Technical documentation

Product name:	**qRE Hypericum perforatum L., flowering tops**
Substance:	Hypericum perforatum L., flowering tops dry extract
Plant source common names:	en: St John's wort ; fr: Millepertuis
Reference:	E0066
Packaging:	100 mg in a 1.5 ml borosilicate amber vial
Storage conditions:	Keep container closed. Protect from light and moisture. Keep at -18 °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 261

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 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.

Organoleptic characteristics of dry extract

Colour: Burgundy
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 rutin, hyperoside, quercetin, chlorogenic acid and quercitrin

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:

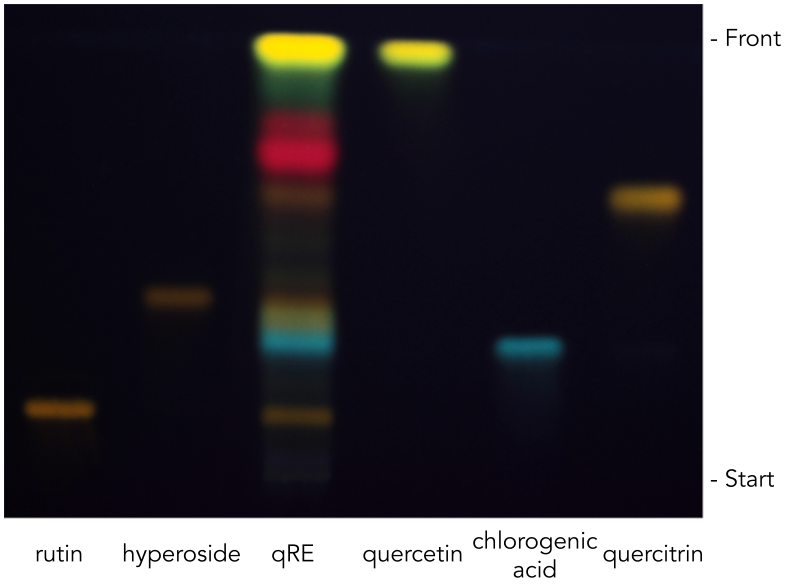
Elution solvent compound	Volume (ml)
ethyl acetate	90
H_2O	9
formic acid	6

Developing distance: 70 mm from the lower edge

Initial spot volume and concentration:

rutin:	1 µl of a 0.1 % (w/v) solution in methanol
hyperoside:	2 µl of a 0.02 % (w/v) solution in methanol
qRE:	4 µl of a 2 % solution in 50 % (v/v) aqueous ethanol
quercetin:	2 µl of a 0.02 % (w/v) solution in methanol
chlorogenic acid:	0.5 µl of a 0.2 % solution in 50 % (v/v) aqueous ethanol
quercitrin:	1.5 µl of a 0.02 % (w/v) solution in ethanol 96 %

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.

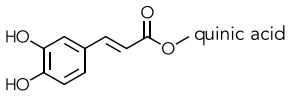
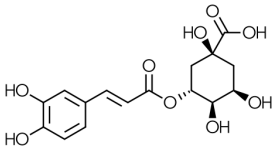
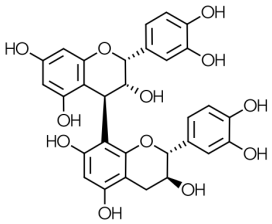
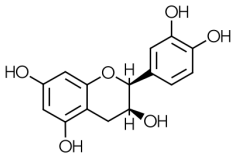


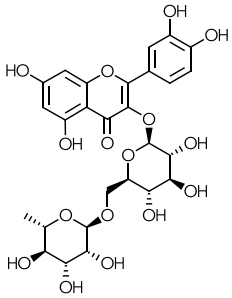
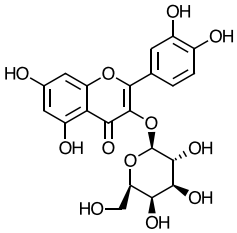
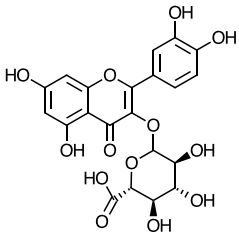
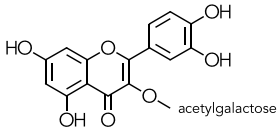
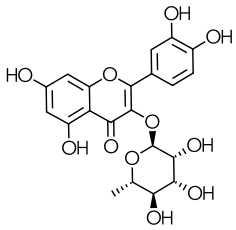
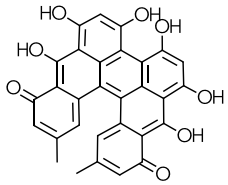
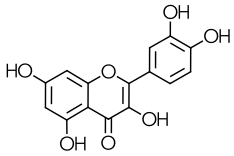
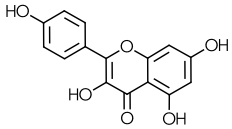
HPLC

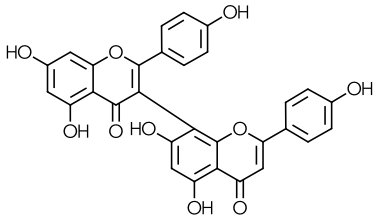
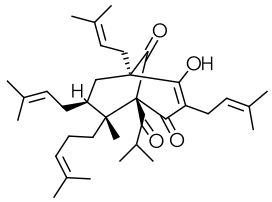
Precolumn: Ascentis® Express C18 0.5 cm × 3.0 mm 2.7 µm
Column: Ascentis® Express C18 15 cm × 3.0 mm 2.7 µm
Sample: 10 µl 1.09 % qRE (w/v) solution in 25 % (v/v) aqueous ethanol
Flow: 0.45 ml/min
Temperature: 25 °C
Mobile phase: A: 0.1 % formic acid (v/v) in water
B: 0.1 % formic acid (v/v) in acetonitrile
Detection: Diode Array Detector, 255 nm
Gradient:

Time (mn)	A %	B %
0	97	3
5	86	14
25	77	23
45	0	100
50	0	100

Quantified substances

Compound	CAS No	2D Structure	Peak No
Chlorogenic acid isomer	NA		1
Chlorogenic acid	327-97-9		3
Catechin or epicatechin dimer	NA		5
Epicatechin	490-46-0		6

Compound	CAS No	2D Structure	Peak No
Rutin	153-18-4		11
Hyperoside	482-36-0		12
Miquelianin	22688-79-5		13
Acetylated hyperoside derivative	NA		15
Quercitrin	522-12-3		16
Protohypericin	548-03-8		18
Quercetin	117-39-5		19
Kaempferol	520-18-3		20

Compound	CAS No	2D Structure	Peak No
3,8'-biapigenin	101140-06-1		21
Unknown	NA	NA	2, 4, 7, 8, 9, 10, 14, 17
Hyperforin	11079-53-1		22