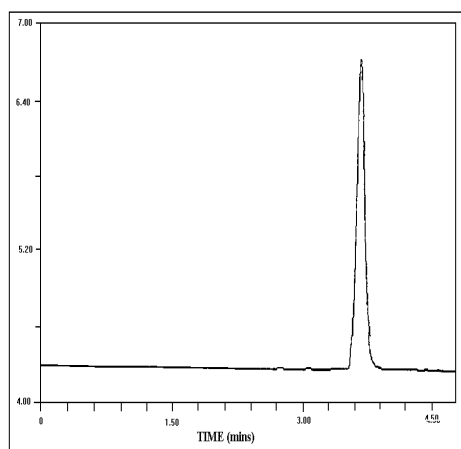


Figure 1 Ascorbic acid standard

Keywords:
Ascorbic Acid, Electrochemical Detection, Vitamin C

Conditions

Column: Spherisorb S5 ODS2, 250 x 4.6 mm ID
Guard: 50 x 4.6 mm ID
Temperature: 30°C
Mobile Phase: 0.1 M sodium acetate containing 0.4 mM disodium DETA and 1 mM octylamine:methanol (90:10) - degassed
Flow Rate: 0.8 ml/min

Sample Preparation

Dilute juice 1:100 with 0.05 M perchloric acid Ultrasonicate solution for five minutes
Filter solution (0.2 micron)
Further dilutions should be made with mobile phase, if necessary.
Inject 20 µl.

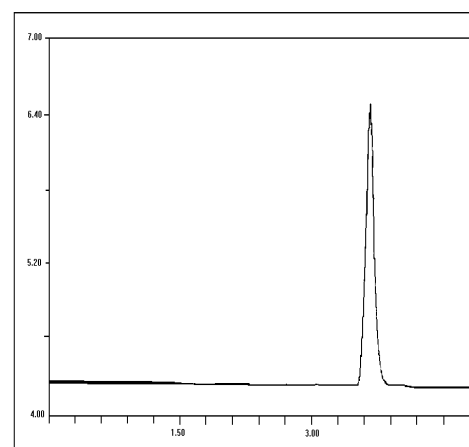


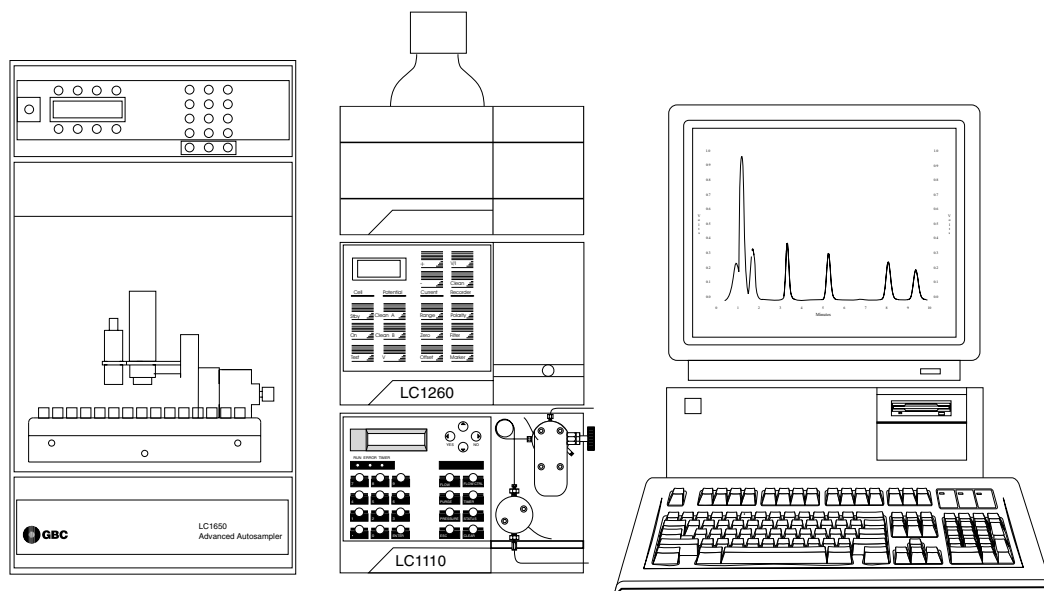
Figure 2 Commercial orange juice

GBC HPLC Instrumentation

LC1110 Dual Piston HPLC Pump
LC1260 Electrochemical Detector
LC1445 System Organiser
LC1650 Advanced Autosampler
LC1120/LC1150 HPLC Column Oven Option
WinChrom Chromatography Data
Management System
Online Degasser

Reference

1. 'Principles of Biochemistry', by White, Handler and Smith, p. 973 (McGraw-Hill, 1964).



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