



Analytical Method OP

Edition: 1	COENZYME A SODIUM FROM YEAST - (C3144)	Number: 10-039-0021
Supersedes: None	% Purity by High Performance Liquid Chromatography	Effective: 9/12/02

Document History

New

1. **OBJECTIVE**

To standardize a liquid chromatographic procedure for COENZYME A SODIUM FROM YEAST, Sigma-Aldrich Product Number **C3144**.

2. **SCOPE**

To define the conditions and procedures for the liquid chromatographic analysis of each lot of C3144 as assigned in Analytical Services.

3. **DEFINITIONS**

Area Percent Normalization: proportion, on a scale of 100, of the total area of integrated peaks in a chromatogram, which is under a given peak, often the major peak.

Gradient: program which describes how 1 or more parameters change with time; examples might be % organic, flow rate, and/or pH for a mobile phase program or temperature for a column oven.

Isocratic: program where 1 or more parameter(s) remain constant over time.

Limit of Detection: lowest observable level of a given component, expressed in units like area or weight percent, corresponding to a specified multiple of the signal to noise ratio for the run.

RI (Refractive Index): form of detection where effluent is monitored for changes in refractive index as analytes pass through.

Wavelength: setting for ultraviolet/visible light absorbance detectors which specifies the band of radiation to which the effluent stream will be exposed; for diode array detectors, this will be the nearest multiple of 5.

Weight Percent: quantitation of a given sample component based upon comparison of its response to that of an external standard.

4. **DISCUSSION**

N/A

5. RESPONSIBILITIES

Personnel in Analytical Services are responsible for compliance with this procedure according to the guidelines set forth in the analytical method operating procedure.

6. SAFETY

HMIS: (Health acute, Health chronic, Flammability, Reactivity) (0, , 0, 0)

QCSS or Online MSDS's state - "Not aware of any hazards", but SAP MSDS states Harmful/Irritant, Eyes, Lungs & Skin

Refer to material data safety sheets (MSDS), standard safety operating procedures, department manuals or protocols, and/or group green sheets, whichever may be applicable, for proper handling and disposal.

7. PROCEDURE

Column: **Supelcosil LC18-T**, 15cm X 4.6mm, 5um

Diluent: **H2O** Concentration: **0.5mg/ml**

Flow Rate: **1.5ml/min**

Injection Volume: **10ul** Immediate Injection: **OK in soln ~20hr@RT***

Detection: **260nm**

Eluants:

Eluant A = **5mM Tetra Butyl Ammonium Phosphate + 10mM NH4H2PO4 pH 5.5**

Eluant B = **Acetonitrile**

Gradient or isocratic conditions:

Time (min)	0	20
%Eluant A	70	50
%Eluant B	30	50

Calculation: **Area Percent Normalization**

8. REFERENCES & ATTACHMENTS

Dissolution Instructions:

Special Instructions:

9. EMERGENCY SHUTDOWN

Refer to current department emergency plan for details.

10. APPROVAL

	Print Name	Title	Date
Prepared by:	Donald L. Price	Originator	9/12/02
Approved by:	Marie E. Hargis	Supervisor, Analytical Services	9/12/02
Approved by:	Jeff D. Heiland	Manager, Quality Assurance	9/12/02

Customers who wish to verify the results obtained by Sigma should consider that variations in equipment, reagents, and technique, may affect the results.