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## Determination of L-ascorbic acid in fruit juice samples

### Introduction

Vitamin C is one of the parameters measured in the German RSK method for assessment of authenticity of juices. Claims for high Vitamin C content are often used in labelling of juices. Many other foods cite Vitamin C content on labelling. However, during storage - especially in ambient or greater temperatures and in the presence of heavy metals - Vitamin C breaks down.

Routine measurement of Vitamin C in foodstuffs is therefore important for substantiating nutritional claims and this can be conveniently accomplished by spectrophotometric measurement of L-ascorbic acid.

### Principle

L-Ascorbic acid (L-ascorbate) and some other reducing substances reduce the tetrazolium salt MTT [3-(4, 5-dimethylthiazoyl-2)-2,5-diphenyltetrazolium bromide] in the presence of the electron carrier PMS (5-methylphenazinium methyl sulfate) at pH 3.5 to a formazan, which is determined by its absorbance at 578 nm. This gives the total reducing substances in the sample.

If other reducing substances are present in the sample for the specific determination of L-ascorbate, for a sample blank determination the L-ascorbate fraction only in the sample can be oxidatively removed by the addition of ascorbate oxidase (AAO) in the presence of oxygen (2). The dehydroascorbate formed does not react with MTT/PMS. Thus the absorbance difference of the sample minus the absorbance difference of the sample blank is equivalent to the quantity of L-ascorbate in the sample. In our analyses of orange and grapefruit juices, the sample blank determinations indicated the absence of other reducing substances and therefore this step could be omitted.



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As mentioned above, ascorbic acid readily breaks down, initially by oxidation to dehydroascorbic acid, which subsequently degrades further. Dehydroascorbic acid is thought to have similar biological activity, as body tissues can convert it to ascorbic acid. The method below can be used to measure dehydroascorbic acid by performing a preliminary oxidation step on the sample in the cuvette using 0.2ml (0.08ml) dithiothreitol (1mg/10ml 0.5mmol phosphate buffer pH 7.5) for 10 mins. Dehydroascorbic acid content is then given by subtraction of the unoxidised value from the oxidised value.

Fruit juices may be analysed directly without any preparation. However, it is recommended that samples containing suspended matter are certified or filtered first, to avoid pipetting difficulties.

For faster analyses, place cell in a thermostat at 37°C.

## Method

The analysis can be rapidly carried out directly on orange juice. It is recommended that the suspended solids are first removed by centrifugation for 0.5 mins or via a syringe filter. The above reaction can be carried out in a stoppered UV grade cuvette of 10mm pathlength; alternatively disposable methacrylate cells can be used (for the UV cell, the volumes shown in brackets should be used). Solutions are conveniently dispensed using an automatic pipette.

1ml (0.4ml) MTT in disodium hydrogen phosphate/citric acid buffer pH 3.5  
1.5ml (0.6ml) distilled water  
0.1ml (0.04ml) sample

Mix by inversion and read absorbance after 6mins (A1).

A blank determination is necessary. The procedure is identical apart from omission of the sample and addition of an equivalent volume of water. Use of the blank as reference automatically subtracts the blank absorbance from the sample.

## Libra S21/S22 operation

- Select Applications (key 2) from the main menu
- Select Reaction rate
- Enter wavelength 578 and press OK (F3)
- Select time in mins
- Enter delay time of 6 and press OK (F3)
- Enter duration time at 20 and press OK (F3)
- Enter factor 0.286 to convert readings directly to concentration units and press OK (F3) [Analysis at Biochrom showed that the concentration of ascorbic acid g/l = 0.286 x absorbance value]
  - To go back and change the parameters press Method (F1)

- A blank determination is necessary. The preparation is identical apart from omission of the sample and addition of an equivalent volume of water.
- Insert blank and press green run key. This reference value is used for subsequent samples until changed.
- Insert samples as required and press the green run key.
- After reading the sample at 6 mins (abs A1), add 0.10ml (0.04ml) PMS solution. Mix and stand for 20mins at ambient temperature, recommended range 20-25°C. Read absorbance (A2).
- The absorbance difference calculated as A2-A1, is shown as delta A on the results screen.

Other information is displayed, which although not required for this calculation is a check on the chemical stability of the assay. The assay is shown graphically as it proceeds and the results show slope (abs/min) and the line quality (a coefficient of determination of > 95 % is expected if the assay was carried out over a linear section).

Print outs may be obtained by setting up the printer options in System Utilities and Preferences (3) prints results. This is automatic with autoprint.

Additionally press . to print result if auto-print is off, or to re-print result if auto-print is on

The above procedure can be easily used with other instruments in the Libra range by using the concentration mode and the 0.268 factor.

## Results

Fruit Juice Sample	Ascorbic acid, mg/l
Sainsbury freshly squeezed orange juice	400
Tesco pure orange juice made from concentrated orange juice	372
Tesco freshly squeezed orange juice	469
Marks and Spencer freshly squeezed orange juice	210
Sainsburys grapefruit juice made from concentrated grapefruit juice	390

## Ordering Details

Libra S5	80-2115-00
Libra S11	80-2115-15
Libra S12	80-2115-10
Libra S21	80-2115-25
Libra S22	80-2115-20
Libra S32	80-2115-30

The reaction can be accelerated for increased sensitivity if warmed. For this purpose the Libra S21/S22 have the following accessories:

- 8 position water heated cell changer (80-2109-70) used with an external heating bath
- 6 position Peltier heated cell changer (80-2106-04) and Temperature Control Unit (80-2112-49)
- Single position water heated cell holder (80-2106-08) used with an external heating bath
- Single position electrical cell holder (80-2106-12), temperatures selectable from 25, 30 and 37°C
- Single position Peltier cell holder, temperatures selectable over the whole range from 20-49°C (80-2106-13).

The Sipper (80-2112-25) enables some automation of the analyses, and can be used together with a heated (not water heated) or non-heated single cell holder.

## Reference

Deneke, U., Michal, G. & Beutler, H.O (1978) Neue Methode zur Bestimmung von Vitamin C in Lebensmitteln, Deutsche Lebensmittel-Rundschau 74, 400-403.