Schools' Analyst Competition 2017

Dublin City University Heat
25th April 2017

Quality control analysis of a new brand of fizzy drink - Orangerina

Laboratory Handbook

Royal Society of Chemistry

Analytical Division

SCHOOL NAME: _____

Welcome to Ireland's inaugural RSC Schools' Analyst Competition, DCU-heat. You are required to complete three experiments today.

Throughout the day assessors will be observing you at work and giving marks for such things as attitude to safety, housekeeping and experimental technique. Most marks will be awarded for your experimental findings and answers reported. The demonstrators are also available to help you so don't be afraid to ask if you are unsure of something; you won't automatically lose marks for asking in fact you may gain some.

Remember, today is a competition so there can only be one winner but the organizers' hope you will all enjoy your afternoon. The judge's decision will be final. Good luck!

Units: In this booklet you will encounter different ways of describing concentration. The first is "molarity". This should be familiar to you and describes the number of moles per cubic decimetre (mol dm^{-3} or M) of each chemical. You will also use mg L^{-1} which is the same as mg dm^{-3} or parts per million (ppm). All of these units are commonly found on food products.

Safety: You must remember that you are in a laboratory setting and therefore tasting of the drinks is **not** allowed. Laboratory coats and safety spectacles are mandatory. Disposable gloves must be worn when necessary and this will be clearly stated in the lab script.

Competition Overview

There are many manufacturers of fizzy drinks across the world and even more retailers. One of the key activities which will take place before a product is sold is a quality assurance check to ensure that the chemical content is correct. In today's competition, your team will be carrying out this function. Your role is to quality check a new brand of fizzy drink, Orangerina. As part of this role, you and your team will check:

1. The vitamin C (also known as ascorbic acid) content using a titration
2. The caffeine content using high performance liquid chromatography (HPLC)
3. The benzoic acid content using a spectrophotometric procedure

How should you plan this task?

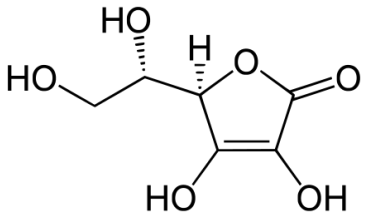
In your team of three decide who is going to do each analysis; then read the experiment you are going to do. Once you are happy with what you are going to do, you should begin the practical work. Pool the results of the three experiments in your 'neat copy' of the answer booklet and hand this in together with any graphs. Make sure your names are correctly and clearly spelled as there is nothing worse than a participation certificate with your name spelled incorrectly! Good luck and enjoy the challenge.

In each section of the workbook, the theory of each analytical method is explained, followed by the procedure that you will work by.

Background:

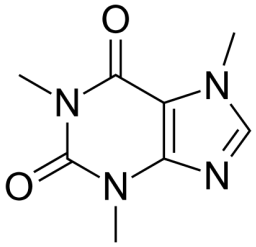
Given the widespread consumption of fizzy drinks in the developed world, quality control and routine analysis of these commercial drinks have an importance for the human health. Apart from sugars, there are many other chemical additives in fizzy drinks that need to be monitored for their concentration levels. In this context, quantitative analysis and quality control of commercial drinks require powerful analytical methods giving reliable, precise, and accurate results with short runtime and low cost of analysis. A description of the chemicals you will be analyzing today is given below.

Vitamin C, also known as ascorbic acid, is one of the most important vitamins, which plays an important role for hydroxylation reactions and antioxidants. Symptoms of lack of ascorbic acid are physical and mental infirmity, fatigue, weight loss, bruising, dry hair and skin, and increased sensibility of infections. Ascorbic acid addition is common in the manufacture of beverages, especially those made from fruit juices. Ascorbic acid not only restores nutritional value lost during processing, but also contributes to the products' appearance and palatability.



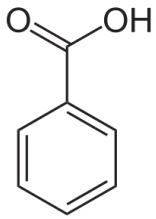
Ascorbic acid is a vitamin found in food and used as a dietary supplement.

Caffeine, which is a xanthine alkaloid, has widely been used in tea (black, white, and green), coffee, guarana, chocolate, cocoa, soft and energy drinks, and pharmaceutical products. In recent years, the use of caffeine in energy drinks has increased significantly due to its excitation and analgesic properties. However, the use of a high dosage of ascorbic acid gives rise to some symptoms such as headache, slowness, fatigue, and depression.



Structure of caffeine which is a central nervous system (CNS) stimulant of the methylxanthine class.

Benzoic acid and its salts are used as a food preservatives, represented by the E-numbers E210, E211, E212, and E213. Benzoic acid inhibits the growth of mold, yeast and some bacteria. Typical levels of use for benzoic acid as a preservative in food are between 0.05–0.1%. Foods in which benzoic acid may be used and maximum levels for its application are controlled by international food law.



Benzoic acid is a colorless crystalline solid and a simple aromatic carboxylic acid often used as a preservative in fizzy drinks.

Task 1
Determination of the ascorbic acid content in Orangerina fizzy drink

1.1 Introduction

Ascorbic acid ($C_6H_8O_6$) is a mild reducing agent that reacts rapidly and quantitatively with iodine (present as the tri-iodide ion, I_3^-) to reduce it to iodide ion (I^-). The ascorbic acid is oxidized to dehydroascorbic acid as shown in reaction (i):

$$I_3^- + C_6H_8O_6 + H_2O \rightarrow C_6H_8O_7 + 3I^- + 2H^+ \quad \text{(i)}$$

The tri-iodide ion used in the titration is generated by adding an excess of potassium iodide, KI, to an acidified solution of potassium iodate, KIO_3, according to reaction (ii):

$$6H^+ + KIO_3 + 8KI \rightarrow 9K^+ + 3I_3^- + 3H_2O \quad \text{(ii)}$$

The amount of I_3^- produced can be calculated from the amount of KIO_3 reacted with excess KI; The excess I_3^-, that does not react with the ascorbic acid in the sample, can be detected by titration with $Na_2S_2O_3$ as shown in reaction (iii):

$$I_3^- + 2S_2O_3^{2-} \rightarrow 3I^- + S_4O_6^{2-} \quad \text{(iii)}$$

{The starch forms a complex with I_3^- (I_3**.**starch), blue in colour; reaction with thiosulphate releases I^- }.

1.2 Apparatus

100 mL conical flask x 3
10 cm^3 graduated cylinder x1
Titration apparatus
10 mL pipette x 2
250 mL beaker x1
150 mL beaker x1
Pipette filler x1

1.3 Reagents

Standard potassium iodate solution
0.5 M H_2SO_4
Sodium thiosulphate
0.1 M potassium iodide solution
Starch indicator
Orangerina sample

***All waste to be collected in labelled waste beaker provided**

1.4 Method

Part A – Standardisation of the sodium thiosulphate solution

1. You are provided with a standardised solution of potassium iodate of known concentration, 0.02 M. This solution was prepared by dissolving 8.56 g of potassium iodate in distilled water and making the volume up to 2 litres.
2. Based on the data given in step 1, show your calculation to prove that this is the correct concentration for this potassium iodate solution.
3. Pipette 10 cm^3 of the KIO_3 solution into a conical flask.
4. Using a graduated cylinder add 5 cm^3 of 1 M KI and 10 cm^3 of 0.5 M H_2SO_4. Note the red-brown colour due to the presence of I_3^- ions.
5. Titrate the solution against $Na_2S_2O_3$ until the colour changes to pale yellow/amber.
6. Add approximately 2 cm^3 of starch indicator. The solution should turn deep blue. Continue titrating until the blue colour disappears.
7. Repeat the titration as many times as you deem necessary to ensure precision.
8. Show your neat stepwise calculations in order to determine the concentration the sodium thiosulphate solution.

Part B – Determination of ascorbic acid content in fizzy drink

9. Pipette 10 cm^3 of the Orangerina solution provided into a conical flask.
10. Using a graduated cylinder, add approximately 5 cm^3 of 1 M KI and 10 cm^3 of 0.5 M H_2SO_4 to the solution.
11. Pipette exactly 10 cm^3 of standard KIO_3 into the sample.
12. Titrate with your sodium thiosulphate solution until the solution has lost its initial reddish-brown colour and has become pale yellow/amber. Add approx. 2 mL starch indicator and continue the titration slowly until end point is reached.
13. Repeat the titration as many times as you deem necessary to ensure precision.
14. Show all your calculations used to determine the moles/L (Molarity; M) of ascorbic acid present in the fizzy drink. All calculations should be written stepwise in a neat manner in your answer sheet.
15. Also report your answer as g/L of ascorbic acid present in the fizzy drink.

ROUGH WORK

(Use this sheet to do any rough calculations for Task 1 if required)

Note: It is advisable to complete the answer sheet as you proceed; the answer sheet is graded

Task 1 Answer Sheet

Part A

Calculations to confirm the concentration of 0.02 M KIO_3 stock solution:

Data / Calculations to determine the concentration of the $Na_2S_2O_3$ solution:

$Na_2S_2O_3$

Burette readings	I	II	III	IV	V
Initial (cm^3)					
Final (cm^3)					
Volume used (cm^3)					

Number of titres as appropriate

Average titre _____.

Calculations and result(s):

Part B

Data / Calculations to determine the concentration of the fizzy drink:

$Na_2S_2O_3$

Burette readings	I	II	III	IV	V
Initial (cm^3)					
Final (cm^3)					
Volume used (cm^3)					

Number of titres as appropriate

Average titre _____.

Calculations and result(s):

Q1. Suggest why the solution changed to blue upon addition of starch?

_____
_____
_____

Q2. A graduated cylinder was used to add KI and H_2SO_4, however a pipette was used to add KIO_3; explain why?

_____
_____
_____

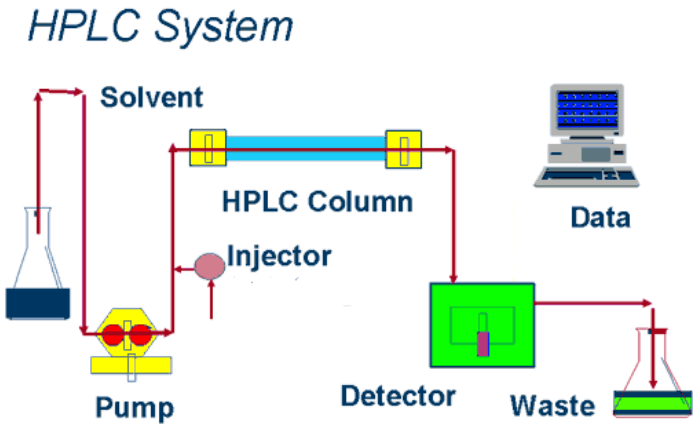
Q3. Name three other foods or drinks that contain ascorbic acid.

_____
_____
_____

Task 2
Analysis of caffeine in Orangerina fizzy drink by high performance liquid chromatography

1.1 Introduction

High performance liquid chromatography (HPLC) is a widely used instrumental method used for separating and quantifying components of lots of types of mixtures. It is widely used in the pharmaceutical industry. The chromatography (or separation) of a mixture takes place on a column 3-25 cm in length, packed with a suitable stationary phase packing, usually based on chemically modified silica. A mobile phase solvent is pumped at high pressure, up to 3000 psi, through this column. The sample mixture is introduced to the mobile phase stream by means of a sample injector. The method of detection depends on the nature of the sample that we want to analyse; the most widely used detectors are based on the absorption of ultraviolet light. A plot of the detector signal versus time is called a chromatogram. The area under a peak is proportional to the concentration of the component in the solution. The compound is identified by the time it takes for the peak to appear after the injection; each different compound appears at a different 'retention' time.



The time taken for a peak to emerge from the column after injection, its retention time, is a measure of analyte identity, when compared with standard analytes run under the same chromatographic conditions. The area underneath the peak can be integrated to give a measure of the amount of analyte present. This needs to be calibrated using a type of standardisation method.

The method used here is a **standard addition** procedure. A *sample solution* is prepared containing a dilution of the Orangerina drink. A second sample solution is prepared and a known quantity of a pure caffeine is added to give the *spiked sample*. Both the *sample solution* and the *spiked sample* are measured and the increased analytical signal is related to the amount of standard added.

If C represents the concentration of caffeine in the drink, and C_s the concentration increase due to the addition of the standard, then the concentration of caffeine may be calculated as follows:

$$C = Y_0 \times C_s / (Y_1 - Y_0)$$

Where:

Y_0 is the peak area obtained for the *sample*

Y_1 is the peak area obtained for the *spiked sample*
C_s is the concentration of the added standard in the *spiked sample*
C is the concentration of caffeine in the Orangerina drink

You will use this equation to calculate C, the concentration of caffeine in the drink.

2.2 Apparatus

Analytical balance capable of weighing 100 mg accurately
High performance liquid chromatograph, isocratic mode, with a UV detector at 275 mm
Distilled water
2 x pasteur pipette
25 mL glass pipette
10 mL glass pipette
5 mL glass pipette
2 x 250 mL volumetric flask
50 mL volumetric flask
25 mL volumetric flask

2.3 Reagents

Pure caffeine
Filtered, degassed Orangerina sample for HPLC analysis
Distilled water
Mobile phase containing water (50 parts) methanol + (50 parts) water (provided for you at the HPLC system)

2.4 Method

Making up the caffeine stock solution

1. In a weigh boat, accurately weigh about 100 mg of caffeine (you will need to use the four figure analytical balances for this).
2. Record the exact weight of the caffeine.
3. Transfer the caffeine into a 250 mL volumetric flask, using distilled water to wash any residues in the weighing boat into the flask. Make up to just below the line and shake the flask until all the caffeine has dissolved. Make up to exact volume using distilled water and a Pasteur pipette. Mix thoroughly again.
4. Using a 25 mL pipette, transfer 25 mL of the caffeine solution into a 250 mL volumetric flask, make up to volume with distilled water and mix thoroughly. This solution is your *caffeine stock*. Calculate the exact concentration of caffeine in this solution.

Making up the *sample A* for HPLC analysis

Using a 5 mL glass pipette, transfer 5 mL of the degassed Orangerina into a 25 mL volumetric flask. Make up to the exact volume with distilled water (add it carefully to avoid foaming) and mix thoroughly. This is your sample for analysis. Label the flask *sample A*.

Making up the *spiked sample B* for HPLC analysis

Using a 10 mL glass pipette, transfer 10 mL of the degassed Orangerina drink into a 50 mL volumetric flask. Using a 25 mL pipette, transfer 25 mL of caffeine stock (see

above) into the same flask. Make up to exact volume with distilled water (add it carefully) using a pasteur pipette and mix thoroughly. This is your sample with added standard. Label the flask *spiked sample B*.

HPLC analysis.

Take your *caffeine stock*, *sample A* and *spiked sample B* to the HPLC instrument. A demonstrator will bring you. Please be patient if you have to wait a few minutes while other samples are run. Using the caffeine stock, you will be able to identify the caffeine peak through the retention time of the standard. Using the chromatograms and data for *sample A* and *spiked sample B*, you can calculate the amount of caffeine in the Orangerina drink (see the Answer sheet to work through this). (If you have to wait for the instruments to become free, we suggest one team member should wait in queue, while the others continue working on the other Tasks).

ROUGH WORK

(Use this sheet to do any rough calculations for Task 2 if required)

Note: It is advisable to complete the answer sheet as you proceed; the answer sheet is graded

Task 2 Answer Sheet

Retention time of caffeine	min

	Peak Area
Sample A	
Spiked sample B	

Concentration of caffeine stock added to *spiked sample B* = _____mg L^{-1}

Remember, the caffeine stock was diluted (25 mL into 50 mL volumetric)
Therefore the concentration of the added standard in *spiked sample B* (C_s) = _____mg L^{-1}

Using the formula:

$$C = (Y_0 \times C_s) / (Y_1 - Y_0)$$

Y_0 is the peak area obtained for *sample A*
Y_1 is the peak area obtained for the *spiked sample B*
C_s is the concentration of the added standard in the *spiked sample B*
C is the concentration of caffeine in *sample A*

Therefore, the concentration of caffeine in *sample A* = _____mg L^{-1}
(Use space above for your calculations of the formula)

Remember, this solution was diluted before injection onto the HPLC. Therefore, the concentration of caffeine in Orangerina = _____mg L^{-1}

Questions:

1. The structure of caffeine is shown earlier. Using the periodic table provided, calculate the molecular weight of caffeine. Remember to include units.

_____
_____
_____

2. Using this molecular weight of caffeine, calculate the concentration of caffeine in moles L^{-1} (or molarity) in the Orangerina drink.

_____
_____
_____

3. Why might caffeine be added to drinks?

_____
_____
_____

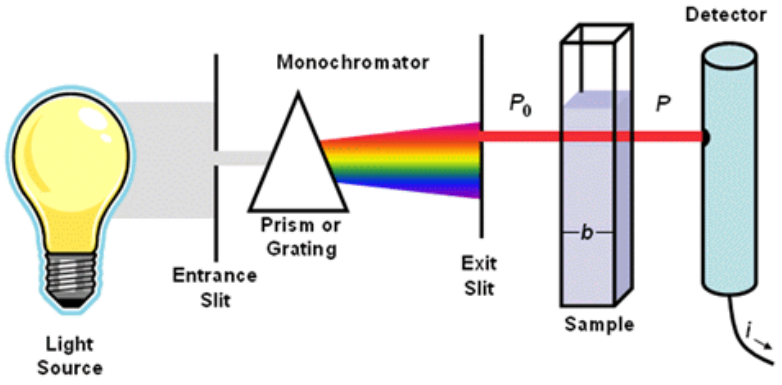
4. Why is it important medically that this substance and its concentration are listed by the manufacturers?

_____
_____
_____

Task 3
Analysis of benzoic acid in Orangerina by UV spectrometry

3.1 Introduction

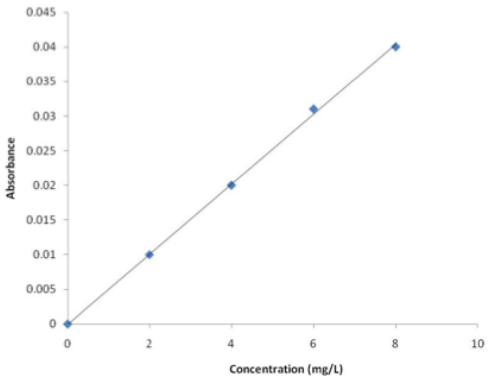
The theory of quantitative spectroscopy is based upon BEER'S LAW which relates to the amount of radiation which is removed from a beam of radiation when it passes through a solution containing an absorbing species. The amount which is removed is termed the amount ABSORBED and is measured in terms of ABSORBANCE. The process may be illustrated as:



$$\text{Absorbance} = \log \frac{\text{intensity of incident radiation}}{\text{intensity of transmitted radiation}} = abc$$

Where a is a constant related to the absorbing species
b is the path length of the cell containing the species
c is the concentration of the absorbing species

Given that a and b remain constant throughout the analysis: ABSORBANCE is proportional to CONCENTRATION.
Therefore a calibration graph can be produced using standard solutions as illustrated here



3.2 Apparatus

3 x 20 mL volumetric flasks
5 mL glass pipette
2 x quartz cuvettes
Beakers
UV spectrometer

3.3 Reagents

10 mg L^{-1} benzoic acid solution (labelled as Standard A)
Orangerina sample (de-colourised to eliminate spectral interference)
Deionized water

3.4 Method

A Preparation of standards

1. A solution of benzoic acid (10 mg L^{-1}) is provided (labelled as Standard A). Pipette 10 mL of this stock solution into a volumetric flask (20 mL) and add deionized water up to the mark.
2. Stopper the volumetric and turn upside down a few times to make sure it is mixed. Label this as Standard B.
3. Take 10 mL of Standard B and add it to another volumetric flask (20 mL) making up to mark with deionized water. Label this as Standard C.

B Preparation of Orangerina sample

4. Take 2 mL of your Orangerina sample and pipette into a volumetric flask (50 mL) and add deionized water up to the mark.
5. Stopper the volumetric and turn upside down a few times to make sure it is mixed. Label this as Sample Dilution 1.
6. Take 5 mL of Sample Dilution 1 and pipette into a volumetric flask (50 mL) and add deionized water up to the mark. Label this as Sample Dilution 2.

C UV Analysis

7. Bring your Standards A, B and C and Sample Dilution 2 over to a UV spectrometer. Once an instrument becomes available, a demonstrator will help you.
8. Record the ultraviolet absorbances of Standards A, B and C and your Sample Dilution 2 at a wavelength of 225 nm. The absorbance reading relates to the concentration of benzoic acid according to the Beer Lambert Law.

ROUGH WORK

(Use this sheet to do any rough calculations for Task 3 if required)

Note: It is advisable to complete the answer sheet as you proceed; the answer sheet is graded

Task 3 Answer Sheet

Solution	Benzoic acid concentration (mg L^{-1})	Absorbance
A		
B		
C		
	Sample Dilution 2	

Data Treatment
Plot a Beer-Lambert calibration graph by hand using graph paper supplied, of absorbance versus concentration for the standard benzoic acid solutions, and use the graph to determine the concentration of benzoic acid in Orangerina.

Attach calibration graph

Concentration of benzoic acid in Sample Dilution 2 is _____ mg L^{-1}
(show your working out on the graph)

Concentration of benzoic acid in Orangerina is _____ mg L^{-1}

Can you express this concentration in parts per million and as a %?

_____ ppm

_____ %

The Periodic Table