



Analytical Method OP

Edition: 1	FORSKOLIN FROM COLEUS FORSKOHLII - (F6886)	Number: 10-039-0023
Supersedes: None	% Purity by High Performance Liquid Chromatography	Effective: 4/23/02

Document History

New

1. OBJECTIVE

To standardize a liquid chromatographic procedure for FORSKOLIN FROM COLEUS FORSKOHLII, Sigma-Aldrich Product Number **F6886**.

2. SCOPE

To define the conditions and procedures for the liquid chromatographic analysis of each lot of F6886 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) (2, *, 0, 0)
Harmful, Eyes, Lungs, Skin, Target Organs: Smooth Muscle, Behavioral (Change in Motor Activity)

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: **Discovery C-18**, 15cm X 4.6mm, 5um
Diluent: **Methanol** Concentration: **5.0mg/ml**
Flow Rate: **1.5ml/min**
Injection Volume: **10ul** Immediate Injection: **Unknown, Needs stability info***
Detection: **215nm**

Eluants:

Eluant A = **10mM NH4H2PO4 pH 3.6**
Eluant B = **Acetonitrile**

Gradient or isocratic conditions:

Time (min)	0	30	5	5
%Eluant A	55	25	20	20
%Eluant B	45	75	80	80

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	4/23/02
Approved by:	Marie E. Hargis	Supervisor, Analytical Services	4/23/02
Approved by:	Jeff D. Heiland	Manager, Quality Assurance	4/23/02

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