Application: 1 µl.

Development: over 2/3 of the plate.

Drying: in air.

Detection: dip the plate into *dimethylaminobenzaldehyde solution R2*. Heat at 90 °C until the spots appear.

System suitability: the chromatogram obtained with reference solution (b) shows 2 clearly separated spots or 2 clearly separated groups of spots.

Results: the principal spot or group of principal spots in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot or group of principal spots in the chromatogram obtained with reference solution (a) and to the spot or group of spots with the highest R_f value in the chromatogram obtained with reference solution (b).

C. Examine the chromatograms obtained in the test for composition.

Results: the 3 principal peaks in the chromatogram obtained with the test solution are similar in retention time to the 3 principal peaks in the chromatogram obtained with reference solution (a).

TESTS

Composition. Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution. Dissolve 25 mg of the substance to be examined in 10 ml of *methanol R* and dilute to 25 ml with the mobile phase.

Reference solution (a). Dissolve 25 mg of *gramicidin CRS* in 10 ml of *methanol R* and dilute to 25 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm,
– *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm),
– *temperature*: 50 °C.

Mobile phase: *water R, methanol R* (29:71 *V/V*).

Flow rate: 1.0 ml/min.

Detection: spectrophotometer at 282 nm.

Injection: 20 µl.

Run time: 2.5 times the retention time of gramicidin A1.

Relative retention with reference to gramicidin A1 (retention time = about 22 min): gramicidin C1 = about 0.7; gramicidin C2 = about 0.8; gramicidin A2 = about 1.2; gramicidin B1 = about 1.9.

System suitability: reference solution (a):

– *resolution*: minimum 1.5 between the peaks due to gramicidin A1 and gramicidin A2,
– the chromatogram obtained is concordant with the chromatogram supplied with *gramicidin CRS*.

Composition:

– *sum of the contents of gramicidins A1, A2, B1, C1 and C2*: minimum 95.0 per cent,
– *ratio of the content of gramicidin A1 to the sum of the contents of gramicidins A1, A2, B1, C1 and C2*: minimum 60.0 per cent,
– *disregard limit*: the area of the peak due to gramicidin A1 in the chromatogram obtained with reference solution (b).

Related substances. Liquid chromatography (*2.2.29*) as described in the test for composition.

Limit:

– *any impurity*: maximum 2.0 per cent and not more than 1 peak is more than 1.0 per cent; disregard the peaks due to gramicidins A1, A2, B1, C1 and C2.

Loss on drying (*2.2.32*): maximum 3.0 per cent, determined on 1.000 g by drying over *diphosphorus pentoxide R* at 60 °C at a pressure not exceeding 0.1 kPa for 3 h.

Sulphated ash (*2.4.14*): maximum 1.0 per cent, determined on 1.0 g.

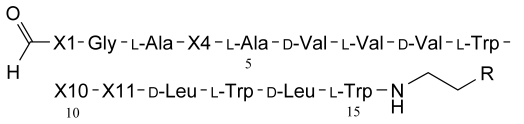
ASSAY

Carry out the microbiological assay of antibiotics (*2.7.2*), using the turbidimetric method. Use *gramicidin CRS* as the reference substance.

STORAGE

In an airtight container, protected from light.

IMPURITIES



Impurity	X1	X4	X10	X11	R
A	L-Val	Met	D-Leu	L-Trp	OH
B	L-Val	D-Leu	D-Leu	L-Trp	CH_2-OH
C	L-Ile	D-Leu	D-Leu	L-Phe	OH
D	L-Val	D-Leu	Met	L-Tyr	OH
E	L-Ile	D-Leu	D-Leu	L-Trp	CH_2-OH

A. [4-methionine]gramicidin A1,

B. gramicidin A1 3-hydroxypropyl,

C. gramicidin B2,

D. [10-methionine]gramicidin C1,

E. gramicidin A2 3-hydroxypropyl.

01/2005:1861

GREATER CELANDINE

Chelidonii herba

DEFINITION

Dried, whole or cut aerial parts of *Chelidonium majus* L. collected during flowering.

Content: minimum 0.6 per cent of total alkaloids, expressed as chelidonine ($C_{20}H_{19}NO_5$; M_r 353.4) (dried drug).

CHARACTERS

Macroscopic and microscopic characters described under identification tests A and B.

IDENTIFICATION

A. The stems are rounded, ribbed, yellowish to greenish-brown, somewhat pubescent, about 3 mm to 7 mm in diameter, hollow and mostly collapsed. The leaves are thin, irregularly pinnate, the leaflets ovate to oblong with coarsely dentate margins, the terminal leaflet often three-lobed; the adaxial surface is bluish-green and glabrous, the abaxial surface paler and pubescent,

especially on the veins. The flowers have 2 deeply concavo-convex sepals, readily removed, and 4 yellow, broadly ovate, spreading petals about 8 mm to 10 mm long; the stamens are numerous, yellow, and a short style arises from a superior ovary; long, capsular, immature fruits are rarely present.

B. Reduce to a powder (355). The powder is dark greyish-green to brownish-green. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: numerous fragments of leaves in surface view, the epidermal cells with sinuous walls; anomocytic stomata (*2.8.3*) occur on the abaxial surface only; covering trichomes long, uniseriate, with thin walls and usually fragmented; vascular tissue from the leaves and stems with groups of fibres, pitted and spirally thickened vessels and associated latex tubes with yellowish-brown contents; occasional fragments of the corolla with thin-walled, partly papillose cells containing numerous pale yellow droplets of oil; spherical pollen grains about 30 µm to 40 µm in diameter with 3 pores and a finely pitted exine.

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

Test solution. To 0.4 g of the powdered drug (710) add 50 ml of *dilute acetic acid R*. Boil the mixture under a reflux condenser in a water-bath for 30 min. Cool and filter. To the filtrate add *concentrated ammonia R* until a strong alkaline reaction is produced. Shake with 30 ml of *methylene chloride R*. Dry the organic layer over *anhydrous sodium sulphate R*, filter and evaporate *in vacuo* to dryness. Dissolve the residue in 1.0 ml of *methanol R*.

Reference solution. Dissolve 2 mg of *papaverine hydrochloride R* and 2 mg of *methyl red R* in 10 ml of *alcohol R*.

Plate: *TLC silica gel plate R*.

Mobile phase: *anhydrous formic acid R, water R, propanol R* (1:9:90 *V/V/V*).

Application: 10 µl as bands.

Development: over a path of 10 cm.

Drying: in air.

Detection: spray with *potassium iodobismuthate solution R* and dry the plate in air; spray with *sodium nitrite solution R* and allow the plate to dry in air; 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 weaker zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
———	———
Methyl red: a red zone	A brown zone
	A brown zone
Papaverine: a greyish-brown zone	A greyish-brown zone
———	———
	A brown zone
	A brown zone
Reference solution	**Test solution**

TESTS

Foreign matter (*2.8.2*): maximum 10 per cent.

Loss on drying (*2.2.32*): maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) by drying in an oven at 100-105 °C for 2 h.

Total ash (*2.4.16*): maximum 13.0 per cent.

ASSAY

Test solution. To 0.750 g of the powdered drug (710), add 200 ml of *dilute acetic acid R* and heat on a water-bath for 30 min, shaking frequently. Cool and dilute to 250.0 ml with *dilute acetic acid R*. Filter. Discard the first 20 ml of the filtrate. To 30.0 ml of the filtrate add 6.0 ml of *concentrated ammonia R* and 100.0 ml of *methylene chloride R*. Shake for 30 min. Separate the organic layer, place 50.0 ml in a 100 ml round-bottomed flask and evaporate to dryness *in vacuo* at a temperature not exceeding 40 °C. Dissolve the residue in about 2-3 ml of *alcohol R*, warming slightly. Transfer the solution to a 25 ml volumetric flask by rinsing the round-bottomed flask with *dilute sulphuric acid R* and dilute to 25.0 ml with the same solvent. To 5.0 ml of the solution, add 5.0 ml of a 10 g/l solution of *chromotropic acid, sodium salt R* in *sulphuric acid R* in a 25 ml volumetric flask, stopper the flask and mix carefully. Dilute to 25.0 ml with *sulphuric acid R* and stopper the flask.

Compensation solution. At the same time and in the same manner, place in a 25 ml volumetric flask 5.0 ml of *dilute sulphuric acid R* and 5.0 ml of a 10 g/l solution of *chromotropic acid, sodium salt R* in *sulphuric acid R*, stopper the flask and mix carefully. Dilute to 25.0 ml with *sulphuric acid R* and stopper the flask.

Place both solutions on a water-bath for 10 min. Cool to about 20 °C and dilute if necessary to 25.0 ml with *sulphuric acid R*. Measure the absorbance (*2.2.25*) of the test solution at 570 nm.

Calculate the percentage content of total alkaloids, expressed as chelidonine, from the expression:

$$\frac{A \times 2.23}{m}$$

i.e. taking the specific absorbance of chelidonine to be 933.

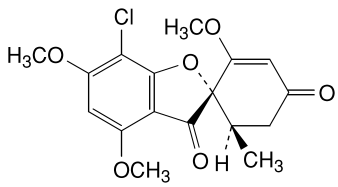
A = absorbance at 570 nm,

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

01/2005:0182

GRISEOFULVIN

Griseofulvinum



$C_{17}H_{17}ClO_6$ M_r 352.8

DEFINITION

Griseofulvin is (1′*S*,3-6′*R*)-7-chloro-2′,4,6-trimethoxy-6′-methylspiro[benzofuran-2(3*H*),1′-[2]cyclohexene]-3,4′-dione, a substance produced by the growth of certain strains of *Penicillium griseofulvum* or obtained by any other means. It contains not less than 97.0 per cent and not more than the equivalent of 102.0 per cent of $C_{17}H_{17}ClO_6$, calculated with reference to the dried substance.