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Factors influencing the separation and quantitation of intact glucosinolates and desulphoglucosinolates by micellar electrokinetic capillary chromatography

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ABSTRACT

Micellar electrokinetic capillary chromatography methods using cetyltrimethylammonium bromide as a surfactant have been developed for the qualitative and quantitative determination of intact glucosinolates and desulphoglucosinolates. The influence of changes in separation conditions has been investigated. A great number of different intact glucosinolates and desulphoglucosinolates have been used for the development of efficient separation conditions for the closely related, but structurally different, compounds. Repeatability and linearity of the quantitative analyses have been evaluated, and critical parameters have been determined. Rapid and efficient separations are possible for glucosinolates in crude extracts and for mixtures of glucosinolates isolated from seeds and the vegetative parts of plants.

INTRODUCTION

Glucosinolates are plant products with well defined structures, and more than 100 glucosinolates are known [1]. Glucosinolates and degradation products of glucosinolates are of great importance for the quality of food and feed based on glucosinolate-containing plants (*e.g.* oilseed rape, cabbage and kale). High concentrations of these compounds in food and feed results in antinutritive, toxic and off-flavour effects [1,2]. This limits the possible use of economically important crops such as oilseed rape. To determine the glucosinolate content in feed, food and the various plant species containing glucosinolates, there is a need for reliable, fast and inexpensive methods of analyses for individual glucosinolates.

Available methods for the determination of individual glucosinolates include gas chromatography (GC) and high-performance liquid chromatography (HPLC), where HPLC has several advantages over GC [1,3]. However, HPLC methods suffer from some disadvantages. Columns are expensive and sensitive to sample impurities, the chemicals are relatively expensive, the time of each analysis is typically 50–60 min, and the peak capacity and resolution obtainable are relatively low compared with that of capillary electrophoresis. An inexpensive, fast, simple and low detection limit method is therefore required.

Since the introduction of micellar electrokinetic capillary chromatography (MECC) by Terabe *et al.* in 1984 [4], this technique has been developed into very powerful and advantageous methods for the separation of various charged and uncharged compounds [5–10]. Over 700 000 theoretical plates per metre of capillary have been obtained [8]. With the experience gained from the use of ion-paired reversed-phase HPLC for the determination of glucosinolates [1,11] it was decided to investigate the use of MECC for the separation of glucosinolates.

The MECC method using cetyltrimethylammonium bromide (CTAB) has been introduced for the separation of glucosinolates [12]. It allows efficient

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separation of various intact glucosinolates in samples obtained after a fast and simple purification and concentration step or even in the crude extracts of plants [12,13]. Furthermore, uncharged desulphoglucosinolates and various phenolic compounds can also be analysed by this technique [13,14].

This paper describes the aspects of qualitative and quantitative determinations of intact glucosinolates and desulphoglucosinolates. This includes the evaluation of the method by studying the influences of different separation conditions on migration times, peak areas, resolution, and the number of theoretical plates obtained with a wide variety of closely related but structurally different glucosinolates. The migration order of various glucosinolates is presented and discussed. Critical parameters for the repeatability and linearity of the qualitative and quantitative analyses have been determined. Furthermore, detection limits and sample solvent effects, from crude extracts or isolated samples from seeds and vegetative parts of plants, are described.

EXPERIMENTAL

Apparatus

Three different capillary electrophoresis instruments were used. The ABI Model 270A capillary electrophoresis system (Applied Biosystems, Foster City, CA, USA) was used with a 720 mm × 50 μm I.D. fused-silica capillary and detection at 500 mm from the injection end of the capillary. Data processing was performed on a Shimadzu Chromatopac C-R3A instrument (Kyoto, Japan). The Dionex capillary electrophoresis system I (Dionex, Sunnyvale, CA, USA) was used with a 650 mm × 50 μm I.D. fused-silica capillary. The detection window was 600 mm from injection end of the capillary, and data were processed by manufacturer-supplied software on an IBM PS/2 computer. The Spectra PHORESIS 1000 System (Spectra-Physics, San Jose, CA, USA) had a 690 mm × 50 μm I.D. fused-silica capillary. The detection was at 620 mm from the injection end of the capillary. Data were processed with manufacturer-supplied software on an IBM PS/2 computer.

Materials and reagents

Glucosinolates (potassium salts) from the collection in this laboratory were used [1]. The com-

pounds have been extracted from various plants and isolated as intact glucosinolates or desulphoglucosinolates [1]. Determination of glucosinolate purity and identification have been based on paper chromatography, high-voltage electrophoresis, UV and NMR spectroscopy, and HPLC [1,15]. The rapeseed used was Danish-grown single and double low spring rape of different varieties (Gulliver, Ceres, Global and Line). Isolation and purification of intact glucosinolates were performed according to Bjerg and Sørensen [16] and Sørensen [1].

Sodium tetraborate and sodium phosphate were from Sigma (St. Louis, MO, USA). CTAB was from BDH (Poole, UK). All chemicals were of analytical-reagent grade.

Procedure

Buffer preparation for the CTAB system consisted of stock solutions of (1) sodium tetraborate (100 mM), (2) sodium phosphate (150 mM) and (3) CTAB (100 mM). The run buffers were mixed from these stock solutions, water was added to the desired concentration, adjusted to pH 7.0, and filtered through a 0.45-μm membrane filter before use.

Buffers were changed manually on the ABI instrument after various numbers of analyses. On the Spectra-Physics instrument, buffers were changed at the detection end of the capillary between each analysis and after various numbers of analyses at the injection end. On the Dionex instrument, buffers were changed at both ends of the capillary between each analysis. Samples were introduced from the cathodic end of the capillary. Sample injections were carried out either by vacuum for 1 s (ABI) or for 6 s (Spectra-Physics), or by a hydrodynamic process at 150 mm for 25 s (Dionex). Separations were performed at 20 kV with negative polarity at the injection end unless stated otherwise. The temperatures were 30°C (ABI and Spectra-Physics) and room temperature (Dionex) unless stated otherwise. On-column UV detection was at 235 nm unless other wavelengths are stated.

The columns were washed with buffer between each analysis for 4–7 min. After various numbers of analyses, the capillary was washed for 2–4 min with 1.0 and 0.1 M NaOH.

Calculations

Migration times were calculated relative to a reference glucosinolate in the mixture:

$$RMT = MT_1/MT_2 \qquad (1)$$

where RMT is the relative migration time, MT_1 is the migration time of the actual glucosinolate, and MT_2 is the migration time of the reference glucosinolate for which RMT has a value of 1.

Peak areas were calculated relative to a reference glucosinolate in the sample:

$$RA = A_1/A_2 \qquad (2)$$

where RA is the relative peak area, A_1 is the measured peak area of the actual glucosinolate and A_2 is the measured peak area of the reference glucosinolate for which RA has a value of 1.

Correct quantitation of compounds by capillary electrophoresis also involves correction of the obtained peak areas by multiplying by peak velocity [17,18]:

$$VA = Al/MT \qquad (3)$$

where VA is the velocity-corrected peak area, A is the measured peak area, l is the length of the capillary to detector, and MT is the migration time of the glucosinolate. If the capillaries have identical lengths between analyses, then the multiplication by l in eqn. 3 can be omitted [18] to obtain eqn. 4 for normalized peak area, NA:

$$NA = A/MT \qquad (4)$$

The number of theoretical plates (N) [19] was calculated as:
$$N = 16(MT/w)^2 = 5.54 \ (MT/w^{1/2})^2 \qquad (5)$$

where N is the number of theoretical plates, $w^{1/2}$ is the peak width at half-height, and w is the peak width at baseline.

Resolution (R_s) [20] was calculated as:

$$R_s = 2 \ (MT_2 - MT_1)/1.699 \ (w_1^{1/2} + w_2^{1/2}) \qquad (6)$$

where MT_1 and MT_2 are the migration times of compounds 1 and 2, $w_1^{1/2}$ and $w_2^{1/2}$ are their corresponding peak widths at half-height, and $w = 1.699 \cdot w^{1/2}$.

Repeatabilities were estimated from the means and relative standard deviations (R.S.D.). The linearity of the method was determined from linear regression analyses based on least-squares estimates.

RESULTS AND DISCUSSION

The names and structures of the glucosinolates used in this study are presented in Fig. 1, together with numbers used in the other figures and tables. Differences between glucosinolates are due to the various types and sizes of R-groups as well as to the numerous substituents on the R-groups and the glucose moiety. More than 100 naturally occurring glucosinolates exist, but most often only a few are quantitatively dominate in the single plant species of the various glucosinolate-containing plants [1].

Preliminary high-performance capillary electrophoresis experiments were performed with buffers containing various concentrations of phosphate and borate as well as sodium dodecylsulphate. The separations obtained with the glucosinolates investigated were not acceptable, and changes of temperature and voltage did not improve the separations sufficiently in relation to expectations. The technique based on CTAB was attractive because of the possibility of both hydrophobic and ion-pairing interactions with glucosinolates [1,3,11].

The MECC separation using CTAB is based on the hydrophobic and ion-pairing interaction of the negatively charged glucosinolates and the positively charged CTAB micelles and the CTAB-coated capillary wall. This results in differential partitioning of glucosinolates in the CTAB phase in a similar way to that described for the reversed-phase HPLC method [1,3]. The electrophoretic mobility of the negatively charged glucosinolates pulls them towards the anode with electro-osmotic flow increasing their speed and CTAB retarding them, because the positively charged micelles move towards the cathode. Separations of glucosinolates can, apart from changes in voltage and temperature [12,13], also be altered by changing the composition of the running buffer (detergent, electrolyte and pH) and various other parameters.

Separation conditions

A systematic investigation of the influence of parameter changes on MT, RMT, NA, R_s and efficiency expressed as N have been carried out. The initial parameters used on the ABI instrument were a running buffer consisting of 18 mM borate, 30 mM phosphate and 50 mM CTAB adjusted to pH 7.0, a temperature of 30°C and the voltage set at 20

Fig. 1. Names and structures of the glucosinolates used in MECC analyses. Numbers are used in all figures and tables, and are the same as those used by Sørensen [1].
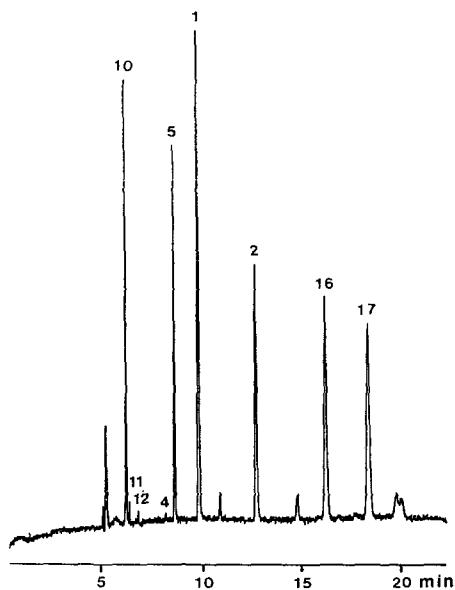
Fig. 2. Electropherogram of the mixture of glucosinolates used in the optimization studies. Numbers as in Fig. 1. Conditions: buffer of 18 mM borate, 30 mM phosphate and 50 mM CTAB adjusted to pH 7.0; temperature 30°C; voltage 20 kV; total length of capillary 720 mm and length from injection end to detector 500 mm; detection wavelength 235 nm. Vacuum injection for 1 s.

Fig. 3. Influence of CTAB concentration on migration times of glucosinolates. Numbers as in Fig. 1. Other separation conditions as in Fig. 2.

kV. The total length of the capillary was 720 mm and the length from the injection end to the detector was 500 mm. The electropherogram in Fig. 2 shows the glucosinolates used for these experiments together with their migration order.

Values of MT generally increased with CTAB concentrations from 10 to 40 mM, whereas small reductions in MT values were seen when moving from 40 to 50 mM (Fig. 3). RMT values with glucosinolate 1 as the reference compound decreased only for glucosinolates 10, 16 and 17. NA values for glucosinolates 10, 5, 1 and 2 were almost unaffected, whereas a reduction in NA values was observed for glucosinolates 16 and 17 when moving from 20 to 40 mM CTAB. NA values for these glucosinolates increased again from 40 to 50 mM CTAB (Fig. 4). R_s and N values were highest when the buffer contained 50 mM CTAB, therefore this concentration was chosen for further study.

An increase in MT values of the more hydrophobic glucosinolates with increasing CTAB concentration is anticipated due to increasing the phase ratio, i.e., the ratio of the volume of the micellar phase to
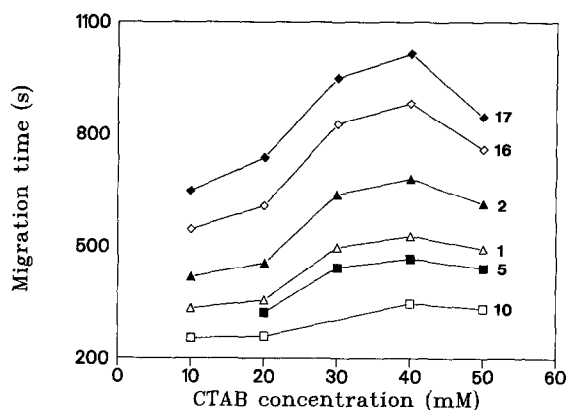
that of the aqueous phase, and may be due to changes in the magnitude of the electro-osmotic flow. The decreases in the MT values of especially the more hydrophobic glucosinolates when increasing the CTAB concentration from 40 to 50 mM may indicate an increase in the temperature of the electrolyte in the capillary [21,22] or perhaps less interaction between the CTAB micelles and glucosinolates, which is probably caused by types of CTAB aggregates other than spherical micelles [23]. In all parameters investigated here, the most profound changes were seen when increasing the CTAB concentration from 40 to 50 mM, showing
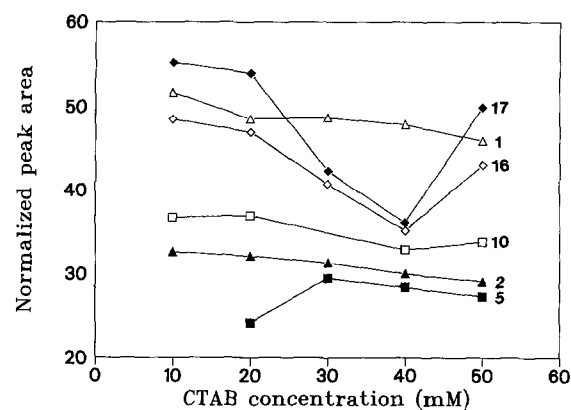

Fig. 4. Influence of CTAB concentration on normalized peak areas of glucosinolates. Numbers as in Fig. 1. Other separation conditions as in Fig. 2.

the absence of a linear response to the higher number of micelles in the buffer. The influence on NA values also indicates altered separation conditions for the hydrophobic glucosinolates. Based on these findings, it is important to note that a quantitative determination of especially the more hydrophobic glucosinolates obtained with one CTAB concentration cannot be compared with results obtained with another CTAB concentration.

Increasing the electrolyte concentration from 16 to 48 mM borate plus phosphate (in a ratio of 3:5) did not affect the MT and RMT values of the more hydrophilic glucosinolates **1**, **5** and **10**, whereas the values increased for the more hydrophobic glucosinolates **2**, **16** and **17** (Fig. 5). Only NA values of glucosinolates **16** and **17** were affected and decreased considerably when moving from 32 to 40 mM of the electrolytes (Fig. 6). R_s values were high and constant at all electrolyte concentrations for the two fastest migrating glucosinolates **10** and **5**, whereas for the other glucosinolates the R_s values increased considerably with increasing electrolyte concentration. With increasing electrolyte concentration, N values decreased for glucosinolate **10**, increased for **5**, **1** and **2**, and were unaffected for **16** and **17**.

It has been reported that electro-osmotic flow decreases with increasing electrolyte concentration [24]. This phenomena is further discussed by Bjergegaard et al. [14]. However, this cannot explain the observed migration times of the more hydrophilic

Fig. 6. Influence of electrolyte concentration (borate/phosphate in a ratio of 3:5) on normalized peak areas of glucosinolates. Numbers as in Fig. 1. Other separation conditions as in Fig. 2.

glucosinolates (Fig. 5). Increased current, due to higher electrolyte concentrations, will increase the temperature in the capillary and thereby reduce the migration times due to the lower viscosity of the buffer. This was not seen for the more hydrophobic glucosinolates. Finally, increased electrolyte concentrations in buffers containing anionic or cationic detergents may lead to an increase in the aggregation number of micelles and a decrease in the critical micelle concentration [23,25]. The same effects seem to be obtained by increasing the CTAB concentration as seen in Fig. 3. The observed behaviour of the glucosinolates is most probably a result of a combination of these effects. From the electropherograms it was seen that the large reductions in NA values for glucosinolates **16** and **17** were a result of reductions in peak height, rather than peak width. A possible explanation could be decreasing response factors (peak area/glucosinolate concentration) at high electrolyte concentrations for these aromatic glucosinolates. Response factors of glucosinolates vary in HPLC according to the glucosinolate and separation conditions [3,26], and the molar extinction coefficients vary from glucosinolate to glucosinolate [1]. Changes in response factors could also explain the variations seen in NA values when increasing the detergent concentration.

Altogether, no single effect can explain the changes observed; however, with the highest electrolyte concentration high R_s and N values were found, therefore a concentration of 48 mM was
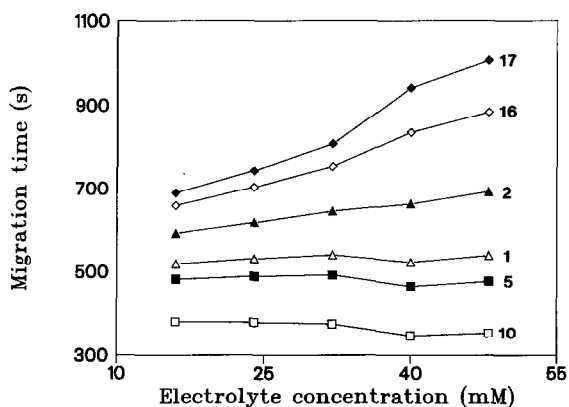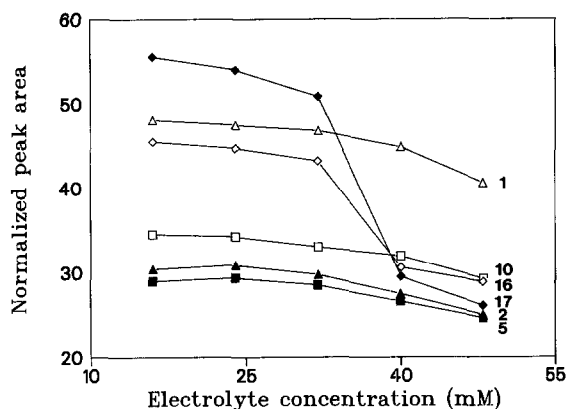

Fig. 5. Influence of electrolyte concentration (borate/phosphate in a ratio of 3:5) on migration times of glucosinolates. Numbers as in Fig. 1. Other separation conditions as in Fig. 2.

chosen. A high electrolyte concentration in the buffer also improves the stacking conditions of sample molecules.

Changing the pH from 6.0 to 8.0 in the separation buffer had relatively little effect on the separation parameters except for NA values. MT values increased slightly, and RMT values decreased slightly, probably because of decreasing electro-osmotic flow with increasing pH [27]. Changes in NA values with pH (Fig. 7) were a result of larger peak heights and not wider peaks, indicating that band-broadening effects are not involved. The fact that the observed NA values increased for the same amount of glucosinolate at different pH values again suggests that the actual MECC response factors also change with pH. A pH of 7.0 was chosen because of slightly higher values of R_s and N at this pH value.

Increasing the voltage from 15 to 26 kV reduced the migration times and had no effect on RMT and R_s values. Only the N values determined for the two fastest migrating glucosinolates were affected. NA values for the more hydrophobic glucosinolates decreased markedly at voltages above 20 kV (Fig. 8). The reductions were a result of both lower peak heights and narrower peaks. According to Jorgenson and Lukacs [19], N would be expected to increase with increasing voltage, whereas the increase in temperature in the capillary will decrease N due to additional band broadening [28]. No clear effect on N was observed here, probably because both ef-
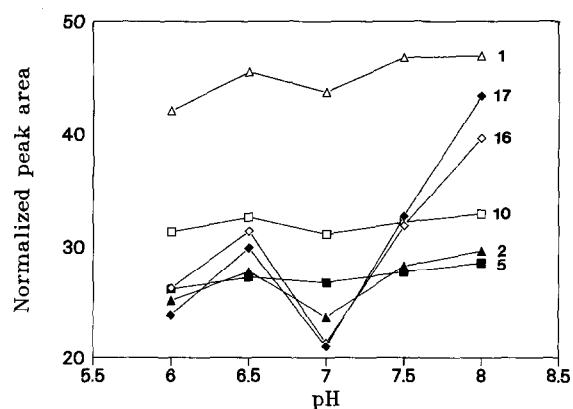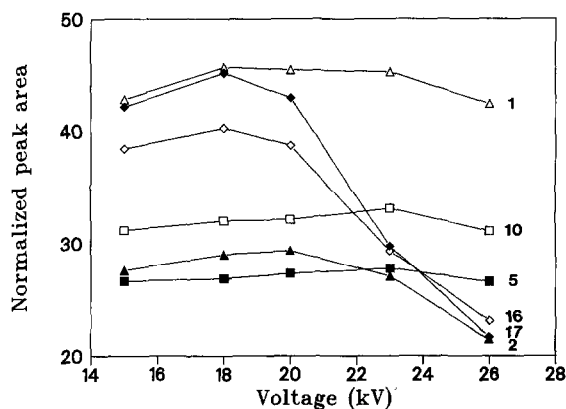

Fig. 8. Influence of applied voltage on normalized peak areas of glucosinolates. Numbers as in Fig. 1. Other separation conditions as in Fig. 2.

fects were taking place. An acceptable set of separation parameters was obtained using a voltage of 20 kV.

As mentioned in the previous discussion of changes in normalized peak areas, a general temperature increase in the capillary due to increased heat produced as a result of higher CTAB concentrations, electrolyte concentrations or voltage applied could explain the similar changes seen in NA values for the hydrophobic glucosinolates. The increase in temperature can be very significant [29], and it might result in other forms of micelles or changes in the interactions between glucosinolates and micelles.

Increasing the temperature from 25 to 45°C gave reduced migration times and had no effect on the RMT, R_s or N values. NA values increased, which again suggests changes in the response factors of glucosinolates and higher injection volume under the applied MECC conditions. These changes in NA values indicate that temperature changes within the capillary are smaller here than the changes obtained by increasing the electrolyte or CTAB concentrations or voltage. A temperature of 30°C was chosen for the separation of glucosinolates as it resulted in an acceptable total analysis time.

N values determined with the chosen separation conditions for the glucosinolates **10**, **5**, **1**, **2**, **16** and **17** were 566 000, 506 000, 296 000, 350 000, 328 000 and 240 000, respectively, of theoretical plates per metre of capillary. R_s values for the glucosinolates

Fig. 7. Influence of buffer pH on normalized peak areas of glucosinolates. Numbers as in Fig. 1. Other separation conditions as in Fig. 2.

10–5, **5–1**, **1–2**, **2–16** and **16–17** were 35.8, 12.1, 22.1, 21.6 and 10.1, respectively, under the chosen separation conditions.

Separation conditions have large influences on MT, RMT, NA, R_s and N values as seen from the previously described results. The mixture of glucosinolates tested represents a wide variation of structurally different glucosinolates, and the recommendations shown for separation conditions are based on these glucosinolates. However, no single set of separation conditions can be used to separate all known glucosinolates, but changes in separation conditions can alter the absolute and relative migration times of glucosinolates. This gives the possibility of separating the actual glucosinolates found in various plant species. Once the conditions are selected, response factors of the different glucosinolates have to be determined for that set of conditions to use the method quantitatively. This is being undertaken now in this laboratory.

Repeatability

Determination of the repeatabilities of MT, RMT, NA and relative normalized peak area (RNA) values included experiments with all three capillary electrophoresis instruments. Selected results are shown in Table I and Fig. 9. The Dionex and Spectra-Physics instruments performed very well with respect to repeatabilities, whereas the ABI instrument yielded less reproducible results in the first test. When the buffer and sample were changed between each analysis, the repeatabilities improved considerably on the ABI instrument, as it is seen in test 2 (Table I). The unsatisfactory repeatabilities in

TABLE I

RELATIVE STANDARD DEVIATIONS (R.S.D.) OF MIGRATION TIMES (MT), RELATIVE MIGRATION TIMES (RMT), NORMALIZED PEAK AREAS (NA) AND RELATIVE NORMALIZED PEAK AREAS (RNA) FOR GLUCOSINOLATES

Conditions: ABI, vacuum injection for 1 s; Dionex, hydrodynamic injection for 25 s; Spectra-Physics, vacuum injection for 6 s. Other separation conditions as in Fig. 2. Numbers in bold are glucosinolate numbers (see Fig. 1).

Instrument	Relative standard deviation							
	10	**5**	**1**	**2**	**16**	**17**		
Dionex (n = 5)								
MT	0.44	0.52	0.56	0.55	0.50	0.57		
RMT[a]	0.22	0.16	0.09	–	0.10	0.14		
	10	**5**	**1**	**2**	**18**	**16**	**17**	
Spectra-Physics (n = 14)								
MT	1.14	1.34	1.38	1.41	1.40	1.43	1.51	
RMT[a]	0.51	0.37	0.26	–	0.32	0.41	0.67	
NA	0.97	0.82	0.90	1.22	1.21	1.93	2.43	
	10	**11**	**12**	**14**	**5**	**2**	**30**	**18**
ABI test 1 (n = 16)								
NA	3.25	8.46	7.64	1.99	1.71	8.57	8.63	9.01
	10	**5**	**1**	**2**	**18**	**16**	**17**	
ABI test 2 (n = 9)								
MT	2.38	2.51	2.50	2.51	2.54	2.50	2.46	
RMT[a]	0.28	0.18	0.10	–	0.08	0.11	0.19	
NA	2.11	1.60	1.91	2.61	1.76	1.82	1.51	
RNA[a]	2.23	1.58	1.45	–	1.33	1.48	1.55	

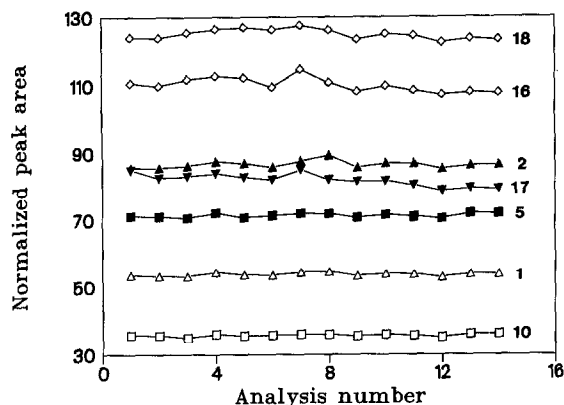[a] Relative to gluconapin (**2**).

Fig. 9. Repeatability of normalized peak areas determined from fourteen analyses performed on the Spectra-Physics instrument. Vacuum injection for 6 s, other separation conditions as in Fig. 2. Numbers as in Fig. 1.

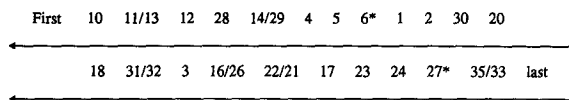| First	10	11/13	12	28	14/29	4	5	6*	1	2	30	20
	18	31/32	3	16/26	22/21	17	23	24	27*	35/33	last	

Fig. 10. Migration order of glucosinolates under standard separation conditions (see Fig. 2). Numbers as in Fig. 1. The asterisk refers to samples of *Brassica napus* seed.

ues and peak areas. R.S.D. values for RNA of between 0.8 and 2.2% were generally obtained using internal standards, showing that repeatabilities can be very high.

Migration order

The migration order of 28 glucosinolates was determined from MECC analyses of single glucosinolates and various glucosinolate mixtures (Fig. 10). An example of an effective separation of glucosinolates within 15 min from a mixture containing eleven different glucosinolates is seen in Fig. 11. It appears that the relatively small differences in the structure of these glucosinolates is sufficient to obtain complete separation. Desulphoglucosinolates (no charge) migrated with a higher velocity than intact glucosinolates (Fig. 12). This indicates that ion-pairing with CTAB is of greater importance than the electrophoretic mobility of the negatively charged intact glucosinolates and the hydrophobic

test 1 were caused by a combination of buffer depletion, running many analyses with the same buffer, and evaporation from sample vials during the test.

No anti-evaporation septa were used on the sample vials in any of the instruments, but the design of the instruments probably resulted in larger problems with evaporation from sample vials on the ABI 270A instrument due to the placement of the autosampler just under the chamber for temperature control of the capillary and fan. Evaporation from sample vials was determined in two tests at a temperature of 30°C and using four vials containing glucosinolates in a volume of 250 μl at the beginning. The evaporation from the eight samples was determined to be 1.10–1.16% per hour. The values were obtained from the weight loss in the vials and quantitative UV measurements of glucosinolates in vials after 13 h. The introduction of the new ABI Model 270-A-MT with several buffer vials and septa for sample vials has probably reduced problems with buffer depletion and evaporation from samples when analysing many samples in a series.

R.S.D. values of similar size to the values obtained here have been reported for the peak areas of cinnamic acid and cinnamic acid analogues [27], and for migration times of various phenols [30]. As seen in Table I, the repeatability expressed as R.S.D. values was reduced considerably when RMT, NA and RNA [calculated relative to glucosinolate (2)] values were used compared with MT val-
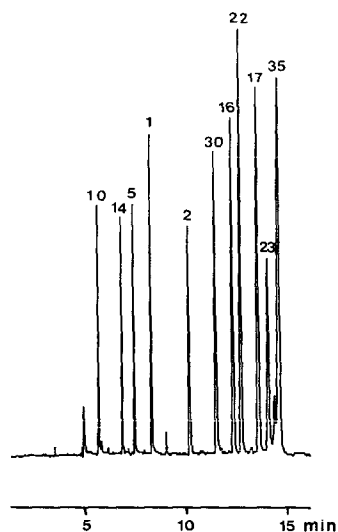

Fig. 11. Separation of eleven glucosinolates in one sample. Separation conditions as in Fig. 2. Numbers as in Fig. 1.
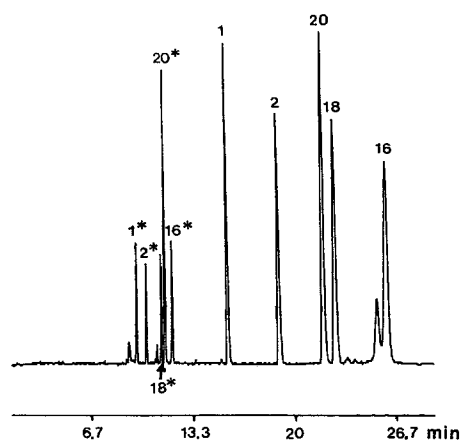
Fig. 12. Electropherogram of intact glucosinolates and desulphoglucosinolates analysed on the ABI instrument. Conditions: voltage, 16 kV; temperature, 25°C; detection wavelenght, 230 nm. Other separation conditions as in Fig. 2. Numbers as in Fig. 1. The numbers with the asterisk refer to the desulphoglucosinolates.

interaction of R-groups in desulphoglucosinolates with CTAB. Furthermore, the desulphoglucosinolates of **18** and **20** changed migration order compared with that of the intact glucosinolates.

The selective retention of solutes in MECC arises from differences in the partitioning of solutes between the micellar and aqueous phases. Partitioning requires solubilization by the micelles through surface interactions or through penetration of the solute into the micelle core [31]. Solubilization of charged solutes in the micelles seems to require net-neutral ion-pairs [1,11], and solubilization may take place simultaneously or after ion-pair formation. Many complex equilibria can occur and this makes the elucidation of the solubilization mechanism difficult [31].

The behaviour of intact glucosinolates and desulphoglucosinolates was in agreement with the importance of ion-pair formation. Futhermore, the hydrophobicity of the R-group as well as steric hindrances because of the size of the R-group will influence the solubilization by micelles. The epimers **4** and **5** as well as **18** and **30** separated well, which is probably caused by differences in the steric hindrance of solubilization inside micelles. The migration order of glucosinolates in homologous series such as **10–11–12**, **5–6**, **1–2** and **16–17** showed higher hydrophobicity with longer side-chains of the

glucosinolates and no steric hindrance of solubilization. The differences in migration order of **17** compared with **18** and **30** were probably caused by the higher hydrophobicity of the glucosinolate without the hydroxygroup. Sinapoyl- (**33**) and isoferoyl- (**35**) derivatives had very high solubilities in micelles as found for reversed-phase HPLC [16]. The importance of ion-pairing for the separation of charged molecules by MECC has also been demonstrated by Nishi et al. [8] in the separation of water-soluble vitamins.

MT and RMT values were found to change as a result of buffer depletion and the state of the capillary. Identification of glucosinolates from RMT values should therefore be carried out with care, and identification should only be performed using standards containing one or more of the glucosinolates present in the actual samples and analysed in the same series of analyses as the samples in question. Furthermore, knowledge of the types of glucosinolates found in the actual plant material and plant species is required. As a result of differences in the number of chromophores and consequently the UV spectra of the various glucosinolates [1], it will be advantageous to use fast-scanning absorption detection for the identification of glucosinolates. Wernly and Thormann [32] demonstrated the advantages in identifying various drugs and their metabolites using fast-scanning absorption detection.

Linearity

The linearity of the method was determined from several tests as the correlation between increasing concentrations of different glucosinolates and the corresponding NA values. The results from one test made on the ABI instrument and one made on the Dionex instrument are shown in Table II. Correlation coefficients for the different glucosinolates were between 0.9723 and 0.9960 with only small differences between the results obtained from the two instruments. The tests were made without an internal standard, which probably would have increased the correlation coefficients [30]. However, the obtained correlation coefficients show that this MECC method gives linear increases in NA values with increasing concentrations of glucosinolates in the samples. The method can therefore be used to quantitate glucosinolates using internal standards once the correct response factors have been determined.

TABLE II

LINEARITY TESTS USING ABI AND DIONEX INSTRU-
MENTS

Correlation coefficients (r^2) from linear regression analyses by
the least-squares method of normalized areas (NA) for various
concentrations of glucosinolates. Conditions as in Table I and
Fig. 2.

Gluco-sinolate	ABI ($n = 7$)		Dionex ($n = 5$)	
	Concentration range (mM)	r^2	Concentration range (mM)	r^2
10	0.146–0.488	0.9960	0.052–0.260	0.9776
5	0.119–0.396	0.9941	0.042–0.211	0.9972
1	0.169–0.566	0.9935	0.060–0.302	0.9784
2	0.160–0.533	0.9864	0.057–0.285	0.9819
16	0.198–0.660	0.9746	0.070–0.352	0.9897
17	0.337–1.123	0.9723	0.107–0.533	0.9882

Detection limits

Approximate detection limits have been deter-
mined from a signal-to-noise ratio of 2:1 using vari-
ous dilutions of glucosinolate samples containing
glucosinolate **1**, **2**, **4**, **13**, **22** and **23**. The detection
limits found here correspond to the conditions ap-
plied, which were a 1-s sample injection into a 720-
mm-long capillary with an internal diameter of 50
μm. Detection limits were between 3 and 8 μM of
each glucosinolate in the sample. According to Har-
baugh *et al.* [33] and Vinther [34], the injected vol-
ume should be 4.6 nl assuming a viscosity in the
electrolyte and sample identical to water and with a
capillary temperature of 30°C. This results in detec-
tion limits between 15 and 40 fmol for each of the
glucosinolates or approximately 10 pg of glucosino-
late, depending on the molecular weight.

Sample solvent effects

The isolation and purification of glucosinolates
before HPLC analyses involve crude extracts of
plant material containing up to 70% methanol [3].
Group separation of compounds in crude extracts
on QMA columns described elsewhere [1] results in
QMA eluates containing 50% acetonitrile, 50 mM
hydrogencarbonate and 50 mM chloride [1,3]. Di-
rect MECC analyses of QMA eluates gave very
poor separations of glucosinolates. However, dilut-
ing 1:3 with water, or evaporating the solvent and
redissolving in water to the same concentration,

solved the problems, showing that the high concen-
trations of acetonitrile in QMA eluates interfered
with the CTAB micelles in spite of the low volume
injected. The solvent in crude extracts also inter-
fered with the CTAB micelles, and a dilution of 1:3
with water solved the problem. Evaporating solvent
from QMA eluates and redissolving in water to the
same concentration did not solve separation prob-
lems completely, whereas a further dilution of 1:1
with water did. This showed that both the solvent
and high concentrations of compounds in crude ex-
tracts caused the problems seen in MECC analyses.
From these results it appears that samples contain-
ing concentrations of up to 12.5% acetonitrile or up
to 12–17% methanol in QMA eluates and crude
extracts of glucosinolates, respectively, will not af-
fect the separation of glucosinolates.

Many interfering compounds are seen in the elec-
tropherograms of crude extracts. However, crude
extracts can, with caution, be used to identify gluco-
sinolates present in samples. For screening purpos-
es in plant breeding, this may in some instances be
sufficient, and certainly more informative, than us-
ing total determinations of glucosinolates. Increas-
ing the voltage or temperature will decrease migra-
tion times and make faster analyses possible.

After the group separation of plant extracts, both
glucosinolates and aromatic carboxylic acids can be
present in the same fractions [12]. It is possible by
the method presented here to separate glucosino-
lates and aromatic carboxylic acids present in the
same sample [12,13].

CONCLUSIONS

In conclusion, the described MECC method gives
fast and reliable determinations of glucosinolates. It
is easy and fast to change the separation conditions
to separate actual glucosinolates in samples from
various plants. MECC analyses are inexpensive
compared with HPLC analyses because of the re-
agents and small amounts used. The capillary is
much less sensitive to impurities in samples than
HPLC columns, and the capillaries are inexpensive
compared with HPLC columns. Crude extracts of
glucosinolates can be analysed directly. If relative
migration times and normalized peak areas are
used, then the repeatability and linearity of the
method are good. This gives the possibility of quan-

titative determinations of glucosinolates from MECC analyses when the response factors have been determined. Critical parameters to repeatability and linearity are evaporation from samples, depletion of buffers and the state of the capillary. Precautions must be taken to avoid evaporation from the samples, and the buffers must be changed often, preferably between each analysis. The capillary must be washed with sodium hydroxide solution when migration times change too much between consecutive analyses, or as a daily routine. The peak capacity of the method is very high and can exceed 600 000 plates/m of capillary compared with typically no more than 50 000 plates/m of column in HPLC analyses of glucosinolates.

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