

**Sulphuric acid
extraction
method C**

Powdered drug (0.4–2 g) is shaken for 15 min with 15 ml 0.1 N sulphuric acid and then filtered. The filter is washed with 0.1 N sulphuric acid to a volume of 20 ml filtrate; 1 ml concentrated ammonia is then added. The mixture is shaken with two portions of 10 ml diethyl ether. The ether is dried over anhydrous sodium sulphate, filtered and evaporated to dryness and the resulting residue dissolved in 0.5 ml methanol. This is the preferred method for leaf drugs, e.g. *Belladonnae folium* (0.6 g), *Stramonii folium* (0.4 g), *Hyoscyami folium* (2 g) or *Fumariae herba* (1 g).

1.2 Thin-Layer Chromatography

Drug extracts

The samples applied to the TLC plate should contain between 50 and 100 µg total alkaloids, which have to be calculated according to the average alkaloid content of the specific drug (see 1.4 Drug List).

Example: Powdered drug (1 g) with a total alkaloid content of 0.3%, extracted with 5 ml methanol by the general method described above will yield 3 mg in 5 ml methanolic solution, containing approximately 60 µg total alkaloids per 100 µl.

**Reference
compounds**

- Commercially available compounds are usually prepared in 1% alcoholic solution and 10 µl is applied for TLC, e.g. atropine, brucine, strychnine, berberine, codeine.
- *Rauvolfia* alkaloids are prepared in 0.5% alcoholic solution, and 10 µl is applied for TLC, e.g. reserpine, rescinnamine, rauwolscine, ajmaline, serpentine.
- Colchicine is prepared as a 0.5% solution in 70% ethanol, and 10 µl is applied for TLC.

Alkaloid references can also be obtained from pharmaceutical products by a simple methanol extraction. The sample solution used for TLC should contain between 50 and 100 µg alkaloid.

- Alkaloid content 10–250 mg per tablet or dragée:
One powdered tablet or dragée is mixed with 1 ml methanol per 10 mg alkaloid and shaken for about 5 min at 60°C. After filtration or centrifugation, the extract is applied directly; 10 µl then corresponds to 100 µg alkaloid.
- Alkaloid content 0.075–1.0 mg per tablet or dragée:
Ten powdered tablets or dragées are mixed with 10 ml methanol, shaken for about 5 min at 60°C and filtered and the filtrate evaporated to dryness. The residue is dissolved in 1 ml methanol and, if necessary, the solution cleared by centrifugation; 10 µl of this solution contains 100 µg alkaloid (1.0 mg/tablet), or 100 µl contains 75 µg alkaloid (0.075 mg/tablet).

Test mixtures

- Cinchona alkaloids test mixture for *Cinchonae* (*Chinae*) cortex (DAB 10)
A mixture of 17.5 mg quinine, 0.5 mg quinidine, 10 mg cinchonine and 10 mg cinchonidine is dissolved in 5 ml ethanol, and 2 µl of this solution is applied for TLC.
- Test mixture for Solanaceae drugs (DAB 10)
A total of 50 mg hyoscyamine sulphate is dissolved in 9 ml methanol and 15 mg scopolamine hydrobromide in 10 ml methanol.

For *Belladonnae folium* (T1): 1.8 ml scopolamine hydrobromide solution is added to 8 ml hyoscyamine sulphate solution; 20 µl is used for TLC.