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Research Article

Identification of desulphoglucosinolates in *Brassicaceae* by LC/MS/MS: Comparison of ESI and atmospheric pressure chemical ionisation-MS

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In order to develop a sensitive method for the detection of desulphoglucosinolates by HPLC-MS, the two most common interfaces for HPLC-MS, atmospheric pressure chemical ionisation (APCI) and ESI, were compared. While working with the APCI-interface the evaporation temperature and corona amperage were optimised. In doing so 300°C and 6 μA proved to be most suitable for aliphatic and indole desulphoglucosinolates. The use of formic acid instead of water in the eluent in HPLC-ESI-MS measurements increased the sensitivity for the indole desulphoglucosinolates in the presence of 1 mM formic acid, while the sensitivity for the aliphatic desulphoglucosinolate desulphoglucoraphanin was substantially increased by the presence of 5 mM formic acid. Using an Agilent ion trap, two optimisation procedures for the MS parameters, smart and expert mode, were available. In smart mode the software optimises several parameters automatically, which is much more time efficient than expert mode, in which the optimisation is done manually. It turned out that ESI-MS is most sensitive in smart mode, while for APCI-MS a higher sensitivity could be gained using the expert mode. Comparing both interfaces, APCI-MS was more sensitive than ESI-MS. However, no additional information, in terms of structure determination, was obtained by APCI-MS.

Keywords: Atmospheric pressure chemical ionisation-MS / *Brassicaceae* / desulphoglucosinolates / ESI-MS / HPLC

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1 Introduction

In recent years glucosinolates (GS), like other bioactive plant constituents, have received increasing attention [1]. GS are a common component of Brassica-type vegetables like broccoli (*Brassica oleracea* L. var. *italica*) and bai cai (*Brassica campestris* L. ssp. *chinensis*), also referred to as pak choi or bok choy. GS are β-thioglycoside *N*-hydroxysulfates (also known as (Z)-*N*-hydroximinosulfate esters or *S*-glucopyranosyl thiohydroximates) with a side chain and a sulfur-linked β-D-glucopyranose moiety. The side chain determines whether the GS is defined as aliphatic, aryl, or indole. Upon disruption of the tissue the GS are brought into contact with the plant-born enzyme myrosinase (β-thioglucosidase) releasing the aglycone, and depending on the reaction conditions (pH, Fe^{2+}) the corresponding isothiocyanates, nitriles or thiocyanates are formed. Isothiocyanates may react to oxazolidinethiones, while aglycones with terminal double bonds may be converted into sulphur-free nitriles in addition to epithionitriles [2].

The GS accumulation in plants is discussed in relation to the anti-fungal and anti-microbial properties of their breakdown products [3]. With respect to human consumption, GS breakdown products show anti-microbial, anti-carcinogenic and cholesterol-reducing effects [4]. These GS are particularly abundant in broccoli, Brussels sprouts [*Brassica oleracea* var. *gemmifera*]) and kale (*Brassica oleracea* var. acephala) [5, 6] but also in Asian leafy vegetables such as mustard greens (*Brassica juncea)* and bai cai (*Brassica campestris* L. ssp. *chinensis*) [7, 8].

In order to investigate the environmental and nutritional factors that affect GS accumulation during plant growth it is essential to establish a suitable analytical procedure. However, purified GS for standardisation are only available for

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Abbreviations: APCI, atmospheric pressure chemical ionisation; **DS,** desulpho; **GS,** glucosinolate(s); **Oct,** octopole very few compounds, for example sinigrin and glucotropaeolin, which calls for MS detection for identification. As GS cannot be analysed by GC-MS without time-consuming and tedious derivatisation HPLC-MS is the method of choice, but this requires evaporation of the solvent during ionisation of the sample before entering the mass spectrometer.

The two most common interfaces are (i) ESI, an ionisation process that uses electrical fields to generate charged droplets and subsequently analyte ions by ion evaporation for MS analysis; and (ii) atmospheric pressure chemical ionisation (APCI), which is a gas phase chemical ionisation process where the evaporating solvent acts as the chemical ionisation reagent to ionise the analytes. Both have been used in the past to determine GS [*e.g.* 9–13].

ESI involves the introduction of a liquid solution directly into the atmospheric pressure source through an emitter, where this atomised spray stream hits the drying gas [14]. A high electrical field at the end of the emitter forms charged droplets that become smaller while the solvent evaporates. The ions produced in the ion source are then extracted to the mass analyser. For APCI the solvent is nebulized (gas assisted) into a hot vaporizer chamber that serves to evaporate the spray droplets rapidly, so that both the HPLC solvent and the analyte molecules are in the gaseous phase, and they are then ionised by the discharge of a corona needle [14]. Due to the lower proton affinity of the solvent a proton is transferred to the analyte, which is transported to the mass analyser.

The ions produced by ESI or APCI are then investigated with various MS techniques, such as quadropole, ion trap, or triple quadropole MS. In this study an ion trap is used, which can also be utilised to identify substances with the same mass, for example 4-methoxyglucobrassicin and neoglucobrassicin, due to the option of MS^n-experiments that result in different fragments of the investigated substances [12]. In order to make full use of these analytical procedures, it is necessary to optimise the interface and the MS parameters. Using an Agilent ion trap system two different approaches, smart and expert mode, are available. Most users optimise their HPLC-MS parameters using the smart mode, in which the software of the MS detector optimises several parameters automatically according to the target mass of interest, because this is more time efficient than the expert mode.

However, it is possible that manual optimisation in the expert mode, which also optimises the MS parameters according to the selected target mass, would provide additional sensitivity. Interestingly the advantages and disadvantages of the two interfaces in relation to the different modes of MS optimisation have not been evaluated in direct comparison for structure determination of different GS groups.

The aim of this study was to establish a highly sensitive HPLC-MS method by comparing ESI and APCI, starting from an existing HPLC-DAD method [8], for the identification and structure elucidation of GS as their desulpho (DS) derivatives. Optimisation was carried out using material from broccoli and bai cai, representing important Brassica-type vegetables in the Western and Asian diets, respectively.

2 Materials and methods

2.1 Reagents and solvents

Reagents and solvents were purchased from Roth (Karlsruhe, Germany) and were of HPLC or analytical grade quality.

2.2 Plant material

Freeze dried broccoli (*Brassica oleracea* L. var. *italica*), variety “Marathon”, was grown in the field of the Institute of Vegetable and Ornamental Crops Groβbeeren/Erfurt e.V., and bai cai (*Brassica campestris* L. ssp. *chinensis*), variety “You Dong Er”, was grown in the field of the Institute of Vegetable Science, Department of Horticulture, Zhejiang University, Hangzhou, China.

2.3 Sample Preparation

DS-GS were determined using the HPLC method described by Krumbein *et al.* [8]. Zero point five grams freeze-dried and ground sample material was brought to 75°C for 1 min to inactivate myrosinase and other enzymes. After addition of 4 mL aqueous methanol (70%, v/v, T = 75°C) and 200 μL 5 mM sinigrin standard (Sigma-Aldrich, Taufkirchen, Germany) the sample was incubated at 75°C for 10 min and occasionally mixed. Subsequently the sample was centrifuged at 4000 rpm (1790 × g) for 10 min after adding 1 mL 0.4 M barium acetate. The supernatant was recovered, the sample mixed again with 3 mL aqueous methanol (70%, v/v, T = 75°C) and subsequently centrifuged again. This procedure was repeated once, and the combined extracts were diluted to 10 mL. For further cleanup and isolation the GS were adsorbed to anion exchange resin, rinsed and subsequently desulphonated as follows. Five millilitres of the crude extract were applied to 200 μL DEAE Sephadex A25 ion-exchanger (Sigma-Aldrich, acetic acid-activated) and rinsed with 10 mL doubly distilled water. Thereafter followed an addition of 250 μL purified aryl sulfatase solution (Th. Geyer Chemie, Berlin, Germany, Concentration ∼14 U/mL at 25°C). Twelve hours later the desulpho compounds were eluted with 5 mL of doubly distilled water (Fig. 1).

2.4 HPLC-DAD-ESI/APCI-MS

All analyses were performed on an Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Ger-

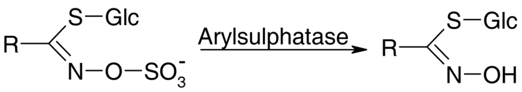

Figure 1. Desulphurisation of GS by arylsulphatase.

many), which consisted of a degasser, a binary HPLC pump, an auto sampler, a thermostat and a photodiode array detector, controlled by ChemStation software (version A.09.01). The LC/MSD trap was controlled by LCMSD software (version 4.1). Ionisation was carried out in the positive mode, which proved to be more sensitive than the negative mode.

The membrane-filtered (0.45 μm, cellulose mixed ester, Roth) extracts were separated on a 125 mm × 4.0 mm id, Prontosil Spheribond ODS2 5.0 μm column using a water ACN gradient at a flow-rate of 0.6 mL/min, a column temperature of 30°C and a detector wavelength of 229 nm [8]. While working with ESI, 1 mM and 5 mM formic acid (VWR International GmbH, Darmstadt, Germany) instead of water were also used. While working with APCI, the eluent was directed to waste after 40 min.

The ion trap includes the following ion optics: A capillary, a skimmer, octopoles (Oct 1 DC, Oct 2 DC, Oct RF), and lenses (Lens 1, Lens 2). Once the sample has been evaporated and ionised, the originated ions enter the capillary, they are directed into the low-pressure ion optics region by electrical fields and pressure drop. The capillary serves to isolate the spray chamber from the low-pressure ion optics region. The skimmer serves to sample the ions while directing the flow of neutrals to the vacuum system and also focuses the ions. The Oct transports the ions to the mass filter. In expert mode the parameters were optimised using "optimise parameter ramping". All optimisations were carried out for the peak of the molecular-ion $(M+H)^+$ since this had the highest intensity. For optimising ESI, formic acid was added to aqueous sinigrin and glucotropaeolin standard solutions (AppliChem GmbH, Darmstadt, Germany), as well as to a broccoli sample.

In all experiments a scan area of 100–450 *m/z* and averages of ten measurements were used and nitrogen was used as drying and nebulizer gas. For HPLC-ESI-MS the gas flow was 10 L/min (325°C) and the nebulizer gas pressure was set to 35 psi. For HPLC-APCI-MS the settings for the nitrogen drying and nebulizer gas were 5 L/min (325°C) and 60 psi. While working with the APCI-Interface the APCI temperature and the capillary amperage were investigated before optimising the numerous parameters. The MS/MS spectra were recorded by isolation and fragmentation of selected pseudo molecular ions.

The syringe pump was set to a flow rate of 0.6 mL/h. When used for ESI-MS the gas flow was 5 L/min (325°C) and the nebulizer gas pressure was set to 15 psi. For APCI-MS the settings for the nitrogen drying and nebulizer gas were 5 L/min (325°C) and 5 psi.

2.5 Ranking order for analysis of results

Individual GS are affected by the interface and MS parameters to different extents and therefore no set of parameters provides optimal detection conditions for all GS under consideration. Consequently the final decision must be considered, a compromise, and in order to evaluate the quality of the optimisation with respect to all GS considered simultaneously, the parameter sets were evaluated on their ranked performance considering both peak area and variability. Optimisation was carried out using two broccoli samples and integrating all peaks of DS-GS. For all GS the average and the variation coefficient (%) were calculated. Ranks were allocated for all averages and variation coefficients depending on the quantity of optimisations that should be compared, beginning with the largest area and the lowest variation coefficient. These numbers were totalled for all DS-GS of the same optimisation, with the allocated rank for the signal area being weighed twice in order to avoid selecting parameter sets of low signal strength due to the correspondingly lower signal variability. The parameter set optimisation with the lowest total number is considered the most suitable.

3 Results and discussion

The optimisation of the MS detector was carried out in smart and in expert mode with the syringe pump using standard solutions of sinigrin and glucotropaeolin as examples of aliphatic GS and aromatic GS, respectively. The settings for ESI and APCI were further optimised using DS-glucoraphanin and DS-glucobrassicin that had been extracted from broccoli by setting the corresponding target masses, as standards were not commercially available. Afterwards bai cai was used to extend the GS spectrum for comparing the ESI and APCI interface (Table 1). Finally, the aliphatic DS-GS, DS-glucoiberin, DS-glucoraphanin, DS-glucoalyssin, DS-gluconapin, DS-glucobrassicanapin, the aromatic DS-GS DS-gluconasturtiin as well as the indole DS-GS DS-glucobrassicin, DS-4-methoxyglucobrassicin and DS-neoglucobrassicin were investigated.

3.1 Developing a highly-sensitive MS method

3.1.1 Evaluation of ESI-MS

As fewer parameters need to be optimised for ESI-MS and as the majority of all MS measurements are done with ESI-MS, this method was tested first. Given that the compounds are ionised while they are still dissolved the solvent influences the ionisation. Therefore an addition of formic acid to the eluent was tested in concentrations of 1 mM and 5 mM. The acid present in the eluent transfers protons to the analyte resulting in an increased degree of ionisation and increased sensitivity (Fig. 2). The use of formic acid instead

Table 1. DS-GS considered in this study.

Rt[a] (min)	Compound (common name)	Systematic names of the DS-GS	MW of DS-GS (Nominal)	Plant
5.8	DS-glucoiberin	3-Methylsulphinylpropyl-DS-GS	343	Broccoli
9.3	DS-glucoraphanin	4-Methylsulphinylbutyl-DS-GS	357	Broccoli
12.4	DS-glucoalyssin	5-Methylsulphinylpentyl-DS-GS	371	Bai Cai
13.1	DS-gluconapin	But-3-enyl-DS-GS	293	Bai Cai
18.9	DS-glucobrassicanapin	Pent-4-enyl-DS-GS	307	Bai Cai
23.0	DS-glucobrassicin	3-Indolylmethyl-DS-GS	368	Broccoli, Bai Cai
26.4	DS-gluconasturtiin	Phenylethyl-DS-GS	343	Bai Cai
27.6	DS-4-methoxyglucobrassicin	4-Methoxy-3-indolylmethyl-DS-GS	398	Broccoli, Bai Cai
33.9	DS-neoglucobrassicin	1-Methoxy-3-indolylmethyl-DS-GS	398	Broccoli, Bai Cai

a) Retention time

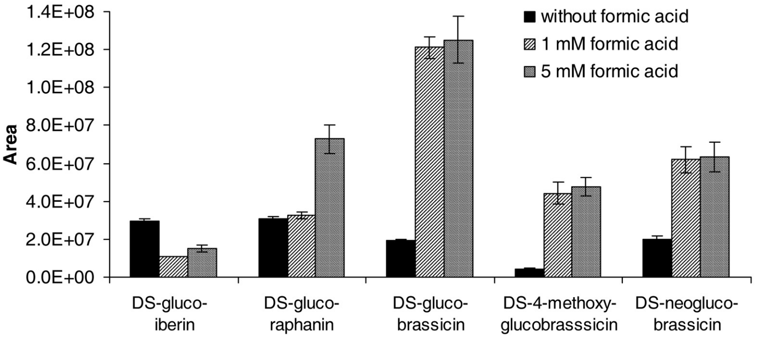

Figure 2. Peak area (means ± SD) obtained for different GS of broccoli in the absence and presence of two different concentrations of formic acid measured with HPLC-ESI-MS.

of water in HPLC-ESI-MS measurements resulted in the expected increase in sensitivity for all the DS-GS except DS-glucoiberin, presumably because its content in the samples was relatively low and it was not separated from a contamination completely. For the indole GS DS-glucobrassicin, DS-4-methoxyglucobrassicin and DS-neoglucobrassicin the sensitivity was increased in the presence of 1 mM formic acid, but there was no further increase with 5 mM formic acid. However, the sensitivity for the aliphatic GS DS-glucoraphanin was substantially increased by the presence of 5 mM formic acid while 1 mM formic acid showed barely any effect (Fig. 2). Also according to the ranking order 5 mM formic acid proved to be the most suitable solvent, and was consequently used for all further ESI-MS experiments including further optimisation procedures. To form protonated species in solution a postcolumn addition of formic acid (10%) was also used for the determination of GS in seeds of different *Brassicaceae* using LC-ESI in combination with triple-quadrupole MS [11].

Conditions for ESI-MS were optimised in both smart and expert mode. The parameters obtained using the target masses for DS-sinigrin, DS-glucotropaeloin and DS-glucoraphanin are shown in Table 2. Measurements with broccoli samples carried out using different approaches showed that the optimisation procedure for DS-sinigrin using the smart mode was the most suitable, resulting in the highest peak areas for the investigated GS in broccoli (Fig. 3). Optimisation using the smart mode with ESI resulted in similar MS parameters. Only the values for the “Cap Exit” were slightly different for the tested molecular weights, leading to small differences in the obtained peak areas. Otherwise, lower values in “Oct 1 DC” and higher values in “Cap exit” obtained by the expert mode in comparison to the smart mode seem to decrease peak areas of most of the GS in broccoli measured with HPLC-ESI-MS.

3.1.2 Evaluation of APCI

Since APCI is a gas phase ionisation technique it is necessary to use an elevated temperature for the evaporation of the spray droplets, which evaporates the analytes without destroying them. Furthermore, it is essential to find the optimal corona amperage required for the complete ionisation of the gas-phase solvent and analyte molecules, by the discharge of the corona needle and for maximal proton transfer from the protonated solvent to the analyte, assuming that the proton affinity of the analyte is greater than that of the solvent. These optimisation procedures were carried out with broccoli samples in smart mode. As shown in Fig. 4, an evaporation temperature of 300°C proved most suitable except for the aliphatic DS-GS DS-glucoiberin and the indole DS-GS DS-4-methoxyglucobrassicin. In the case of DS-glucoiberin the sensitivity could be slightly increased by using 250°C instead. Nevertheless this would imply a substantial decrease of sensitivity for the other DS-GS. For DS-4-methoxyglucobrassicin sensitivity could be slightly increased by using 400°C, but this is counterproductive as

Table 2. Optimised parameter sets for ESI-MS obtained using expert and smart mode for the reference material sinigrin and glucotropaeolin and for the glucoraphanin in a broccoli sample (identified based on retention time and MS/MS spectra).

Parameter	Range	Expert mode			Smart mode		
		DS-sinigrin	DS-gluco-tropaeolin	DS-gluco-raphanin	DS-sinigrin	DS-gluco-tropaeolin	DS-gluco-raphanin
Target Mass $(M+H)^+$ (*m/z*)		280	330	358	280	330	358
Oct 1 DC (V)	20 to 1.8	10.40	8.90	7.40	12.00	12.00	12.00
Oct 2 DC (V)	7.1 to 0	1.63	1.98	1.75	1.70	1.70	1.70
Trap Drive		41.87	44.33	43.51	43.1	43.1	43.1
Cap Exit (V)	300 to 50	140.16	152.46	123.77	113.7	117.4	119.5
Capillary (V)		−3467.22	−3319.67	−3418.03	−3500	−3500	−3500
Oct RF (Vpp)	50 to 300	172.95	127.87	209.84	150.0	150.0	150.0
Skimmer (V)	100 to 15	41.48	48.44	21.97	40.0	40.0	40.0
Lens 2 (V)	−10 to −100	−71.97	−71.97	−69.02	−60.0	−60.0	−60.0
Lens 1 (V)	0 to −15	−4.67	−6.64	−4.67	−5.0	−5.0	−5.0

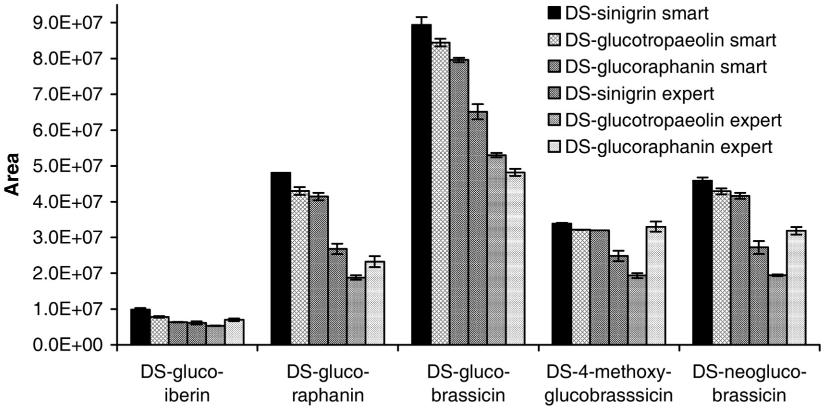

Figure 3. Peak area (means ± SD) obtained for different GS of broccoli with diverse optimised parameter-sets for ESI-MS.

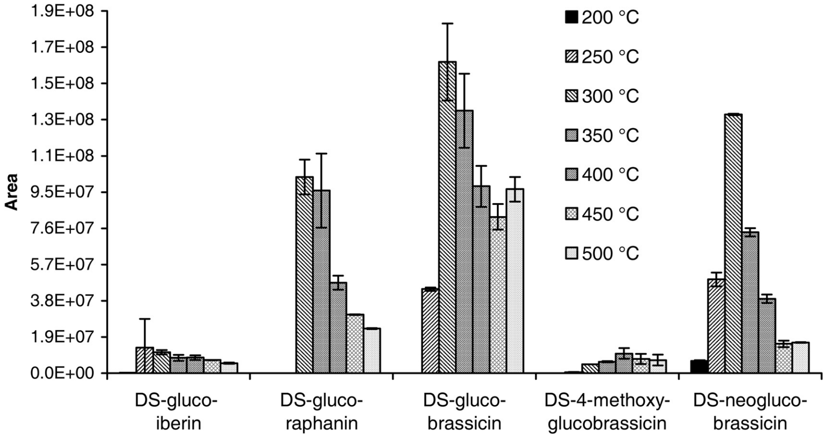

Figure 4. Peak area (means ± SD) obtained for different GS of broccoli with varying evaporation temperatures.

the sensitivity for DS-glucoraphanin, -brassicin and -neoglucobrassicin decreases strongly. Obviously the stability of indole GS is decreased at higher temperatures [15]. Overall, considering the ranking order, which considers both peak area and peak variability (see Section 2.5), reveals that an evaporation temperature of 300°C is most appropriate. This is similar to the result found by Tolrà *et al.* [10] by an optimisation procedure for the aliphatic GS sinigrin who found 290°C to be optimal.

The results for the investigations using different corona amperages are shown in Fig. 5. As demonstrated for the evaporation temperature no corona amperage could be identified that was optimal for all DS-GS. For DS-4-methoxyglucobrassicin all corona amperages under investi-

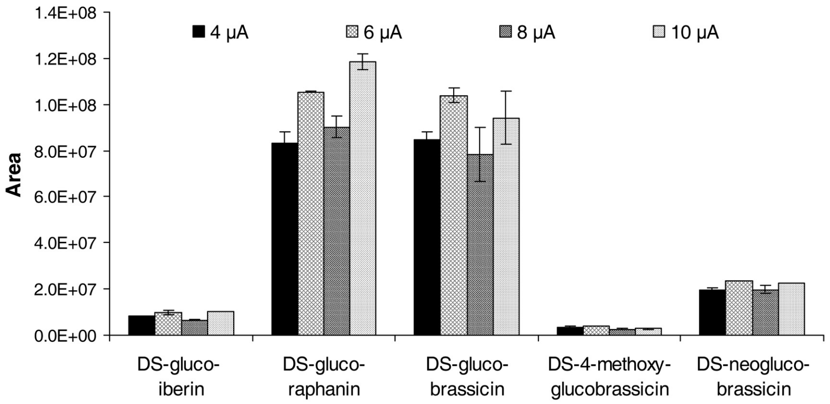

Figure 5. Peak area (means ± SD) obtained for different GS of broccoli with the corona amperages under consideration.

Table 3. Optimised parameter sets for APCI-MS obtained using expert and smart mode for the reference material sinigrin and glucotropaeolin, and for the glucobrassicin in a broccoli sample (identified based on retention time and MS/MS spectra).

Parameter	Range	Expert mode			Smart mode		
		DS-sinigrin	DS-gluco-tropaeolin	DS-gluco-brassicin	DS-sinigrin	DS-gluco-tropaeolin	DS-gluco-brassicin
Target Mass $(M+H)^+$ (*m/z*)		280	330	369	280	330	369
Oct 1 DC (V)	20 to 1.8	7.17	7.47	7.47	12.0	12.0	12.0
Oct 2 DC (V)	7.1 to 0	1.86	2.10	1.86	1.7	1.7	1.7
Trap Drive		35.31	40.23	43.51	43.1	43.1	43.1
Cap Exit (V)	300 to 50	62.30	74.59	90.98	112.0	115.8	118.7
Capillary (V)		−4500	−4500	−4500	−4500	−3500	−3500
Oct RF (Vpp)	50 to 300	50.00	82.79	121.87	148.8	150.0	150.0
Skimmer (V)	100 to 15	15.00	19.18	15.00	40.0	40.0	40.0
Lens 2 (V)	−10 to −100	−55.74	−70.49	−60.16	−60.0	−60.0	−60.0
Lens 1 (V)	0 to −15	−4.43	−4.92	−4.43	−5.0	−5.0	−5.0

gation resulted in similar sensitivity, which might be due to its low concentration in the samples. For DS-glucoiberin and DS-neoglucobrassicin the sensitivity could be increased by using either 6 µA or 10 µA, but for DS-glucoraphanin 10 µA was most effective. Considering the ranking order reveals a corona amperage of 6 µA to be most suitable. This is in the range of results used by Griffiths *et al.* [12], who used 5 µA and Tolrá *et al.* [10] who found 8 µA to be most suitable.

All further optimisation procedures for APCI were done using an evaporation temperature of 300°C and a corona amperage of 6 µA.

Conditions for APCI-MS were optimised in smart and expert mode. The parameters obtained using the target masses for DS-sinigrin, DS-glucotropaeloin and DS-glucobrassicin are shown in Table 3. Measurements with broccoli samples showed that the optimisation for DS-glucobrassicin using the expert mode was most suitable (Fig. 6). Peak areas of GS of broccoli measured in expert mode were generally higher than the peak areas measured in smart mode. Lower values in "Oct 1 DC" with 7 V, in "Cap Exit" with 91 V, in "Oct RF" with 122 V and a Skimmer voltage with 15 V obtained within expert mode in comparison with smart mode seem to increase the peak areas of GS measured with HPLC-APCI-MS.

3.1.3 Comparison of the sensitivity of HPLC-ESI-MS and HPLC-APCI-MS

Although ESI-MS is a powerful tool for identifying highly polar, heat labile compounds such as GS present as ions in solution [9] the direct comparison of the peak areas of DS-GS of a broccoli and a bai cai sample, each obtained with optimised HPLC-APCI-MS parameters and HPLC-ESI-MS parameters, demonstrates that APCI-MS is much more sensitive for the investigated aliphatic, aromatic and indole DS-GS than ESI-MS under the investigated conditions (Figs. 7 and 8). With the exception of DS-4-methoxyglucobrassicin, for which the peak areas obtained by both interfaces were in the same range, employing HPLC-APCI-MS resulted in 3- (DS-glucobrassicanapin) to 7-fold (DS-glucoraphanin) larger peak areas in comparison to HPLC-ESI-MS.

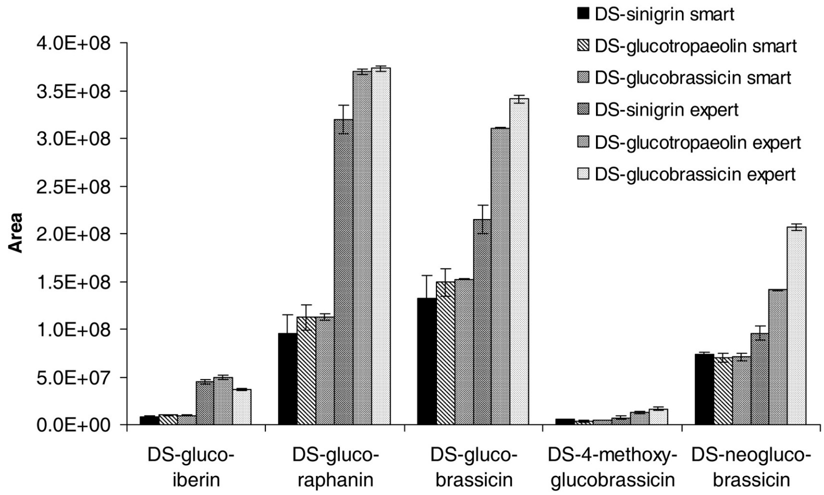

Figure 6. Peak area (means ± SD) obtained for different GS of broccoli with diverse optimised parameter sets for APCI-MS.

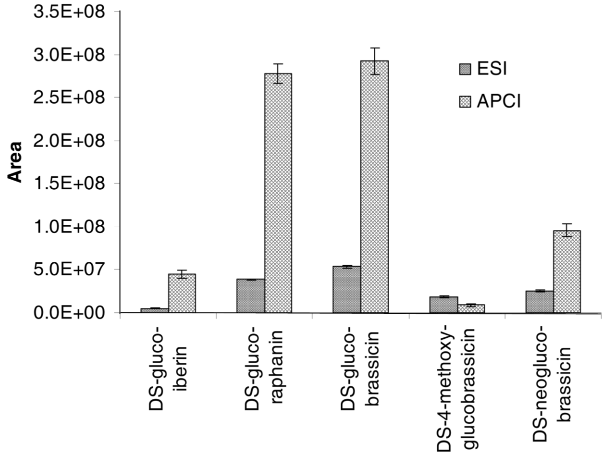

Figure 7. Peak area obtained for different GS of broccoli employing either HPLC-ESI-MS or HPLC-APCI-MS.

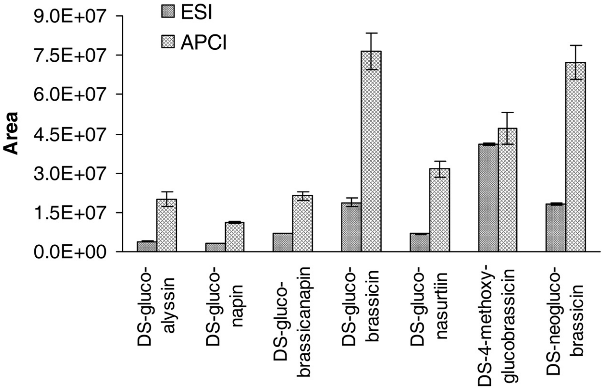

Figure 8. Peak area (means ± SD) obtained for different GS of bai cai employing either HPLC-ESI-MS or HPLC-APCI-MS.

3.2 Comparison of HPLC-ESI-MS/MS and HPLC-APCI-MS/MS for structure determination

As APCI-MS proved to be much more sensitive for DS-GS as ESI-MS it was investigated whether APCI-MS would also be advantageous for structure determination. The mass spectra of each DS-GS measured with HPLC-ESI-MS and HPLC-APCI-MS were characterised by the protonated molecular ions $(M+H)^+$ and the group-specific fragment ions $(M+H\ -C_6H_{10}O_5)^+$ which dissociate the glucose molecule (Tables 4 and 5). These typical ions were also found by other authors [12, 16–18]. Using HPLC-ESI-MS $(M+Na)^+$ and $(M+K)^+$ were also found. The biggest advantage of the ion trap is its possibility to perform MS^n measurements, which are essential for structure determination of unknown substances, or for identification in cases where either standards are not available or substances have the same molecular weight. The results of the MS/MS measurements after fragmentation of $(M+H)^+$ are shown in Tables 4 and 5. The most intense signal in the MS/MS spectra, irrespective of the interface, is the fragment ion corresponding to the protonated molecular ion with the loss of a sugar group $(M+H\ -C_6H_{10}O_5)^+$. The relative intensities, with respect to the dominant peak, of the observed fragments are identical for both interfaces in the MS and MS/MS spectra in the majority of cases. Interestingly, APCI-MS/MS does not yield to more fragments than ESI-MS/MS, although the intensity of the fragments gained with APCI-MS is higher than of those obtained with ESI-MS/MS except DS-4-methoxyglucobrassicin.

The aliphatic sulphinyl-containing DS-GS (DS-glucoiberin, DS-glucoraphanin, DS-glycoalyssin) showed more fragments than the aliphatic alkenyl DS-GS (DS-gluconapin, DS-glucobrassicanapin). Depending on the side chain R the MS/MS spectra of sulphinyl DS-GS contained the corresponding empirical ion $(RCNH)^+$ besides the $(M+H\ -C_6H_{10}O_5)^+$ ion. Kiddle *et al.* [16] found $(CH_3SO(CH_2)_3CNH)^+$ and $(CH_3SO(CH_2)_4CNH)^+$ for DS-glucoiberin (3-methylsulphinylpropyl-DS-GS) and DS-glucoraphanin (4-methylsulphinylbutyl-DS-GS), respectively,

Table 4. Comparison of the signal intensity of the fragments obtained with ESI-MS and APCI-MS for the DS-GS in a broccoli sample.

DS-GS	*m/z*	Signal intensity		Fragment[a]	References
		ESI	APCI		
DS-glucoiberin					
	MS 382	316 391	–	$(M+K)^+$	
	366	126 205	–	$(M+Na)^+$	
	344	464 338	2 095 294	$(M+H)^+$	[12]
	182	388 872	394 802	$(M+H-C_6H_{10}O_5)^+$	[12]
	148	–	145 263	$(RCNOH)^+$	[12, 17]
	118	122 860	101 568		
	105	–	102 676	$(R)^+$	[12, 17]
	MS/MS 311	12 539	39 375	$(M+H-33)^+$	
	182	116 117	324 525	$(M+H-C_6H_{10}O_5)^+$	[12]
	118	26 581	60 418		
	132	10 668	31 227	$(RCNH)^+$	[16]
DS-glucoraphanin					
	MS 396	519 053	–	$(M+K)^+$	
	380	325 159	–	$(M+Na)^+$	
	358	4 016 249	20 664 609	$(M+H)^+$	[12]
	325	218 418	–	$(M+H-33)^+$	
	196	3 175 827	3 118 067	$(M+H-C_6H_{10}O_5)^+$	[12]
	162	–	1 060 510	$(RCNOH)^+$	[12]
	146	–	3 052 708	$(RCNH)^+$	[16]
	136	–	2 338 048	$R(OH)^+$	[12]
	132	216 956	–		
	MS/MS 325	161 630	981 575	$(M+H-33)^+$	
	196	1 135 611	6 856 294	$(M+H-C_6H_{10}O_5)^+$	[12]
	146	56 872	296 590	$(RCNH)^+$	[16]
DS-glucobrassicin					
	MS 407	116 647	–	$(M+K)^+$	[19]
	391	137 390	–	$(M+Na)^+$	[19]
	369	3 142 921	16 904 047	$(M+H)^+$	[12]
	207	3 766 510	4 083 987	$(M+H-C_6H_{10}O_5)+$	[12, 17]
	174	93 459	–	$(RCNOH+H)^+$	[17]
	130	263 939	2 961 801	$(R)^+$	[17]
	MS/MS 207	1 519 408	9 100 522	$(M+H-C_6H_{10}O_5)+$	[12, 17]
	174	58 426	281 897	$(RCNOH+H)^+$	[17]
	130	109 059	548 564	$(R)^+$	[17]
DS-4-methoxyglucobrassicin					
	MS 437	49 526	–	$(M+K)^+$	[19]
	421	65 045	–	$(M+Na)^+$	[19]
	399	1 642 834	919 110	$(M+H)^+$	[12]
	244	–	70 097		
	237	680 632	54 452	$(M+H-C_6H_{10}O_5)^+$	[12]
	160	173 134	302 304	$(R)^+$	[12, 17]
	MS/MS 237	833 230	366 798	$(M+H-C_6H_{10}O_5)^+$	[12]
	160	260 774	122 492	$(R)^+$	[12, 17]
DS-neoglucobrassicin					
	MS 437	118 724	–	$(M+K)^+$	
	421	142 718	–	$(M+Na)^+$	
	399	393 638	6 594 568	$(M+H)^+$	[12]
	237	855 179	493 184	$(M+H-C_6H_{10}O_5)^+$	[12]
	160	–	288 609	$(R)^+$	[12]
	130	206 994	151 369	$(R-CH_3O+H)^+$	[12]
	MS/MS 237	659 775	2 181 042	$(M+H-C_6H_{10}O_5)^+$	[12]
	205	44 306	136 816	$(RCNOH+2H)^+$	[17]
	177	48 309	165 565	$(ROH)^+$	[12]
	144	95 774	277 851		
	130	207 654	586 727	$(R-CH_3O+H)^+$	[12]

a) tentative identification

Table 5. Comparison of the signal intensity of the fragments obtained with ESI-MS and APCI-MS for the DS-GS in a bai cai sample.

DS-GS	*m/z*	Signal intensity		Fragment[a)]	References
		ESI	APCI		
DS-glucoalyssin					
	MS 410	86 587	–	$(M+K)^+$	
	394	120 668	–	$(M+Na)^+$	
	372	639 142	2 150 777	$(M+H)^+$	
	210	366 691	244 865	$(M+H-C_6H_{10}O_5)^+$	
	MS/MS 339	36 919	159 623	$(M+H-33)^+$	
	210	165 194	722 960	$(M+H-C_6H_{10}O_5)^+$	
	160	7 247	–	$(RCNH)^+$	
	142	18 397	67 001		
DS-gluconapin					
	MS 332	100 737	–	$(M+K)^+$	
	316	159 282	–	$(M+Na)^+$	
	294	115 862	805 421	$(M+H)^+$	[12, 16]
	132	344 762	908 174	$(M+H-C_6H_{10}O_5)^+$	[12, 16]
	MS/MS 132	124 585	134 360	$(M+H-C_6H_{10}O_5)^+$	[12, 16]
DS-glucobrassicanapin					
	MS 346	99 758	–	$(M+K)^+$	
	330	162 366	–	$(M+Na)^+$	
	308	232 442	1 155 039	$(M+H)^+$	[12, 16]
	146	489 985	1 017 046	$(M+H-C_6H_{10}O_5)^+$	[12, 16]
	MS/MS 146	80 661	211 729	$(M+H-C_6H_{10}O_5)^+$	[12, 16]
DS-gluconasturtiin					
	MS 382	82 888	–	$(M+K)^+$	
	366	132 823	–	$(M+Na)^+$	
	344	427 843	2 041 270	$(M+H)^+$	[12, 16]
	182	547 034	959 034	$(M+H-C_6H_{10}O_5)^+$	[12, 16]
	105	83 819	–		
	MS/MS 182	169 985	1 128 831	$(M+H-C_6H_{10}O_5)^+$	[12, 16]

a) tentative identification

DS-indole GS were also identified in bai chai. These are the same DS-indole GS as already described for broccoli (Table 4).

as base peaks using chemical ionisation MS. In contrast, Griffiths *et al.* [12] found the base peak in the spectrum of DS-glucoiberin (3-methylsulphinylpropyl-DS-GS) at *m/z* 149, which corresponded to an ion with the empirical formula $(RCNOH+H)^+$, while in the APCI spectrum of DS-glucoraphanin (4-methylsulphinylbutyl-DS-GS) the base peak was at *m/z* 136, which corresponded to an empirical ion $(ROH)^+$. Furthermore, we found ions with relative high intensity at *m/z* 311, *m/z* 325 and *m/z* 339 in the MS/MS spectra of DS-glucoiberin (3-methylsulphinylpropyl-DS-GS), DS-glucoraphanin (4-methylsulphinylbutyl-DS-GS) and DS-glucoalyssin (5-methylsulphinylpentyl-DS-GS) respectively, which could correspond to an empirical ion $(M+H-33)^+$ with the loss of SH- from the parent ion.

The fragmentation patterns of the indole GS DS-glucobrassicin (3-indolymethyl-DS-GS) and DS-4-methoxyglucobrassicin (4-methoxyindol-3-ylmethyl-DS-GS) were also characterised by fragment ions at *m/z* 130 and *m/z* 160, respectively, which correspond to the ion $(R)^+$. This $(R)^+$ ion was found by Hogge *et al.* [17] using thermospray-LC-MS and Griffiths *et al.* [12] using APCI-LC-MS as the major fragments for these indole GS. In contrast to DS-4-methoxyglucobrassicin (4-methoxy-3-indolylmethyl-DS-GS), in the MS/MS spectrum of DS-neoglucobrassicin (1-methoxy-3-indolylmethyl-DS-GS) the most intense fragment beside the group-specific fragment ions $(M+H - C_6H_{10}O_5)^+$ was a fragment ion at *m/z* 130 that has previously been attributed to the loss of CH_3O- from the side chain R [12]. This fragment ion resulted in *m/z* to that produced by DS-glucobrassicin (3-indolylmethyl-DS-GS). This indicates that the CH_3O- attached to the nitrogen atom in the indole group was lost more easily than in position 4. Further ions related to the side chain R were found in the MS/MS spectrum of DS-neoglucobrassicin (1-methoxy-3-indolylmethyl-DS-GS) while the corresponding $(R)^+$ ion was found only in the MS spectrum obtained by APCI-MS.

4 Concluding remarks

The present study shows that the sensitivity of the APCI interface is superior to the ESI interface under the conditions investigated, although the latter is more appropriate for the analysis of highly polar, heat labile compounds. The more extensive optimisation required for APCI is rewarded by a gain in sensitivity. However both interfaces lead to fragments that allow for the identification of aliphatic, aromatic and indole DS-GS and the fragments observed with both interfaces are generally identical. Thus the higher sensitivity of APCI-MS appears to be the only benefit.

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