

***Rheum palmatum L.* extract preparation**

3500 Kg of underground parts of *Rheum palmatum* (European Pharmacopoeia quality) are extracted with ethanol (70-80) - water (30-20) (v/v) at 65-75 °C under exhaustion conditions using decanter system. In these conditions from 20 to 30 volumes of solvent with respect to the weight of the starting dried plant materials are used. The extract is concentrated under vacuum at a temperature <50 °C and the resulting soft mass is subsequently dried under vacuum at 60-70 °C. The extract is standardized by mixing with an amount of dehydrated glucose to get a final content of hydroxyanthracene derivatives in the range 5.0-6.1% (w/w), according to the European Pharmacopoeia monograph.

Provided by Bernett s.r.l. (Indena Group)

***Rhamnus frangula L.* extract preparation**

2400 Kg of *Rhamnus frangula L.* bark (European Pharmacopoeia quality) are extracted with ethanol-water 90-10 (v/v) at 75 °C under exhaustion conditions using static percolators system. In these conditions from 20 to 30 volumes of solvent with respect to the weight of the starting dried plant material are used. The extract is concentrated under vacuum at a temperature <50 °C and the resulting soft mass is subsequently dried under vacuum at 60 °C. The extract is standardized by mixing with an amount of dehydrated glucose to get a final content of glucofrangulins >15% (w/w), according to the European Pharmacopoeia monograph.

Provided by Bernett s.r.l. (Indena Group)

***Cassia senna fructus* powdered extract preparation**

2700 Kg of Senna pods (European Pharmacopoeia quality) are extracted with ethanol-water (60-40) (v/v) at 40 °C under exhaustion conditions using static percolators and decanter system. The extract is concentrated under vacuum at a temperature <55 °C. The resulting soft extract is dried <100 °C. The dried extract is finally homogenized. The extract is a native extract without excipients and a drug to extract ratio of 7-12:1.

Provided by Finzelberg GmbH & Co.CKG.

***Rhamnus purshianae* dry extract preparation.**

3400 Kg of cut *Rhamnus purshianae* bark (European Pharmacopoeia quality) are extracted with ethanol-water (75-25) (v/v) at 45 °C under exhaustion conditions using static percolators system. The extract is concentrated under vacuum at a temperature <55 °C and the resulting soft mass is subsequently dried. The extract is standardized by mixing with an amount of lactose-monohydrate Ph. Eur. to get a final content of hydroxyanthracene glycosides 18-22% (w/w), according to the European Pharmacopoeia monograph cascara dry extract. The genuine drug to extract ration is 4-6:1.

Provided by Finzelberg GmbH & Co.CKG.

***Cassia senna leaf* extract preparation**

82 Kg of *Cassia angustifolia Vahl.* leaf (European Pharmacopoeia quality) are extracted with ethanol-water 60-40 (v/v) at 25 °C under exhaustion conditions using mixer extractor system. The extract is concentrated under vacuum at a temperature < 35 °C and the resulting soft mass is subsequently dried. The extract is standardized by mixing with an amount of maltodextrin to get a final content of hydroxyanthracene glycosides, expressed as sennoside B >5.0% (w/w), according to the European Pharmacopoeia monograph. The genuine drug to extract ratio is 7-9:1.

Provided by Martin Bauer SpA.

Extracts characterization

The loss on drying in dry extract was measured for all the matrix using the same method described in the Pharmacopoeia. The determination of the active molecule content was also performed according to Pharmacopoeia. Moreover, for all the matrix, HPLC/UV was used to determine the content of some hydroxyanthracenes, as listed here:

Loss on drying of dry extracts (gravimetry), Ph.Eur. 10.0, 2.8.17.

Hydroxyanthracene glycosides, sennoside B (photometry), Ph.Eur. 10.0 (mod.), (Senna leaf dry extract).

Total hydroxyanthracene glycosides, expressed as 1) sennoside B, referred to the dried drug (HPLC-UV), Ph. Eur. 10.1, monograph Senna leaflet 04/2020:0206 and Senna pods 04/2020:0207.

Hydroxyanthracene glycosides and Cascarosides, expressed as Cascaroside A, with reference to the dried extract, (UV/VIS), according to Ph. Eur. 10.0 (Rhamni purshianae extractum siccum normatum).

Glucofrangulins, expressed as glucofrangulin A, with reference to the dried substances, (UV/VIS) according to Ph.Eur. 10.0 (Frangulae cortices extractum siccum normatum).

Hydroxyanthracene derivatives, expressed as rhein, with reference to the dried substance, (UV/VIS), according to Ph.Eur. 10.0 (Rhubarb).

Hydroxyanthracenes HPLC determination HPLC/UV internal method (Aloin, Aloe emodin, Rhein, Emodin Danthron).

We added this additional info in the supplementary materials