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DETERMINATION OF THE PHENOLIC PROFILE IN BRASSICA JUNCEA OF DIFFERENT SPECIES BY COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY

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Brassica vegetables are known to contain a high concentration of bioactive compounds such as hydroxycinnamic acids and flavonoid derivatives, which play an important role in the prevention of several pathologies e.g. coronary heart diseases and cancer, especially of the gastrointestinal tract. In particular, the molecules responsible for the beneficial effects are polyphenols and glucosinolates.
For their characterization, different studies have been carried out by conventional one-dimensional liquid chromatography even though it can present some limits especially in terms of resolving power.
A powerful alternative is represented by comprehensive two-dimensional liquid chromatography (LC×LC), where two columns of different selectivity are separated by means of two switching valves. The aim of this study was to investigate three different cultivars by using reversed phase conditions in both dimensions and specifically cyano column (250 × 1 mm I.D, 5 μm dp) and a RP-Amide column (50 x 4.6 mm, 2.7 μm dp) in the first (^{1}D) and second (^{2}D) dimensions, respectively. Moreover, to improve the separation efficiency in the ^{2}D a segmented in-fraction gradient was employed, this allowing a further increase of the overall theoretical, effective and corrected peak capacity of the LC×LC system.
Interestingly, one of the most recent achievements is the possibility to employ a micro LC pump in the first dimension of the LC×LC, allowing high reproducibility and stable retention times was also evaluated.
The samples, represented by dried and powdered leaves, chosen for their complexity, were extracted by liquid-liquid extraction procedure using MeOH/Water (60:40 v/v). The recovery yield was calculated by adding apigenin as internal standard at the beginning of the extraction procedure.

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Forty-five compounds were separated and identified through PDA and MS (ESI^-) detection, in three different cultivar of Brassica juncea.
Quantification was carried out through three different standards (quercetin-3-O-glucopyranoside, isorhamnetin-3-O-glucoside and kaempferol-3-O-glucoside), the calibration curves were created automatically by the use of Cromsquare software and the method was validated yielding satisfactory LODs and LOQs values.
The comprehensive approach demonstrated its validity in the analysis of complex matrices, such as Brassica extracts. Furthermore, the characterization of these samples will aid to confirm their potential use for the human health.

The authors thank Shimadzu and Merck KGaA for the continuous support.
This work has been carried out within the framework of the Research Project PRIN 2015: Securing and ensuring sustainable use of agriculture waste, co- and by-products: an integrated analytical approach combining mass spectrometry with health effect-based biosensing, supported by the Italian Ministry of University and Scientific Research, no. 2015FFY97L.