Method for estimation of Coumarin content in Cinnamon

(Method by Spices Board, Cochin)

Objective: To estimate the Coumarin content in Cinnamon and Cassia by HPLC

A. Apparatus:

1. Measuring Cylinders, 50 mL, 100 mL capacity
2. Conical Flask, 250 mL capacity
3. HAPC system with accessories as mentioned under Instrument conditions
4. Micro litre syringe capable of injecting 1-20 mL
5. Balance, readable to 0.001gm
6. Whatman No. 1 filter paper (90 mm)/ syringe filter 0.45 mm.
7. Sample powdering mill or equivalent

B. Reagents:

1. Methanol HPLC grade
2. Acetonitrile HPLC grade
3. Water HPLC grade
4. Acetic and HPLC grade.
5. Ammonium acetate
6. Coumarin Standard (>90%)

Standard stock solution:

1. Weight accurately 0.1 gm of the above standard and dissolve and make upto 100 mL with HPLC Methanol.
2. Keep this solution as stock solution (1000 ppm) in standard flask wrapped in black cover. Shelf life is one year under refrigeration.
3. Working standard 10 ppm- From the stock solution pipette 1 mL to the 100mL standard flask and make up to the mark with HPLC Methanol.

4. Keep under refrigeration in standard flask wrapped in black cover. Shelf life is six months under refrigeration.

C. Procedure:

Sample preparation:

Whole Cassia & Cinnamon: After mixing & quartering, powder 100gm of the sample and pass through the sieve ASTM No. 20. (850 mm)

Ground Cassia & Cinnamon: Take a subsample of 100 gm by mixing and quartering of the entire sample

1. Weight accurately 1.0gm of the above sample in duplicate into 250mL conical flask.
2. Add 50 mL 90 % (v/v) Methanol using Calibrated measuring cylinder.
3. Shake for 30 minutes.
4. Filter 3 to 4 mL through whatman no.1 filter paper or 0.45mm syringe filter into stoppered test tubes.

D. Instrumentation Conditions

HPLC System – HPLC System with UV-Detector

HPLC Column – 5mm C18 (4.6X250 mm) steel column.

Mobile phase A: Water, 5mm Ammonium acetate buffer with 0.2% (v/v) acetic acid.

Mobile phase B: Acetonitrile: Methanol 1: 2 (v/v).

All solvent should be HPLC grade.

Flow rate: 0.8 mL/minute in a gradient program. The gradient program is as follows.

Time	Conc of B in A
14’	22%
16’	70%

22’	70%
25’	30%
30’	Stop

UV Absorbance- 279.8 nm

Volume for injection – 5 to 20 µL

E. Calculations :

Coumarin Contect is calculated as follows:

$$\text{Coumarin contect (mg/Kg)} = \frac{\text{x}(nG)\ X\ 50mL}{5\mu L\ X\ 1g} = \text{x X 10 mg/Kg}$$

F. Result and Reporting :

Report Coumarin content to an accuracy of 0.0 mg/kg.

G. Environmental aspects:

Coumarin is harmful. Handle with care.