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Convenient identification of desulfoglucosinolates on the basis of mass spectra obtained during liquid chromatography–diode array–electrospray ionisation mass spectrometry analysis: Method verification for sprouts of different *Brassicaceae* species extracts☆

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a b s t r a c t

Over the past decade, glucosinolates (GLs) present in different tissues of Brassicaceae and their breakdown products, especially isothiocyanates formed after myrosinase catalyzed hydrolysis, have been regarded as not only environment friendly biopesticides for controlling soilborne pathogens, but most impor-tantly as promising anticarcinogenic compounds. For these reasons, the identification and quantitative determination of the content of individual glucosinolates in plant material is of great interest. Among the different analytical approaches available today for determining GLs in brassica plant samples, HPLC analysis of their desulfo derivatives (DS–GLs) according to ISO 9167-1, 1992, method is the most widely used. However, the notorious lack of commercially available standards limits its usefulness. To overcome these limitations, liquid chromatography–electrospray ionisation–mass spectrometry was investigated as a potential method for the identification of DS–GLs. The characteristic pattern of fragmentation either in positive or negative ionisation was established based on mass spectra of 11 DS–GL standards, then pro-posed for additional over 30 most common desulfated GLs. The applicability of MS detection of DS–GLs was verified for real plant samples, the extracts of 14 kinds of brassica sprouts. The results indicated that this methodology combines a convenient identification of DS–GLs with the well established analytical procedure preferred by many researchers. Thus, incorporation of MS detection into popular ISO method seems to result in an improved and more reliable approach to GLs determination.

Keywords: Desulfoglucosinolates, HPLC–DAD–ESI–MS, Brassica sprouts

1. Introduction

Glucosinolates (GLs) are sulfur rich secondary metabolites, derived from protein and nonprotein L-amino acids, found almost exclusively within the plant genus Brassica of the family *Brassicaceae*. In conjunction with the enzyme myrosinase, these compounds constitute a protective system that provides brassicas with the natural defence against attacks by herbivores as the products of enzymatic GLs hydrolysis, mainly isothiocyanates, show broad biocidal activity including insecticidal, nematicidal and fungicidal effects [1–3]. However, following the discovery of exceptional anticarcinogenic properties of broccoli sprouts resulting from the presence of sulforaphane [4], the degradation product of GL glucoraphanin, the interest in these phytochemicals shifted from environmental [5,6] to medical applications. Over the past two decades, numerous studies have been focused on GLs and their breakdown products as the most promising dietary means in cancer chemoprevention and treatment. The reason of this unprecedented interest stems from the inverse association between the consumption of cruciferous vegetables and risk of many types of cancer documented in a number of human studies [7–10]. It has been also realised that GL derivatives are not equal in their biological potential. Consequently, the identification and quantitative determination of the composition and content of individual glucosinolates in plant tissues have become of great importance.

Several chromatographic methodologies have been developed to quantify total and individual GLs in plants, such as determination of either desulfated or intact GLs or measurement of their enzymatic breakdown products [11,12]. The current tendency observed in this field steers towards analysis of the intact GLs [13–19]. However, the separation of intact GLs by liquid chromatography is not straightforward due to the relatively high

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polarity of such thioglucosides. Therefore, a common approach, still preferred by most laboratories, is to convert the intact GLs into desulfo derivatives, as they can be more easily resolved by reversed phase liquid chromatography. Also this approach has become the basis for the development of a standardised method, ISO 9167-1 (1992) [20], that was set up at the end of the eighties, with the view to analyzing of rapeseed oil correctly and in an uniform way among different laboratories. Gradually, the ISO 9167-1 method began to be also used to determine the GLs content in aerial parts and roots of different *Brassicaceae* species. However, the broader use of this method revealed its shortages, frequent overlaps of analytes and notorious lack of standard DS–GLs. Only a few commercial reference standards are available, and because the identification of individual analytes in the standard ISO method is based solely on the comparison of retention times, order of elution and UV spectra, the determination of unknown GLs is difficult or even not possible. It follows that, MS techniques combined with HPLC would appear to be the ideal methods for confirming the identity of the constituent GLs providing the MS spectra are published.

Several studies have been undertaken in this area. Some of them employed LC–thermospray–MS [21,22], but the majority have been based on the atmospheric pressure chemical ionisation (APCI) [23–25]. Only very few attempted to use the liquid chromatography–electrospray ionisation (ESI) for the identification of DS–GLs [26–28]. In the case of characterization of GL composition in less investigated species, where unknown structures may appear demanding more sophisticated identification, application of LC–MS/MS may be needed. For instance, such an approach was used successfully in studies on determination of bioactive compounds from *Capparis spinosa* [28] or ecotypes of *Brassica rapa* L. subsp *sylvestris* [25]. However, more routine tasks neither require such elaborate equipment nor the expert staff to run and interpret analyses. With the view to these routine applications, the objective of the present study was to establish whether combining current, widely applied ISO method with ESI–MS would improve the detection and widen the range of DS–GLs that can be unequivocally identified. In order to assess the usefulness of the proposed approach, positive and negative ion spectra for several reference DS–GLs were recorded. The fragmentation diagnostic ions determined were then used during LC–ESI–MS analysis of sprout extracts derived from various brassica plants known to differ in phytochemical composition to detect and identify GLs in these samples.

2. Materials and methods

2.1. Reagents and chemicals

HPLC grade acetonitrile and methanol, imidazole ACS, acetic acid (glacial), and formic acid were purchased from Merck (Germany). Glucotropaeolin was from AppliChem (Germany), sulphatase isolated from *Helix Pomatia* H1 (22,400 units/g solid) and DEAE-Sephadex A-25 anion-exchange resin from Sigma (Germany). Glucosinolates (GLs) were isolated at the Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di Ricerca per le Colture Industriali (CRA-CIN), Bologna, Italy, using the procedure based on Thies [29] with some modifications as reported by Baasanjav-Gerber et al. [30]. The following vegetables (seeds or leaves) were the sources of purified GLs: glucoiberin from *Iberis amara*, progoitrin from *Brassica napus* cv. JetNeuf, epi-progoitrin from *Crambe abyssinica* cv. Belenzian, glucoraphanin from *Brassica oleracea* L. var. *acephala sabellica*, sinalbin from *Sinapis alba* cv. Maxi, gluconapin from *B. rapa* cv. Silla, glucoerucin from *Eruca sativa*, glucobrassicin from *Isatis tinctoria*, gluconasturtiin from *Nasturtium officinales*. The purified GLs were subsequently enzymatically desulfated as described elsewhere [31] to obtain standards that were used to generate MS spectra for this study: desulfo-glucoiberin (GIB), desulfo-progoitrin (PRO), desulfo-epi-progoitrin (ePRO), desulfo-glucoraphanin (GRA), desulfo-sinigrin (SIN), desulfo-sinalbin (SNB), desulfo-gluconapin (GNA), desulfo-glucotropaeolin (GTL), desulfo-glucoerucin (GER), desulfo-glucobrassicin (GBS), desulfo-gluconasturtiin (GST).

2.2. Plant material

Brassica seeds used in the investigations were obtained from the following seed producers: garden cress (*Lepidium sativum*), rocket salad (*E. sativa*), rutabaga (*B. napobrassica*), red cabbage (*B. oleracea* var. *capitata* f. *rubra*), kale (*B. oleracea* var. *acephala*), cauliflower (*B. oleracea* var. *botrytis*), kohlrabi (*B. oleracea* var. *gongylodes*) by Polan (Kraków, Poland), Brussels sprouts (*B. oleracea* var. *gemmifera*), radish (*Raphanus sativus* var. *sativus*), mustard (*Sinapsis alba*), white cabbage (*B. oleracea* var. *capitata f. alba*), broccoli (*B. oleracea* L. var. *italica*) by PNOS (Ożarowice Mazowieckie, Poland), rapa (*B. rapa* var. *rapa*) by Plantico (Zielonki, Poland) and savoy cabbage (*B. oleracea* var. *capitata* f. *sabauda*) by Green-Land Service (Michałowice, Poland). Seeds were placed in germination plates and rinsed twice a day with tap water. The germination was performed in the phytotron with controlled temperature (25 °C) and photoperiod including 16 h of light, 8 h in the dark. After 7 days of germination, the sprouts were harvested and freeze-dried.

2.3. Preparation of plant samples

The GLs extraction from plant material was carried out in duplicate according to the EU official procedure [20]. Briefly, 100 mg samples of freeze-dried plant material were extracted twice with boiling methanol (70%, 3 mL) and pooled. The water solution of glucotropaeolin (5 mM, 0.2 mL) was added to each sample just before the first extraction as an internal standard for the quantitative analysis. The extracted GLs were purified on 1 mL column filled with 0.5 mL of DEAE-Sephadex A-25 anion-exchange resin. The column was pre-washed with 2 mL of imidazole formate (6 M), then twice with 1 mL of water and loaded with 6 mL of each extract. The sulphatase was dissolved before use in deionised water at the concentration of 1.67 mg/mL and 0.2 mL of this solution was added onto the column. Desulfatation reaction was carried out overnight at room temperature. Next day, the DS–GLs were eluted with deionised water (2×0.75 mL).

2.4. LC–DAD–ESI–MS analysis

DS–GLs were analyzed using a HPLC Agilent 1200 series system with a Grace Altima HP AQ RP-C18 column (150 mm × 4.6 mm, 3 μm). The separation and UV detection conditions were as recommended by EU official procedure [20]. The mobile phase contained water (A) and acetonitrile/water (20:80, v/v, B). Chromatography was performed with 1 mL/min flow rate at 30 °C and the following gradient program: linear gradient from 5% B to 100% B for 10 min and isocratically 100% B for 15 min, finally the system was equilibrated for 7 min. The injection volume of samples was 30 μL. DS–GLs were detected by DAD (Agilent 1200 series) with monitoring the absorbance at 229 nm, then subsequently by API–ES–MS (Agilent 6130 Quadrupole LC/MS). MS parameters were as follows: capillary voltage, 3000 V; fragmentor, 120 V; drying gas temperature, 350 °C; gas flow (N_2), 12 L/min; nebulizer pressure, 35 psig. The instrument was operated both in positive and negative ion

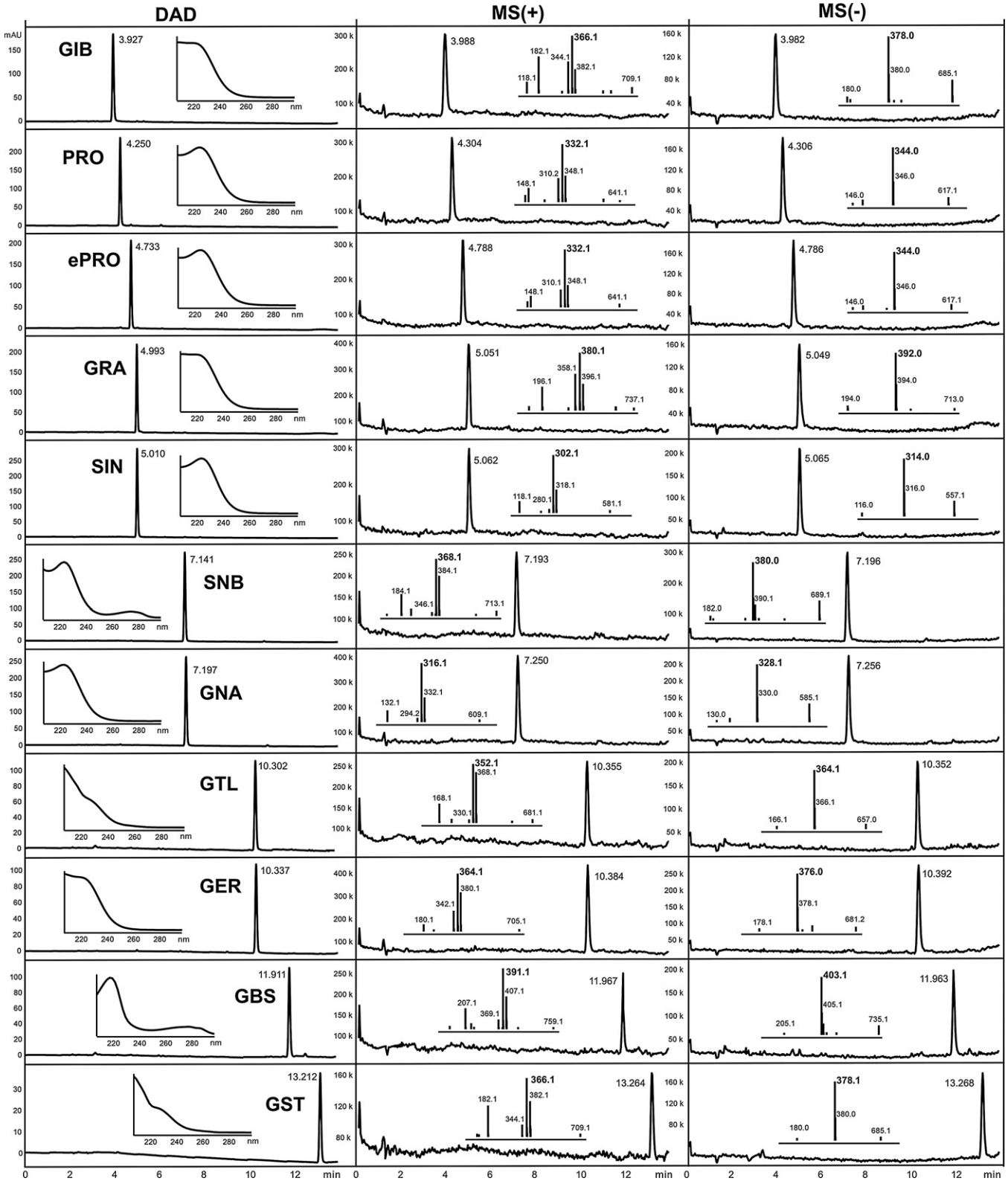

Fig. 1. The chromatograms and spectra obtained during analyses of standard desulfoglucosinolates by LC–DAD–ESI–MS system. The acronyms on top of the panels refer to: DAD–detection at 229 nm; MS(+)–detection in full scan mode with positive ionisation; MS(−)–detection in full scan mode with negative ionisation. The characterised desulfoglucosinolates include: desulfo-glucoiberin (GIB), desulfo-progoitrin (PRO), desulfo-epi-progoitrin (ePRO), desulfo-glucoraphanin (GRA), desulfo-sinigrin (SIN), desulfo-sinalbin (SNB), desulfo-gluconapin (GNA), desulfo-glucotropaeolin (GTL), desulfo-glucoerucin (GER), desulfo-glucobrassicin (GBS), desulfo-gluconasturtiin (GST).

Table 1
The trivial and chemical names, molecular formula and mass of examplary GLs with the fragmentation diagnostic ions which could be expected during HPLC–ESI–MS analyses of their desulfo derivatives either in positive or negative mode.

Glucosinolate		Molecular formula	M	M-80 = M_{DS}	Fragmentation diagnostic ions							
Trivial name	Type of side chain				Positive ionisation					Negative ionisation		
					M_{DS} + H-162	M_{DS} + H	M_{DS} + Na	M_{DS} + K	2M_{DS} + Na	M_{DS} − H-162	M_{DS} + Cl	2M_{DS} − H
Glucocapparin	Methyl	$C_8H_{15}NO_9S_2$	333	253	92	254	276	292	529	90	288	505
Sinigrin	2-Propenyl	$C_{10}H_{17}NO_9S_2$	359	279	118	280	302	318	581	116	314	557
Glucoibervirin	3-Methylthiopropyl	$C_{11}H_{21}NO_9S_3$	407	327	166	328	350	366	677	164	362	653
Glucoiberin	3-Methylsulfinylpropyl	$C_{11}H_{21}NO_{10}S_3$	423	343	182	344	366	382	709	180	378	685
Glucocheirolin	3-Methylsulfonylpropyl	$C_{11}H_{21}NO_{11}S_3$	439	359	198	360	382	398	741	196	394	717
Glucoputranjivin	1-Methylethyl	$C_{10}H_{18}NO_9S_2$	360	280	119	281	303	319	583	117	315	559
Glucosisymbrin	2-Hydroxy-1-methylethyl	$C_{10}H_{19}NO_{10}S_2$	377	297	136	298	320	336	617	134	332	593
Glucoerysimumhieracifolium	3-Hydroxypropyl	$C_{10}H_{19}NO_{10}S_2$	377	297	136	298	320	336	617	134	332	593
Gluconapin	3-Butenyl	$C_{11}H_{19}NO_9S_2$	373	293	132	294	316	332	609	130	328	585
Progoitrin	(2R)-2-Hydroxy-3-butenyl	$C_{11}H_{19}NO_{10}S_2$	389	309	148	310	332	348	641	146	344	617
Epiprogoitrin	(2S)-2-Hydroxy-3-butenyl	$C_{11}H_{19}NO_{10}S_2$	389	309	148	310	332	348	641	146	344	617
Glucoerucin	4-Methylthiobutyl	$C_{12}H_{23}NO_9S_3$	421	341	180	342	364	380	705	178	376	681
Glucoraphasatin	4-Methylthio-3-butenyl	$C_{12}H_{21}NO_9S_3$	419	339	178	340	362	378	701	176	374	677
Glucoraphanin	4-Methylsulfinylbutyl	$C_{12}H_{23}NO_{10}S_3$	437	357	196	358	380	396	737	194	392	713
Glucoraphenin	4-Methylsulfinyl-3-butenyl	$C_{12}H_{21}NO_{10}S_3$	435	355	194	356	378	394	733	192	390	709
Glucoarabidopsithalianain	4-Hydroxylbutyl	$C_{11}H_{21}NO_{10}S_2$	391	311	150	312	334	350	645	148	346	621
Glucoconringiin	2-Hydroxy-2-methylpropyl	$C_{11}H_{21}NO_{10}S_2$	391	311	150	312	334	350	645	148	346	621
Glucoalyssin	5-Methylsulfinylpentyl	$C_{13}H_{25}NO_{10}S_3$	451	371	210	372	394	410	765	208	406	741
Glucobrassicanapin	Pent-4-enyl	$C_{12}H_{21}NO_9S_2$	387	307	146	308	330	346	637	144	342	613
Gluconapoleiferin	2-Hydroxy-pent-4-enyl	$C_{12}H_{21}NO_{10}S_2$	403	323	162	324	346	362	669	160	358	645
Glucocleomin	2-Hydroxy-2-methylbutyl	$C_{12}H_{23}NO_{10}S_2$	405	325	164	326	348	364	673	162	360	649
Glucolesquerellin	6-Methylthiohexyl	$C_{14}H_{27}NO_9S_3$	449	369	208	370	392	408	761	206	404	737
Glucohesperin	6-Methylsulfinylhexyl	$C_{14}H_{27}NO_{10}S_3$	465	385	224	386	408	424	793	222	420	769
Glucoarabishirsutain	7-Methylthioheptyl	$C_{15}H_{28}NO_9S_3$	462	382	221	383	405	421	787	219	417	763
Glucoarabishirsuin	8-Methylthiooctyl	$C_{16}H_{30}NO_9S_3$	476	396	235	397	419	435	815	233	431	791
Glucohirsutin	8-Methylsulfinyloctyl	$C_{16}H_{31}NO_{10}S_3$	493	413	252	414	436	452	849	250	448	825
Glucobrassicin	3-Indolylmethyl	$C_{16}H_{20}N_2O_9S_2$	448	368	207	369	391	407	759	205	403	735
Hydroxyglucobrassicin	4-Hydroxy-3-indolylmethyl	$C_{16}H_{20}N_2O_{10}S_2$	464	384	223	385	407	423	791	221	419	767
Methoxyglucobrassicin	4-Methoxy-3-indolylmethyl	$C_{17}H_{22}N_2O_{10}S_2$	478	398	237	399	421	437	819	235	433	795
Neoglucobrassicin	N-Methoxy-3-indolylmethyl	$C_{17}H_{22}N_2O_{10}S_2$	478	398	237	399	421	437	819	235	433	795
Glucotropaeolin	Benzyl	$C_{14}H_{19}NO_9S_2$	409	329	168	330	352	368	681	166	364	657
Sinalbin	p-Hydroxybenzyl	$C_{14}H_{19}NO_{10}S_2$	425	345	184	346	368	384	713	182	380	689
Gluconasturtiin	2-Phenethyl	$C_{15}H_{21}NO_9S_2$	423	343	182	344	366	382	709	180	378	685
Glucobarbarin	(2S)-2-Hydroxy-2-phenethyl	$C_{15}H_{21}NO_{10}S_2$	439	359	198	360	382	398	741	196	394	717
Glucomalcomiin	3-Benzoyloxypropyl	$C_{17}H_{23}NO_{11}S_2$	481	401	240	402	424	440	825	238	436	801

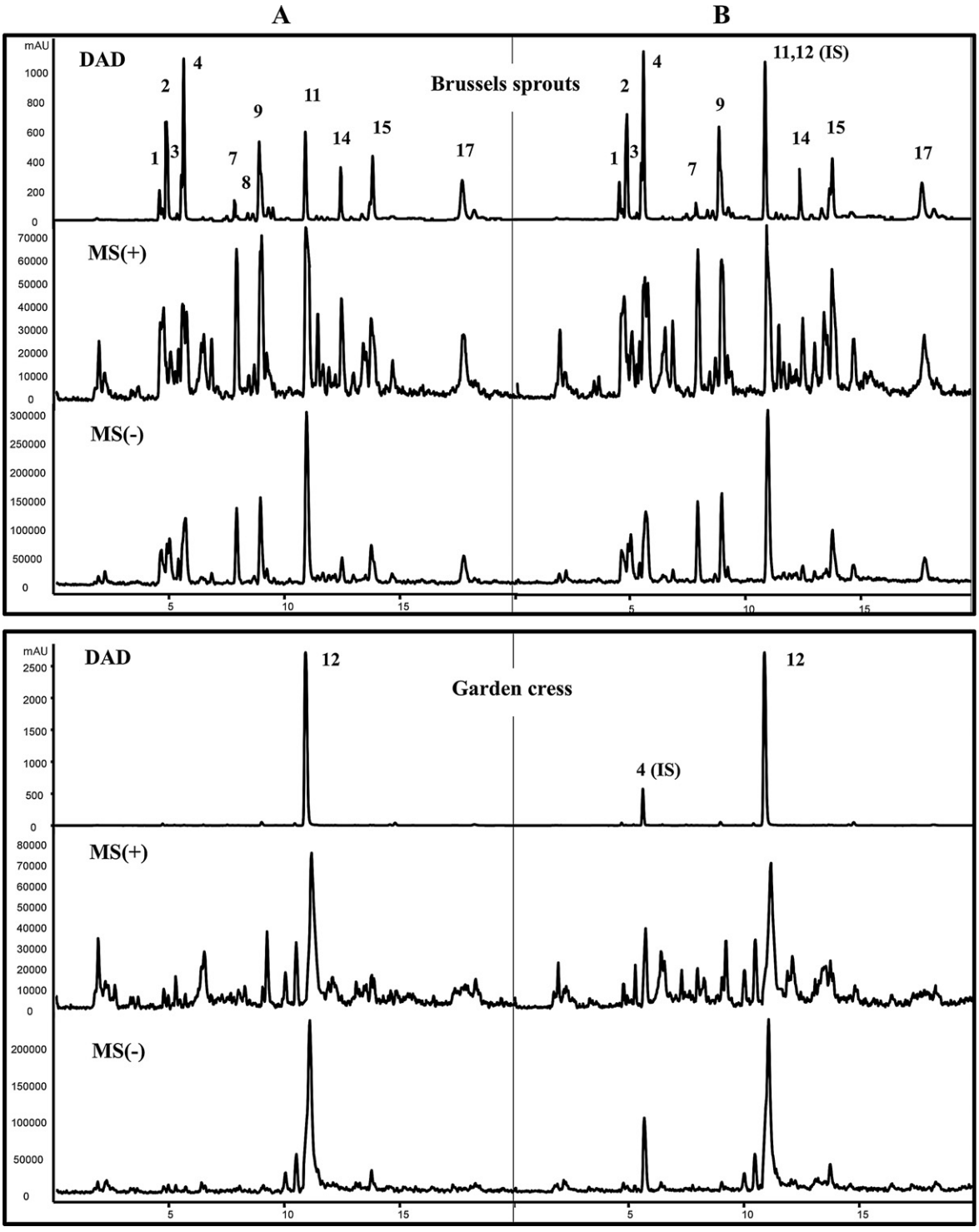

Fig. 2. The chromatograms of desulfoglucosinolates obtained during analyses of brassica sprouts extracts without (A) and with addition of internal standard (B). The internal standards were either glucotropaeolin or sinigrin in the case of Brussels sprouts and garden cress, respectively. The acronyms in the panels refer to: DAD–detection at 229 nm; MS(+)–detection in full scan mode with positive ionisation; MS(−)–detection in full scan mode with negative ionisation. (Peak numbers refer to: 1, glucoiberin; 2, progoitrin; 3, glucoraphanin; 4, sinigrin; 7, gluconapin; 8, glucoibervirin; 9, 4-hydroxyglucobrassicin; 11, glucoerucin; 12, glucotropaeolin; 14, glucobrassicin; 15, 4-methoxyglucobrassicin; 17, neoglucobrassicin.

modes, scanning from m/z 100 to 800. The GLs content of each sample was quantified by the internal standard (glucotropaeolin or sinigrin) method according to ISO protocols [20]. However, in calculations of the content of individual GLs, the updated UV response factors proposed by Clark [11] were used. GLs concentrations are expressed in micromoles per gram of fresh weight.

3. Results and discussion

The aim of the presented study was to incorporate MS detection into highly popular ISO 9167-1 method adopted in the EU initially for the analysis of GLs content and composition in rape seeds (*B. napus* var. *oleifera*) [20]. Despite official recommendations, neither specific information, nor DS–GLs are available today as reference

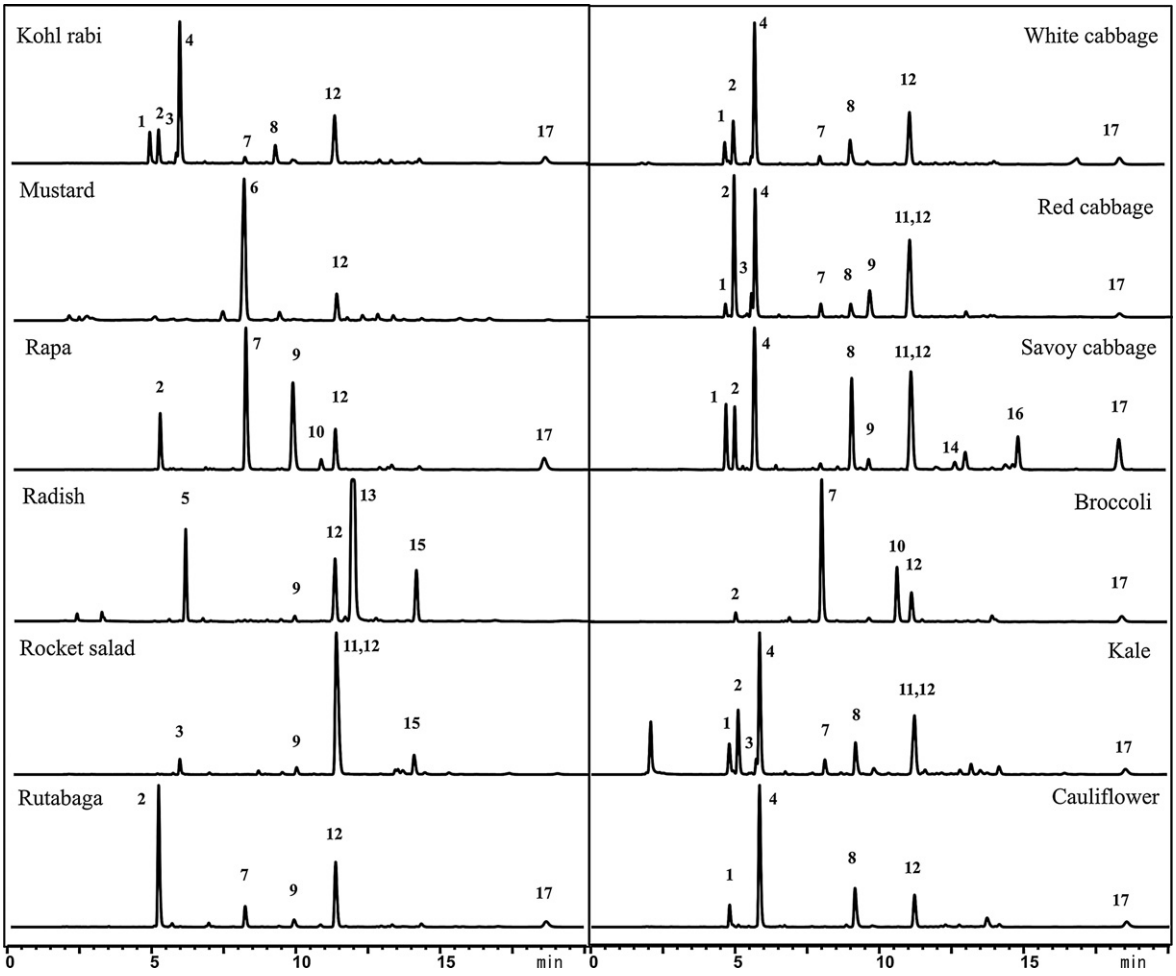

Fig. 3. The HPLC chromatograms of desulfoglucosinolates recorded at 229 nm during analyses of brassica sprout extracts. Peak numbers refer to: 1, glucoiberin; 2, progoitrin; 3, glucoraphanin; 4, sinigrin; 5, glucoraphenin; 6, sinalbin; 7, gluconapin; 8, glucoibervirin; 9, 4-hydroxyglucobrassicin; 10, glucobrassicanapin; 11, glucoerucin; 12, glucotropaeolin, internal standard; 13, glucoraphasatin; 14, glucobrassicin; 15, 4-methoxyglucobrassicin; 16, gluconasturtiin; 17, neoglucobrassicin.

compounds for optimising the analysis conditions and identifying without ambiguity even the 12 GLs present in this crop. For the determination of the total GLs content in rape seeds and the calibration of the ISO analytical method, the Institute for Reference Materials and Measurements (IRMM) of the European Commission provides seeds of three reference rape varieties CRM 366, CRM 190, and CRM 367. When this method began to be widely employed for analysis of samples derived from other brassica plants, its limitations have become even more obvious. The additional analytical tool – ESI–MS – proposed in this study is meant to render the identification of GLs, especially those for which standards are difficult to obtain, more reliable. The methodology is thus based on the DS–GLs resolution by RP–HPLC with UV detection of peaks, additionally coupled to ESI–MS detector for identification of resolved analytes. As a first step, the fragmentation patterns for 11 standard DS–GLs were established on the basis of mass spectra recorded in positive as well as in negative ion modes. In Fig. 1, all recorded chromatograms along with UV and mass spectra for standard compounds are presented.

The mass spectra collected during analysis of standard DS–GLs show typical fragmentation patterns characterized by a major molecular ion peak that in the case of positive ionisation occurs as sodium adduct [M_{DS} + Na] and in negative ionisation as a chloride adduct [M_{DS} + Cl] [26,27,32]. Additionally, in the case of both ionisation modes, the ions formed as a result of the loss of glucose were observed: [M_{DS} + H-162] or [M_{DS} − H-162], respectively. The positive ion mass spectra of DS–GLs included molecular ions [M_{DS}] and their potassium adducts [M_{DS} + K]. The recorded spectra contained also peaks of dimers of molecular ions: [$2M_{DS}$ − H] in negative and [$2M_{DS}$ + Na] in positive ionisation. The formation of [M+ Na, K or Cl] type adducts has been reported previously in the presence of traces of respectively sodium, potassium or chloride ions in solvents [33]. The most frequent ions observed in the mass spectra of analysed standard DS–GLs are listed in Table 1. presents also positive charge and negative charge ions expected for DS–GLs whose standards are not available, but could be found in literature. Another problem of EU recommended ISO method that can be alleviated by the use of ESI–MS is the shortage of affordable internal standards. Basically, only two purified GLs that may serve this purpose are available – sinigrin and glucotropaeolin. Unfortunately, both are frequently found in brassica plant samples. To make matters worse, it often happens also that other GLs co-elute with these two compounds. As shown in Fig. 2, the application of ESI–MS makes it possible to pinpoint such difficulties. In the case of Brussels sprouts sample, sinigrin is its natural component, while glucotropaeolin used as an internal standard co-elutes with glucoerucin. It would be impossible, based only on the retention time, to recognize this overlap. The MS spectra reveal the identity of substances in the peak (marked 11, 12) while performing the analysis with and without glucotropaeolin enables quantification of the analyte. The different situation occurs in the garden cress sample. Its major glucosinolate (in our sample the only) detected

Table 2
Glucosinolate content in different brassica sprouts.

Peak	GLs	Brussels sprouts	Garden cress	Rapa	Radish	Rocket salad	Rutabaga	Mustard	White cabbage	Red cabbage	Savoy cabbage	Broccoli	Kale	Cauliflower	Kohlrabi
1	**GIB**	0.13	–	–	–	–	–	–	0.45	0.13	0.69	–	0.42	0.56	0.42
2	**PRO**	1.16	–	1.12	–	–	2.79	–	0.91	1.74	0.56	0.19	0.78	–	0.52
3	**GRA**	0.03	–	–	–	0.22	–	–	–	0.22	–	–	0.14	–	0.12
4	**SIN**	1.01	–	–	–	–	–	–	3.08	1.52	1.80	–	1.72	3.86	2.24
5	**GRE**	–	–	–	0.51	–	–	–	–	–	–	–	–	–	–
6	**SNB**	–	–	–	–	–	–	2.33	–	–	–	–	–	–	–
7	**GNA**	0.17	–	3.72	–	–	0.38	–	0.20	0.21	–	3.44	0.23	–	0.12
8	**GIV**	0.11	–	–	–	–	–	–	0.50	0.17	0.68	–	0.36	1.00	0.26
9	**4OHGBS**	0.24	–	0.75	0.01	0.04	0.05	–	–	0.15	0.03	–	–	–	–
10	**GBN**	–	–	0.28	–	–	–	–	–	–	–	1.44	–	–	–
11	**GER**	0.66	–	–	–	2.79	–	–	–	0.05	0.81	–	0.07	–	–
12	**GTL**	–	4.82	–	–	–	–	–	–	–	–	–	–	–	–
13	**GRH**	–	–	–	0.79	–	–	–	–	–	–	–	–	–	–
14	**GBS**	0.24	–	–	–	–	–	–	–	–	0.06	–	–	–	–
15	**MeOHGBS**	0.19	–	–	0.05	0.10	–	–	–	–	–	–	–	–	–
16	**GST**	–	–	–	–	–	–	–	–	–	0.36	–	–	–	–
17	**neoGBS**	0.10	–	0.11	–	–	0.05	–	0.04	0.03	0.12	0.05	0.03	0.07	0.04
	TOTAL	**4.04**	**4.82**	**5.98**	**1.36**	**3.15**	**3.27**	**2.33**	**5.18**	**4.22**	**5.11**	**5.12**	**3.75**	**5.49**	**3.72**

The number of a peak corresponds to peaks on chromatograms presented in Figs. 2 and 3. The acronyms refer to the following GLs: glucoiberin (GIB), progoitrin (PRO), glucoraphanin (GRA), sinigrin (SIN), glucoraphenin (GRE), sinalbin (SNB), gluconapin (GNA), glucoibervirin (GIV), 4-hydroxyglucobrassicin (4OHGBS), glucobrassicanapin (GBN), glucoerucin (GER), glucotropaeolin (GTL), glucoraphasatin (GRH), glucobrassicin (GBS), metoxyglucobrassicin, gluconasturtiin (GST), neoglucobrassicin (neo-GBS). Each value is a mean of three replicates and standard deviations do not exceed 15%. All values are given in μmol/g FW.

is glucotropaeolin. In such a case, sinigrin should be used as an internal standard. All in all, it is not always possible to predict the composition or chromatographic properties of GLs found in a sample before analysis. Especially, that GLs composition may vary substantially depending not only on brassica species or variety, but even location or growing conditions. As demonstrated by the examples presented in Fig. 2, combining the current standardised method of DS–GLs determination with ESI–MS ensures the right choice of internal standard, as well as identification and even quantification (e.g. in SIM mode) of co-eluting analytes.

In order to confirm the usefulness of MS detection of DS–GLs in real samples, the extracts of other 12 kinds of brassica sprouts were analyzed. The representative chromatograms recorded at 229 nm are shown in Fig. 3. The comparison of positive and negative ion fragmentation patterns obtained for chromatographic peaks detected for plant samples studied in combination with the retention times enabled the identification of 17 different GLs (Table 2), 7 of them were those for which reference compounds are not accessible. Moreover, based on mass spectra, it was also possible to determine co-eluting DS–GLs that in chromatograms appeared as a single peak (e.g. Fig. 3., peaks marked 11, 12). As expected, the mass spectra of DS–GLs present in brassica samples displayed similar patterns of charged fragments as were detected for standard compounds. Only in some cases, the intensity of ion peaks was different, e.g. ions of dimers $[2M_{DS} - H]$ in negative and $[2M_{DS} + Na]$ in positive ionisation were the most intensive in some real samples. For standard DS–GLs, the sodium and chloride adducts were always the most prominent peaks detected.

The quantitative determination of the DS–GLs identified was based on the internal standard method. The application of ESI–MS enabled also indirect quantitative estimation of the content of those analytes that co-eluted, i.e. GTL and GER in red or savoy cabbage, kale and rocket salad sprouts (Fig. 3). The MS detection of GER suggested that the additional analysis without GTL spike should be performed, as was done for sprout samples naturally containing glucotropaeolin, e.g. garden cress.

The GLs contents in brassica samples investigated are assembled in Table 2. It is known that concentration of GLs is strongly affected by seedling age [19,34]. According to Baenas et al. [34], the general trend for the majority of GLs is a decrease over germination time and these authors conclude that 8-days-old-sprouts could be considered as at their optimum for consumption. The plant material used for the presented experiments was 7-days-old-sprouts. Thus, the sprouts used in this study can be regarded as being at the optimal "consumption stage" with the total concentration of GLs ranging from 1.36 to 5.98 μmol/g FW (21.42–75.56 μmol/g DW). However, their composition, that is equally if not more important from healthiness perspective, was very diversified. Progoitrin and sinigrin were the most predominant GLs in the case of Brussels sprouts, kale, kohlrabi, as well as in white, red and savoy cabbages (54, 67, 74, 77, 77 and 46% of the total, respectively). Sprouts of most broccoli cultivars studied in recent years contained glucoraphanin as the main thiofunctionalised GL, with its concentration depending upon genotype and duration of the sprouting period [19,34,35]. In the experiments reported here, broccoli sprouts contained no glucoraphanin, while the major GLs was gluconapin (67% of the total). Gluconapin was also the major GL in the case of rapa (62% of the total). As could be expected, some amounts of glucoraphanin were observed in the case of Brussels sprouts, rocket salad, red cabbage, kale and kohlrabi. On the other hand, another beneficial compound – glucoraphenin, was found to be the one of the dominant GLs in radish sprouts (38% of the total), together with glucoraphasatin being the radish characteristic GLs (58% of the total), that is in agreement with literature data [36]. In rocket salad sprouts, glucoerucin prevailed (38.15 μmol/g DW) and represented 89% of the total GLs. Similar results were reported by Barillari et al., showing glucoerucin content in rocket salad sprouts to represent 79% of the total GLs [37]. Finally, garden cress and white mustard sprouts were rich in characteristic for these species aromatic GLs [34]: glucotropaeolin (95% of the total) and glucosinalbin (90% of the total), respectively.

4. Conclusions

The HPLC–DAD–ESI–MS analysis applied in this study for the determination of composition and content of GLs in different brassica sprouts confirmed the usefulness of mass spectrometer for the identification of DS–GLs. The incorporation of MS detection into popular ISO method does not force the researchers to resign from the analytical method well established in a number of laboratories, offering at the same time the improved and more reliable approach to GLs identification, which in the case of coeluting analytes is a

prerequisite of their proper quantitation. The ion fragmentation patterns included in this report may be used for the identification of GLs composition in different plant or food samples even by laboratories that do not have access to standard desulfo derivatives. GLs profiles differ among genotypes both qualitatively and quantitatively; their accurate determination in conjunction with biological assessments may help to pinpoint the most beneficial composition of these phytochemicals for prevention of human disease.

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