Technical documentation

Product name:	**qRE Crataegus monogyna Jacq., flowering aerial parts**
Substance:	Crataegus monogyna Jacq., flowering aerial parts dry extract
Plant source common names:	en: Hawthorn; fr: Aubépine
Reference:	E0085
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 75

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: Orange brown
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 chlorogenic acid, epicatechin, hyperoside and rutin

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	50
butanone	30
water	10
formic acid	10

Developing distance: 70 mm from the lower edge

Initial spot volume and concentration:

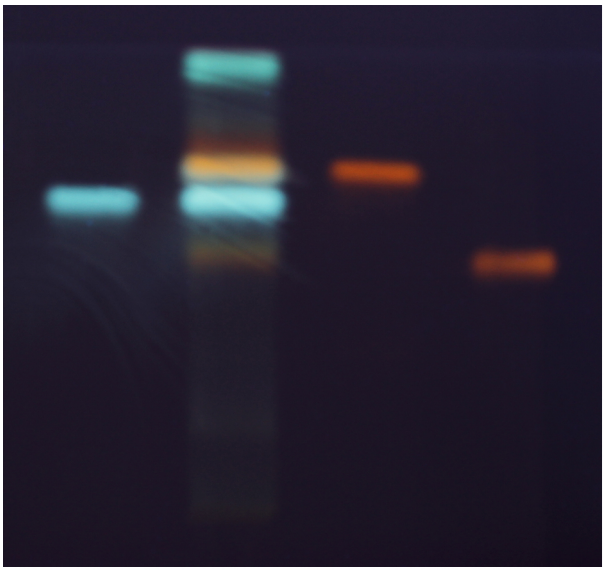
epicatechin:	2 µl of a 0.02 % (w/v) solution in 50 % (v/v) aqueous ethanol
chlorogenic acid:	0.5 µl of a 0.02 % (w/v) solution in 50 % (v/v) aqueous ethanol
qRE:	5 µl of a 2 % (w/v) solution in 50 % (v/v) aqueous ethanol
hyperoside:	1.5 µl of a 0.02 % (w/v) solution in methanol
rutine:	1.5 µl of a 0.02 % (w/v) solution in methanol

Detection of chlorogenic acid, hyperoside and rutin

Reagent mixture: Natural products - polyethylene glycol reagent (NP/PEG)

Preparation: Dissolve 0.25 g of diphenylboric acid 2-aminoethylester and 1.25 g of polyethylene glycol 400 in 25 mL of methanol.

Dip the plate in the reagent mixture and dry for 15 minutes at room temperature. Expose to UV light at 365 nm.



Detection of epicatechin

Reagent mixture: Dimethylaminocinnamaldehyde

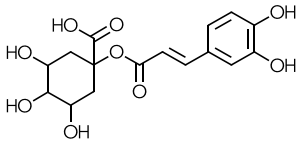
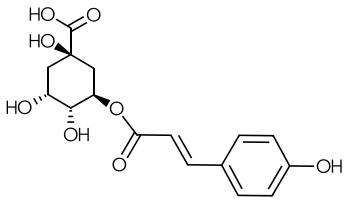
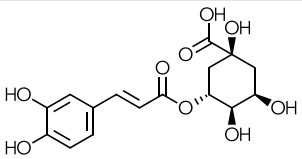
Preparation: Dissolve 0.060 g of 4-dimethylaminocinnamaldehyde in 187 mL of ethanol and add slowly 13 mL of hydrochloric acid.
Dip the plate in the reagent and dry for 15 minutes at room temperature.
Expose to visible light.

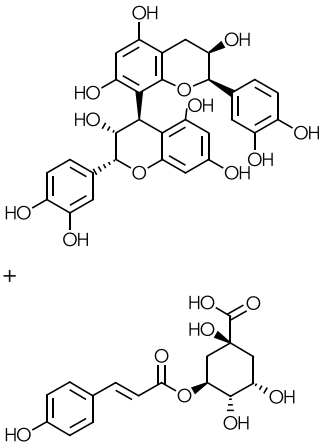
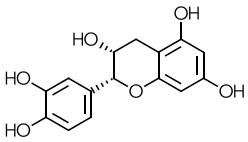
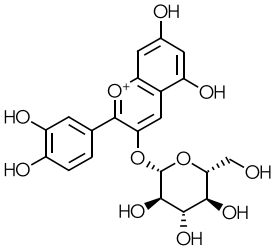
HPLC

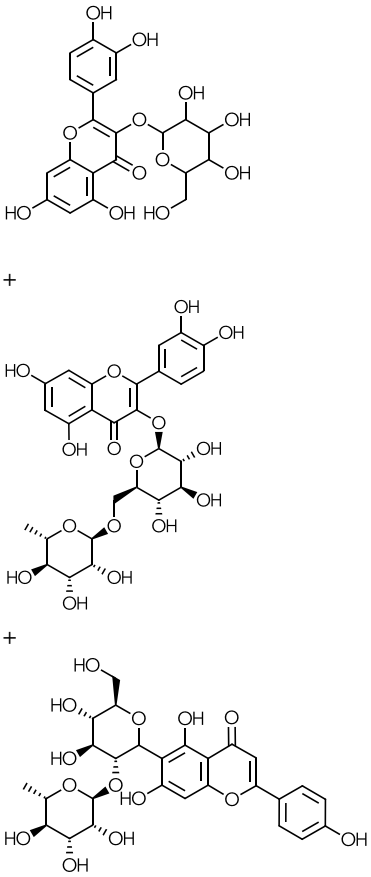
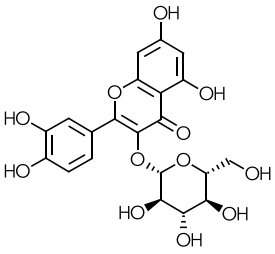
Precolumn: Ascentis® Express C18 0.5 cm × 3.0 mm 2.7 µm
Column: Ascentis® Express C18 15 cm × 3.0 mm 2.7 µm
Sample: 8 µl 1.5 % qRE® (w/v) solution in 25 % (v/v) aqueous ethanol
Flow: 0.45 ml/min
Temperature: 25 °C
Mobile phase: A: 0.1 % formic acid (v/v) in water
B: 0.1 % formic acid (v/v) in acetonitrile
Detection: Diode Array Detector, 280 nm
Gradient:

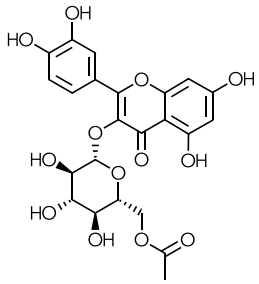
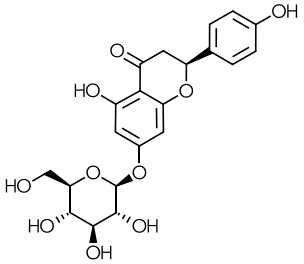
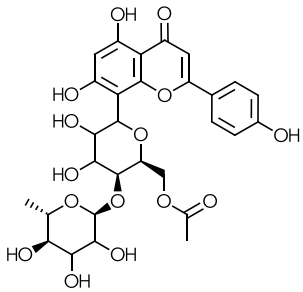
Time (mn)	A %	B %
0	97	3
100	77	23
113	70	30

Quantified substances

Compound	CAS No	2D Structure	Peak No
Caffeoylquinic acid	NA		1
cis-5-O-p-coumaroylquinic acid	NA		2
Chlorogenic acid	327-97-9		3

Compound	CAS No	2D Structure	Peak No
Procyanidin B1 + cis-3-O-p-coumaroylquinic acid	20315-25-7 + 1899-30-5		4
Epicatechin	490-46-0		5
Procyanidin derivate	NA	NA	6, 7
Cyanidin-3-O-glucoside	7084-24-4		8

Compound	CAS No	2D Structure	Peak No
Hyperoside + rutin + isovitexin-2"-O-rhamnoside	482-36-0 + 153-18-4 + NA		9
Isoquercitrin	482-35-9		10

Compound	CAS No	2D Structure	Peak No
Quercetin-O-acetyl hexoside + naringenin-7-O-glucoside	NA + 529-55-5	 + 	11
Cratenacin	28329-82-0		13
Unknown	NA	NA	12