

# Determination of Vitamin C in Fruits and Vegetables

## Brief Notes for Preparing and Running the Practical

### Introduction

The HPLC procedure described arose from the need to have an instrumental method for the determination of vitamin C in green peppers. The practical was intended to replace a titrimetric method using 2,6-dichloro-phenolindophenol (DICPIP) undertaken as part of a Stage 2 (second year) analytical instrumentation module.

The starting point was the titrimetric method and the HPLC methodology and adjustments to the work-up methodology were largely developed as part of a Stage 3 (final year) project. Further adjustments have taken place as a result of running the session in a Stage 2 environment.

### Files provided

- Vitamin C (FSA protocol).doc  
Pro-forma sheet on which student can record the data obtained from the titrimetric determination of vitamin C.
- Vitamin C (HPLC - non-specific instrumentation).doc  
Practical script for the determination of vitamin C using HPLC, without reference to any particular make or model of equipment.
- Vitamin C (HPLC - specific instrumentation).doc  
Practical script for the determination of vitamin C using HPLC, using specific equipment that has been used in developing the practical.
- Vitamin C (notes).doc  
This document.
- Vitamin C (titration).doc  
Practical script for the determination of vitamin C using titrimetry with 2,6-dichloro-phenolindophenol (DICPIP).

### Equipment – For Each Student

Beaker (100 mL) x 2

Beaker (50 mL) x 2

Centrifuge tube and top (50 mL; plastic)

Filter funnels x 2

Measuring cylinder (25 mL)

Pasteur pipette (plastic)

Pestle and mortar

Volumetric flask (50 mL)

Wash bottle

Weighing boat

1/3 of the cohort will require: Beaker (400 mL)

Bunsen burner  
Gauze  
Tipod  
Tubing (for gas to Bunsen burner)

### **Equipment – For General Use**

Ascorbic acid solution (1000 mg/L) – 1 L will be sufficient for preparation of calibration standards  
Aluminium foil  
Balance (2 decimal place)  
Balance (two-pan; for balancing centrifuge tubes)  
Centrifuge and appropriate accessories  
HPLC column (reversed phase C18)  
HPLC UV/Vis detector (capable of measurement at 254 nm)  
HPLC injector  
HPLC pump (isocratic)  
Knife  
Microwave oven  
Orthophosphoric acid (0.02%; mobile phase) – allow at least 50 mL per student  
Orthophosphoric acid (5%; extraction medium) – allow at least 200 mL per student  
Oven (capable of maintaining 180-200°C)  
Parafilm®  
Sand  
White tile

### **Reagents for HPLC – Prepared Beforehand**

1. Orthophosphoric acid (0.02% v/v) – HPLC mobile phase  
Pipette 0.2 mL of orthophosphoric acid into a 1000 mL volumetric flask. Make up to the mark with Milli-Q water. Mix. Filter under vacuum through GF/F to remove any particulate matter. Degas with nitrogen gas for about 5 minutes before use.
2. Orthophosphoric acid (5% v/v) – extraction medium  
Pipette 25 mL of orthophosphoric acid into a 500 mL volumetric flask. Make up to the mark with Milli-Q water. Mix thoroughly.
3. Ascorbic acid standard stock solution (1000 mg/L)  
Dissolve accurately approximately 0.1 g of ascorbic acid in about 20 mL of orthophosphoric acid (5% v/v) in a beaker. Transfer the solution to a 100 mL volumetric flask and wash the beaker with about 10 mL of orthophosphoric acid (5% v/v). Add the washings to the flask. Repeat the wash and make up to the mark with orthophosphoric acid (5% v/v). Mix thoroughly.  
  
From the 1000 mg/L ascorbic acid standard stock solution, prepare a calibration series of ascorbic acid (e.g. 0, 25, 50, 75, 100 mg L<sup>-1</sup>) in orthophosphoric acid (5% v/v) in 50 mL volumetric flasks. Calibration solutions

should be prepared no more than 24 hours in advance and kept in a cool dark place. If refrigerated ensure solutions return to room temperature before use.

### **Sample Preparation and Analysis**

Knives need to be reasonably sharp but not excessively so!

White tiles useful for cutting up peppers.

When grinding the pepper, the skins will tend to remain intact. This does not appear to be a problem.

Cover beaker for microwave cooking with Parafilm<sup>®</sup>. Do not use Parafilm<sup>®</sup> to cover beakers being placed in the regular oven – use aluminum foil instead.

Method states that samples should be diluted into a 50 mL volumetric flask. In certain circumstances a further dilution may be necessary (e.g. ten-fold dilution) ensure data falls within the calibration range.

Three replicate analyses of each sample are ideal and with each analysis taking about 4 minutes sufficient time should be allowed for the analyses. Factor this into planning along with the number of students in cohort and the number of HPLC instruments available.

### **Column Care**

If using an Ascentis column, flush overnight at 0.2 mL min<sup>-1</sup> before use that day. After use, flush with a high percentage aqueous mobile phase, e.g. 95:5 water:acetonitrile to remove traces of acid and store in 100% methanol or 50:50 methanol:water.

### **SWOT – Weaknesses and Threats**

HPLC equipment needs to be well maintained. The major issues tend to be with the Rheodyne<sup>®</sup> injector and column viability. It is a good idea to check out the prospective system well in advance of the practical session to allow sufficient time to address poorly performing systems. Students expect analytical equipment to work properly all the time and do not always understand that this is not always the usual state of affairs! Working systems will ensure everything runs smoothly.

### **Additional Thoughts**

Use the Vitamin C (FSA protocol).doc as a basis for a “real” role-play analytical situation.

Students should think about why the vitamin C content measured after microwave cooking (and sometimes oven cooking) is the same or greater than that measured in the raw fruit. Vitamin C is not being created by the cooking procedure but is being more efficiently extracted from cells that have been lysed by the cooking procedure. The vitamin C content of boiled pepper tends to be lower than the raw fruit, even when the amount in the water is also quantified.

Other scenarios of investigating the vitamin C content in different part of the fruit (near stalk, middle, bottom, *etc.*) or in different coloured peppers (green, orange, red, yellow, *etc.*) are possible.

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