DETERMINATION OF INTACT GLUCOSINOLATES IN BRASSICA EXTRACTS BY HPLC/FRIT -FAB MASS SPECTROMETRY

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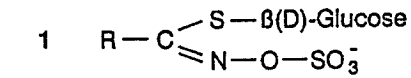
INTRODUCTION

Intact glucosinolate anions (1) in rapeseed extracts can be separated and quantified by means of HPLC without desulphation of the glucosinolates. The separation can be achieved by reversed-phase (RP) HPLC using programmed elution with aqueous solution of a neutral salt and acetonitrile (ACN) and by UV-monitoring of the eluent (Björkqvist and Hase 1988; Kokkonen et al. 1989).



This simple separation method of the plant extracts produces HPLC chromatograms in which all extracted compounds with reasonable UV-extinction coefficient are present. The known UV-spectra of different types of glucosinolates and the retention times can be used to distinguish between individual glucosinolates in the extracts. Some of the non-glucosinolate compounds in the extracts however have similar UV-spectra as the glucosinolates and the differences in retention times are small and confusing. In this study combined HPLC/frit-FAB mass spectrometry was used to screen glucosinolate anions from other compounds and to distinquish between different glucosinolates. Quantitation was done by UV monitoring using the published (Möller et al. 1985) relative response factors of glucosinolates and desulfoglucosinolates.

Positive ion and negative ion FAB mass spectra of separated glucosinolates and glucosinolate mixtures have been published earlier (Fenwick et al. 1982) and the HPLC/frit-FAB technique was used in identification of three intact glucosinolates in Brussel sprouts (Kokkonen et al. 1989).

EXPERIMENTAL

Liquid Chromatography

The HPLC system consisted of a Hewlett-Packard (HP) 1090 chromatograph and a UV detector operating with a filter at 230 nm wavelength. A 20 µl constant volume loop injector was used for sample injection. Column was a LiChrospher 100 RP-18 (250 mm x 4 mm ID, 5 µm particle size) column from HP. The glucosinolates were separated using an eluent flowrate of 1,5 ml/min. Mobile phase A contained 1 % of glycerol and 99 % of aqueous 0.1 M ammonium acetate (NH_4Ac) solution. Mobile phase B contained 1 % of glycerol, 49.5 % of 0.1 M aqueous NH_4Ac solution and 49,5 % of ACN (vol/vol %). The gradient used was as follows: 1 min A 100 %, 10 min B from 0 % to 50 % and 5 min B from 50 % to 100 %. The column was at ambient temperature. A pneumatic Jeol MS-PNS splitter was used to reduce the flow going into the mass spectrometer to 2 µl/min.

Sinigrin monohydrate was used as internal and external standard. Sinigrin was dissolved in deionized water.

Mass spectrometry

The HPLC splitter system was connected to the stainless steel Jeol frit-FAB probe by a 60 μm i.d. fused silica capillary tube. A Jeol SX 102 double focusing mass spectrometer equipped with a HP 9000/375+ computer system was used for data collection. The FAB spectra were obtained in the negative ion mode by scanning from mass 350 to mass 550 with a speed of 3 s/cycle. Acceleration voltage of 10 kV was used and the resolution of 1000 was observed. The Jeol FAB gun with xenon gas was operated at 3 kV. Liquid nitrogen trap was used at the top of the diffusion pump and the temperature of the ion source was kept at 57 °C to improve the evaporation of the mobile phase.

Materials

NH_4Ac and glycerol (p.a. and anhydrouds, extra pure) were from Merck, Darmstadt, F.R.G and ACN (HPLC grade) was from Rathburn Chemicals Ltd, Walkesburn, G.B. Sinigrin monohydrate was from Karl Roth, Karlsruhe, F.R.G. One variety of *Brassica napus* seeds (sample 1, total glucosinolate content of 28.2 μmol/g) and pad-walls and two varieties of *Brassica campestris* seeds (sample 2 and sample 3 with total glucosinolate content of 12.4 and 28.2 μmol/g, respectively, determined by X-ray fluorescence method (Schnug and Haneklaus 1990; Creutz and Hase 1990)) were used in screening and examination of the glucosinolates. The recommended method (Colza and Rape Seed 1986) was used in the extraction of the plant material (200 mg). The extraction according to this method is performed using 70% aqueous methanol solution (2 ml) at 80 °C. The extraction is repeated after the centifugion and separation of the first extract. The combined extracts are diluted to 10 ml using deionized water. This solution was used for analysis without further treatment.

Determination of the Total Glucosinolate Content in the Samples by X-Ray Fluorescence Spectroscopy

The total glucosinolate content of the samples was determined by the indirect X-ray fluorescence method that is based on the linear correlation of sulphur content and total glucosinolate content in the rapeseed material (Schnug and Haneklaus 1990).

RESULTS AND DISCUSSION

Fig. 1 shows the HPLC chromatogram of sample 2 recorded by UV detector at 230 nm vawelength. The chromatogram shows many peaks including identified and unidentified intact glucosinolates. Intact glucosinolates give mass spectra with the glucosinolate anion (1) molecular ion as the strongest peak in the spectrum in negative-ion mode. Thus FAB mapping of the negative ions was performed by direct inlet of the dried extracts to screen for the glucosinolates present in the samples. Fig. 2 shows the FAB mapping diagram of negative ions in sample 3 between m/z 300 and 600, the m/z area of intact glucosinolate anions. Many anions of known intact glucosinolate anions, i.e. 372, 386, 420, 434, 463 are seen in Fig. 2, but also anions that can not easily be interpreted are present. M/z 307 and 341 are probably fragment ions of other anions.

The ions present in FAB mapping diagram can be plotted as a function of the HPLC retention time using the HPLC/frit-FAB MS system in the SCAN mode. Fig. 3 shows the HPLC/frit-FAB chromatogram of the most eminent ions present in sample 2. The retention times and identification of the ions are also showed in Table 1. The peaks in Fig.3 indicating the most abundant glucosinolates are quan-

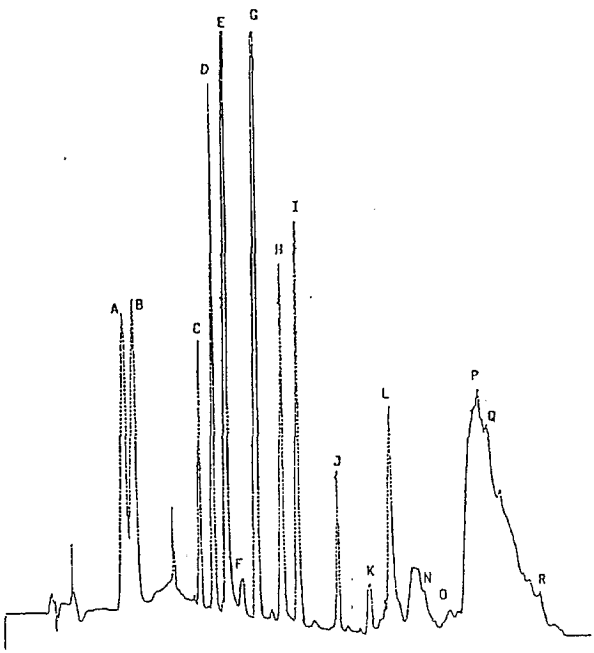


Fig.1. An UV detected liquid chromatogram of sample 2. Peak labeling is interpreted in Table 1.

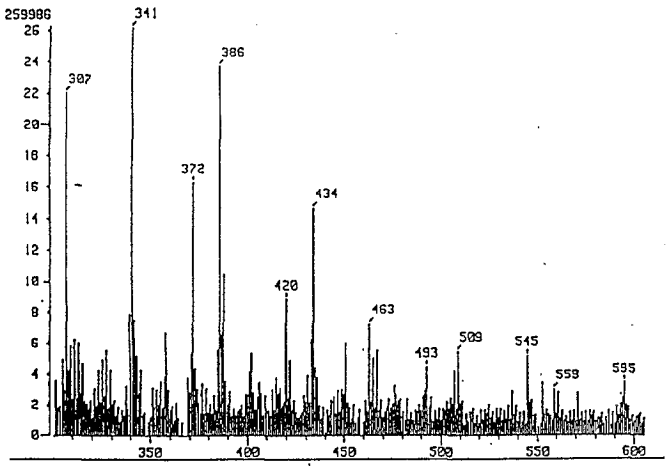


Fig.2. Direct inlet FAB mapping of sample 3. Negative ions between m/z 300 and 600 are shown.

tified from the corresponding UV detected HPLC chromatogram of Fig.1 using the published relative response factors. The results and labeling of Fig. 1 are shown in Table 1. Ions 465, 467, 493, 507, 509, 552, 559, 561, 571 and 595 detected in FAB mapping are not shown in Table 1.

Negative ion FAB mass spectra of intact glucosinolates consist mainly of the glucosinolate ion (1) (Kokkonen et al. 1989; Fenwick et al. 1982). The HSO_4^- (m/z 97) is mostly the second largest peak. Since the HSO_4^- should be a common fragment ion for all glucosinolates the screening of the glucosinolates can also be done by monitoring of the ion m/z 97. In some glucosinolate FAB spectra in negative ion mode the m/z 97 however is absent as can be seen of the published FAB mass spectrum of glucotropaeolin (Fenwick et al. 1982). Thus the monitoring of m/z 97 can not alone be used in screening of the glucosinolates in plant extracts. It is striking that compounds corresponding peaks E, F, J and L in Fig. 1 give no anions and not a fragment anion m/z 97 which shows that the corresponding compounds are not glucosinolates.

This paper indicates the HPLC/frit-FAB MS technique to be a relevant and fast identification method for intact glucosinolates, especially when the obtained chromatographic resolution for the mass chromatograms is now relatively good do to improved interface and column. We are also attempting to quantify the glucosinolates using this technique.

A draw-back of the HPLC/frit-FAB MS technique is that the SCAN mode has a relatively low sensitivity. Injected amounts of 0.5 to 2 µg of individual glucosinolates were used to obtain HPLC mass chromatograms. This was a magnitude smaller than raported earlier (Kokkonen et al. 1989) and in the same level as used in the thermospray MS (Hogge et al. 1988). For plant samples with very low content of glucosinolates i.e. the pad-walls of *Brassica napus* this technique is too insensitive to produce any results. The monitoring by UV spectrometry is by 2 orders of magnitude more sensitive technique in detection and quantitation of the glucosinolates. To extend the HPLC/frit-FAB MS technique for quantitation of the glucosinolates real time selected ion monitoring (SIM) mode of the technique was used. Fig. 4 shows the result of monitoring ions m/z 372 (gluconapin), 386 (glucobrassica napin) and 388 (progoitrin) in sample 2. M/z 358 (sinigrin) was added in the sample as internal standard. The retention times in Fig. 4 are not identical with those in Fig. 1 and 3, since in the SIM screening overlapping of the peaks is not hazardous and the chromatography can be done using a faster eluent program.

The experiments performed using the HPLC/frit-FAB MS SIM technique for glucosinolate anions selected by FAB mapping shows that this technique is by a magnitude more sensitive than the corresponding technique in the SCAN mode and sensitive enough to be used for quantitative analysis of glucosinolates in plant extracts.

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Table 1.

Anions detected by direct inlet FAB MS mapping and their corresponding retention times in scan mode. Peak labeling correspond to Fig. 1 and Fig. 3.

Ion m/z	Ret. time in ionizati-on cham-ber, min	Labelling from Fig. 1 and Fig. 3b	Identification	Quantified as µmol/g samples		
				1	2	3
358	3.9	B[a]	sinigrin			
372	5.7	D	gluconapin	2.6	5.5	1.2
386	7.4	H	glucobrassica-napin	2.4	4.8	3.0
388	3.7	A	progoitrin	10.4	11.5	1.2
402	5.4	C	napoleiferin	0.5	1.3	0.3
402	10.4	M		0.3[b]	-	-
420	7.7	I	glucoerucin	2.1	1.5	1.0
422	9.3	K	gluconasturtin	-	0.3	0.1
434	9.2	K	glucoberteroin	0.2	0.2	0.2
435	10.7	N		-	1.0[b]	0.5[b]
450	5.2	C	glucoalyssin	0.5	1.0	0.3
451	12.7	Q		-	0.2[b]	0.1[b]
463	6.9	G	4-hydroxy-glucobrassicin	3.8	4.0	3.5
530	13.7	R			0.2[b]	0.1[b]
537	11.0	O		0.1[b]	0.5[b]	0.9[b]
545	11.6	P		0.2[b]	0.2[b]	0.1[b]

a) internal standard

b) estimated using relative response factor compared to sinigrin as 1.00

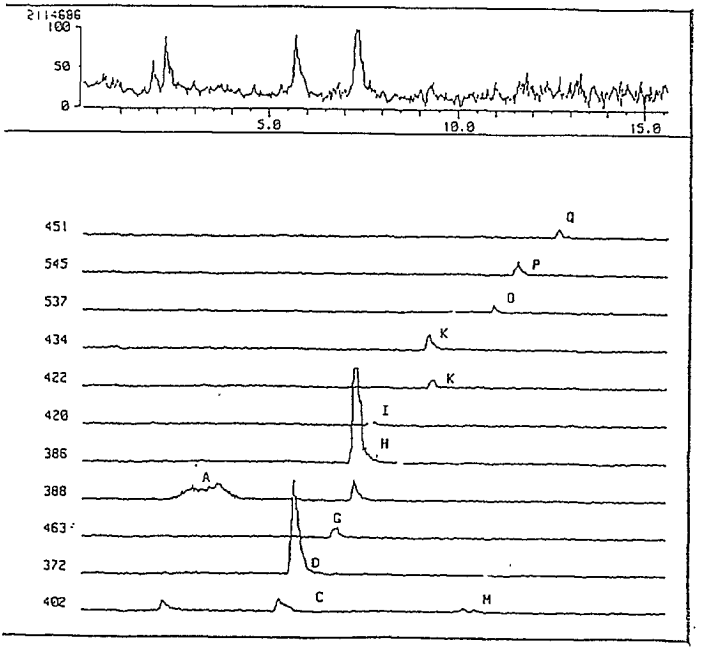

Fig.3. An HPLC/FAB mass chromatogram of sample 2. Glucosinolate anions found are shown.

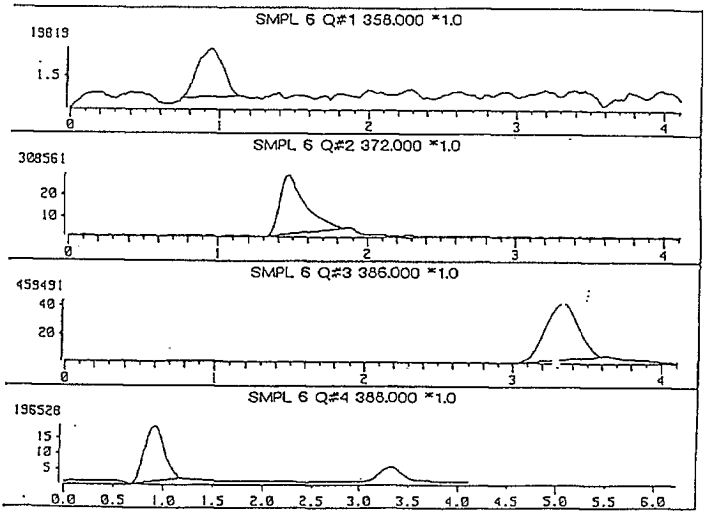

Fig.4. An HPLC/FAB selected ion monitoring of four main glucosinolate anions (m/z 358, 372, 386 and 388).