Technical documentation

Product name:	**qRE Cynara cardunculus L., leaves**
Substance:	Cynara cardunculus L., leaves dry extract
Plant source common names:	en: Cardoon; fr: Cardon
Reference:	E0030
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, 1976, Vol 4, p 248

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: 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, luteolin-7-glucoside and luteolin

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
acetic acid	11
formic acid	11

Developing distance: 70 mm from the lower edge

Initial spot volume and concentration:

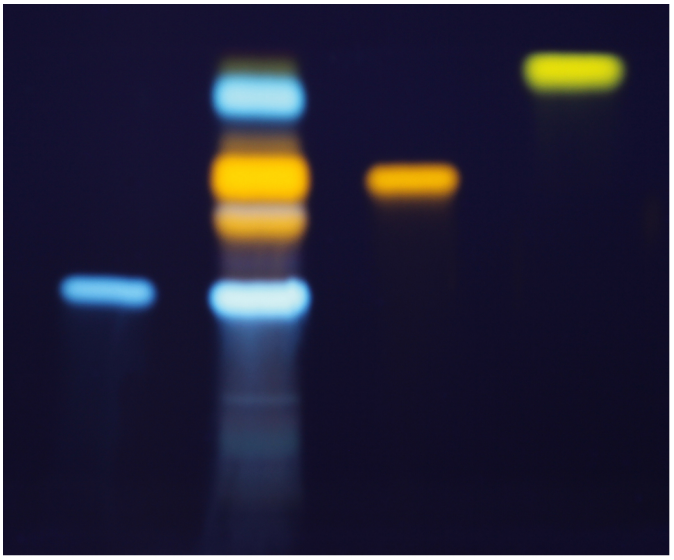
chlorogenic acid:	1.5 µl of a 0.02 % (w/v) solution in ethanol 96 %
qRE:	5 µl of a 1.55 % (w/v) solution in 50 % (v/v) aqueous ethanol
luteolin-7-glucoside:	1.5 µl of a 0.04 % (w/v) solution in ethanol 96 %
luteolin:	2 µl of a 0.02 % (w/v) solution in methanol

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 cynaropicrin

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	20
acetone	10
ethyl acetate	10
methanol	1.2

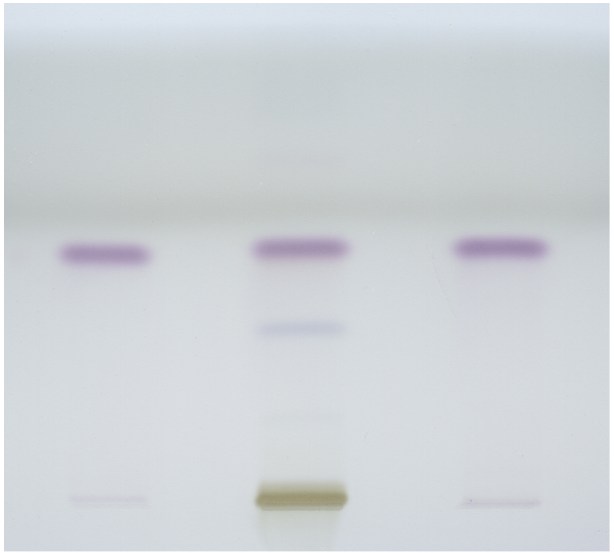
Developing distance: 70 mm from the lower edge

Initial spot volume and concentration:

cynaropicrin 1: 2 µl of a 0.15 % (w/v) solution in 60 % (v/v) aqueous ethanol
qRE: 5 µl of a 1.55 % (w/v) solution in 50 % (v/v) aqueous ethanol
cynaropicrin 2: 3 µl of a 0.15 % (w/v) solution in 60 % (v/v) aqueous ethanol

Reagent mixture: Vanillin - sulphuric acid reagent

Preparation: Dissolve 1 g of vanillin in 98 mL of methanol then add with caution 2 mL of sulphuric acid.
Dip the plate in the reagent mixture and dry for 10 minutes at 105 °C.
Expose to visible light.

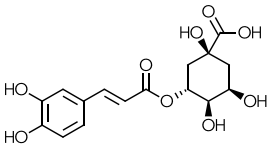
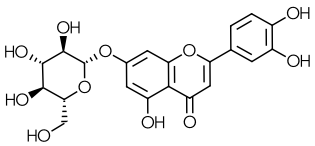
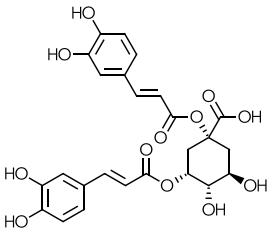
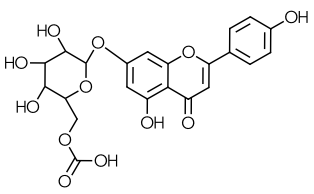


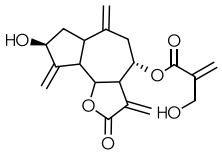
HPLC

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: 8 µl 0.40 % 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, 210 nm
Gradient:

Time (mn)	A %	B %
0	97	3
10	85	15
40	85	15
65	36	64

Quantified substances

Compound	CAS No	2D Structure	Peak No
Chlorogenic acid	206-325-6		1
Luteolin-7-O-glucoside	68321-11-9		3
Unknown	NA	NA	2, 4
Flavonoid hexoside	NA	NA	5
1,5-dicaffeoylquinic acid	30964-13-7		6
Apigenin-7-O-(6"-acetylglucoside)	520-36-5		7

Compound	CAS No	2D Structure	Peak No
Cynaropicrin	35730-78-0		8