Optimization and validation of analytical RP-HPLC methods for the analysis of glucosinolates and isothiocyanates in *Nasturtium officinale* R. Br.

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INTRODUCTION

Nasturtium officinale R. Br. (watercress) is a plant that belongs to the *Brassicaceae* and is growing mainly in Europe and Asia. The plant contains a considerable amount of vitamins, minerals and secondary metabolites and is used in food and for its medicinal properties. These properties are mainly attributed to the glucosinolates which are precursors of bioactive compounds such as the isothiocyanates. Glucosinolates are sulphur containing secondary metabolites, containing a β-D-thioglucose and an aglucone. The main glucosinolate in *Nasturtium officinale* R. Br. is gluconasturtiin. The main isothiocyanate is phenylethylisothiocyanate (PEITC). Since the quality of a food supplement of watercress depends on the content of its glucosinolates and isothiocyanates, two quantitative methods were developed: one to analyse the glucosinolates and one to analyse PEITC.

Gluconasturtiin

METHOD OPTIMIZATION

The method was optimized, starting from the ISO method "Rapeseed – determination of glucosinolates content"[1] and the method described by Heyerick A.[2]

Volume extraction solvent (mL)	Gluconasturtiin (µmol/g)
2	15,52
3	19,00
5	18,68

Table 1: Extraction volume.

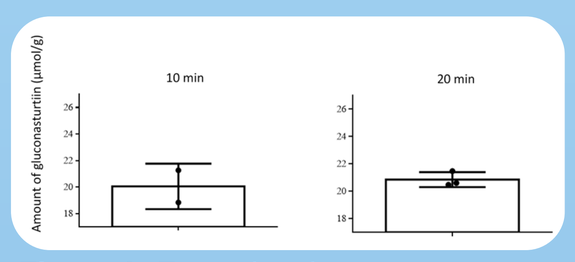


Figure 1: Extraction time.

HPLC	Agilent 1200 series, DAD	
	Column:	Lichrosphere RP C18
	Solvents:	(A) 0.025% FA / (B) ACN + 0.025% FA
	Gradient:	3 min 2%B, 3-38 min 3 → 25% B, 38-40 min 25% B, 40-43 min 2% B
	flow 1 mL/min	
UV	242 nm	
Standard	Sinigrin monohydrate	

Table 2: HPLC conditions .

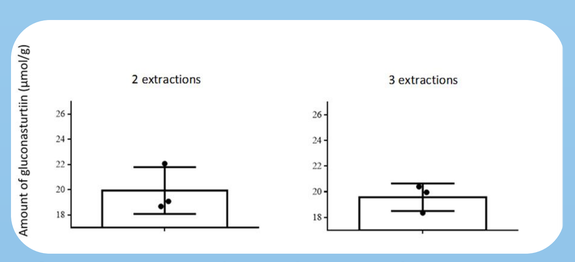


Figure 2: Number of extractions.

PEITC

METHOD OPTIMIZATION

The method was optimized, starting from the method described by Heyerick A.[2]

Volume extraction solvent (mL)	Gluconasturtiin (µmol/g)
2	15,52
3	19,00
5	18,68

Table 3: Extraction volume.

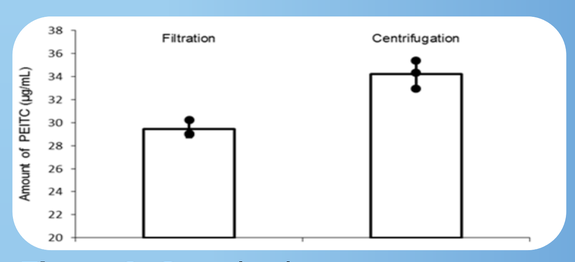


Figure 3: Sample cleanup.

HPLC	Agilent 1200 series, DAD	
	Column:	Lichrosphere RP C18
	Solvents:	(A) 0.025% FA / (B) ACN + 0.025% FA
	Gradient:	Isocratic
	flow 1 mL/min	
UV	242 nm	

Table 4: HPLC conditions .

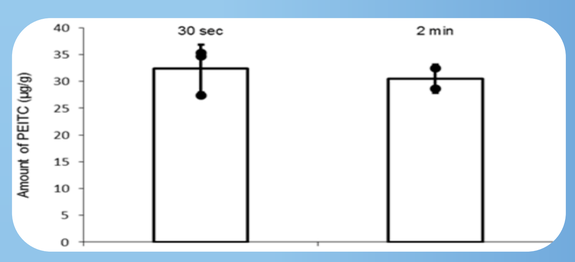


Figure 4: Time of extraction.

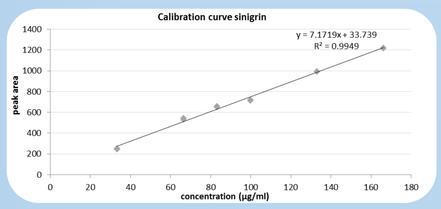
RESULTS

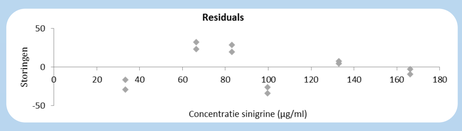
Final Method

150 mg watercress powder is heated at 75°C in a waterbath. 5 mL boiling MeOH (70% v/v) and 200 µL internal standard (sinigrin monohydrate, 20 mmol/L) are added. The mixture is stirred for 10 min and then sonicated for 10 min at 75°C. The mixture is centrifuged and the supernatant transferred. The residu is extracted a 2nd and a 3rd time with 5 mL boiling MeOH 70%. Purification and desulfatation of the glucosinolates is done on a DEAE Sephadex A-25 ion exchange column, on which the extract and sulfatase are brought and incubated overnight at 37°C. The analysis is performed with HPLC-DAD.

VALIDATION

Calibration model



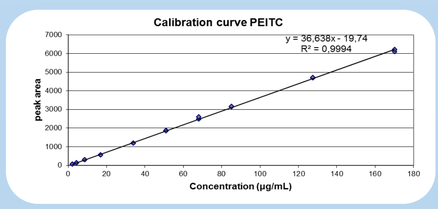


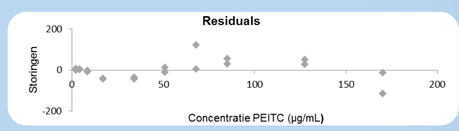
Precision

	Between days	Between levels
Mean (µmol/g)	20.56	20.13
$RSD_{between}$	9.74%	8.96%
Cochran	0.60 (C_{crit} 0.71)	0.45 (C_{crit} 0.51)
F-test	1.73 (F_{crit} 3.68)	1.81 (F_{crit} 2.76)

VALIDATION

Calibration model





Precision

	Between days	Between levels
Mean (µg/g)	144.74	147.55
$RSD_{between}$	12.67%	13.10%
Cochran	0.39 (C_{crit} 0.71)	0.31 (C_{crit} 0.51)
F-test	1.81 (F_{crit} 3.68)	1.12 (F_{crit} 2.76)

DISCUSSION

Two quantitative HPLC-DAD methods to analyse glucosinolates and isothiocyanates in watercress were optimized and validated. The extraction of glucosinolates is more complete if (1) the extraction volume is increased to 5 mL, (2) the sample is homogenized by stirring and sonication, (3) the extraction procedure is repeated 3 times and (4) the extraction time is prolonged to 20 min. The final method was validated whereas the concentration of gluconasturtiin was 20,13 ± 1,80 µmol/g which is higher than the amount analysed with the original method (17,70 µmol/g). The linearity of the method was proven from 75 to 225 mg of lyophilized watercress. The variation remained relatively high, but is acceptable because of the complexity of the method. Probably an internal standard more related to gluconasturtiin could reduce the variation, but since the high costs of more related glucosinolates it would not be adequate for method development and routine analysis.

For PEITC, the extraction is more complete if (1) an extraction volume of 5 mL is used, (2) the sample is vortexed for 30 seconds and (3) centrifugation is used instead of filtration. A linear range was proven from 2,2 to 170,2 µg/mL. The precision of the method (3 days) was 12,67%. The precision on 3 different concentration levels was 13.10%. These values are high but acceptable due to the volatility of PEITC.

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References

[1] International standardization organization. Rapeseed – determination of glucosinolates content – part 1: Method using high-performance liquid chromatography 9167-1. Genève; 1992

[2] Heyerick A. Waterkers (Nasturtium officinale): onderzoek naar de invloed van procesparameters op de inhoud aan bioactieve bestanddelen. University of Ghent, report IWT-KMO portefeuille; 2010:1-27.