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Identification and quantification of glucosinolates in different tissues of *Raphanus raphanistrum* by liquid chromatography tandem-mass spectrometry

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ABSTRACT

This study aims to identify and quantify the glucosinolates from different parts of wild radish *R. raphanistrum* (leaves, flowers, fruits, roots) using liquid chromatography-tandem mass spectrometry. Glucoraphenin is the predominant compound, accounting for about 87% (w/w) of total glucosinolate content, followed by glucobrassicin, glucoraphasatin and glucoraphanin (153 mg 100 g⁻¹, 149 mg 100 g⁻¹ and 141 mg 100 g⁻¹ FW, respectively) in fruits; followed by glucoraphasatin (3 mg 100 g⁻¹ FW) in flowers and by glucobrassicin, 4-hydroxyglucobrassicin and glucoraphasatin (145 mg 100 g⁻¹, 27 mg 100 g⁻¹ and 24 mg 100 g⁻¹ FW, respectively) in leaves. In roots the major glucosinolate is glucoraphasatin (56 mg 100 g⁻¹ FW) followed by the glucoraphenin and methoxyglucobrassicin (16 mg 100 g⁻¹ and 7 mg 100 g⁻¹ FW, respectively). Principal component analysis allowed the discrimination of fruit samples from other parts of the plant for the majority of glucosinolates and the fruits are highlighted as sources of glucosinolates. The results are interesting given that wild radish is one of the most important weeds of crops in the Mediterranean region and is popular for home vegetable production and for its employment in human nutrition both as a food as well as for medicinal purposes.

1. Introduction

Raphanus genus, belonging to the family of Brassicaceae, consists of two species: *Raphanus sativus* L. (edible radish) and *Raphanus raphanistrum*, which includes three subspecies: *R. r. raphanistrum*, *R. r. landra* and *R. r. rostratus*. Wild radish (*Raphanus raphanistrum* L.) is an annual broadleaf plant widely distributed in the southeast of United States, (Schroeder, 1989) in south of Australia, southern Europe and across the Mediterranean region. *R. raphanistrum* is also an edible plant commonly consumed in the Mediterranean region in countries such as Italy (Conti et al., 2005; Scott et al., 2002). All subspecies of *R. raphanistrum* are found in the Mediterranean area. The plant and its subspecies are known in human nutrition both as a food as well as for medicinal purposes. In fact, the young leaves and the aerial parts, slightly spicy, are

consumed fresh or cooked as a salad, with olive oil and lemon juice like spinach, or boiled, like those of cultivated radish (*Raphanus sativus*), broccoli (*Brassica oleracea*) or edible brassicaceae, to prepare side dishes or in vegetable and legume soups (Hedge, 1965). In some areas, the roots are consumed grated or boiled or fried like radish. Moreover, *R. raphanistrum* is a component of a typical Sardinian dish, namely "Ramolaccio", a soup composed of 18 wild herbs of Barbagia. (Atzei, 2003). Moreover the aerial parts of the plant are used in traditional medicine for their anti-rheumatic and hypoglycemic activity and for the treatment of various ailments such as gastrointestinal diseases (Conforti et al., 2008). Anti-inflammatory and antioxidant activity of hydro-alcoholic extracts of *R. raphanistrum* has also been reported (Conforti et al., 2011; El and Karakaya, 2004; Küçükboyacı et al., 2012; The Local Food-Nutraceuticals Consortium, 2005). In addition, wild radish showed biofumigation potential when aqueous extracts and soil-incorporated air-dried biomass were tested in controlled environments (Bones and Rossiter, 1996; Norsworthy, 2003; Rosa et al., 1996).

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Previous studies on the chemical composition of radish have reported the presence of numerous beneficial compounds (poly-phenols, anthocyanins) and, in particular, the majority of the studies have been conducted on a class of phytochemicals, characteristic of cruciferous vegetables, known as glucosinolates (GLs) (Hanlon and Barnes, 2011).

Glucosinolates are thioglucoside compounds, that contain a sulfated aldoxime moiety and a variable side chain derived from amino acids, more than 200 different side-groups have been identified (Agerbirk and Olsen, 2012; Deng et al., 2015; Franco et al., 2016). Although glucosinolates themselves possess limited biological activity, their enzymatic degradation by myrosinase leads to the formation of a number of biologically active compounds including ionic thiocyanate, isothiocyanates, nitriles, oxazolidinethione, epithionitriles and organic thiocyanates. Glucosinolates hydrolysis products are thought to be responsible for the characteristic pungent smell and flavour of this class of vegetables (Bennett et al., 2002; D'Antuono et al., 2009; Deng et al., 2015). In particular, it has been demonstrated that isothiocyanates can induce phase 2 detoxification enzymes and inhibit phase 1 activation enzymes, and this activity can be a key element in reducing risk of cancer (Kusznierewicz et al., 2013). It has been also demonstrated that all GL derivatives are not equal in their biological potential. Consequently, the identification, but also the quantitative determination, of individual glucosinolates in plant and plant tissues is extremely important (Kusznierewicz et al., 2013; Maldini et al., 2014; Rosa et al., 1996). Several HPLC-MS based methods have recently been used for the analysis of intact glucosinolates (Bennett et al., 2004; Maldini et al., 2012; Maldini et al., 2014). These methods can be used for both qualitative and quantitative analysis, especially for the characterization of GLs in less explored species, where the presence of unknown structures may require more sophisticated identification techniques (Grata-cos-Cubarsi et al., 2010; Maldini et al., 2012; Maldini et al., 2014; Mellon et al., 2002; Tian et al., 2005).

The aim of this work was to identify and quantify the occurrence of glucosinolates in the different parts of *R. raphanistrum* i.e. in the leaves, flowers, fruits and roots. To the best of our knowledge, there are no studies in literature that report analysis, either qualitative and quantitative, on "glucosinoloma" of the radish *R. raphanistrum* grown in La Maddalena (Sardinia, Italy). In this paper, glucosinolates were analysed and determined in different samples with the use of ultra-high performance liquid chromatography-triple quadrupole/linear ion trap tandem mass spectrometry (UHPLC-QTRAP/MS/MS). Furthermore, the quantitative results were analysed by principal component analysis (PCA) to compare extracts from different tissues and to identify the variables responsible for the differences and the similarities among samples. The reason for analysing this species is that it could be a good source of glucosinolates, since wild radish is one of the most important weeds of crops in the Mediterranean region and is popular for home vegetable production and for its employment in human nutrition both as a food as well as for medicinal uses.

2. Material and methods

2.1. Materials

LC-MS grade acetonitrile, methanol and formic acid, were obtained from Sigma-Aldrich Chemical Company (St Louis, MO). HPLC grade water (18 mV) was produced using a Millipore Milli-Q (Bedford, MA, USA) purification system. Glucosinolate standards (glucobrassicin, glucoraphanin, glucoiberin, glucotropaeolin, glu-conapin, progoinin, glucoerucin and glucoraphenin potassium salts) were purchased from PhytoLab GmbH & Co. KG (Vestenbergsgreuth, Germany). Standard purity was more than 80%.

2.2. Plant material and extraction

Three samples (biological triplicates) of mature wild *Raphanus raphanistrum* were collected in July 2014 in Spargiotto island, La Maddalena archipelago, Sardegna region (Italy), and authenticated by Doctor M. Chessa. Voucher specimens were dried and deposited at the Erbarium Sassa of Sassari University (n 60). The plants were gently harvested, without cutting, with whole roots and transported in cold water from the field to the laboratory. Plants were separated into leaves, flowers, fruits and roots, giving 12 biological samples, and immediately frozen in liquid nitrogen and stored at 80 C to avoid hydrolysis of the GLs. Samples were stored for no more than one week before sample preparation. On the day of extraction, each sample (12) was rapidly ground to a fine powder in a Waring blender cooled with liquid nitrogen and, approximately, 1 g was weighed into several 50 mL tubes and promptly extracted at 70 C for 30 min under vortex mixing, using 25 mL methanol: water (70:30 v/v); sample to solvent ratio (1:25 w/v). The samples were centrifuged at 4000 rpm for 30 min (4 C) and the supernatants were collected. After collection the solvent was entirely removed using a rotary evaporator under vacuum at 40 C. The samples were reconstituted using ultrapure water and filtered through 0.20 µm syringe PVDF filters (Whatman International Ltd., UK). The measurements were done in triplicate on 3 different samples, each of them obtained by combining material coming from at least 3 different plants.

2.3. ESI-MS and ESI-MS/MS analyses

MS analysis was performed using an ABSciex (Foster City, CA, USA) API4000 Q-Trap spectrometer operating in the negative ion mode (ion spray voltage at 4500 V). The experimental conditions were optimised by infusing a standard solution of Glucoraphanin (1 mg mL⁻¹ in methanol:water 50:50 (v/v)) into the source (flow rate: 10 mL min⁻¹). The declustering potential (DP) was set at 70 eV, the entrance potential (EP) was set at 12.2 eV, the collision energy (CE) was set at 28.5 eV and the collision cell exit potential (CXP) was set at 21.8 eV.

2.4. HPLC-ESI-MS and HPLC-ESI-MS/MS analyses

Qualitative UHPLC-ESI-MS/MS analysis was performed using an UHPLC system interfaced to an ABSciex (Foster City, CA, USA) API4000 Q-Trap instrument in ion trap mode. Liquid chromatography was performed with a Flexar UHPLC AS system (Perkin-Elmer, USA) consisting of degasser, Flexar FX-10 pump, autosampler and PE 200 column oven. Five microliters of each sample were injected into a XSelect CSH C18 column (Waters, Milford, MA) (100 2.1 mm i.d., 2.5 mm d) (kept at 47 C) and eluted at 300 mL min⁻¹ with mobile phase A (H₂O containing 0.1% formic acid) and mobile phase B (acetonitrile containing 0.1% formic acid) according to the following gradient: 0–8 min, from 0% to 6% B; 8–25 min, linear gradient 6–10% B; 25–35 min, linear gradient 10–40%. IDA (Information Dependent Acquisition) was used to perform the qualitative analysis. The IDA method included: IDA criteria (specifying the charge state and the mass range), enhanced MS scan, enhanced resolution, enhanced product ion scan or MS/MS scan. The source temperature was held at 450 C, and MS parameters were those optimised for the ESI-MS and ESI-MS/MS analyses. MS data were acquired using Analyst software (1.6.2 version), and extracted ion chromatograms (XIC) were analysed in order to identify glucosinolates from their deprotonated molecular ions and retention time.

Quantitative on-line HPLC-ESI-MS/MS analyses were performed in Multiple Reaction Monitoring (MRM) mode (using the mass spectrometer working as a triple quadrupole analyser).

Standard solutions (1 mg mL⁻¹ in methanol:water 50:50 (v/v)) were infused at 10 mL min⁻¹ for tuning, the optimised parameters (fragmentation reactions selected for each compound, dwell times, and Declustering Potential, Entrance Potential, Collision Energy, Collision Cell Exit Potential values) are reported in Supplementary Material (Table S1).

2.5. Calibration and quantification of glucosinolates

1 mg of each standard was accurately weighed and dissolved into a 1 mL of methanol:water (50:50 (v/v)) to prepare a stock solution at 1 mg mL⁻¹. The stock solution was diluted with methanol in order to obtain work solutions containing 0.01, 0.1, 0.5, 1, 5, 10 mg mL⁻¹ of standards. The calibration curves for each compound were calculated by regression analysis, by plotting the peak area obtained after standards injection (3 replicates at each concentration) against the known standard concentrations.

2.6. Method validation

The LC–MS/MS method was validated according to the European Medicines Agency (EMA) guidelines relating to the validation of analytical methods (EMA, 1995). Three concentration levels for each compound were measured.

Precision was evaluated through intra-day and inter-day triplicate over 3 days; the intra-day precision (coefficient of variance) was within 6%, while the inter-day precision was within 11% for all analytes (Table 1). Specificity was defined as the non-interference of other analytes detected in the region of interest. With regard to the LC–MS/MS method, which was developed on the basis of the characteristic fragmentation of detected glucosinolate, no other peaks interfered with the analytes in the MS/MS

detection mode. The recoveries (assessed through the addition of pre-determined quantities of standards to known amounts of plant samples) were determined to be 100%. The sensitivity was estimated as both limit of quantification (LOQ) and limit of detection (LOD) and was calculated by injecting a series of increasingly dilute standard solutions until the signal-to-noise ratio was reduced to 2 (Table 1).

2.7. Statistical analyses

Data were expressed as mean SD and analyzed by a non-parametric test, as they showed a non-Gaussian distribution. Differences in the level of GSL among the different parts of plant were assessed by using Friedman's test and multiple comparison tests (MCT). Analysis was performed using XLSTAT software.

A m n matrix (where m is the number of samples, and n is the number of variables) was used in PCA. Thus, quantitative data of each chemical marker were used to define a data set with 12 observations and 15 variables. The resulting metabolomics data were analysed by Principal Component Analysis (PCA).

PCA was carried out by Statgraphics Centurion¹ software (Version 15.0, Statpoint Inc., Herndon, VA, USA) and performed on data scaled by unit variance. The results of the analysis are presented in term of score- and bi-plot.

3. Results and discussion

3.1. LC–MS/MS analysis and fragmentation study

To qualitatively determine the glucosinolates occurring in different parts of *R. raphanistrum*, a convenient IDA method with EMS survey scans, ER and EPI scans was developed. The MS² mode

Table 1
Accuracy and precision at three concentration levels, linearity, LOQ and LOD of LC-ESI-QQ-MS/MS MRM method for the analysis of eight standard compounds.

Compound	Concentration mg mL ⁻¹	Precision Intra-day CV% ^a	Precision Inter-day CV% ^a	Calibration curve equation	R ²	LOQ (mg 100 g ⁻¹ FW)	LOD (mg 100 g ⁻¹ FW)
Glucobrassicin	0.1	5.9	2.9	$y = 8.38e^4x + 3.1e^3$	0.999	0.0305	0.0102
	0.5	4.1	4.9				
	1	3.7	4.4				
Glucotropaeolin	0.1	4.1	5.3	$y = 3.67e^5x - 8.26e^3$	0.997	0.03	0.009
	0.5	0.8	7.6				
	1	4.5	1.9				
Glucoiberin	0.1	2.3	4.3	$y = 1.03e^6x - 2.13e^4$	0.997	0.0082	0.0025
	0.5	2.7	4.3				
	1	2.1	5.6				
Glucoraphenin	0.1	4.9	6.1	$y = 1.29e^5x + 3.13e^4$	0.998	0.0051	0.0015
	0.5	4.0	8.5				
	1	4.4	1.9				
Glucoraphanin	0.1	2.7	2.8	$y = 4.73e^5x - 2.87e^4$	0.999	0.0163	0.0048
	0.5	1.7	3.5				
	1	1.2	2.6				
Gluconapin	0.1	4.7	11.1	$y = 8.79e^4x - 1.04e^3$	0.994	0.0038	0.0011
	0.5	9.3	4.7				
	1	6.2	2.0				
Progoitrin	0.1	1.5	4.0	$y = 2.13e^5x + 1.26e^3$	0.999	0.0042	0.0013
	0.5	2.5	3.9				
	1	1.1	3.1				
Glucoerucin	0.1	1.7	5.2	$y = 1.69e^6x - 579$	0.997	0.0055	0.0017
	0.5	6.5	5.5				
	1	3.5	0.5				

^a Precision and accuracy were evaluated at three concentration levels for each compound through triplicate intra-day assays and inter-day assays over 3 days.

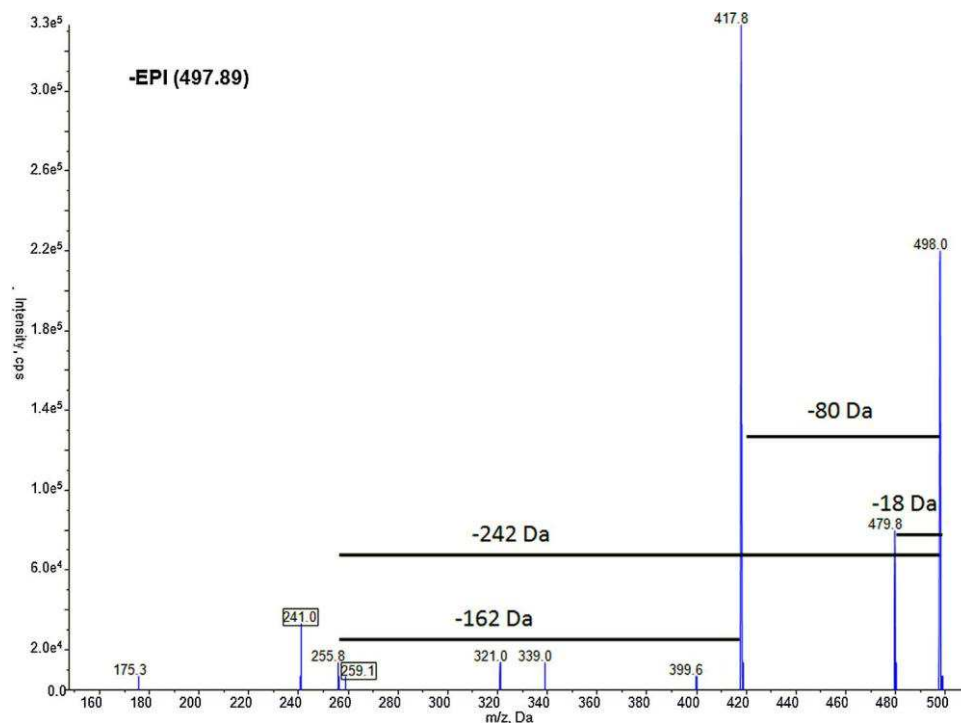


Fig. 1. UHPLC-MS/MS of [M-H] ion of the compound at m/z value of 498.

is a useful tool to provide information on the nature of metabolites analyzed. In particular, for this class of compounds, it is widely reported that the negative ion tandem mass spectrometry gives typical daughter ions. In fact, the MS/MS of the deprotonated molecule [M-H] of intact glucosinolates produces characteristic and diagnostic fragments at m/z values of 259, 195 and 97, which can be assigned to the sulfated glucose moiety, the thioglucose anion and the sulfate group, respectively (Bialecki et al., 2010; Cataldi et al., 2010; Ediage et al., 2011; Glauser et al., 2012; Lelario et al., 2012). Other significant fragment ion peaks detected in glucosinolate product ion spectra are observed at m/z 275, corresponding to the neutral loss of R N $\frac{1}{2}$ C $\frac{1}{2}$ O from the [M-H] ion and at m/z 241 corresponding to C₆H₉O₈S (Ediage et al., 2011; Kokkonen et al., 1991; Velasco et al., 2011).

Thus, this typical collision-induced fragmentation and the typical sulfur isotopic pattern were considered to characterise the presence of glucosinolates in plant extracts. Different parts of *R. raphanistrum* (leaves, fruits, flowers and roots) were analysed and 17 different glucosinolates were detected. Identification was achieved by comparison in LC-ESI-MS/MS of both their MS² spectra, and their retention times, with those observed for the analytical standards. When pure analytical standards were not available, a comparison was made with data reported in literature (Bennett et al., 2004; Cataldi et al., 2007; Kiddle et al., 2001; Mellon et al., 2002; Prestera et al., 1996). Fig. 1 shows the product ion spectrum of the compound at m/z value of 498. The MS/MS spectrum exhibits product ions at m/z 480 [M-18], due to a neutral loss of H₂O; at m/z 418 [M-80] corresponding to the loss of

Table 2
Qualitative analyses by LC-ESI/MS, LC-ESI/MS/MS and occurrence of glucosinolates in the extracts of *R. raphanistrum*.

Compound	TR	[M-H] (m/z)	MS/MS	tentative identification	Presence
1	5.06	422	422, 378, 358, 330, 259	Glucobrassicin ^a	Leaves, Fruits, Flowers, Roots
2	8.53	436	436, 372, 259, 195	Glucoraphanin ^a	Leaves, Fruits, Flowers, Roots
3	9.31	434	434, 419, 259, 97	Glucoraphenin ^a	Leaves, Fruits, Flowers, Roots
4	9.44	388	388, 341, 332, 259, 195	Progoitrin ^a	Leaves, Fruits, Flowers, Roots
5	9.42	374	374, 332, 257, 97	N-butyl- or Isobutyl- or Methyl propyl-glucosinolate	Leaves, Flowers, Roots
6	11.5	450	450, 386, 183, 97	Glucosylsucin	Leaves, Fruits, Flowers, Roots
7	13.3	372	372, 259	Glucoraphanin ^a	Fruits
8	15.7	466	466, 288, 271, 259, 97	5-methylsulfonylpentyl-glucosinolate	Leaves, Fruits, Flowers, Roots
9	17.9	464	464, 400, 375, 356, 256, 97	Glucosylsucin	Leaves, Fruits, Flowers
10	18.0	463	463, 269	4-hydroxyglucobrassicin	Leaves, Fruits, Flowers, Roots
11	19.6	408	408, 328, 275, 259	Glucotropaeolin ^a	Flowers, Roots
12	20.7	420	420, 275, 259, 96	Glucobrassicin ^a	Leaves, Fruits, Flowers, Roots
13	20.8	418	418, 338, 275, 259, 241, 175, 97	Glucoraphasatin	Leaves, Fruits, Flowers, Roots
14	20.9	498	498, 480, 418, 400, 339, 321, 259	3,4,5-trimethoxybenzyl glucosinolate	Leaves, Fruits, Flowers, Roots
15	22.6	447	447, 367, 275, 259, 205, 172, 97	Glucobrassicin ^a	Leaves, Fruits, Flowers, Roots
16	30.0	477	477, 377, 259, 97	Methoxyglucobrassicin	Leaves, Fruits, Flowers, Roots
17	30.7	402	402, 195, 97	Glucoraphanin or C6-aliphatic glucosinolate ^b	Leaves, Fruits, Flowers, Roots

^a Identified using corresponding authentic standards.

^b Tentative annotation of compound class.

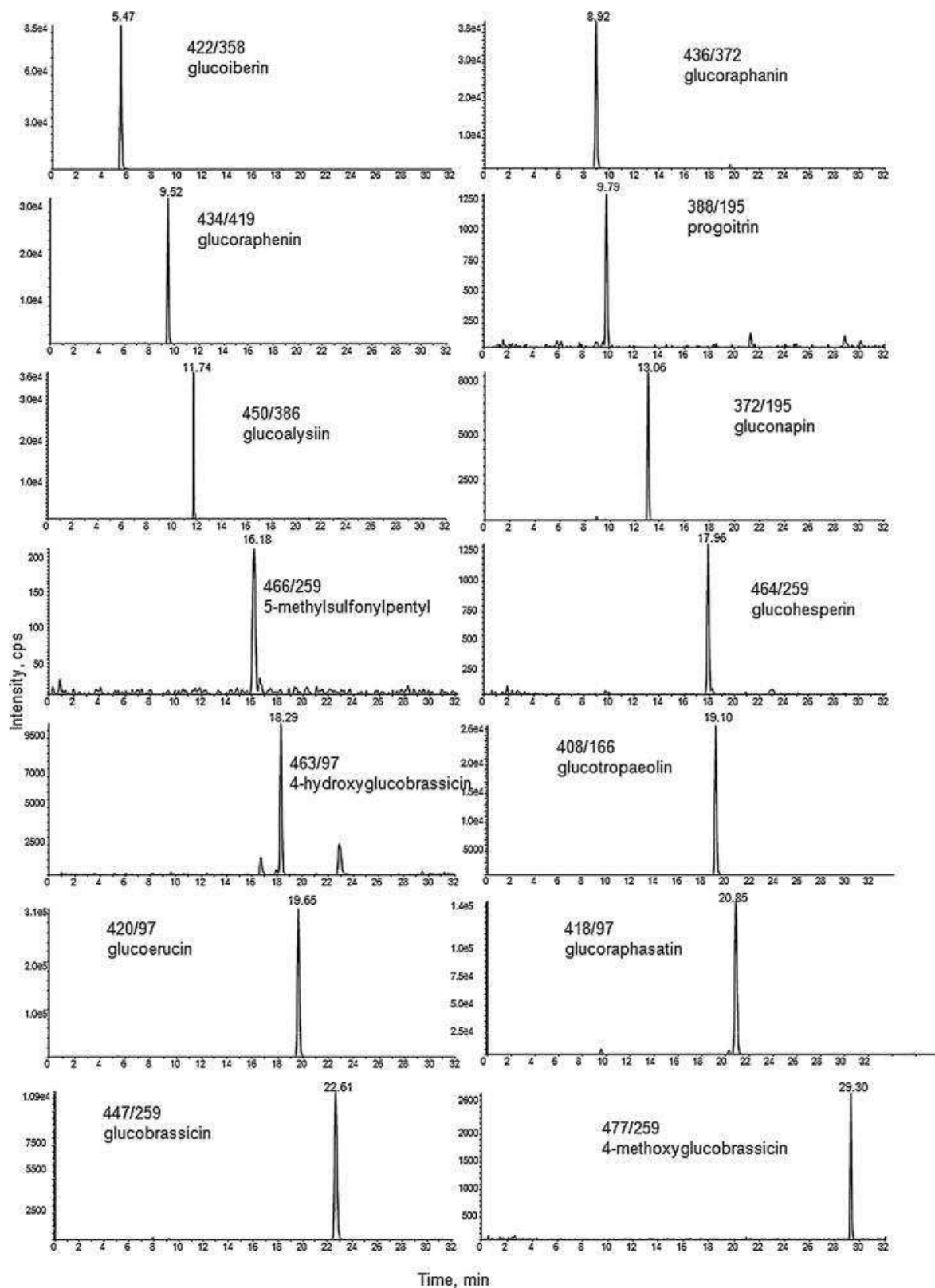


Fig. 2. MRM analysis of glucosinolates in *R. raphanistrum*.

SO₃ and at m/z 256 [M-242 (M-80-162)] attributable to the consequent loss of SO₃ and the glucose moiety. The losses of 18, 80, 162 and 242 Da from the parent ion are typical behavior characteristic of neutral losses found in daughter ion spectra of glucosinolates (Kokkonen et al., 1991). However, the fragment ion

at m/z 175 is probably dependent on side chain R [R = C₄N-SO₄] (Fabre et al., 2007). Furthermore, the fragmentation spectrum of the deprotonated precursor at m/z 498 displays diagnostic ion peaks at m/z 259 and 241, typical of glucosinolate fragmentation. Thus this compound could be identified as 3,4,5-trimethoxybenzyl

glucosinolate; the 3,4,5-trimethoxybenzyl glucosinolate has been previously detected in *Lepidium* (Bennett et al., 2004) and *Juncea* (Kim et al., 2016) genus, however it has never reported in *Raphanus* (Chevolleau et al., 2002; Fabre et al., 2007; Frank et al., 2010; Velasco et al., 2011).

Table 2 summarizes the results on the individual glucosinolates data, including retention times, molecular ions, MS/MS fragments obtained from IDA method together with the presence (or absence) in each plant tissue.

3.2. Glucosinolates content in different parts of the plant

Due to the great importance of the biological activities widely reported for glucosinolates, a quantitative MRM method was developed. Selected transitions, dwell time, DP, EP, CE and CXP parameters are reported in Supplementary Material (Table S1).

The calibration curve equations were linear in the range of 0.01–10 mg mL⁻¹. The LC-ESI MS/MS method was validated according to Quality Guidelines: Validation of Analytical Procedures Text and Methodology (ICH Q2). Calibration curves equations and LOQ and LOD values for each external standard are reported in Table 1.

Fig. 2 shows the LC-ESI-MS/MS MRM analyses of glucosinolates detected in different tissues of *R. raphanistrum*. The chromatographic profile contains all the peaks corresponding to the compounds under investigation.

Quantitative data are summarized in Table 3. As expected glucosinolates occurred in all parts of the plant, but they showed different pattern and concentration. Among tissues, the fruits present the highest total and individual glucosinolate content, followed by the leaves, roots and flower. Also the concentration of individual glucosinolates varied greatly in the different plant tissues. Glucoraphenin is the most predominant compound in fruits, flowers and leaves, accounting for about 88, 86 and 46% (w/w) of total glucosinolate content, respectively.

On the other hand, the main glucosinolate in roots was glucoraphasatin, that accounted for about 61% of total glucosinolate content, while glucoraphenin represented only the 17% (w/w) of total glucosinolate content. Despite the big difference in glucosinolate level (90-fold greater in fruits than in flowers), fruits and flowers showed a similar glucosinolate profile

characterized by the main presence of glucoraphenin, glucobras-sicin, glucoraphasatin and glucoraphenin. On the contrary, in leaves the most predominant glucosinolates were glucoraphenin, glucobrassicin, 4-hydroxyglucobrassicin and glucoraphasatin and in roots the major glucosinolate were glucoraphasatin, methoxy-glucobrassicin and glucoerucin. The glucosinolate profile and level found in our study was different from those observed in *R. raphanistrum* plants obtained from seeds collected across United States (Malik et al., 2010a, 2010b). In these studies, in fact, the main glucosinolates found in roots, leaves and flowers were glucoerucin and glucoraphenin. The observed differences could be ascribable to genetics (cultivar), variations in growth stage, environmental conditions and geographical origins. A great genetic influence on glucosinolate pattern and level of *R. raphanistrum* was already observed among different accessions grown under the same environmental conditions (Malik et al., 2010a).

It is noteworthy that the edible part of the plant (leaves) showed a glucosinolate level of about 410 mg/100 g FW, that is much higher than those reported for many different Brassica species (Verkerk et al., 2009), then these plants could represent an important source of glucosinolates for human diet.

3.3. Principal component analysis

For a better data visualization, principal component analysis (PCA) was performed. Fig. 3 shows the 3D bi-plot of *R. raphanistrum*. The first component (PC1) explains 73% of variance, and the second (PC2) another 17% and the third (PC3) another 9.1%. The 3D diagram showed limited cluster areas, each representing a link to a different part of the plant extracted; in fact, we can observe separated regions related to samples of roots (R), fruits (Fr), leaves

(L) and flowers (Fl). Along the first principal component of the score plot it is possible to discriminate between three main groups: the first one is made up of fruits (in the highest negative values), the second one of leaves, whereas roots and flowers are located in the highest positive PC1 values. As expected, the main discriminating variable among plant tissues is the glucosinolate total amount, which reaches 4602 mg 100 g⁻¹ in the fruits.

Successively was evaluated the contribution of each variable to the differences among samples (Fig. 3). As shown, along the highest negative values of PC1 are located the majority of

Table 3
Quantitative results for glucosinolates detected in extracts of fruits, flowers, roots and leaves (mg 100 g⁻¹ 'fresh' weight) of *R. raphanistrum*.

COMPOUND	FRUITS ^c	FLOWERS ^c	ROOTS ^c	LEAVES ^c
Glucoerucin	3.05 0.61 ^a	0.08 0.04 ^{a,b}	4.96 2.17 ^a	1.28 0.92 ^{a,b}
Glucotropaeolin	1.26 0.04 ^a	0.06 0.01 ^{b,c}	ND ^c	ND ^c
Glucobrassicin	1.52 0.05 ^a	0.05 0.01 ^{b,c}	0.05 0.01 ^b	0.68 0.02 ^{a,b}
Glucoraphenin	4045 263 ^a	0.06 0.01 ^{b,c}	16.8 6.44 ^c	190 14.2 ^{a,b}
Glucoraphenin	141 8.43 ^a	44.6 8.29 ^b	1.31 0.13 ^b	12.2 2.57 ^a
Glucobrassicin	154 7.49 ^a	1.37 0.53 ^b	1.81 0.97 ^{a,b}	145 12.2 ^a
Gluconapin	1.69 0.45 ^a	1.37 0.53 ^b	2.52 0.10 ^c	145 12.2 ^a
Progoitrin	1.69 0.45 ^a	ND ^{b,c}	ND ^c	ND ^{a,b}
Gluconapoleiferin	8.61 0.55 ^a	0.10 0.02 ^{b,c}	0.03 0.01 ^b	0.39 0.05 ^{a,b}
Glucoraphasatin	8.44 0.28 ^a	0.07 0.01 ^c	0.66 0.08 ^{a,b}	1.16 0.24 ^{b,c}
4-hydroxybrassicin	150 12.5 ^a	3.30 1.24 ^b	56.3 21 ^b	24.1 11 ^{a,b}
Methoxyglucobrassicin	49.6 16.8 ^a	0.06 0.03 ^b	0.35 0.34 ^a	27.1 7.25 ^{a,b}
Glucoraphasatin	8.67 0.71 ^a	ND ^b	7.47 5.51 ^b	2.34 0.73 ^{a,b}
Glucosylsin	18.5 1.13 ^a	0.28 0.03 ^{b,c}	0.29 0.08 ^c	2.78 0.23 ^{a,b}
Glucosylsin	4.11 0.14 ^a	0.16 0.01 ^{b,c}	0.12 0.01 ^c	1.80 0.02 ^{a,b}
5-methylsulfopentyl glucosinolate	6.82 0.71 ^a	0.17 0.01 ^{b,c}	0.13 0.01 ^c	2.02 0.02 ^{a,b}
Total	4602 312 ^a	51.6 10.3 ^{b,c}	91.5 36.7 ^b	411 49.4 ^{a,b}

ND: not determined (below LOD).

^a quantified as equivalent of glucobrassicin.

^b quantified as equivalent of glucoerucin.

^c quantified as equivalent of progoitrin.

^d quantified as equivalent of glucoraphenin.

^e Each data is the mean of three replicates (n = 3) (mean SD). Data in a row with different letters are significantly different at p < 0.05 by Friedman's test and multiple comparison tests.

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