

OPTIMIZATION AND VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF ISOTHIOCYANATES AND GLUCOSINOLATES IN BROCCOLI USING HPLC ANALYSIS

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INTRODUCTION

Broccoli is a vegetable of the family *Brassicaceae*. It is an edible vegetable that is rich in vitamins and secondary metabolites. Because of the biological activity of its secondary metabolite sulforaphane, an isothiocyanate with antioxidative effects, broccoli became a popular food supplement. However, broccoli also contains the compounds progoitrin and goitrin, which can enlarge the thyroid gland. To avoid these effects, the Royal Decree of August 29, 1997 limits the daily intake of progoitrin and goitrin with 20 and 5 mg respectively¹. Here, we describe two optimized and validated analytical methods for the quantification of progoitrin and goitrin in broccoli powder. The ICH guidelines on the validation of analytical methods were used for the validation of both methods².

PROGOITRIN

Method optimization

The method for the analysis of progoitrin was based on the validated method for the analysis of glucosinolates in watercress, which was previously developed in the research group NatuRA (UAntwerp). The HPLC conditions are written in table 1.

Agilent 1200 series, DAD
 Column: Licrospher® RP C18
 Solvents: (A) 0.025% FA / (B) ACN + 0,025% FA
 Gradient: 3 min 2% B, 3 - 38 min 25% B, 38 - 40 min 25% B, 40 - 43 min 2% B
 Flow: 1 mL/min
 UV: 242 nm
 Standard: Glucotropaeolin

Table 1: HPLC conditions progoitrin-analysis

Extraction on 0.5 g of the sample was compared with 1 g and 0.25 g. The results are shown in figure 1.

The robustness of the method was tested by carrying out the extraction on different temperatures. The prescribed temperature is 75°C. Figure 2 shows the results.

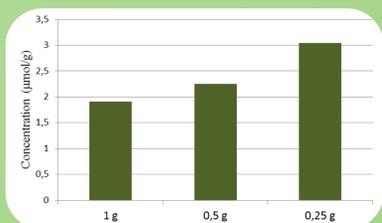


Figure 1: Weighed mass of sample.

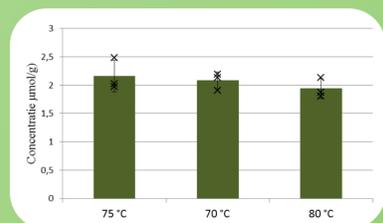


Figure 2: Extraction temperature.

Results

Final Method

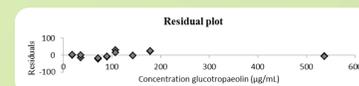
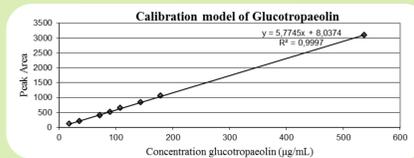
250 mg broccoli seed powder is heated at 75°C in a waterbath. 5 mL boiling MeOH (70% v/v) and 200 µL internal standard (glucotropaeolin, 20 mmol/L) are added. This mixture is stirred for 10 min and then sonicated for 10 min at 75°C. This mixture is centrifuged and the supernatant transferred. The residu 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. 2 mL of the extract and 100 µL sulfatase are brought on the ion exchange column and incubated overnight at 37°C. The analysis is performed with HPLC-DAD.

DISCUSSION

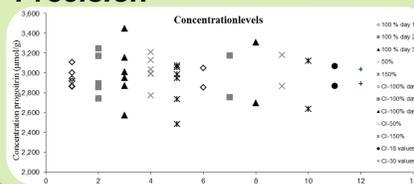
An analytical method for the quantification of progoitrin in broccoli seed powder was optimized and validated. Glucotropaeolin was used as internal standard. The standard curve of glucotropaeolin was linear in the range of 17.9 – 537 µg/mL. The precision of the method for time and concentration gave relative standard deviation (RSD) values higher than 5% (6.55% and 6.56% respectively) but is still accepted because of the complexity of sample preparation. The broccoli powder that was tested contained an average of 1.27 mg/g progoitrin. The method of Wang *et al.* (2013) was used for the determination of goitrin. The standard curve of goitrin was linear in the range 1 µg/mL – 400 µg/mL. The precision of the method for time (3 days) and concentration (3 levels: 10%, 100%, 200%) was tested by spiking broccoli powder with goitrin, where the 100% level was 5.0 mg/g broccoli powder. The precision of the method with respect to time and concentration is accepted with RSD values of 4.3% and 3.5% respectively. The recovery of the method was determined to be 99.1%.

Validation

Calibration model



Precision



Progoitrin	Between days	Between levels
Mean (µmol/g)	2.97	2.96
RSD _{between}	6.55%	6.56%
Cochran	0.64 (C _{crit} 0.71)	0.41 (C _{crit} 0.51)
F-test	0.095 (F _{crit} 3.68)	0.43 (F _{crit} 2.76)

GOITRIN

For the analysis of goitrin, the sample preparation described by Wang *et al.* (2013) was used³. The HPLC conditions are shown in table 2.

Agilent 1200 series, DAD
 Column: Licrospher® RP C18
 Solvents: (A) Water / (B) MeOH
 Gradient: 10 min 5% B, 10 - 25 min 15% B, 25 - 34 min 35% B, 34 - 40 min 95% B, 40 - 45 min 5% B
 Flow: 0,8 mL/min
 UV: 254 nm

Table 2: HPLC conditions goitrin-analysis

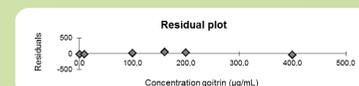
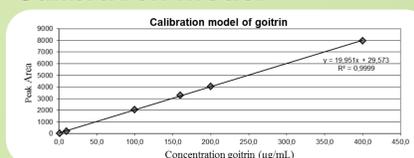
Results

Final Method

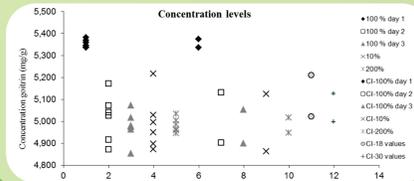
The broccoli seed powder is spiked with goitrin standard to validate the method. To 100 mg of broccoli seed powder, 5mL water is added, the sample is sonicated for 20 minutes and centrifuged for 10 minutes. The analysis on the supernatant is performed with HPLC-DAD.

Validation

Calibration model



Precision



Goitrin	Between days	Between levels
Mean (mg/g)	5.12	5.06
RSD _{between}	4.3%	3.5%
Cochran	0.68 (C _{crit} 0.71)	0.46 (C _{crit} 0.51)
F-test	44,7 (F _{crit} 3.68)	23.5 (F _{crit} 2.76)
RSD _{max} (Fixed)	5%	5%
Recovery	99.0%	99.1%

References

- Koninklijk besluit van 29 augustus 1997 betreffende de fabricage van en de handel in voedingsmiddelen die uit planten of uit plantenbereidingen samengesteld zijn of deze bevatten. (1997, 21 november). Belgisch staatsblad: 36.
- ICH, Text on validation of analytical procedures – ICH Harmonised Tripartite Guideline, 1994.
- Wang X, Xie Y, Hu X, Li Y, Hu P, Wang Y, et al. Qualitative and quantitative analysis of glucosinolates and nucleosides in Radix Isatis by HPLC and liquid chromatography tandem mass spectrometry. *Acta Pharmaceutica Sinica B* 2013; 3 (5): 337-344.