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Rapid profiling of intact glucosinolates in *Arabidopsis thaliana* leaves by UHPLC-QTOFMS using a charged surface hybrid column

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Short abstract

A rapid method based on ultra-high pressure liquid chromatography-quadrupole time-of-flight mass spectrometry is proposed for the analysis of twenty-one intact glucosinolates (GS) in plant Arabidopsis leaf samples. Several sub-2 µm supports were tested and a novel charged surface hybrid column (CSH) was found highly suitable for the adequate retention and separation of GS. The developed method was successfully applied to quantify changes in GS levels in response to insect herbivory in Arabidopsis.

Abstract

Introduction – The analysis of glucosinolates (GS) is traditionally performed by reverse-phase liquid chromatography coupled to ultraviolet detection after a time-consuming desulfation step, which is required for increased retention. Simpler and more efficient alternative methods that can shorten both sample preparation and analysis are much needed.

Objective – To evaluate the feasibility of using ultra-high pressure liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UHPLC-QTOFMS) for the rapid profiling of intact GS.

Methodology – A simple and short extraction of GS from *Arabidopsis thaliana* leaves was developed. Four sub-2 μm reverse-phase columns were tested for the rapid separation of these polar compounds using formic acid as chromatographic additive. High resolution QTOFMS was used to detect and identify GS.

Results – A novel charged surface hybrid (CSH) column was found to provide excellent retention and separation of GS within a total running time of 11 minutes. Twenty-one GS could be identified based on their accurate mass as well as isotopic and fragmentation patterns. The method was applied to determine the changes in GS content that occur after herbivory in Arabidopsis. In addition, we evaluated its applicability to the profiling of other Brassicaceae species.

Conclusion – The developed method can profile the full range of GS, including the most polar ones, in a shorter time than previous methods, and is highly compatible with mass spectrometric detection.

INTRODUCTION

Glucosinolates (GS) are sulfur-containing secondary metabolites that are almost exclusively found in the plant order Capparales. They all share a similar basic structure, namely a sulfonated oxime group, a thioglucose moiety and a variable side chain derived from amino acids (Halkier and Gershenzon 2006). More than 120 individual GS have been identified, mainly in plant species of the Brassicaceae family (Fahey *et al.* 2001). In plant cells, intact GS are stored separately from the activating enzyme myrosinase (Bones and Rossiter 2006). Upon tissue disruption, e.g. following herbivory, both components are brought into contact, which results in the liberation of high amounts of biologically active breakdown products, such as nitriles, isothiocyanates, and thiocyanates (Rask *et al.* 2000). In addition to their fundamental role in plant-insect relationships (Hopkins *et al.* 2009; Muller *et al.* 2010), GS are also known for their anticarcinogenic properties (Verhoeven *et al.* 1997; Shapiro *et al.* 2001).

Different methods for the analysis of GS have been reported in the literature. Among them, reverse-phase HPLC-UV of enzymatically desulfated GS is a well-established and efficient technique (Reichelt *et al.* 2002). The desulfation step decreases the polarity of GS and improves their chromatographic resolution. However, the sample preparation is time-consuming, and faster methods have therefore been evaluated for the analysis of intact GS. The separation of intact GS is challenging because these compounds are poorly retained on reverse-phase material and their sulfated group makes them chromatographically unfavorable. In particular the most polar glucoiberin and glucoraphanin are problematic (West *et al.* 2002). Hydrophilic interaction chromatography (HILIC) (Troyer *et al.* 2001; Wade *et al.* 2007) or ion pairing LC-MS using triethylamine/formate as an additive (Zrybko *et al.* 1997) have been successfully used to overcome this issue. Trifluoroacetic acid has also been reported to be a suitable buffer for GS separation (Mellon *et al.* 2002). Although volatile and compatible with MS detection, these additives can lead to strong ion suppression and as a result minor GS may be overlooked. Capillary electrophoresis has been proposed as an interesting alternative to liquid chromatography, in combination with UV (Karcher and El Rassi 1999) or MS (Bringmann *et al.* 2005) detection. Overall, a major limitation still remains: separations take long (25-80 min for a single analysis) and prevent the high throughput analysis of numerous samples.

In this study, ultra-high pressure liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry (UHPLC-QTOFMS), a more powerful but also more expensive technology than HPLC-UV, was evaluated for the rapid profiling of intact GS in *Arabidopsis thaliana*. To shorten the whole analytical process, sample preparation time was reduced to a minimum and various sub-2µm columns were compared for their ability to adequately retain and separate GS in the shortest possible time. Using state-of-the-art QTOFMS, 21 GS could be identified and absolutely or relatively quantified in Arabidopsis leaves within a total running time of 11 minutes.

EXPERIMENTAL

Chemicals: HPLC grade methanol (MeOH, Sigma, Buchs, Switzerland) and milliQ water (Millipore, Zug, Switzerland) were used for extraction and semi-preparative LC. The solvents and additive used for UHPLC-QTOFMS were water, acetonitrile (ACN),

and formic acid ULC/MS from Biosolve (Valkenswaard, The Netherlands). Sinalbin and glucobrassicin were obtained from Applichem (Darmstadt, Germany). Glucoraphanin was purchased at Chemos GmbH (Regenstauf, Germany). Glucoerucin and glucohirsutin were purified from an Arabidopsis extract according to the method described below.

Plant treatment: *Arabidopsis thaliana* accessions Col-0 and L*er*, *Eruca sativa*, and *Brassica nigra* were grown as described previously (Reymond *et al.* 2000). *Brassica oleracea* var. *gemmifera* was grown for 2 months in a greenhouse. *Brassica oleracea* var. *italica* was obtained from a local grocery shop. Eggs of *Spodoptera littoralis* were obtained from Syngenta (Stein, Switzerland). For treatment with insects, 7 three-week-old plants were challenged for 48 h with 2 neonate *S. littoralis* larvae per plant. 200 mg of leaves (at least one leaf per plant) was weighed, transferred in a 2 mL Eppendorf tube and immediately flash-frozen in liquid nitrogen for GS extraction. Unchallenged plants were used as controls. Three biological replicates were done.

Extraction: The protocol for GS extraction was adapted from Schlaeppi et al. (Schlaeppi *et al.* 2008). 200 mg of frozen leaf powder was ground with a glass rod in a 13 mL tube (Sarstedt, Nümbrecht, Germany) and 25 µL of a solution of sinalbin at 1.56 mM (internal standard, IS) as well as 1.975 mL of ice-cold MeOH/water (70:30, v/v) were immediately added. After homogenization for 30 sec at full speed (Polytron Kinematica, Lucerne, Switzerland), samples were incubated for 15 min at 80°C in a block heater (Techne dri-block, Staffordshire, UK). Extracts were cooled down at room temperature, centrifuged at 3500 g for 10 min and the supernatant was transferred to an appropriate vial for analysis.

Glucosinolate analysis: UHPLC-QTOFMS analyses were performed on an Acquity UPLC^{TM} from Waters (Milford, MA) interfaced to a Synapt G2 QTOF from Waters with electrospray ionization. Four sub-2 µm columns were evaluated: Inertsil ODS-4, 100x2.1mm i.d., 2 µm (GL Sciences, Tokyo, Japan), Pinnacle DB C18, 100x2.1mm i.d., 1.9 µm (Restek, Bellefonte, PA), Acquity BEH C18 100x2.1mm i.d., 1.7 µm (Waters), and Acquity CSH C18 100x2.1mm i.d., 1.7 µm (Waters). Glucosinolates were separated using gradient elution under the following conditions: Solvent A = water + 0.05 % formic acid; Solvent B = ACN + 0.05% formic acid; 2-45% B in 6 min, 45-100% B in 0.5 min, holding at 100% B for 2 min followed by re-equilibration at 2% B for 2.5

min. The flow rate was 400 µL/min. The temperature of the column was maintained at 25°C. One µL was injected. Negative ion data were acquired from 85 to 1200 Da in the so-called MS^E mode using alternating scans of 0.3 s at a collision energy of 4 eV and 0.3 s at a collision energy of 25 eV applied on the trap region of the T-wave cell. The electrospray capillary voltage was set to –2500 V and the cone voltage to –40 V. The source temperature was maintained at 120°C and the desolvation gas temperature at 350°C. The desolvation gas flow was set to 800 L/hr. Argon was used as a collision gas and infused at a flow of 2.1 mL/min into the collision cell. The mobile phase was diverted to waste from 7 to 11 min at the end of the gradient. Exact mass measurements were provided by infusing a solution of leucin-enkephalin at 400 ng/mL at a flow rate of 10 µL/min through the Lock Spray™ probe. Glucosinolates were quantified using standard curves from standard solutions containing each the IS at a concentration of 19.5 µM. Matrix effects were estimated by comparing responses of control extracts, extracts spiked with GS solutions and standard solutions dissolved in MeOH/water (70:30 v/v).

Purification of glucoerucin and glucohirsutin: About 500 g of fresh Arabidopsis leaves were extracted in 2.5 L of MeOH containing 0.5% formic acid. After filtration and evaporation to dryness, the residue was partially redissolved in 5 mL of MeOH/water (30:70, v/v), centrifuged and filtered through a 0.2 µm filter (Agilent Technologies, Palo Alto, CA). GS purification was carried out on an XTerra MS C18 semi-preparative column (150x19 mm i.d., 5 µm) with a 1525 EF HPLC pump from Waters equipped with a UV detector (2487, Waters). The flow rate was 8 mL/min. GS were eluted using a gradient mobile phase composed of A: water + 0.05% formic acid and B: MeOH; 0-3 min 2% B, 3-40 min 2-30% B, 40-45 min 30-100% B, 45-55 min 100% B, 55-75 min 2% B. Eight successive injections of 500 µL were performed. Eight mL fractions were collected every minute in a FC203B fraction collector (Gilson, Madison, WI) and subsequently analyzed by UHPLC-QTOFMS. Fractions containing glucoerucin and glucohirsutin were pooled, evaporated and their purity was verified by nuclear magnetic resonance using a Bruker Avance 400 (400 MHz) spectrometer.

Data processing: Data were processed using Masslynx™ v.4.1. Peak picking was performed using Markerlynx™ XS with the following parameters: initial and final retention times 0.0-7.0 min, mass range 85-1200 Da, intensity threshold 500 counts,

XIC window 0.03 Da, retention time window 0.1 min. MassFragment^{TM} was employed for matching collision-induced fragments with known GS structures.

RESULTS AND DISCUSSION

Sample preparation

First, a rapid and reproducible extraction procedure was devised. Arabidopsis Col-0 leaves were harvested, weighed and instantly flash frozen under liquid nitrogen. The internal standard (IS) as well as ice-cold methanol 70% were added and the samples were immediately ground, heated at 80°C and extracted for 15 min to deactivate myrosinase. Care was taken that the plant material did not thaw before the solvent was added. Alternative grinding and extraction methods may be used to increase throughput, e.g. use of a 96-well shaker (Kliebenstein *et al.* 2001), provided that myrosinases remain inactive. Samples were centrifuged and the supernatant was finally recovered and directly injected in UHPLC-QTOFMS. Contrary to usual procedures, we decided to avoid a time-consuming evaporation-dissolution step, which may increase the variability of the extraction. Skipping this step might affect the sensitivity (because of dilution factor) and chromatographic performances (because of high solvent strength). However, we hypothesized that the high sensitivity of QTOFMS allows for direct injection of limited volumes of crude extracts that can minimize peak broadening and distortion (see below).

Optimization of chromatography

To our knowledge, few studies have reported the use of UHPLC-MS for GS analysis. Gratacos-Cubarsi et al. proposed a targeted UHPLC-DAD-MS/MS method for the simultaneous determination of GS and phenolics (Gratacos-Cubarsi *et al.* 2010). However, the running time that was required was still quite long (30 min) due to the broad range of detected compounds. Another study used UHPLC coupled to a single quadrupole for GS relative quantification, but little information about the methodology was given (Sawada *et al.* 2009). In the present study, we aimed at taking advantage of UHPLC performances to develop a short and simple screening method for GS analysis using QTOFMS detection. A commercial UHPLC system withstanding a backpressure of 1000 bars was employed. Four C18 columns of different selectivity

were evaluated (Table 1). The choice of the mobile phase was driven by the need for speed and acceptable retention and resolution. Water and ACN were selected as elution solvents since this combination leads to the lowest possible backpressure, allowing for higher flow rates. A flow rate of 400 µL/min was applied to maintain the pressure at max. 800 bars throughout the gradient. Formic acid, which is a common additive highly compatible with MS detection, was tested at a concentration of 0.05% (v/v) to minimize possible ion suppression effects. An injection volume of only 1 µL was selected because the injection of a solution stronger than the initial mobile phase composition can lead to important peak broadening and distortion, in particular of early-eluting peaks. Figure 1 presents the base peak intensity (BPI) chromatograms for the four columns tested. Visible peaks corresponding to known glucosinolates are numbered according to their elution order on the CSH column. BEH, IODS and PDB columns gave similar separations although some peak inversions were observed. The main limitation of these 3 columns was the poor retention of glucoiberin (**1**), glucoraphanin (**2**), and of the internal standard sinalbin (IS). Glucoiberin almost eluted in the solvent peak and glucoraphanin, which is very concentrated in Arabidopsis, even split in two distinct peaks (Figure 1A-C). The internal standard sinalbin gave broad and somewhat distorted peaks. As a result, precise measurements of peak areas were not possible for these early-eluting compounds. While appropriate for less polar GS, these 3 columns were clearly not suitable for such polar compounds under the conditions employed. Moreover, the IODS column exhibited severe bleeding even at the low % of ACN employed, which increased baseline noise (Figure 1B). On the other hand, the charged surface hybrid (CSH) column gave quite contrasting results: the retention for GS was much stronger and also the selectivity was different (Figure 1D). While the column dead time was about 0.5 min, the first eluting GS, glucoiberin (**1**), eluted at 1.91 min. Glucoraphanin (**2**) gave one symmetrical and sharp peak, as did sinalbin (IS). This was attributed to the fact that with CSH technology, low-ionic strength additives such as formic acid can advantageously replace TFA and still preserve peak shapes that otherwise would get distorted (Fountain and Hewitson 2011). Using a linear gradient from 2-45% B, the majority of peaks were baseline separated and all GS eluted in less than 7 min (Figure 2). The total chromatographic run took only 11 min, including washing and re-equilibration steps. Hence, the CSH column was found highly suitable for the rapid separation of GS including the most polar glucoiberin and glucoraphanin.

Detection and identification of GS in Arabidopsis leaf extracts

QTOFMS was selected for the detection and identification of GS in Arabidopsis Col-0 extracts because i) it is able to measure masses with high accuracy, enabling the determination of elemental composition, ii) the rapid acquisition rate of QTOFMS makes it an ideal detector in combination with UHPLC, which usually provides very sharp peaks, iii) the dynamic range of the most recent QTOFMS has been much extended, which is an essential feature for GS analysis (the variation in GS concentration in Arabidopsis can be as large as three orders of magnitude), and iv) due to the way it operates, QTOFMS is adapted to perform non-targeted analyses of natural compounds and may potentially lead to the discovery of new GS. Since glucosinolates contain characteristic nitrogen and sulfur that produce typical molecular formula as well as isotopic and fragmentation patterns, it is possible to identify them by QTOFMS with a high degree of confidence even in the absence of pure standards.

To detect GS in Arabidopsis leaf extracts, UHPLC-QTOFMS data were first submitted to a peak picking procedure (see Experimental). This led to a list of 540 ions at given retention times. Each peak was then manually processed to assess whether it could be attributed to a GS. The evaluation criteria were i) typical sulfur isotopic pattern, ii) elemental composition containing both sulfur and nitrogen atoms (a tolerance of 3 ppm was accepted between experimental and calculated masses), iii) typical collision-induced fragments such as *m/z* 96.9596 for the sulfate moiety and others (e.g. *m/z* 259.0124, $C_6H_{11}O_9S$). Online databases (CHEMnetBASE-dictionary of natural products, KNapSAcK etc.) and previous publications (Bringmann *et al.* 2005; Cataldi *et al.* 2007) were consulted to identify known GS. MassFragment^{TM} was finally used to match the identified chemical structures with the obtained collision-induced fragments. Using this procedure, 21 GS were identified in Arabidopsis leaf extracts (Table 2). The mass spectra of glucoraphanin obtained at low and high collision energy are shown as an example in Figure 3A and B respectively. The main fragments are displayed in Figure 3C. Extracted ion chromatograms (EIC) for every GS are presented in Figure 4. In three EIC (*m/z* 477.063, 402.089 and 416.104), two isomers were present. Thanks to different mass spectra (Figure 5), the first eluting peak at *m/z* 477.0633 (RT 4.58 min) could be identified as methoxyglucobrassicin (**13a**) while the second eluting peak

at *m/z* 477.0632 corresponded to neoglucobrassicin (**13b**, RT 5.16 min) with a prominent (M-H-OCH3)$^-$ fragment at *m/z* 446.0453 (Mellon *et al.* 2002; Cataldi *et al.* 2007). The intensity of the ions at *m/z* 402.089 (**14a** and **14b**) and 416.104 (**16a** and **16b**) was very low and fragmentation spectra gave no useful information. Based on reported data (Botting *et al.* 2002; Bringmann *et al.* 2005; Cataldi *et al.* 2007), **14a/b** and **16a/b** were tentatively annotated as aliphatic GS with straight or branched chains containing 6, respectively 7 carbons.

Extraction recovery and quantification of GS

GS quantification was done with the use of internal standard (IS) calibration. Sinalbin was selected as an appropriate IS since i) it is naturally absent from Arabidopsis and ii) its structure is similar to that of the studied GS. To determine the extraction recovery, samples were spiked with the IS before or after extraction of Arabidopsis samples at identical concentration. Yields superior to 97% were obtained. Four major GS, namely glucoraphanin, glucoerucin, glucohirsutin and glucobrassicin could be absolutely quantified based on calibration curves obtained from pure standards. Calibration points were at 0.2, 1, 5, and 20 µg/mL. The response was linear over this range of concentrations (R^2>0.999). Limits of quantification were 60 pg for glucoerucin and glucohirsutin and 50 pg for glucobrassicin and glucoraphanin. No matrix effect was observed for either these four molecules or the IS. It should be noted that the difference in ionization efficiency for the four standards and the IS did not exceed 20%, which raises the possibility that all GS may be quantified based on a single calibration curve. However, this could not be verified and the analysis of the other 17 GS remained semi-quantitative since pure standards for these natural products were not available. Methylsulfinylalkyl-glucosinolates were quantified as glucoraphanin equivalents, methylthioalkyl-glucosinolates as glucoerucin equivalents and indole glucosinolates as glucobrassicin equivalents. For gluconasturtiin (**12**) and aliphatic GS with straight or branched chains (**14a/b**, **16a/b**), relative quantification was performed. By using pure calibration standards for each GS, a fully quantitative method for all GS could be easily implemented. Care was taken that all GS from every analyzed sample did not exceed the linearity domain of QTOFMS.

Analysis of Arabidopsis leaves challenged with *Spodoptera littoralis* larvae

To validate the developed method, GS levels were measured in Arabidopsis leaves that were challenged for two days with neonate larvae of the noctuid moth *S. littoralis*. It is known that GS accumulate in response to insect attack (Mewis *et al.* 2005; Schlaeppi *et al.* 2008) and these data provide a good baseline for comparison. In accordance with published data, herbivory caused a significant increase of the major aliphatic- and indole-GS, including glucoraphanin (4MSOB), glucohirsutin (8MSOO), glucoerucin (4MTB), and glucobrassicin (I3M, Figure 6). In addition, we could also detect a significant increase for less abundant GS, illustrating the power of the detection method, and indicating that herbivory leads to an important accumulation of the majority of GS in Arabidopsis.

Profiling of other plant tissues and species

To verify whether the method may be applied to other plant tissues and species, leaves of *Brassica oleracea* var. *gemmifera*, *Brassica nigra*, *Eruca sativa*, *Arabidopsis thaliana* accession L*er*, infloresence of *Brassica olerace* var. *italica*, and seeds of *Arabidopsis thaliana* accession Col-0 were analyzed. Several known GS which were not present in Arabidopsis Col-0 leaves could be detected and identified. For instance, the very polar 3-hydroxypropyl-glucosinolate and 4-hydroxybutyl-glucosinolate were well retained on the CSH column, eluting as symmetrical peaks at 1.85 min and 1.97 min, respectively. BPI chromatograms as well as a list of all GS found in the different samples are presented in Supporting Information. It should be noted that, in some cases, the concentrations of certain predominant GS exceeded the linearity domain of the mass spectrometer (e.g. sinigrin in *Brassica nigra*, or 4-mercaptobutyl-glucosinolate in *Eruca sativa*). To properly quantify them, smaller amounts of starting plant material should therefore be used.

CONCLUSION

We are not aware of any report on the use of charged surface hybrid (CSH) particles for the UHPLC analysis of plant metabolites. This new technology was found to be very efficient for the separation of intact GS in extracts from Brassicaceae species, including the most polar ones, without the need for ion-pairing agents or buffers that are poorly

compatible with MS detection. Compared to conventional methods, a significant reduction of the chromatographic time was obtained. By combining UHPLC with QTOFMS, we could identify 21 GS in Arabidopsis leaves and precisely measure their accumulation in wild-type plants after insect feeding. The developed method will provide the analytical support for the characterization of several Arabidopsis mutants and for studying the effect of herbivory on their GS contents. GS biosynthesis has been shown to display a large natural variation between Arabidopsis accessions (Kliebenstein *et al.* 2001). A further exploration of this diversity in Arabidopsis and other Brassicaceae will inevitably require the analysis of a large number of samples and we expect that our accurate and fast method will be a useful tool for this kind of study.

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FIGURE LEGENDS

Figure 1: Base peak intensity (BPI) UHPLC-QTOFMS chromatograms of an Arabidopsis Col-0 leaf extract using four different sub-2µm columns. A. Acquity BEH C18, B. Inertsil ODS-4, C. Pinnacle DB C18, D. Acquity CSH C18. Peaks corresponding to glucosinolates are labeled according to Table 2. IS, internal standard.

Figure 2: Base peak intensity (BPI) UHPLC-QTOFMS chromatogram at 20% intensity obtained on the Acquity CSH C18 column. Peaks corresponding to glucosinolates are labeled according to Table 2. IS, internal standard.

Figure 3: High resolution mass spectra of glucoraphanin obtained at A. low collision energy (4 eV), and B. high collision energy (25 eV). C. Mass spectral fragmentation pattern for glucoraphanin.

Figure 4: Extracted ion chromatograms (EIC) for the 21 identified GS. EIC are numbered according to the list of glucosinolates presented in Table 2.

Figure 5: High resolution mass spectra obtained at low collision energy (4 eV) for A. methoxyglucobrassicin (**13a**), and B. neoglucobrassicin (**13b**).

Figure 6: Quantification of Arabidopsis Col-0 GS levels in response to herbivory. Plants were challenged for two days with *S. littoralis* larvae (black bars). Unchallenged plants were used as controls (white bars). Values (±SE) are the mean of three biological replicates. Significant differences between control and treated plants are indicated (Student's t-test, * P<0.05, **P<0.01, ***P<0.001). A. Methylsulfinylalkyl-GS were quantified as glucoraphanin (4MSOB) equivalents, except glucohirsutin (8MSOO) for which a pure standard was available. B. Methylthioalkyl-GS were quantified as glucoerucin (4MTB) equivalents. C. Indole-GS were quantified as glucobrassicin (I3M) equivalents. D. 2-phenylethyl-, 4-methylpentyl-, n-hexyl-, iso-hexyl-, and n-heptyl-GS were quantified relative to the IS sinalbin. For GS abbreviations see Table 2. FW, fresh weight.

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Table 1: sub-2µm chromatographic supports employed in this study. Abbreviations reported in this table are used throughout the manuscript.

Column	Abbreviation	Manufacturer	dimensions (mm)	particle size (µm)
Acquity BEH C18	BEH	Waters	2.1x100	1.7
Inertsil ODS-4	IODS	GL Sciences	2.1x100	2
Pinnacle DB C18	PDB	Restek	2.1x100	1.9
Acquity CSH C18	CSH	Waters	2.1x100	1.7

Table 2: List of glucosinolates identified in Arabidopsis accession Col-0 leaves. For isomers, “a” and “b” labels have been arbitrarily employed. For compound structures, readers can refer to (Cataldi *et al.* 2007) or (Bringmann *et al.* 2005). RT, retention time. IS, internal standard.

No	RT (min)	*m/z*	Formula	error (ppm)	fragments	Systematic name (abbreviation)	Common name
1	1.86	422.0248	$C_{11}H_{20}NO_{10}S_3$	0.1	358, 259, 196, 96	3-Methylsulfinylpropyl- (3MSOP)	Glucoiberin
2	2.03	436.0402	$C_{12}H_{22}NO_{10}S_3$	0.9	372, 259, 178, 96	4-Methylsulfinylbutyl- (4MSOB)	Glucoraphanin
3	2.30	450.0564	$C_{13}H_{24}NO_{10}S_3$	0.4	386, 192, 96	5-Methylsulfinylpentyl- (5MSOP)	Glucoalyssin
4	2.68	464.0725	$C_{14}H_{26}NO_{10}S_3$	1.2	400, 96	6-Methylsulfinylhexyl- (6MSOH)	Glucohesperin
5	3.13	478.0878	$C_{15}H_{28}NO_{10}S_3$	0.6	414, 96	7-Methylsulfinylheptyl- (7MSOH)	Glucoibarin
6	3.20	406.0298	$C_{11}H_{20}NO_9S_3$	0.5	96	3-Methylthiopropyl- (3MTP)	Glucoiberverin
7	3.62	492.1036	$C_{16}H_{30}NO_{10}S_3$	0.8	428, 234, 96	8-Methylsulfinyloctyl- (8MSOO)	Glucohirsutin
8	3.70	420.0460	$C_{12}H_{22}NO_9S_3$	0.7	259, 96	4-Methylthiobutyl- (4MTB)	Glucoerucin
9	4.09	447.0530	$C_{16}H_{19}N_2O_9S_2$	0.4	259, 205, 96	Indol-3-ylmethyl- (I3M)	Glucobrassicin
10	4.09	463.0486	$C_{16}H_{19}N_2O_{10}S_2$	1.1	96	4-Hydroxyindol-3-ylmethyl- (OH-I3M)	Hydroxyglucobrassicin
11	4.32	434.0602	$C_{13}H_{24}NO_9S_3$	2.5	259, 96	5-Methylthiopentyl- (5MTP)	Glucoberteroin
12	4.40	422.0582	$C_{15}H_{20}NO_9S_2$	0.7	96	2-Phenylethyl- (2PE)	Gluconasturtiin
13a	4.46	477.0633	$C_{17}H_{21}N_2O_{10}S_2$	1.0	259, 96	4-Methoxyindol-3-ylmethyl- (4MOI3M)	Methoxyglucobrassicin
14a	4.75	402.0895	$C_{13}H_{24}NO_9S_2$	0.5	96	C6-aliphatic glucosinolate[a]	
14b	4.87	402.0894	$C_{13}H_{24}NO_9S_2$	0.7	96	C6-aliphatic glucosinolate[a]	
15	5.00	448.0760	$C_{14}H_{26}NO_9S_3$	2.2	96	6-Methylthiohexyl- (6MTH)	Glucolesquerellin
13b	5.02	477.0632	$C_{17}H_{21}N_2O_{10}S_2$	1.2	446, 96	1-Methoxyindol-3-ylmethyl- (1MOI3M)	Neoglucobrassicin
16a	5.52	416.1044	$C_{14}H_{26}NO_9S_2$	1.2	96	C7-aliphatic glucosinolate[a]	
16b	5.64	416.1047	$C_{14}H_{26}NO_9S_2$	0.5	96	C7-aliphatic glucosinolate[a]	
17	5.69	462.0922	$C_{15}H_{28}NO_9S_3$	0.9	245, 96	7-Methylthioheptyl- (7MTH)	
18	6.36	476.1075	$C_{16}H_{30}NO_9S_3$	1.7	336, 96	8-Methylthiooctyl- (8MTO)	
IS	2.75	424.0377	$C_{14}H_{18}NO_{10}S_2$	1.2	259, 182, 96	4-Hydroxybenzyl-	Sinalbin

[a] tentative annotation of compound class

Figure 1

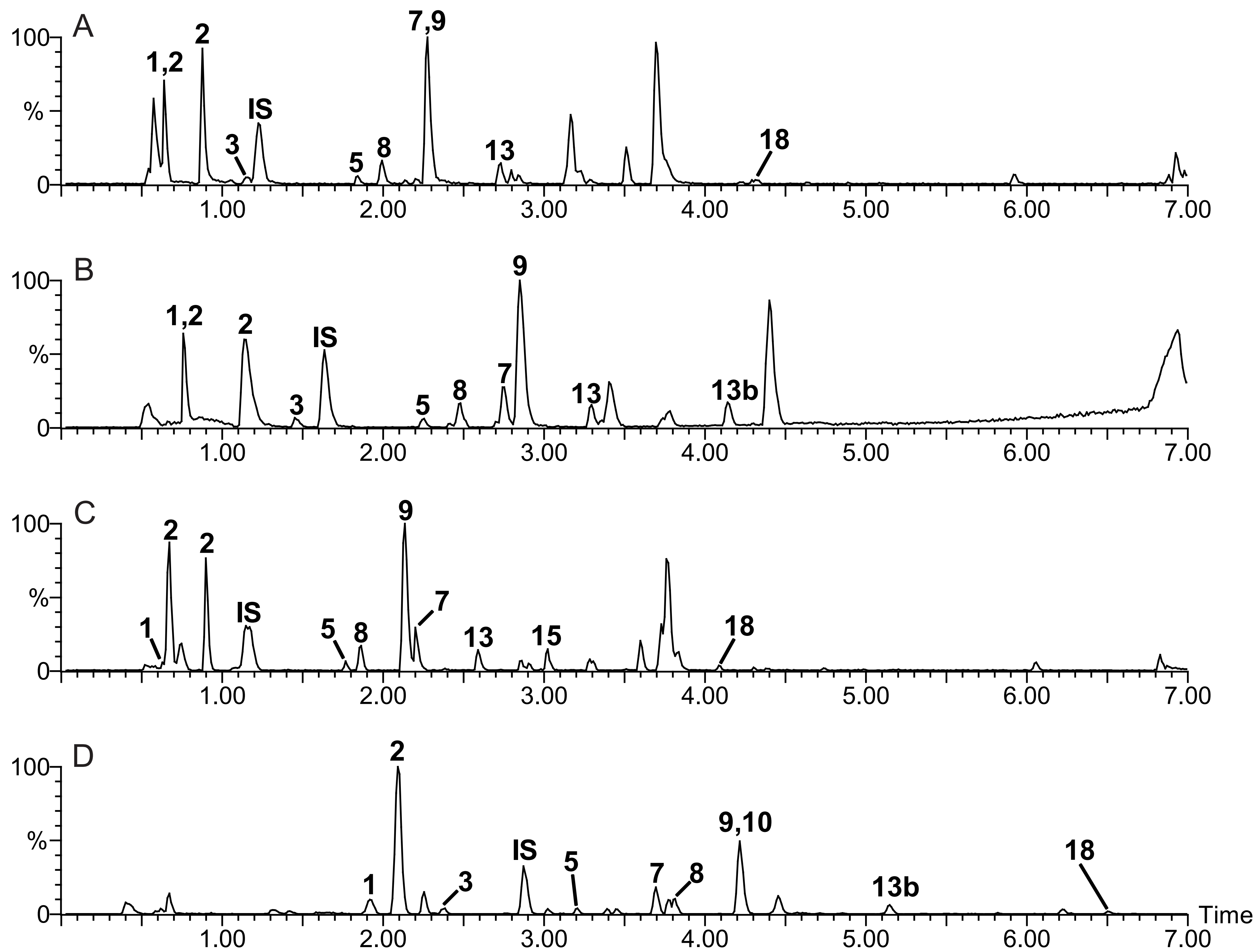


Figure 2

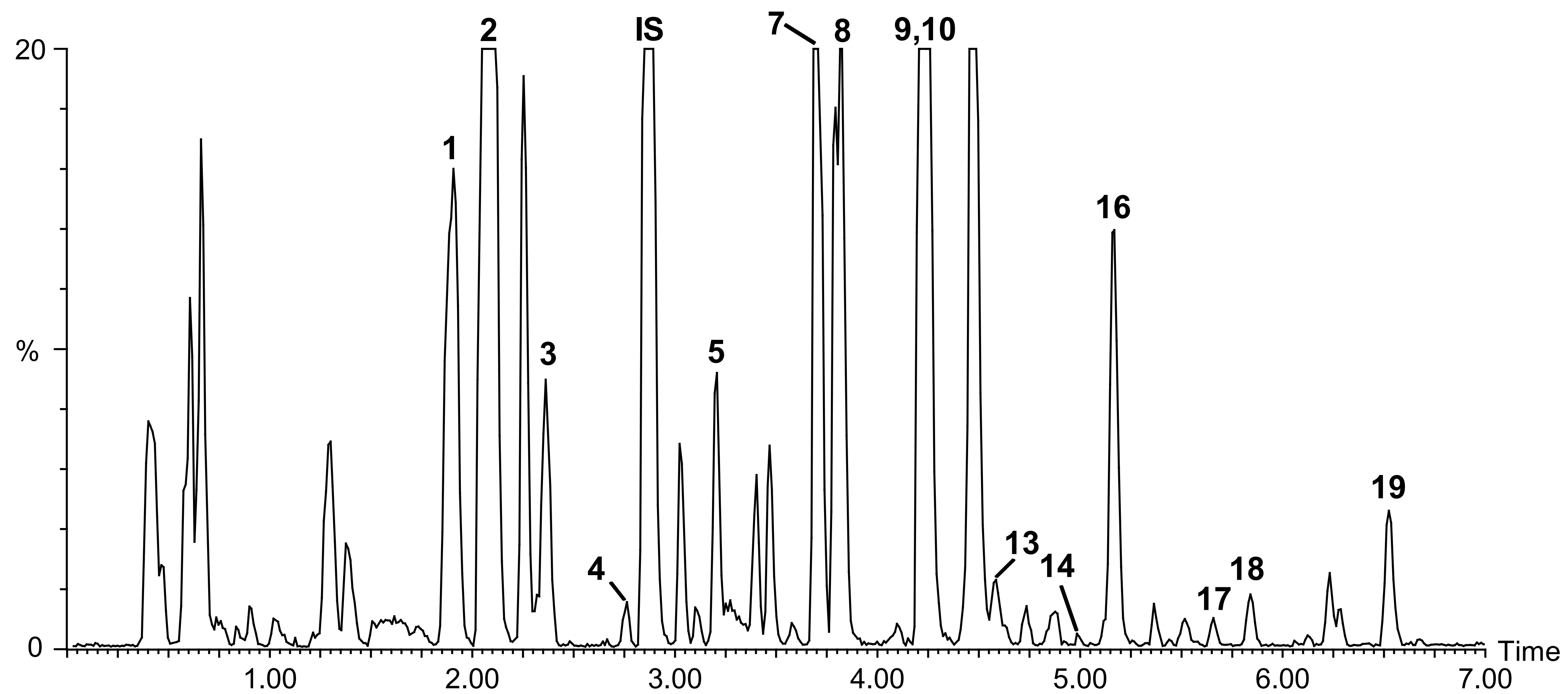


Figure 3

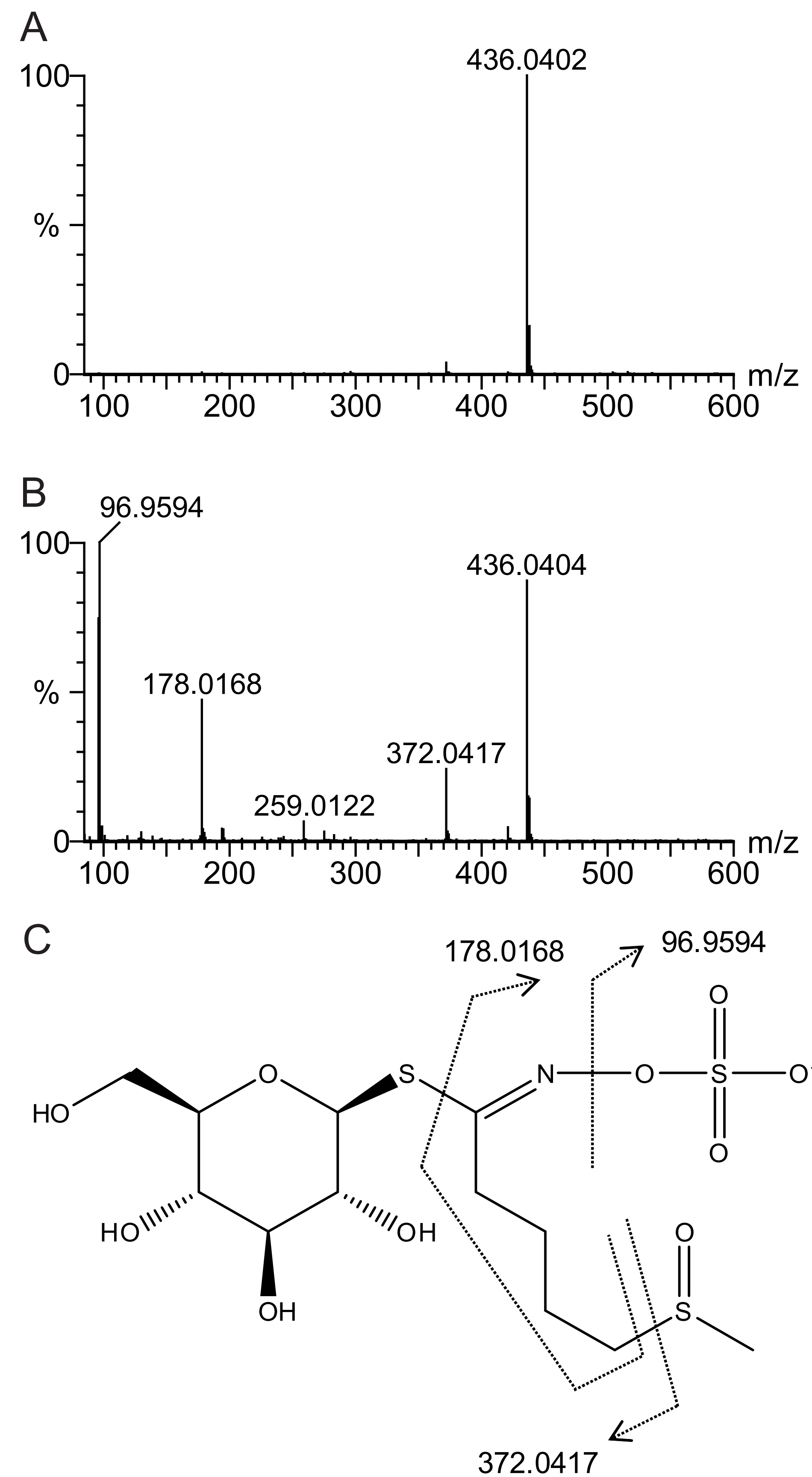


Figure 4

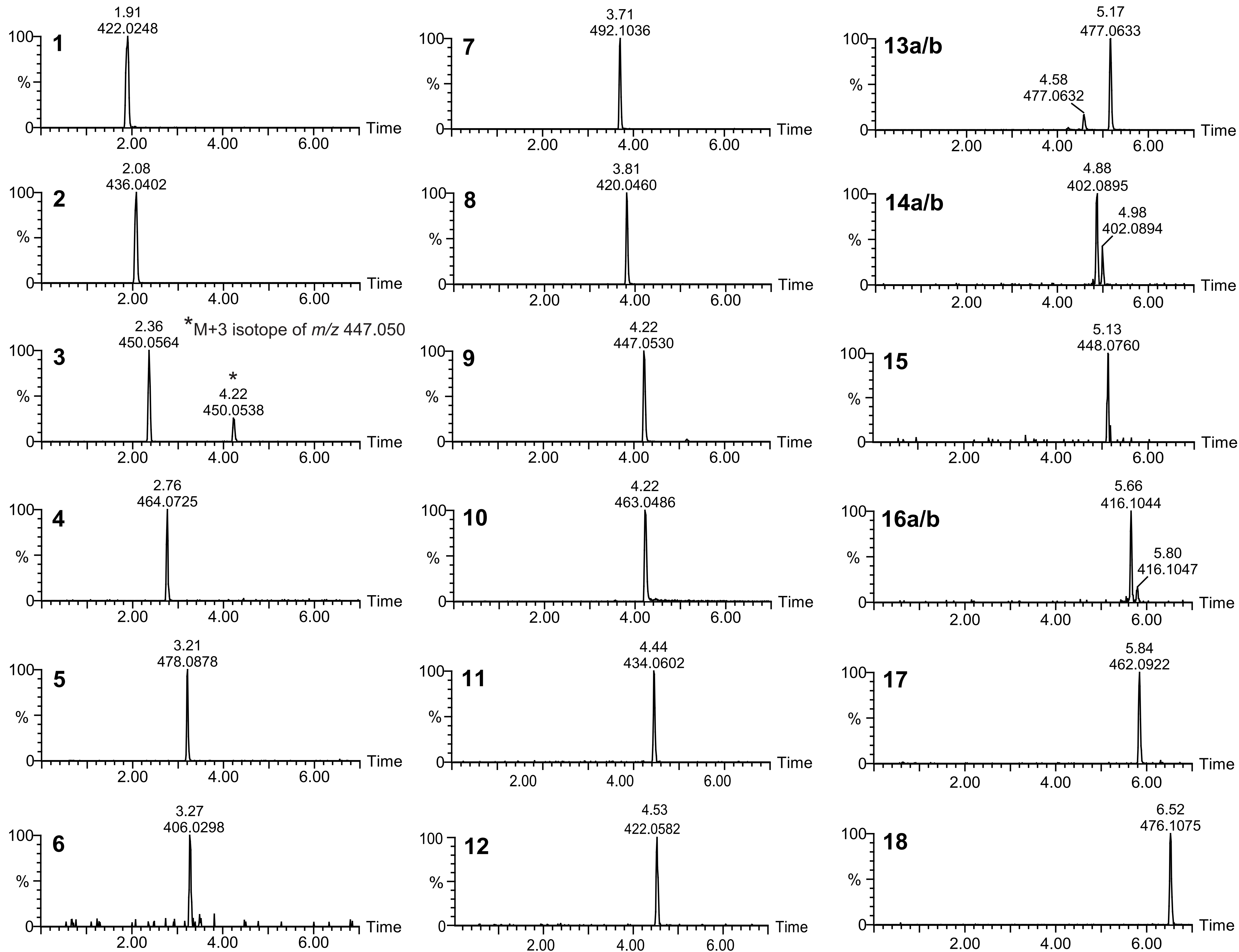
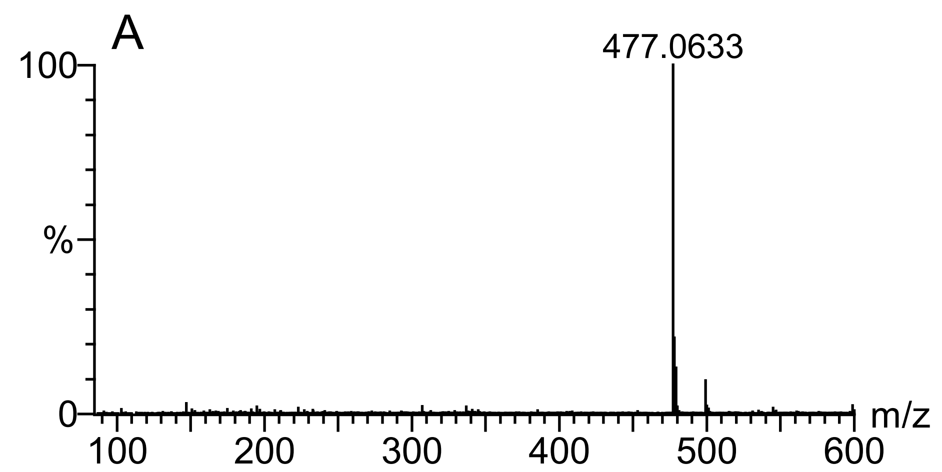


Figure 5



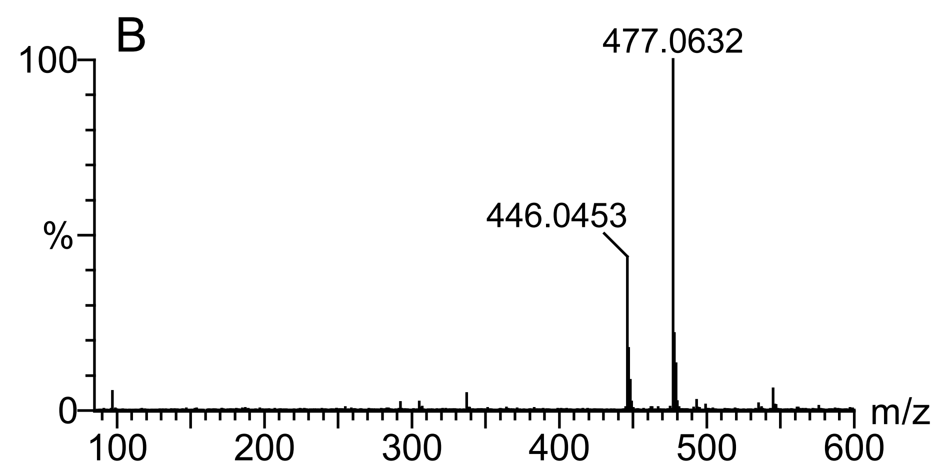


Figure 6