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

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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 respectively [1]. 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 methods [2].

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). 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.

For the analysis of goitrin, the sample preparation described by Wang et al. (2013) was used [3]. 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%.