

# Technical documentation

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Product name:	<b>qRE Vitis vinifera L., leaves</b>
Substance:	Vitis vinifera L., leaves dry extract
Plant source common names:	en: Wine grape ; fr: Vigne rouge
Reference:	E0082
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

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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 246

## Method of production of dry extract

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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

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Colour: Raspberry red

Odour: Non characteristic

Form: Fine powder

## Recommended methods for use

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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, quercetin, kaempferol and isoquercitrin

**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

<b>Elution solvent:</b>	<u>Elution solvent compound</u>	<u>Volume (ml)</u>
	ethyl acetate	15
	formic acid	1
	H <sub>2</sub> O	1

**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
quercetin:	3 µl of a 0.02 % (w/v) solution in methanol
qRE:	10 µl of a 2 % (w/v) solution in 50 % (v/v) aqueous ethanol
kaempferol:	2 µl of a 0.02 % (w/v) solution in 50 % (v/v) aqueous ethanol
isoquercitrin:	2 µl of a 0.08 % (w/v) solution in 50 % (v/v) aqueous ethanol

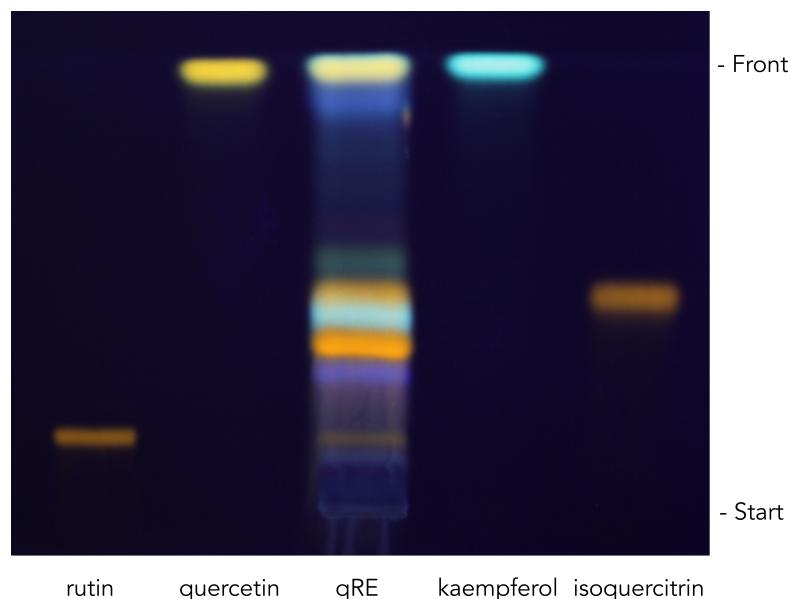
**Reagent mixture:**

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

## 2nd detection of quercetin and kaempferol

**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

<b>Elution solvent:</b>	<u>Elution solvent compound</u>	<u>Volume (ml)</u>
	toluene	10
	ethyl acetate	8
	formic acid	2

**Developing distance:** 70 mm from the lower edge

**Initial spot volume and concentration:**

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

qRE: 10 µl of a 2 % (w/v) solution in 50 % (v/v) aqueous ethanol

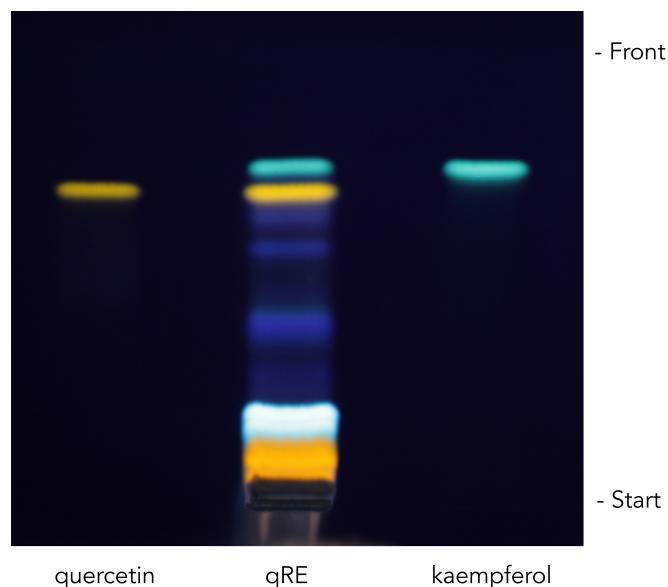
kaempferol: 2 µl of a 0.02 % (w/v) solution in 50 % (v/v) aqueous ethanol

**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.



## 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 0.25 % qRE (w/v) solution in 50 % (v/v) aqueous ethanol

**Flow:** 0.45 ml/min

**Temperature:** 25 °C

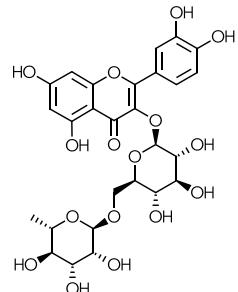
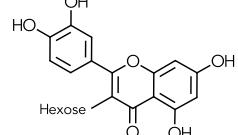
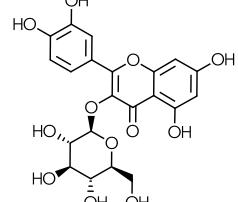
**Mobile phase:** A: 0.1 % formic acid (v/v) in water  
B: 0.1 % formic acid (v/v) in acetonitrile

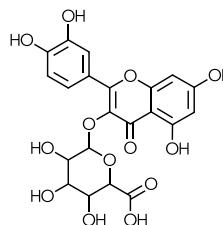
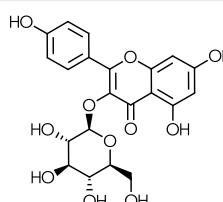
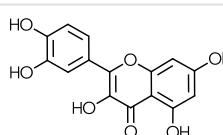
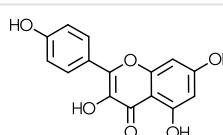
**Detection:** Diode Array Detector, 330 nm

**Gradient:**

Time (mn)	A %	B %
0	97	3
23	81	19
25	81	19
33	75	25
43	63	37
53	0	100
61	0	100

## Quantified substances

Compound	CAS No	2D Structure	Peak No
Rutin	153-18-4		1
Quercetin hexoside	NA		2
Quercetin-3-O-glucoside	21637-25-2		3

Compound	CAS No	2D Structure	Peak No
Quercetin-3-O-glucuronide	22688-79-5		4
Kaempferol-3-O-glucoside isomer	NA		5, 6
Quercetin	117-39-5		7
Kaempferol	520-18-3		8