Technical documentation

Product name:	**qRE Trigonella officinalis (L.) Coulot & Rabaute, flowering aerial parts**
Substance:	Trigonella officinalis (L.) Coulot & Rabaute, flowering aerial parts dry extract
Plant source common names:	en: Yellow sweet clover; fr: Mélilot
Reference:	E0094
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 Gallica, Flore de France, Biotope Ed. 2014, p 769

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: Dark green
Odour: 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 robinin

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	100
H_2O	27
formic acid	11
acetic acid	11

Initial spot volume and concentration:

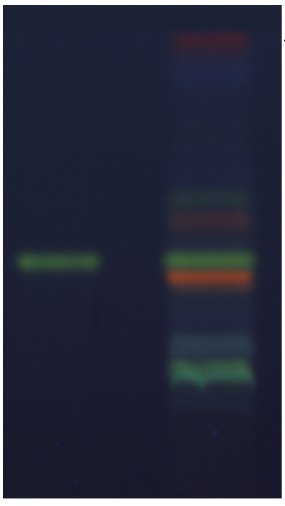
robinin: 2 µl of a 0.02 % (w/v) solution in methanol

qRE: 5 µl of a 1.5 % (w/v) solution in 50 % (v/v) aqueous ethanol

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.



HPTLC

Detection of coumarin

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)
toluene	7
ethyl acetate	3

Initial spot volume and concentration:

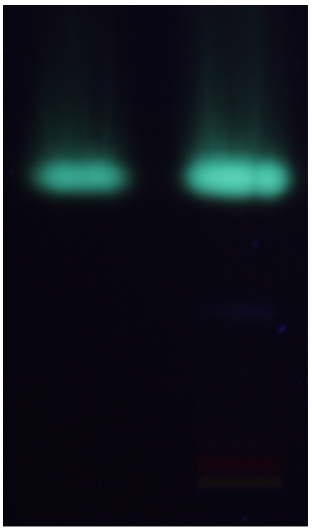
coumarin: 1 µl of a 0.05 % (w/v) solution in 50 % (v/v) aqueous ethanol

qRE: 5 µl of a 1.5 % (w/v) solution in 50 % (v/v) aqueous ethanol

Reagent mixture: Potassium hydroxyde reagent :

Preparation: Dissolve 2 g of potassium hydroxide (KOH) in 25 mL of ethanol.

Dip the plate in the reagent mixture and dry for 5 minutes at room temperature. Expose to UV light at 365 nm.

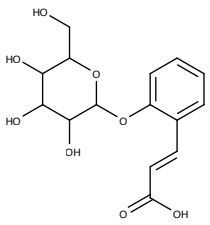
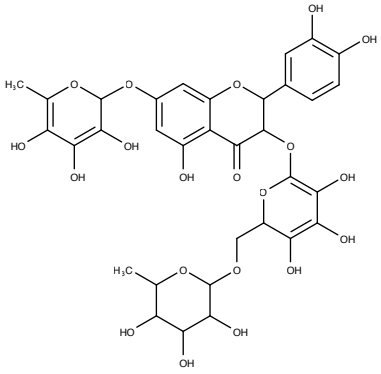


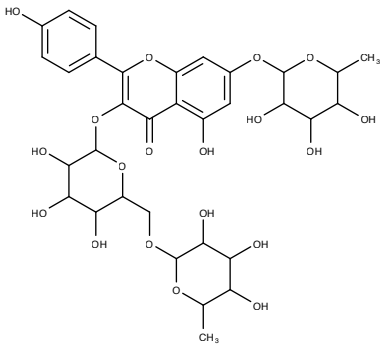
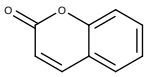
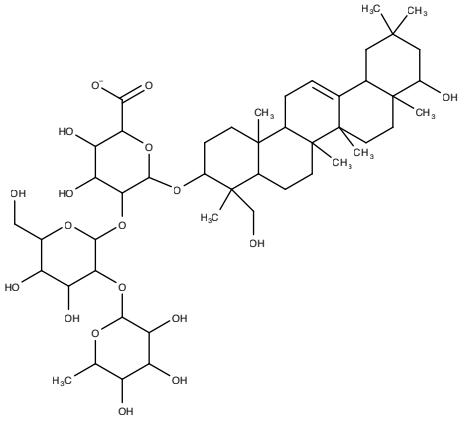
HPLC

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: 8 µl 0.5 % 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
10	88	12
27	80	20
47	50	50
50	40	60

Quantified substances

Compound	CAS No	2D Structure	Peak No
Melilotoside	618-67-7		3
Clovin	81970-00-5		5

Compound	CAS No	2D Structure	Peak No
Robinin	301-19-9		7
Coumarin	91-64-5		8
Azukisaponin V carboxylate	119556-1-3		9
Unknown	NA	NA	1, 2, 4, 6, 10