GX2111

OLLSCOIL NA hÉIREANN, GAILLIMH

THE NATIONAL UNIVERSITY OF IRELAND, GALWAY

SUMMER EXAMINATIONS, 2000

Higher Diploma in Applied Science, (Analytical Biochemistry/Chemistry)

Master of Science, (Analytical Biochemistry/Chemistry)

Third paper

Professor D. Cardin
Professor N.C. Price
Dr. T. Higgins
Dr. J. Phelan

Time Allowed: ***Three*** hours

(Answer ***Five*** Questions)

1. Discuss the use (or not) of separation steps in the operation of immunoassays

2. Write notes on **all** of the following

   (a) Describe the Mohr and Volhard titrations.

   (b) Outline the difficulties associated with potassium chromate as an indicator in precipitation titrations.

   (c) Describe how fluorescein functions as an indicator in precipitation titrations.

3. Give a brief description of **three** of the following:

   (a) Gel permeation chromatography.

   (b) Bio-specific affinity chromatography.

   (c) Ion-exchange chromatography.

   (d) Two methods to determine protein purity.

   (e) The main components in a typical bio-chromatography system.

4. Answer **all** of the following:

(a) What is the most suitable absorbance range for normal analytical work?

(b) Briefly outline the difference between a diode array spectrometer and the more conventional type. What are the advantages offered by diode array spectrometers?

(c) What is NIR spectroscopy? What are the applications of NIR spectroscopy in industry?

(d) What is an isosbestic point?

(e) What is the simplest use of derivative spectra?

5. Discuss the use of Dionex chromatography for the analysis of **either** carbohydrates **or** cations and anions under the following headings:

(i) mechanism of separation

(ii) column

(iii) eluent

(iv) detector.

6. Outline the methods you would use to determine (a) glucose, and (b) reducing sugar content of a sample of barley β-glucan (a polysaccharide) incubated with β-glucanase for a fixed reaction time. Which of the methods are more sensitive?

7. Answer (a) **and** (b)

(a) Describe the types of interference that affect atomic spectrometry measurement.

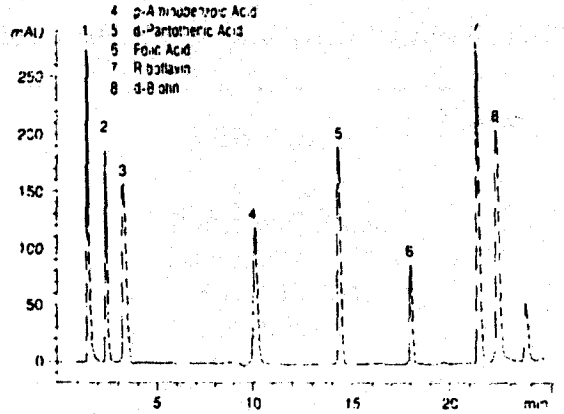
(b) Read the following passage and comment on the suitability or otherwise of the experimental procedure.

"The glassware was detergent washed. A 1000ppm calcium standard solution was prepared by dissolution of calcium carbonate in dilute acid. This stock solution was stored in a dark glass bottle, and made up fresh every fourth week. Working standards, 1, 5, 10 and 20ppm, were prepared by dilution with distilled water and stored in volumetric flasks. The working standards were made up fresh once a week. The sample was dissolved in 50ml of concentrated sulphuric acid, and aspirated directly into a fuel rich air/acetylene flame. Strontium chloride was added to all samples to suppress ionisation. The dissolved samples gave calcium readings between 1 and 2ppm."

8. The experimental details relating to a HPLC analysis of water-soluble vitamins are provided below.

WATER-SOLUBLE VITAMINS

Column. Luna 5µ C18(2)
Dimensions: 150 x 4.6mm
Order No: 00F-4252-E0
Mobile Phase A Water with 0.1% H_3PO_4
B 80% Acetonitrile with 0.1% H_3PO_4
Gradient. A/B 98 2 for 5 minutes ramped to A/B 72 28 in 17 minutes and held constant at A/B 72 28 for 3 minutes
Flow Rate. 0.8 mL/min
Detection: uV @ 210nm
Sample
1 Thiamine
2 Nicotinamide
3 Pyridoxine
4 p-Aminobenzoic Acid
5 d-Pantothenic Acid
6 Folic Acid
7 Riboflavin
8 5-B ohm



(a) Describe the HPLC system that would be required to carry out the analysis, with particular reference to the pumping arrangement.

(b) Draw a mobile phase composition–time profile for the gradient used.

(c) What would happen if the analysis were carried out isocratically using the gradient's initial mobile phase composition.

(d) Solid phase extraction is to be used to prepare a blood sample containing folic acid for analysis. Explain what would be involved and why this sample preparation technique is so attractive.

(e) The packing material in the 5µ C18(2) is end-capped. Draw a small section of the surface of a particle of packing material and explain what is meant by the term end-capped.

(f) The following data were obtained in an analysis of d-pantothenic acid using p-aminobenzoic acid as internal standard. What is the concentration of d-pantothenic in the sample of unknown concentration?

Concentration of d-panthotenic acid (mg/100ml)	**9.5**	**24.9**	**39**	**Unknown**
Peak Area Ratio (Analyte/IS)*	**0.284**	**0.676**	**1.060**	**0.490**
	0.276	**0.664**	**1.068**	**0.484**

* Samples were analysed in duplicate