Detection: spray the warm plate with a 10 g/l solution of diphenylboric acid aminoethyl ester R in methanol R, using about 10 ml for a plate 200 mm square; subsequently spray with the same volume of a 50 g/l solution of macrogl 400 R in methanol R; allow to stand for about 30 min and examine in ultraviolet light at 365 nm.

Results: the chromatogram obtained with the reference solution shows in the upper third a yellowish-green fluorescent zone (apigenin) and in the middle third a yellowish fluorescent zone (apigenin-7-glucoside). The chromatogram obtained with the test solution shows a yellowish-green fluorescent zone and a yellowish fluorescent zone similar in position and fluorescence to the zones due to apigenin and apigenin-7-glucoside in the chromatogram obtained with the reference solution; above the apigenin-7-glucoside zone there is a brownish fluorescent zone (luteolin); immediately below the apigenin-7-glucoside zone there is a light brownish fluorescent zone (apiin); immediately below the apiin zone there is a bright blue fluorescent zone and below this zone a bright blue fluorescent zone; other faint zones may be present.

ASSAY

Carry out the determination of essential oils in herbal drugs (2.8.12). Use 20.0 g of whole drug, a 500 ml round-bottomed flask, 250 ml of water R as the distillation liquid and 0.50 ml of xylene R in the graduated tube. Distil at a rate of 3-3.5 ml/min for 3 h.

Fluorescent substances. In an intermittent-extraction apparatus, treat 1.0 g with 100 ml of cyclohexane R1 for 2 h. Collect the liquid and dilute to 100 ml with cyclohexane R1. Examine in ultraviolet light at 365 nm. The fluorescence of the solution is not more intense than that of a solution of 83 µg of quinine R in 1000 ml of 0.005 M sulphuric acid examined under the same conditions.

Sulphides. To 1.0 g in a conical flask add 5 ml of hydrochloric acid R1 and 20 ml of water R. Heat to boiling. The fumes released do not turn lead acetate paper R brown.

Copper: maximum 25.0 ppm.

Atomic absorption spectrometry (2.2.23, Method I).

Test solution. Use solution S.

Reference solutions. Prepare the reference solutions using copper standard solution (0.1 per cent Cu) R and diluting with 0.1 M hydrochloric acid.

Source: copper hollow-cathode lamp.

Wavelength: 325.0 nm.

Atomisation device: air-acetylene flame.

Lead: maximum 10.0 ppm.

Atomic absorption spectrometry (2.2.23, Method I).

Test solution. Use solution S.

Reference solutions. Prepare the reference solutions using lead standard solution (100 ppm Pb) R and diluting with 0.1 M hydrochloric acid.

Source: lead hollow-cathode lamp.

Wavelength: 283.3 nm; 217.0 nm may be used depending on the apparatus.

Atomisation device: air-acetylene flame.
Zinc: maximum 25.0 ppm.
Atomic absorption spectrometry (2.2.23, Method I).

Test solution. Use solution S.

Reference solutions. Prepare the reference solutions using zinc standard solution (100 ppm Zn) R and diluting with 0.1 M hydrochloric acid.

Source: zinc hollow-cathode lamp.

Wavelength: 214.0 nm.

Atomisation device: air-acetylene flame.

Loss on drying (2.2.32): maximum 15 per cent, determined on 1.00 g by drying in an oven at 120 °C for 4 h.

Sulphated ash (2.4.14): maximum 5.0 per cent, determined on 1.0 g.

Adsorption power. To 0.300 g in a 100 ml ground-glass-stoppered conical flask add 25.0 ml of a freshly prepared solution of 0.5 g of phenazone R in 50 ml of water R. Shake thoroughly for 15 min. Filter and reject the first 5 ml of filtrate. To 10.0 ml of the filtrate add 1.0 g of potassium bromate R and 20 ml of dilute hydrochloric acid R. Using 0.1 ml of methyl red solution R as indicator, titrate with 0.0167 M potassium bromate until the red colour is discharged. Titrate slowly (1 drop every 15 s) towards the end of the titration. Carry out a blank titration using 10.0 ml of the phenazone solution.

Calculate the quantity of phenazone adsorbed per 100 g of activated charcoal from the following expression:

\[
\frac{2.353 (a - b)}{m}
\]

\(a\) = number of millilitres of 0.0167 M potassium bromate used for the blank,

\(b\) = number of millilitres of 0.0167 M potassium bromate used for the test,

\(m\) = mass in grams of the substance to be examined.

Minimum 40 g of phenazone is adsorbed per 100 g of activated charcoal, calculated with reference to the dried substance.

Microbial contamination. Total viable aerobic count (2.6.12) not more than 10^3 micro-organisms per gram, determined by plate count.

STORAGE
In an airtight container.

DEFINITION
Chenodeoxycholic acid contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 3α,7α-dihydroxy-5β-cholan-24-oic acid, calculated with reference to the dried substance.

CHARACTERS
A white or almost white powder, very slightly soluble in water, freely soluble in alcohol, soluble in acetone, slightly soluble in methylene chloride.

IDENTIFICATION
First identification: A.
Second identification: B, C.

A. Examine by infrared absorption spectrophotometry (2.2.24), comparing with the spectrum obtained with chenodeoxycholic acid CRS. Examine the substances prepared as discs using potassium bromide R.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve about 10 mg in 1 ml of sulphuric acid R. Add 0.1 ml of formaldehyde solution R and allow to stand for 5 min. Add 5 ml of water R. The suspension obtained is greenish-blue.

TESTS
Specific optical rotation (2.2.7). Dissolve 0.500 g in methanol R and dilute to 25.0 ml with the same solvent. The specific optical rotation is +11.0 to +13.0, calculated with reference to the dried substance.

Related substances. Examine by thin-layer chromatography (2.2.27), using a suitable silica gel as the coating substance.
Test solution (a). Dissolve 0.40 g of the substance to be examined in a mixture of 1 volume of water R and 9 volumes of acetone R and dilute to 10 ml with the same mixture of solvents.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with a mixture of 1 volume of water R and 9 volumes of acetone R.

Reference solution (a). Dissolve 40 mg of chenodeoxycholic acid CRS in a mixture of 1 volume of water R and 9 volumes of acetone R and dilute to 10 ml with the same mixture of solvents.

Reference solution (b). Dissolve 20 mg of lithocholic acid CRS in a mixture of 1 volume of water R and 9 volumes of acetone R and dilute to 10 ml with the same mixture of solvents.

Reference solution (c). Dissolve 20 mg of ursodeoxycholic acid CRS in a mixture of 1 volume of water R and 9 volumes of acetone R and dilute to 50 ml with the same mixture of solvents.

Reference solution (d). Dissolve 20 mg of cholic acid CRS in a mixture of 1 volume of water R and 9 volumes of acetone R and dilute to 100 ml with the same mixture of solvents.

Reference solution (e). Dilute 0.5 ml of test solution (a) to 20 ml with a mixture of 1 volume of water R and 9 volumes of acetone R. Dilute 1 ml of the solution to 10 ml with a mixture of 1 volume of water R and 9 volumes of acetone R.

Reference solution (f). Dissolve 10 mg of chenodeoxycholic acid CRS in reference solution (c) and dilute to 25 ml with the same solution.