Technical documentation

Product name:	**qRE Olea europaea L., leaves**
Substance:	Olea europaea L., leaves dry extract
Plant source common names:	en: Olive tree; fr: Olivier
Reference:	E0020
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, 1972, Vol 3, p 55

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: Yellow-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 luteolin-7-O-glucoside and oleuropein

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	80
formic acid	13
water	7

Developing distance: 70 mm from the lower edge

Initial spot volume and concentration:

luteolin-7-glucoside:	1.5 µl of 0.04 % (w/v) solution in 96 % ethanol
qRE:	10 µl of 1.5 % (w/v) solution in 50 % (v/v) aqueous ethanol
oleuropein:	3.5 µl of 0.2 % (w/v) solution in methanol

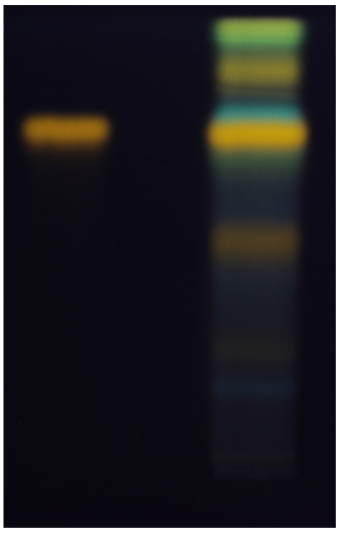
Detection of luteolin-7-O-glucoside:

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.



luteolin-7-O-glucoside qRE

Detection of oleuropein:

To do in less than 20 minutes after the detection of luteolin-7-O-glucoside.

Reagent mixture:

Anisaldehyde reagent

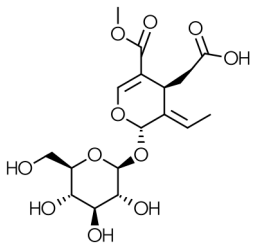
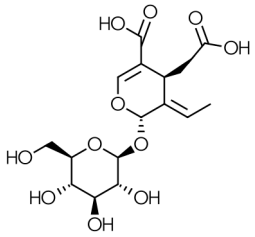
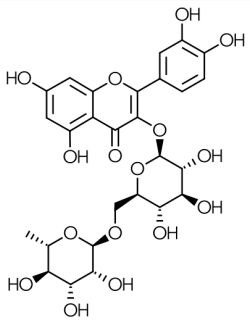
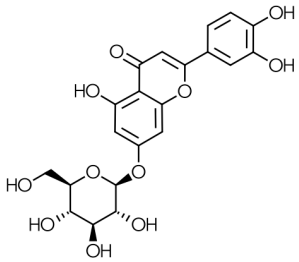
Preparation: Slowly mix 85 mL of ice-cooled methanol with 10 mL of glacial acetic acid and 5 mL of sulfuric acid. Allow the mixture to cool to room temperature, then add 0.5 mL of anisaldehyde (p-methoxy benzaldehyde)
Dip the plate in the reagent mixture and dry for 10 minutes at 110 °C.
Expose to visible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

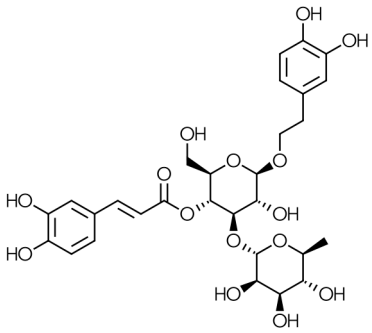
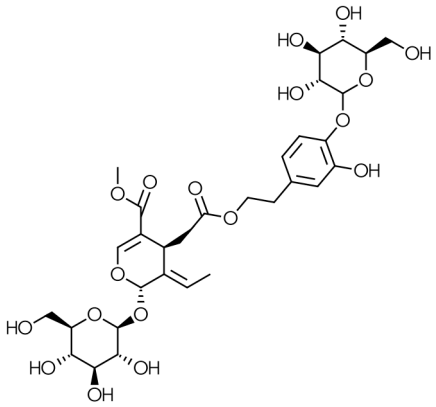
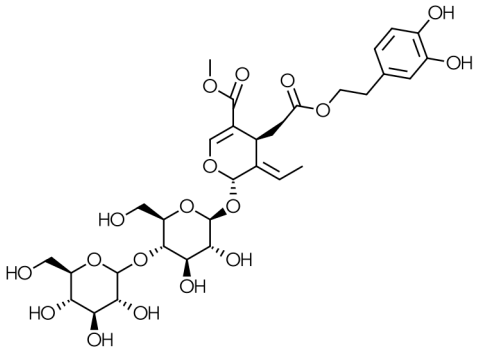
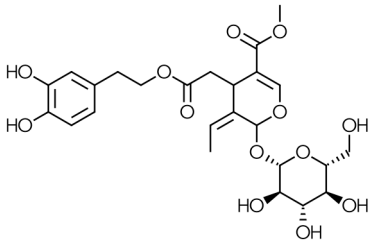
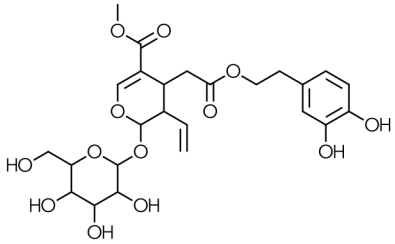
HPLC

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: 10 µl 0.33 % 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, 210 nm
Gradient:

Time (mn)	A %	B %
0	97	3
60	70	30

Quantified substances

Compound	CAS No	2D Structure	Peak No
Elenolic acid glucoside	60539-23-3		1
Oleoside	178600-68-5		2
Rutin	153-18-4		3
Luteolin-7-glucoside	5373-11-5		4

Compound	CAS No	2D Structure	Peak No
Verbascoside	61276-17-3		5
Oleuropein diglucoside isomer 1	NA		6
Oleuropein diglucoside isomer 2	NA		7
Oleuropein	32619-42-4		8
Oleuroside	116383-31-4		9