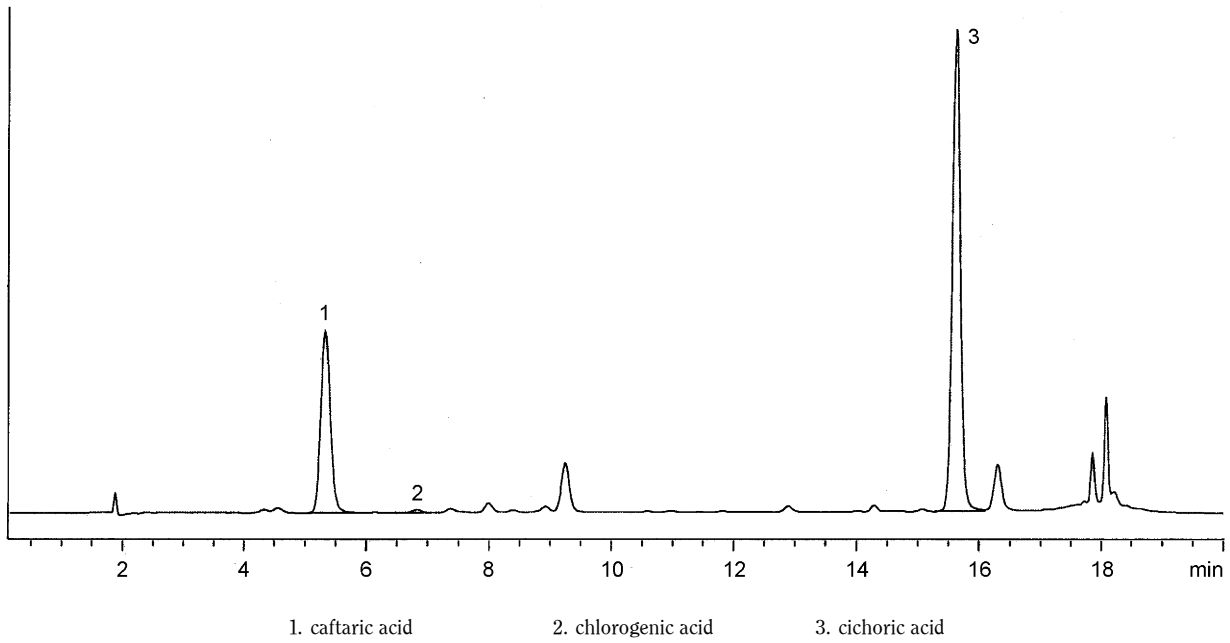

Figure 1823.-1. – *Chromatogram for the assay of caftaric acid and cichoric acid in purple coneflower herb*

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corrected 6.0

PURPLE CONEFLOWER ROOT

Echinaceae purpureae radix

DEFINITION

Dried, whole or cut underground parts of *Echinacea purpurea* (L.) Moench.

Content: minimum 0.5 per cent for the sum of caftaric acid ($C_{13}H_{12}O_9$; M_r 312.2) and cichoric acid ($C_{22}H_{18}O_{12}$; M_r 474.3) (dried drug).

IDENTIFICATION

First identification: A, B, C, E.

Second identification: A, B, D, E.

A. The rhizome is up to 15 cm long, branched, reddish-brown to dark brown on the surface and carries many stem bases; the inside is fibrous and white. The numerous roots are spirally twisted, light to dark brown and show a fine cross structuring on the surface.

B. Reduce to a powder (355) (*2.9.12*). The powder is light yellow to pinkish-beige. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: numerous light-brown spindle-shaped fibres that are joined together in long bundles without black deposits; rare sclereids from the rhizomes and roots, usually occuring singly, those from the rhizomes being isodiametric, about 60 µm in diameter, with black deposits, those from the roots being 50-120 µm in length with no black deposits; secretory cavities up to 180 µm in diameter with yellow oil droplets; squarish to rectangular cells of the outer layers, some with reddish walls; bordered-pitted vessels from the rhizome, 30-40 µm in diameter.

C. Examine the chromatogram obtained in the test for other *Echinacea* species and *Parthenium integrifolium*.

Results: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, faint greenish fluorescent zones may be present just below the zone situated in the middle of the chromatogram obtained with the test solution.

Top of the plate	
Caffeic acid: a strong blue fluorescent zone	A strong blue fluorescent zone
Cynarin: a strong greenish fluorescent zone	A blue fluorescent zone
Echinacoside: a strong greenish fluorescent zone	
Reference solution	**Test solution**

D. Examine the chromatograms obtained in the assay. The principal peak in the chromatogram obtained with the test solution is due to cichoric acid and a smaller peak is due to caftaric acid. Peaks due to caffeic acid and chlorogenic acid are minor or may be absent.

E. Thin-layer chromatography (*2.2.27*).

Test solution. To 1.0 g of the powdered drug (355) (*2.9.12*) add 10 ml of *methylene chloride R* and sonicate for 5 min. Centrifuge and use the supernatant solution.

Reference solution. Dissolve 1 mg of *β-sitosterol R* and a volume of *N-isobutyldodecatetraenamide solution R* corresponding to 1 mg of *N-isobutyldodecatetraenamide R* in 5.0 ml of *methanol R*.

Plate: *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase: *anhydrous formic acid R, cyclohexane R, ethyl acetate R, toluene R* (0.9:3:6:24 *V/V/V/V*).

Application: 25 µl [or 5 µl], as bands.

Development: over a path of about 15 cm [or 5 cm].

Drying: in a stream of cold air for about 10 min.

Detection: dip the plate into *anisaldehyde solution R* for 1 s and heat at 100-105 °C for 3 min; examine in daylight.

Monographs P

Results: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
_____	_____
	A bluish-violet zone
β-Sitosterol: a violet or pink zone	A violet or pink zone (β-sitosterol)
N-Isobutyldodecatetraenamide: a greyish-blue zone	A greyish-blue zone (*N*-isobutyldodecatetraenamide)
_____	_____
	A dark greyish-blue zone
Reference solution	**Test solution**

TESTS

Other *Echinacea* species and *Parthenium integrifolium*. Thin-layer chromatography (*2.2.27*).

Test solution. To 1.0 g of the powdered drug (355) (*2.9.12*) add 10 ml of *methanol R* and sonicate for 5 min. Centrifuge and use the supernatant solution.

Reference solution. Dissolve 1 mg of *echinacoside R*, 1 mg of *cynarin R* and 0.5 mg of *caffeic acid R* in 5.0 ml of *methanol R*.

Plate: *TLC silica gel plate R* (5-40 μm) [or *TLC silica gel plate R* (2-10 μm)].

Mobile phase: *anhydrous formic acid R, water R, methyl ethyl ketone R, ethyl acetate R* (3:3:9:15 *V/V/V/V*).

Application: 10 μl [or 5 μl] of the test solution and 5 μl [or 2 μl] of the reference solution, as bands.

Development: over a path of 10 cm [or 5 cm].

Drying: in a stream of cold air for about 10 min, then at 105 °C for 2 min.

Detection: spray the still-warm plate with a 5 g/l solution of *diphenylboric acid aminoethyl ester R* in *ethyl acetate R*; after 30 min, examine in ultraviolet light at 365 nm.

Results: the chromatogram obtained with the test solution shows no greenish fluorescent zone corresponding to the zone due to echinacoside in the chromatogram obtained with the reference solution, and no greenish fluorescent zone corresponding to the zone due to cynarin in the chromatogram obtained with the reference solution. No other zones apart from very faint dark blue fluorescent zones are seen in the lower half of the chromatogram of the test solution.

Foreign matter (*2.8.2*): maximum 3 per cent.

Loss on drying (*2.2.32*): maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*): maximum 9.0 per cent.

Ash insoluble in hydrochloric acid (*2.8.1*): maximum 2.0 per cent.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution. In a 100 ml volumetric flask place 0.500 g of the powdered drug (355) (*2.9.12*) and add 80 ml of *ethanol (70 per cent V/V) R*. Sonicate for 15 min and dilute to 100.0 ml with *ethanol (70 per cent V/V) R*. Mix the suspension and allow to stand for a few minutes to allow visible solids to settle.

Reference solution. Dissolve 10.0 mg of *chlorogenic acid CRS* and 10.0 mg of *caffeic acid R* in *ethanol (70 per cent V/V) R*, sonicate for 15 min and dilute to 10.0 ml with the same solvent. Dilute 4.0 ml of this solution to 100.0 ml with *ethanol (70 per cent V/V) R*.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 μm);
- *temperature*: 35 °C.

Mobile phase:

- *mobile phase A*: *phosphoric acid R, water R* (1:999 *V/V*);
- *mobile phase B*: *acetonitrile R*;

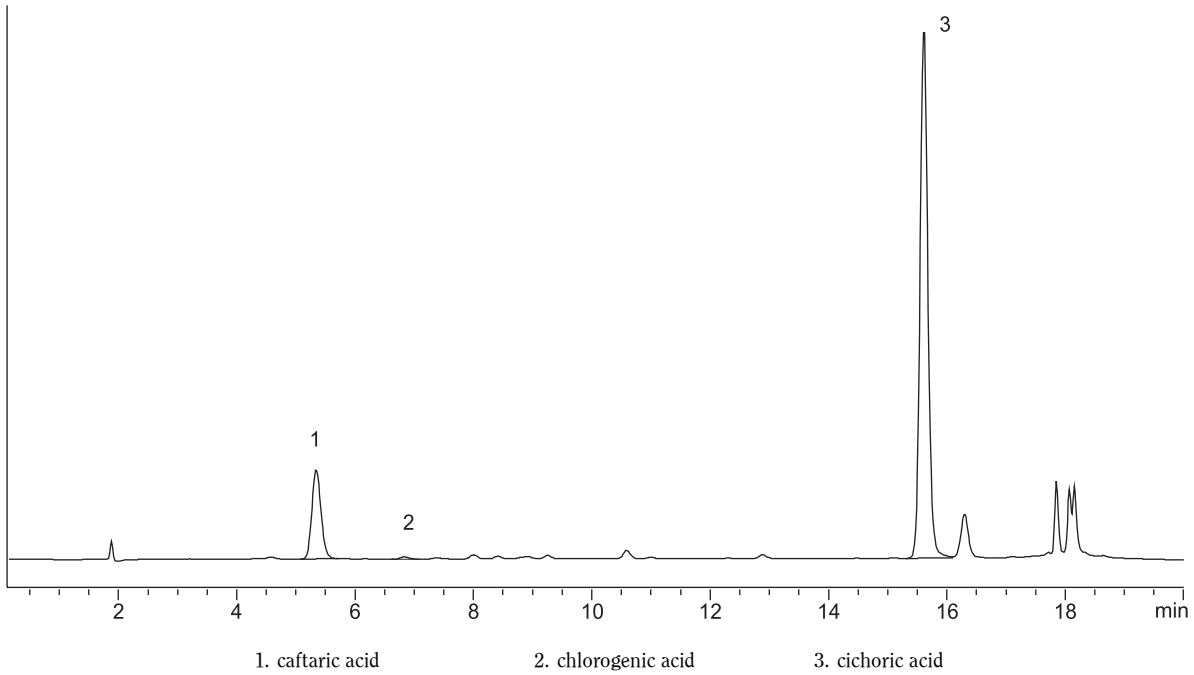

Figure 1824.-1. – *Chromatogram for the assay of caftaric acid and cichoric acid in purple coneflower root*

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0	90	10
0 - 13	90 → 78	10 → 22
13 - 14	78 → 60	22 → 40
14 - 20	60	40

Flow rate: 1.5 ml/min.

Detection: spectrophotometer at 330 nm.

Injection: 10 µl.

Relative retention with reference to chlorogenic acid (retention time = about 7 min): caftaric acid = about 0.8; caffeic acid = about 1.5; cynarin = about 1.6; echinacoside = about 1.7; cichoric acid = about 2.3.

System suitability: reference solution:

– *resolution*: minimum 5 between the peaks due to caffeic acid and chlorogenic acid.

Locate the peaks due to caffeic acid and chlorogenic acid using the chromatogram obtained with the reference solution. Locate the peaks due to caftaric acid and cichoric acid using the chromatogram in Figure 1824.-1.

Calculate the percentage content of caftaric acid using the following expression:

$$\frac{A_1 \times C_2 \times 100 \times 0.881}{A_2 \times C_1}$$

Calculate the percentage content of cichoric acid using the following expression:

$$\frac{A_3 \times C_2 \times 100 \times 0.695}{A_2 \times C_1}$$

A_1 = area of the peak due to caftaric acid in the chromatogram obtained with the test solution;

A_2 = area of the peak due to chlorogenic acid in the chromatogram obtained with the reference solution;

A_3 = area of the peak due to cichoric acid in the chromatogram obtained with the test solution;

C_1 = concentration of the dried drug in the test solution, in milligrams per millilitre;

C_2 = concentration of chlorogenic acid in the reference solution, in milligrams per millilitre;

0.695 = peak correlation factor based upon the liquid chromatography response observed;

0.881 = peak correlation factor between caftaric acid and chlorogenic acid.

STORAGE

Uncomminuted.

01/2008:1886
corrected 6.0

PYGEUM AFRICANUM BARK

Pruni africanae cortex

DEFINITION

Whole or cut, dried bark of the stems and branches of *Prunus africana* (Hook f.) Kalkm. (syn. *Pygeum africanum* Hook f.).

IDENTIFICATION

A. The dark brown to reddish-brown bark occurs in curved, hard, irregular pieces. The outer surface has a wrinkled dark reddish-brown cork with areas of adhering lichen. The reddish-brown to dark brown inner surface bears longitudinal striations. It may also occur in rolled fragments with a fibrous fracture.

B. Reduce to a powder (355) (*2.9.12*). The powder is reddish-brown. Examine under a microscope using *chloral hydrate solution R*. The powdered drug shows thick-walled sclereids, solitary or in groups; calcium oxalate cluster crystals of different size; numerous lignified fibres, thick-walled and with narrow lumen, some of them solitary and most in groups with forked ends; fragments of pigmented polygonal cells of reddish-brown colour; fragments of cork. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*, the powder shows some isolated small starch grains that stain bluish-black against *iodine solution R1*.

C. Thin-layer chromatography (*2.2.27*).

Test solution. Extract 15.0 g of powdered drug (250) (*2.9.12*) with *methylene chloride R* for 30 min in a continuous extraction apparatus (Soxhlet type). Filter. Evaporate the solvent to dryness under reduced pressure. Dissolve the residue in 1 ml of *methylene chloride R*.

Reference solution. Dissolve 20 mg of *β-sitosterol R* and 20 mg of *ursolic acid R* in 10 ml of a mixture of equal volumes of *methanol R* and *methylene chloride R*.

Plate: *TLC silica gel plate R*.

Mobile phase: *methanol R, methylene chloride R* (10:90 *V/V*).

Application: 10 µl, as 1 cm bands.

Development: over a path of 15 cm.

Drying: in air.

Detection: spray with *vanillin reagent R*. Heat the plate at 100-105 °C for 10 min and allow to cool; examine in daylight.

Results: see below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
	A violet zone
	Several weak violet, blue or grey zones
———	———
β-Sitosterol: a violet zone	A violet zone (β-sitosterol)
Ursolic acid: a blue zone	A blue zone (ursolic acid)
	Several weak violet, blue or grey zones
———	———
	A violet zone (β-sitosterol glucoside)
Reference solution	**Test solution**

TESTS

Foreign matter (*2.8.2*): maximum 3.0 per cent.

Loss on drying (*2.2.32*): maximum 12.0 per cent, determined on 1.000 g of powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*): maximum 10.0 per cent.

Monographs P