

## OPTIMIZATION AND VALIDATION OF ANALYTICAL RP-HPLC METHODS FOR THE ANALYSIS OF GLUCOSINOLATES AND ISOTHIOCYANATES IN *NASTURTIUM OFFICINALE* R. BR.

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*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 are mainly attributed to the glucosinolates, which are precursors of bioactive compounds such as the isothiocyanates. Glucosinolates are sulphur containing secondary metabolites, containing a  $\beta$ -D-thioglucose and an aglycon. The main glucosinolate in *Nasturtium officinale* R. Br. is gluconasturtiin. The main isothiocyanate in *Nasturtium officinale* R. Br. 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 another one to analyse PEITC.

An existing method for the determination of gluconasturtiin [1,2] was optimized by changing the volume of the extraction solvent, extraction time and number of extraction steps.

Sinigrinmonohydrate was used as internal standard. For the analytical method of PEITC we adapted a method described by Heyerick [2]. Both methods were validated conform the ICH guidelines on the validation of analytical methods [3]. The standard curve of sinigrinmonohydrate was linear in the concentration range of 33.2 – 166.2  $\mu\text{g/mL}$ . The precision of the method with respect to time (3 days) and concentration (3 concentration levels) was respectively 9.74% and 8.96%, although relatively high, it was still accepted because of the complexity of the method. The mean concentration of gluconasturtiin was 8.5 mg/g lyophilized watercress. For PEITC a linear range was proven from 2.2 to 170.2  $\mu\text{g/mL}$ . The precision of the method (3 days) was 12.67%. The precision on 3 different concentration levels was 7.77%. These values are high but acceptable due to the volatility of PEITC.

[1] ISO 9167-1:1992. Method using high-performance liquid chromatography 9167-1, Genève.

[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.

[3] ICH, Text on validation of analytical procedures – ICH Harmonised Tripartite Guideline, 1994.