Results A: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. The chromatogram obtained with the test solution shows other distinct zones, mainly above the zone due to harpagoside. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
Harpagoside: a quenching zone	A quenching zone: harpagoside
Reference solution	**Test solution**

Detection B: spray with a 10 g/l solution of *phloroglucinol R* in *ethanol (96 per cent) R* and then with *hydrochloric acid R*; heat at 80 °C for 5-10 min and examine in daylight.

Results B: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. The chromatogram obtained with the test solution also shows several yellow to brown zones above the zone due to harpagoside. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
Harpagoside: a green zone	A green zone (harpagoside)
	A yellow zone
	A light green zone
Fructose: a yellowish-grey zone	A yellowish-grey zone may be present (fructose)
	A brown zone
Reference solution	**Test solution**

TESTS

Starch. Examine the powdered drug (355) (*2.9.12*) under a microscope using *water R*. Add *iodine solution R1*. No blue colour develops.

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

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

ASSAY

Liquid chromatography (*2.2.29*).

Test solution. To 0.500 g of the powdered drug (355) (*2.9.12*) add 100.0 ml of *methanol R*. Shake for 4 h and filter through a membrane filter (nominal pore size: 0.45 µm).

Reference solution. Dissolve the contents of a vial of *harpagoside CRS* in *methanol R* and dilute to 10.0 ml with the same solvent.

Column:

- *size*: *l* = 0.10 m, Ø = 4.0 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: *methanol R, water R* (50:50 *V/V*).

Flow rate: 1.5 ml/min.

Detection: spectrophotometer at 278 nm.

Injection: 10 µl.

Run time: 3 times the retention time of harpagoside.

Retention time: harpagoside = about 7 min.

Calculate the percentage content of harpagoside using the following expression:

$$\frac{m_2 \times A_1 \times 1000}{A_2 \times m_1}$$

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

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

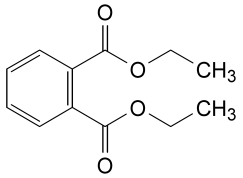
m_1 = mass of the drug to be examined used to prepare the test solution, in grams;

m_2 = mass of *harpagoside CRS* in the reference solution, in grams.

04/2008:0897

DIETHYL PHTHALATE

Diethylis phthalas



$C_{12}H_{14}O_4$ M_r 222.2

[84-66-2]

DEFINITION

Diethyl benzene-1,2-dicarboxylate.

Content: 99.0 per cent *m/m* to 101.0 per cent *m/m*.

CHARACTERS

Appearance: clear, colourless or very slightly yellow, oily liquid.

Solubility: practically insoluble in water, miscible with ethanol (96 per cent).

IDENTIFICATION

First identification: B, C.

Second identification: A, D, E.

A. Relative density (*2.2.5*): 1.117 to 1.121.

B. Refractive index (*2.2.6*): 1.500 to 1.505.

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: thin films.

Comparison: *diethyl phthalate CRS*.

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

Test solution. Dissolve 50 mg of the substance to be examined in *ether R* and dilute to 10 ml with the same solvent.

Reference solution. Dissolve 50 mg of *diethyl phthalate CRS* in *ether R* and dilute to 10 ml with the same solvent.

Plate: *TLC silica gel GF_{254} plate R*.

Mobile phase: *heptane R, ether R* (30:70 *V/V*).

Application: 10 µl.

Development: over 2/3 of the plate.

Drying: in air.

Detection: examine in ultraviolet light at 254 nm.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

E. To about 0.1 ml add 0.25 ml of *sulphuric acid R* and 50 mg of *resorcinol R*. Heat on a water-bath for 5 min. Allow to cool. Add 10 ml of *water R* and 1 ml of *strong sodium hydroxide solution R*. The solution becomes yellow or brownish-yellow and shows green fluorescence.

TESTS

Appearance. The substance to be examined is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Acidity. Dissolve 20.0 g in 50 ml of *ethanol (96 per cent) R* previously neutralised to *phenolphthalein solution R1*. Add 0.2 ml of *phenolphthalein solution R1*. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Related substances. Gas chromatography (*2.2.28*).

Internal standard solution. Dissolve 60 mg of *naphthalene R* in *methylene chloride R* and dilute to 20 ml with the same solvent.

Test solution (a). Dissolve 1.0 g of the substance to be examined in *methylene chloride R* and dilute to 20.0 ml with the same solvent.

Test solution (b). Dissolve 1.0 g of the substance to be examined in *methylene chloride R*, add 2.0 ml of the internal standard solution and dilute to 20.0 ml with *methylene chloride R*.

Reference solution. To 1.0 ml of test solution (a) add 10.0 ml of the internal standard solution and dilute to 100.0 ml with *methylene chloride R*.

Column:

– *material*: glass;
– *size*: *l* = 2 m, Ø = 2 mm;
– *stationary phase*: *silanised diatomaceous earth for gas chromatography R* (150-180 µm) impregnated with 3 per cent *m/m* of *polymethylphenylsiloxane R*.

Carrier gas: *nitrogen for chromatography R*.

Flow rate: 30 ml/min.

Temperature:

– *column*: 150 °C;
– *injection port and detector*: 225 °C.

Detection: flame ionisation.

Injection: 1 µl.

Run time: 3 times the retention time of diethyl phthalate.

Elution order: naphthalene, diethyl phtalate.

System suitability:

– *resolution*: minimum 10 between the peaks due to naphthalene and diethyl phthalate in the chromatogram obtained with the reference solution;
– in the chromatogram obtained with test solution (a), there is no peak with the same retention time as the internal standard.

Limit:

– *total*: calculate the ratio (*R*) of the area of the peak due to diethyl phthalate to the area of the peak due to the internal standard from the chromatogram obtained with the reference solution; from the chromatogram obtained with test solution (b), calculate the ratio of the sum of the areas of any peaks, apart from the principal peak and the peak due to the internal standard, to the area of the peak due to the internal standard: this ratio is not greater than *R* (1.0 per cent).

Water (*2.5.12*): maximum 0.2 per cent, determined on 10.0 g.

Sulphated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Introduce 0.750 g into a 250 ml borosilicate glass flask. Add 25.0 ml of *0.5 M alcoholic potassium hydroxide* and a few glass beads. Boil in a water-bath under a reflux condenser for 1 h. Add 1 ml of *phenolphthalein solution R1* and titrate immediately with *0.5 M hydrochloric acid*. Carry out a blank titration. Calculate the volume of *0.5 M alcoholic potassium hydroxide* used in the saponification.

1 ml of *0.5 M alcoholic potassium hydroxide* is equivalent to 55.56 mg of $C_{12}H_{14}O_4$.

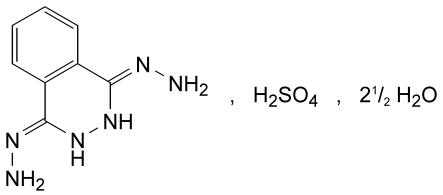
STORAGE

In an airtight container.

01/2008:1310
corrected 6.1

DIHYDRALAZINE SULPHATE, HYDRATED

Dihydralazini sulfas hydricus



$C_8H_{12}N_6O_4S,2^1/_2H_2O$ M_r 333.3
[7327-87-9]

DEFINITION

(Phthalazine-1,4(2*H*,3*H*)-diylidene)dihydrazine sulphate 2.5-hydrate.

Content: 98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or slightly yellow, crystalline powder.

Solubility: slightly soluble in water, practically insoluble in anhydrous ethanol. It dissolves in dilute mineral acids.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *Ph. Eur. reference spectrum of dihydralazine sulphate hydrated.*

B. Dissolve about 50 mg in 5 ml of *dilute hydrochloric acid R*. The solution gives reaction (a) of sulphates (*2.3.1*).

TESTS

Appearance of solution. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Dissolve 0.20 g in *dilute nitric acid R* and dilute to 10 ml with the same acid.