PRODUCTION

The extract is produced from the herbal drug and ethanol (40 per cent *V/V* to 90 per cent *V/V*), methanol (60 per cent *V/V*) or acetone (40 per cent *V/V*) by an appropriate procedure.

CHARACTERS

Appearance: greenish-brown amorphous powder.

IDENTIFICATION

Thin-layer chromatography (*2.2.27*).

Test solution. To 0.25 g of the extract to be examined add *methanol R*. Shake, filter and dilute to 5 mL with *methanol R*.

Reference solution. Dissolve 2.0 mg of *hyperoside R* and 2.0 mg of *rutin R* in *methanol R* and dilute to 10 mL with the same solvent.

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* (10:10:30:50 *V/V/V/V*).

Application: 10 µL [or 5 µL] as bands.

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

Drying: at 100-105 °C.

Detection: spray with a 10 g/L solution of *diphenylboric acid aminoethyl ester R* in *methanol R*. Subsequently spray with a 50 g/L solution of *macrogol 400 R* in *methanol R*. Allow the plate to dry in air for about 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. Other fluorescent zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
———	———
Hyperoside: a yellowish-orange fluorescent zone	
	A green fluorescent zone
	A yellow fluorescent zone
Rutin: a yellowish-orange fluorescent zone	
———	———
	A green fluorescent zone
Reference solution	**Test solution**

TESTS

Loss on drying (*2.8.17*): maximum 5.0 per cent, determined on 0.500 g.

ASSAY

Stock solution. To 50 mg of the extract to be examined add *ethanol (60 per cent V/V) R*. Shake, filter and dilute to 100.0 mL with *ethanol (60 per cent V/V) R*.

Test solution. Introduce 5.0 mL of the stock solution into a round-bottomed flask and evaporate to dryness under reduced pressure. Take up the residue with 8 mL of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into a 25 mL volumetric flask. Rinse the round-bottomed flask with 3 mL of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into the 25 mL volumetric flask. Add 10.0 mL of a solution containing 25.0 g/L of *boric acid R* and 20.0 g/L of *oxalic acid R* in *anhydrous formic acid R* and dilute to 25.0 mL with *anhydrous acetic acid R*.

Compensation liquid. Introduce 5.0 mL of the stock solution into a round-bottomed flask and evaporate to dryness under reduced pressure. Take up the residue with 8 mL of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into a 25 mL volumetric flask. Rinse the round-bottomed flask with 3 mL of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into the 25 mL volumetric flask. Add 10.0 mL of *anhydrous formic acid R* and dilute to 25.0 mL with *anhydrous acetic acid R*.

After 30 min, measure the absorbance (*2.2.25*) of the test solution at 401 nm.

Calculate the percentage content of total flavonoids, expressed as vitexin, from the following expression:

$$\frac{A \times 0.8}{m}$$

i.e. taking the specific absorbance of vitexin to be 628.

A = absorbance at 401 nm,

m = mass of the extract to be examined, in grams.

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corrected 6.0

PELARGONIUM ROOT

Pelargonii radix

DEFINITION

Dried, usually fragmented, underground organs of *Pelargonium sidoides* DC and/or *Pelargonium reniforme* Curt.

Content: minimum 2.0 per cent of tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1) (dried drug).

IDENTIFICATION

A. The root is covered with dark, partly reddish-brown, longitudinally fissured bark. The transverse section shows, underneath the cork layer, yellow or white wood, which clearly shows partly brownish medullary rays.

B. Reduce to a powder (355 (*2.9.12*)). The powder is brownish-red. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: multilayer cork cells consisting of almost uniform, rectangular cells; fragments of parenchyma underneath the cork containing sclereids with a wide lumen; numerous calcium oxalate cluster crystals. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. The powder shows simple starch granules without striations or cracks.

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

Test solution. To 0.5 g of the powdered herbal drug (355) (*2.9.12*) add 10 mL of *methanol R*, shake for 15 min and filter.

Reference solution. Dissolve 1 mg of *scopoletin R* and 2 mg of *esculin R* in 20 mL of *methanol R*.

Plate: *TLC silica gel F_{254} plate R* (5-40 µm) [or *TLC silica gel F_{254} plate R* (2-10 µm)].

Mobile phase: *water R*, *methanol R*, *ethyl acetate R* (10:14:76 *V/V/V*).

Application: 10 µL [or 5 µL] as bands.

Development: over a path of 10 cm [or 6 cm].

Drying: in air.

Detection: spray with *alcoholic potassium hydroxide solution R*. 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 blue fluorescent zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
	A blue fluorescent zone
Scopoletin: a very bright blue fluorescent zone	A weak blue fluorescent zone (scopoletin)
_____	_____
	One or two bright blue fluorescent zones
Esculin: a very bright blue fluorescent zone	
	A blue fluorescent zone
	A weak blue fluorescent zone
_____	_____
	A blue fluorescent zone
Reference solution	**Test solution**

TESTS

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

Total ash (*2.4.16*): maximum 12.0 per cent.

Ash insoluble in hydrochloric acid (*2.8.1*): maximum 3.0 per cent.

ASSAY

Tannins (*2.8.14*). Use 0.750 g of the powdered herbal drug (180) (*2.9.12*).

01/2013:2477
corrected 7.8

PEPPER

Piperis fructus

DEFINITION

Dried, ripe or nearly ripe fruit of *Piper nigrum* L. with an unbroken pericarp (black pepper) or with the outer layers of the pericarp removed (white pepper).

Content:

– *essential oil*: minimum 25 mL/kg (anhydrous drug);
– *piperine* ($C_{17}H_{19}NO_3$; M_r 285.3): minimum 3.0 per cent (anhydrous drug).

IDENTIFICATION

A. *White pepper*. Spheroid berries, 3-5 mm in diameter, slightly flattened at one pole and with a small protuberance at the other, with smooth, externally matt, brownish-grey, greyish-white or pale yellowish-white surface, with numerous pale, linear striations between apex and base.

Black pepper. Spheroid berries, 3-6 mm in diameter, externally blackish-brown, with raised reticular wrinkles, bearing fine remains of the style at the apex and a scar of the peduncle at the base. The texture is hard, the epicarp can be stripped, the endocarp is greyish-white or pale yellow. The fracture is greyish-white, starchy, possessing a small space at the centre.

B. Microscopic examination (*2.8.23*).

White pepper. The powder is light grey. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters (Figure 2477.-1): fragments of the endocarp in surface view, consisting of more or less polygonal sclereids about 20-30 µm in diameter, which have irregularly thickened walls [Ac, C, Fa] and which may or may not be associated with the testa [A, F], consisting of a layer of indistinct, reddish-brown pigmented cells constituting the 'pigmented layer' [Ab, Fb] and a layer of very thin-walled polygonal cells constituting the 'hyaline layer' [Aa]; fragments of the endocarp, in transverse section [G], showing sclereids with thickened inner walls on the 3 lower sides [Ga], usually associated with the testa (pigmented layer [Gb] and hyaline layer [Gc]); fragments of the parenchyma of the mesocarp [D] containing large oil cells 50-75 µm in diameter [Da]; numerous thin-walled, ovoid or polygonal cells of the parenchyma of the seed [E]; rare, elongated sclereids, with thickened walls, from the fruit peduncle [B]; a few fragments of vascular tissue with narrow spiral vessels [J]. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. Rounded, compound starch granules [H], about 30 µm in diameter, made up of tiny individual granules, ovoid or polyhedral by compression, free [Hb] or included in the parenchymatous cells of the seed [Ha].

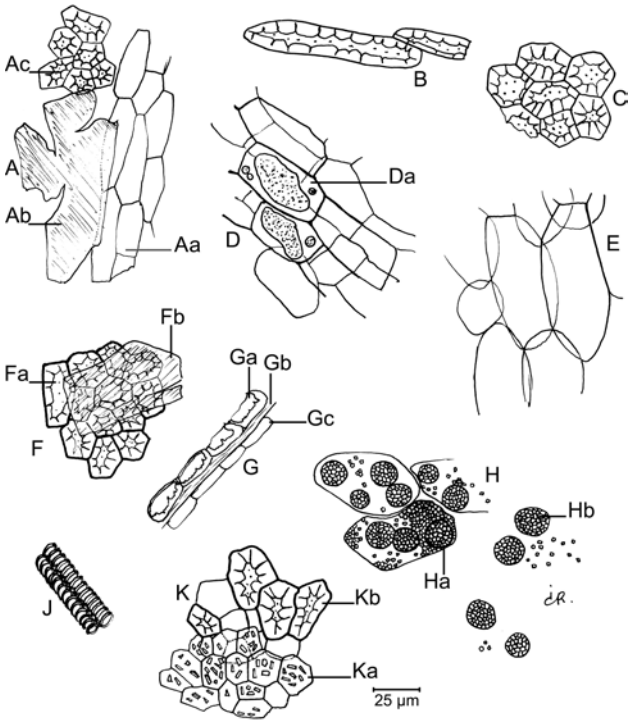

Figure 2477.-1. – *Illustration for identification test B of powdered herbal drug of pepper*

Black pepper. The powder is grey. Examine under a microscope using *chloral hydrate solution R*. In addition to the diagnostic characters described for white pepper, the powdered black pepper shows the following diagnostic characters (Figure 2477.-1): fragments of the epicarp [K] with extremely thin-walled, brownish-red pigmented, polygonal or ovoid cells, which contain small prisms of calcium oxalate [Ka], and which are associated with the outer layers of the mesocarp consisting of groups of sclereids with strongly thickened walls [Kb].

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

Test solution. To 0.5 g of the powdered herbal drug (355) (*2.9.12*) add 5 mL of *methanol R*. Sonicate for 10 min, centrifuge and use the supernatant.

Reference solution. Dissolve 10 mg of *borneol R* and 15 mg of *piperine R* in 10 mL of *methanol R*.

Plate: *TLC silica gel F_{254} plate R* (5-40 µm) [or *TLC silica gel F_{254} plate R* (2-10 µm)].

Mobile phase: *ethyl acetate R*, *cyclohexane R* (30:50 *V/V*).