Loss on drying (*2.2.32*): maximum 14.0 per cent, determined on 1.000 g of drug by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*): maximum 4.0 per cent.

STORAGE

Store protected from moisture.

01/2008:1823
corrected 6.0

PURPLE CONEFLOWER HERB

Echinaceae purpureae herba

DEFINITION

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

Content: minimum 0.1 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.

Second identification: A, B, D.

A. The herbaceous perennial plant is 60-150 cm, rarely up to 180 cm high. The stem is green to red, upright and slightly branched. The leaves are alternate, ovate to ovate-lanceolate, irregularly serrate, rugose on both surfaces, dark green with prominent light green veins; the lamina is thick and shiny. The involucral bracts of the large capitulum are arranged in 2 or 3 rows. The solid receptacle is slightly convex. Each of the outer violet ligulate florets (4-6 cm) and of the inner violet-pink tubular florets is attached to a reddish acute and coriaceous bract, which overtops the tubular florets. The calyx is reduced to a very short crown, one of the sepals is up to 1 mm long.

B. Reduce to a powder (355) (*2.9.12*). The powder is green. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: whitish-green groups of fibres, 150-200 µm in length, 10-15 µm in diameter, sometimes with black deposits; fragments of leaves in surface view showing anomocytic or anisocytic stomata (*2.8.3*) (about 35-40 µm in length); uniseriate covering trichomes or fragments thereof consisting mainly of 3 or 4 thick-walled cells of which the apical cell is markedly longer than the others; fragments of leaves with rosette-like arranged epidermal cells around the base of the covering trichomes; uniseriate glandular trichomes composed of very thin-walled cells; pitted parenchymatous cells from the pith of the stem as well as pitted elongated cells from the mesocarp of the achenes; fragments of parenchyma from the seeds with oil droplets; fragments of the epidermis of ligulate florets composed of red to violet papillous cells; spheroidal pollen grains, 30-40 µm in diameter, with a spiny exine.

C. 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 0.5 mg of *caffeic acid R* and 0.5 mg of *chlorogenic 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: 25 µL [or 5 µL] of the test solution and 10 µL [or 2 µL] of the reference solution, as bands.

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

Drying: in a stream of cold air for about 10 min, then at 100 °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: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint blue fluorescent zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
	An intense red fluorescent zone
Caffeic acid: a strong blue fluorescent zone	A blue fluorescent zone
	A blue fluorescent zone
Chlorogenic acid: a strong blue fluorescent zone	A faint yellow-orange 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.

TESTS

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 12.0 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*;

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.

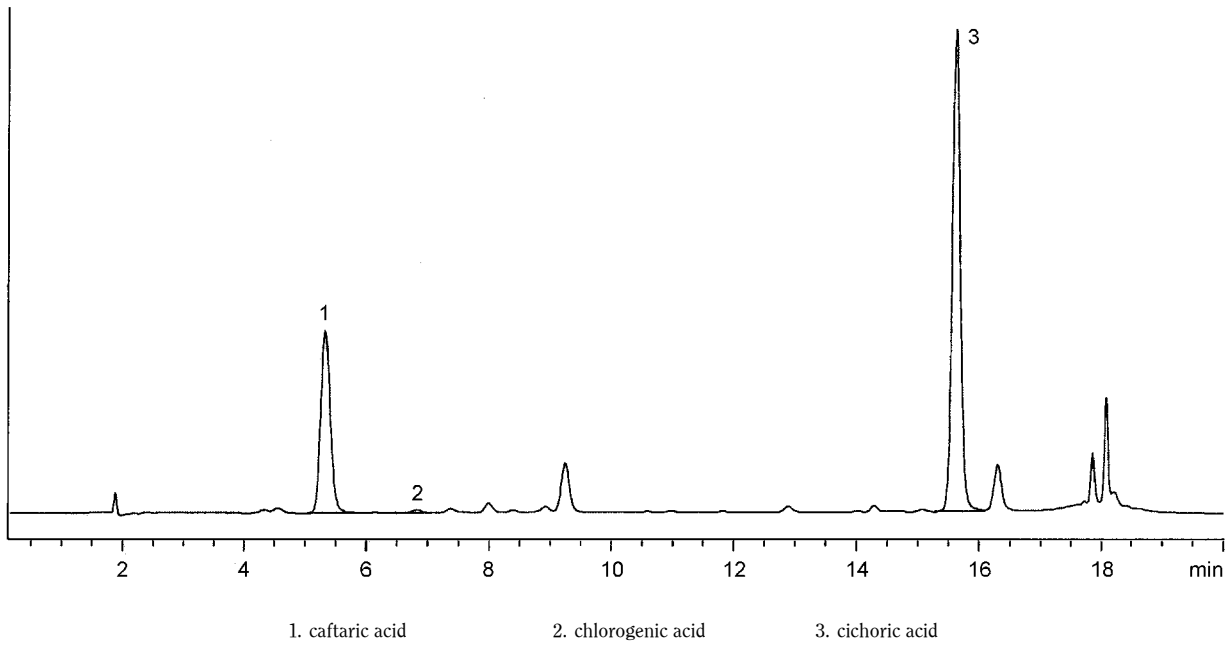

Figure 1823.-1. – *Chromatogram for the assay of caftaric acid and cichoric acid in purple coneflower herb*

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 1823.-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 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.

01/2008:1824
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.