Technical documentation

Product name:	**qRE Aesculus hippocastanum L., seeds**
Substance:	Aesculus hippocastanum L., seeds dry extract
Plant source common names:	en: Horse chestnut; fr: Marron d'inde
Reference:	E0015
Packaging:	100 mg in a 1.5 ml borosilicate amber vial
Storage conditions:	Keep container closed. Protect from light and moisture. Keep inferior to -15 °C.
Retest:	12 months

Botanical identification of plant source

Plants in our botanical garden are identified and a herbal voucher is prepared by an expert botanist.
Each batch collected for extraction is verified and identified.
Reference: Flora Europaea, Cambridge University Press, 1968 ,Vol 2, p 240

Method of production of dry extract

Whole plant or plant parts are collected, freeze-dried and coarsely ground. Extraction is performed by maceration in 50 % (v/v) aqueous ethanol (v/v) for 48 hours at room temperature. Ethanol is then evaporated under reduced pressure at less than 40 °C and the aqueous residue is freeze-dried.
Residual water content measurement is done by Karl Fischer titration.

Organoleptic characteristics of dry extract

Colour: Very light beige
Odour: Non characteristic
Form: Fine powder

Recommended methods for use

Weight a precise weight of qRE and solubilise in the recommended solvent at the concentration indicated in the HPLC or HPTLC method described in this document.
Sonicate for 90 seconds (70 W).
Filter on a 0.45 µm PVDF membrane and put the resulting solution into HPLC dispenser or apply on the HPTLC plate.
Dose and analyse your extract with qRExtract using the HPLC / HPTLC methods described in this document or using your own methods.

HPTLC

Detection of escin Ia

Layer: 10 × 10 cm HPTLC Nano-Sil-20 UV 254 (Carl Roth ref. N084.1)

Thin layer conditionnement: 1 h at room temperature and 33 % relative humidity

Elution solvent:

Elution solvent compound	Volume (ml)
ethyl acetate	40
isopropanol	40
H_2O	30

Initial spot volume and concentration:

escin Ia 1: 1.5 µl of a 0.1 % (w/v) solution in 50 % (v/v) aqueous ethanol
qRE 1: 5 µl of a 1 % (w/v) solution in 50 % (v/v) aqueous ethanol
qRE 2: 5 µl of a 1 % (w/v) solution in 50 % (v/v) aqueous ethanol
escin Ia 2: 1.5 µl of a 0.1 % (w/v) solution in 50 % (v/v) aqueous ethanol

Reagent mixture: Anisaldehyde reagent

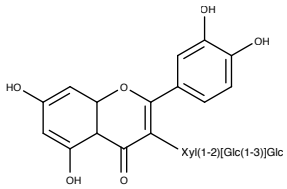
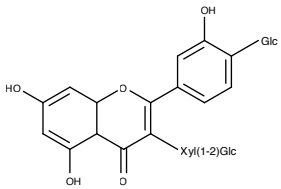
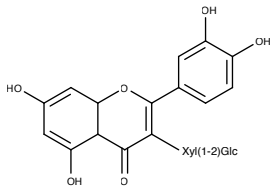
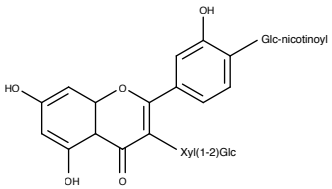
Preparation: Slowly mix 85 mL of ice-cooled methanol with 10 mL of glacial acetic acid and 5 mL of sulfuric acid. Allow the mixture to cool to room temperature, then add 0.5 mL of anisaldehyde (p-methoxy benzaldehyde) Dip the plate in the reagent mixture and dry for 10 minutes at 110 °C. Expose to visible light.

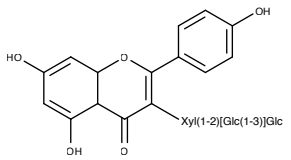
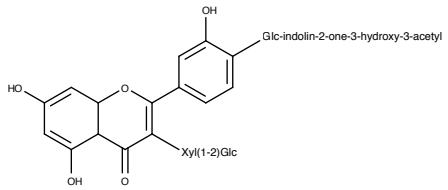
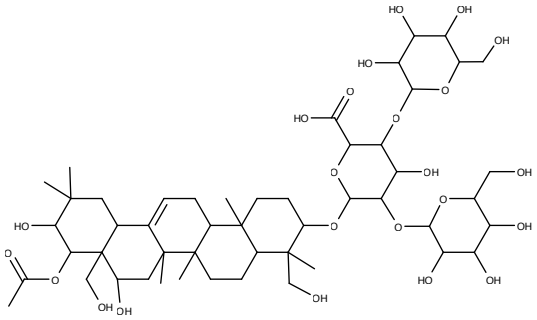
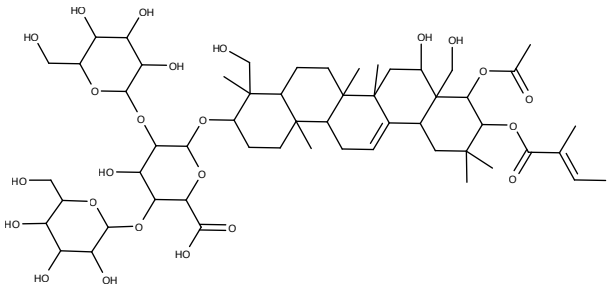
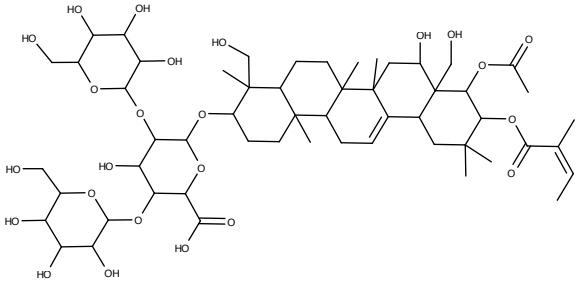
HPLC

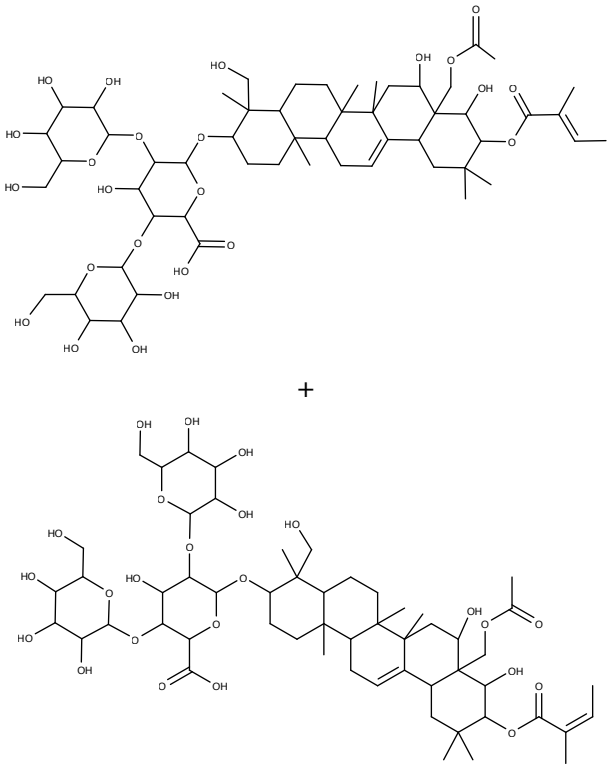
Precolumn: Ascentis® Express C18 0.5 cm × 3.0 mm 2.7 µm
Column: Ascentis® Express C18 15 cm × 3.0 mm 2.7 µm
Sample: 8 µl 1.5 % qRE® (w/v) solution in 35 % (v/v) aqueous ethanol
Flow: 0.45 ml/min
Temperature: 25 °C
Mobile phase: A: 0.1 % formic acid (v/v) in water
B: 0.1 % formic acid (v/v) in acetonitrile
Detection: Diode Array Detector, 210 nm
Gradient:

Time (mn)	A %	B %
0	97	3
8	92	8
60	86	14
95	67	33
125	67	33
142	50	50

Quantified substances

Compound	CAS No	2D Structure	Peak No
Quercetin-3-xylosyl(1-2)[glucosyl(1-3)]glucoside	NA		4
Quercetin-3-xylosyl(1-2)glucosyl-4'-glucoside	NA		5
Quercetin-3-xylosyl(1-2)glucoside	NA		6
Quercetin-3-xylosyl(1-2)glucosyl-4'-nicotinoyl glucoside	NA		7

Compound	CAS No	2D Structure	Peak No
Kaempferol-3-xylosyl(1-2)[glucosyl(1-3)] glucoside	NA		8
Quercetin-3-xylosyl(1-2)glucosyl-4'-indolin-2-one-3-hydroxy-3-acetyl glucoside	NA		9
Desacylescin I	26339-92-4		10
Escin Ia	123748-68-5		13
Escin Ib	26339-90-2		14

Compound	CAS No	2D Structure	Peak No
Isoescin Ia + Isoescin Ib	219944-39-5 + 219944-46-4		15
Unknown	NA	NA	1, 2, 3, 11, 12, 16