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Analysis of eleven iridoid glycosides by micellar electrokinetic capillary chromatography (MECC) and screening of plant samples by partial filling (MECC)–electrospray ionisation mass spectrometry

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Abstract

Of ammonium, lithium and sodium salts of dodecyl sulfate studied as surfactants in the separation of iridoid glycosides by micellar electrokinetic capillary chromatography (MECC), the last one gave the best results. Eleven neutral iridoid glycosides were separated by MECC with sodium dodecyl sulfate as surfactant, and the water–micelle partition coefficients of the compounds were calculated. The separation system was coupled via a coaxial sheath flow electrospray interface to a mass spectrometer, and the partial filling technique was used in the on-line analysis. Seven plant species belonging to five genera (*Plantago*, *Veronica*, *Melampyrum*, *Succisa* and *Valeriana*) were screened for the iridoid glycosides by the new method that was developed. The findings confirmed those of an earlier study on five of the iridoid glycosides. Some new iridoid glycosides were found in *Plantago lanceolata*, *Veronica spicata* and *V. chamaedrys*.

Keywords: Iridoid glycosides

1. Introduction

Iridoids are cyclopentanoid monoterpene derivatives, which are formed in plants as secondary compounds. They fall into four groups: iridoid glycosides, nonglycosidic iridoids, secoiridoids and bisiridoids. The most numerous group of these is the iridoid glycosides, several hundreds of which have been identified to date [1–3]. Some butterflies use these compounds in their chemical defence. The butterfly larvae gather iridoid glycosides into their bodies from the plants they eat, and the bitter tasting iridoids make the larvae unpalatable to generalist predators such as birds. Knowledge of the amounts of these compounds in plants and in larvae feeding on them can assist in the study of the behaviour of butterflies and their parasitoids.

We have been studying iridoid glycosides in plant extracts, as well as extracts of *Melitaea cinxia* butterfly larvae, with the help of capillary electromigration techniques [4–7]. The conditions for iridoid glycoside analysis need to be basic, since even under mildly acidic conditions the compounds are easily hydrolysed and undergo rearrangement [8,9]. Some of the more unstable iridoid compounds are also hydrolysed under basic conditions [9], but the compounds of this work did not show signs of rearrangement, with formation of a black precipitate, under the conditions we used.

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Iridoid glycosides have usually been analysed by chromatographic methods [10–13]; only a few articles describe their analysis by capillary electromigration techniques [4–7,14–16]. Our previous study [6] evidently is the only one in which iridoids have been analysed by capillary electrophoresis (CE) coupled on-line to a mass spectrometer (CE–MS). CE provides effective separation of the iridoid glycosides, and, in general, MS improves the reliability of analyte identification. Electrospray ionisation (ESI) is the most popular ionisation technique in CE–MS. For the coupling, there are several interfacing techniques available, such as the sheath flow technique, the liquid–liquid junction and the sheathless technique. The sheath flow technique was applied in this work.

Volatile buffers of low ionic strength are preferred in CE–ESI-MS to ensure a stable electrospray and to preserve the sensitivity of the mass spectrometer. Nonvolatile micelles are a problem in on-line micellar electrokinetic capillary chromatography (MECC)–ESI-MS experiments as they soil the MS system. Several approaches that prevent the micelles from reaching the MS system have been developed to separate neutral compounds. One of the easiest to apply is the partial filling (PF) technique, as first described by Valtcheva et al. [17]. In PF-MECC the capillary is filled with an electrolyte solution devoid of nonvolatile compounds, and a plug of micellar solution is injected. After the micelles have been introduced to the capillary, the sample is injected and the separation voltage is applied. The analytes are partitioned into the micelles according to their partition coefficients, and after separation they migrate out of the capillary with the velocity of the electroosmotic flow. Thus, the analytes enter the MS system well before the micelle plug and the run can be interrupted before the surfactants reach the MS system. Between analyses the capillary is flushed with electrolyte solution to rinse the micelles to waste.

PF-MECC has been successfully coupled to MS via an electrospray ionisation interface in several different studies [18–20]. Koezuka et al. have studied peptides [18] and Nelson et al. triazine herbicides [19] under acidic conditions with acidic sheath liquid. Wiedmer et al. [20] have used basic conditions and acidic sheath liquid in the analysis of corticosteroids.

The primary aim of this study was to develop a MECC separation method for 11 iridoid glycosides: unedoside (UNE), harpagide (HARP), methyl catalpol (MEC), morroniside (MOR), asperuloside (ASP), griselinoside (GRIS), catalpol (CAT), ketologanin (KET), verbenalin (VERB), loganin (LOG) and 10-cinnamoyl catalpol (CIN). The last five compounds were studied in our earlier work [6], where they were analysed by a PF-MECC–ESI-MS method. The presence of some iridoid glycosides in the plants have been earlier reported. Bianco et al. [21] have found asperuloside in flowers of *Plantago lanceolata*, and Handjieva et al. [12] asperuloside in the plant in addition to globularin (10-*trans*-cinnamoyl catalpol).

In this study the method for the analysis of the eleven iridoid glycosides was studied in the screening of seven plant species belonging to five genera, *Plantago*, *Veronica*, *Melampyrum*, *Succisa* and *Valeriana*. We were especially interested in the genera *Plantago* and *Veronica*, since the *Melitaea cinxia* butterfly feeds exclusively on plants of these genera.

2. Materials and methods

2.1. Reagents and samples

The iridoid glycosides used as standards were donated by Dr. Søren Rosendal Jensen (Danish Technical University, Lyngby, Denmark). The compounds had been extracted from plant material with ethanol [13], purified by reversed-phase high-performance liquid chromatography (RP-HPLC) and identified by UV detection at wavelengths of 206 and 254 nm. The standards were dissolved in purified water. Fig. 1 shows the structures of the compounds and Table 1 their molar masses.

Ammonium acetate was purchased from Fisher Scientific (Loughborough, UK) and disodium tetraborate decahydrate (borax) from Merck (Darmstadt, Germany). Sodium dodecyl sulfate (SDS) was manufactured by Bio-Rad Labs. (Hercules, CA, USA). Lithium dodecyl sulfate (LDS) was from Sigma (St. Louis, MO, USA). Ammonium dodecyl

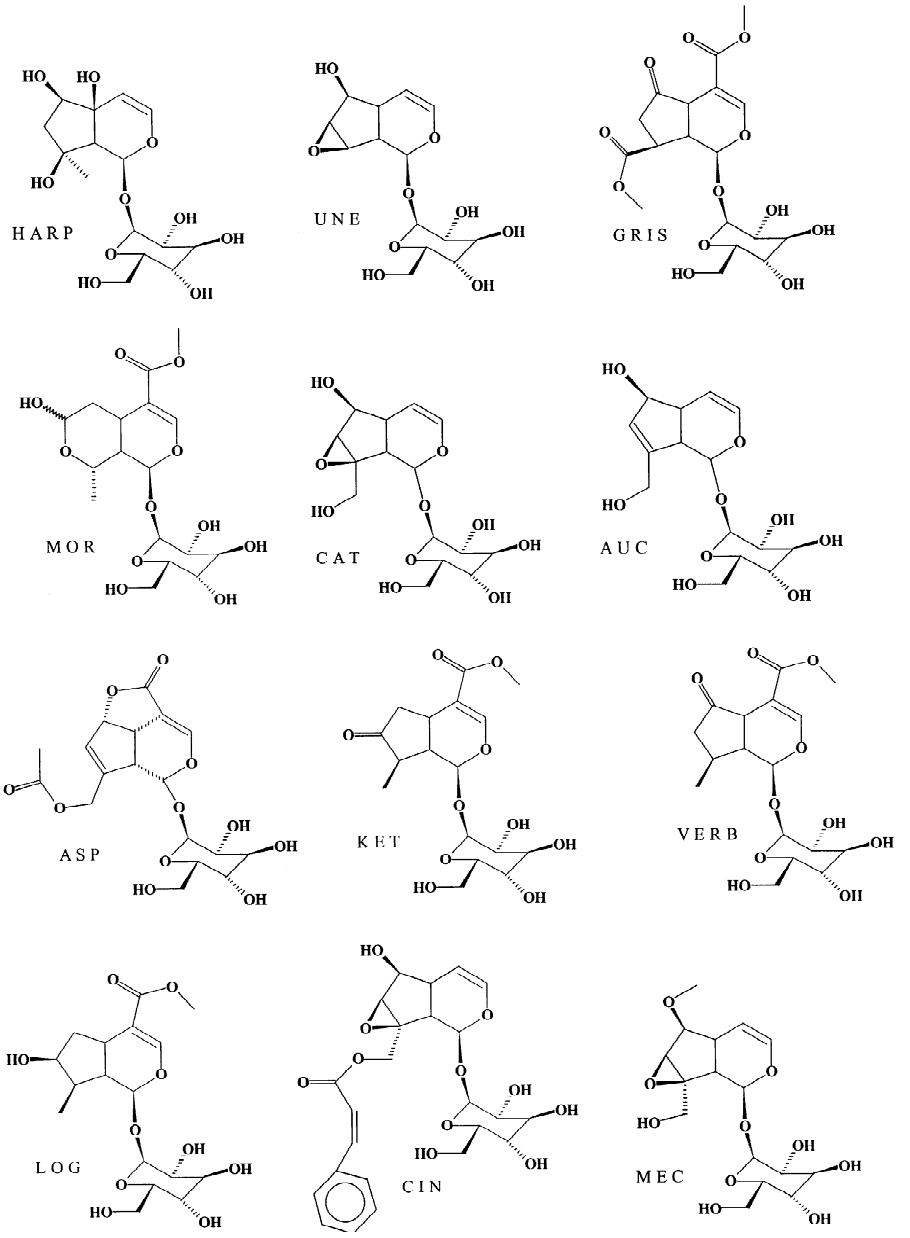

Fig. 1. Structures of the iridoid glycosides studied in this work [1,2]. The iridoid glycosides are CAT, catalpol; UNE, unedoside; HARP, harpagide; MEC, methyl catalpol; MOR, morroniside; ASP, asperuloside; GRIS, griselinoside; KET, 7-ketologanin; VERB, verbenalin; LOG, loganin; CIN, 10-cinnamoyl catalpol; AUC, aucubin.

sulfate was purchased as a 30% water solution from Fluka (Buchs, Switzerland). Lithium acetate used in the sheath liquid was purchased from Acros Organics (Geel, Belgium). Water used in the experiments was first distilled and then further purified with a Millipore Milli-Q apparatus (Molsheim, France) until its conductance was 0.054 μS. The methanol used in the sheath liquid was of HPLC grade and manufactured

Table 1
Molar masses and calculated water–micelle partition coefficients of the iridoids with their relative standard deviations ($n=6$)

Iridoid	M/g/mol	Log P	RSD (%)
Catalpol	362.1	0.21	2.8
Unedoside	332.1	0.21	2.8
Harpagide	364.1	1.15	7.0
Methyl catalpol	376.1	1.22	10.2
Morroniside	406.1	1.40	1.9
Asperuloside	414.1	1.53	0.6
Griselinoside	432.1	1.56	0.6
7-Ketologanin	388.1	1.66	0.4
Verbenalin	388.1	1.80	0.4
Loganin	390.2	1.96	0.5
10-Cinnamoylcatalpol	492.5	2.47	0.7

The electrolyte solution in which the measurements were performed contained 100 m*M* SDS in 20 m*M* ammonium acetate, pH 9.5.

by J.T. Baker (Deventer, The Netherlands).

In all electrolyte solutions the pH was adjusted with aqueous ammonium solution. The ammonium solution was diluted with purified water from a 25% solution manufactured by Riedel-de Haën (Seelze, Germany).

Leaf samples of seven plant species, *V. spicata*, *V. chamaedrys*, *P. lanceolata*, *M. pratense*, *M. sylvaticum*, *S. pratensis* and *Valeriana sambucifolia*, were gathered from southern and southwestern Finland.

2.2. Preparation of samples and electrolyte solutions

The pure iridoid glycosides were dissolved in purified water. The stock solutions contained 1000 to 2000 mg/l of the iridoid in question. Mixtures of the analytes were prepared from these stock solutions and diluted with purified water when necessary.

The leaf samples were pretreated by hot water extraction as described in our previous article [4] unless otherwise specified. Briefly, the dry leaves (on average 100 mg) were crushed and weighed in vials and purified water was added. The mixture was left at room temperature for 40 min to wet the leaves thoroughly and then heated at 100 °C for 60 min. The crushed leaves were removed and the extract was evaporated to dryness overnight at atmospheric pressure and then dissolved in a known volume of purified water, filtered, and injected to the capillary electrophoresis equipment.

A stock solution of 100 m*M* ammonium acetate was used for the preparation of the 20 m*M* electrolyte solution used in the PF-MECC experiments, and the pH was adjusted to 9.5 with ammonia. The solution of 100 m*M* SDS was prepared in 20 m*M* ammonium acetate solution at pH 9.5.

2.3. Apparatus

The capillary electrophoresis system was a Hewlett-Packard ^{3D}CE system (Agilent Technologies, Waldbronn, Germany) with an air-cooling device for the capillary and a diode array detector. The dimensions of the uncoated fused-silica capillary (Composite Metal Services, The Chase, Hallow, UK) were 50 μm I.D.×375 μm O.D. The total length of the capillary was 80 cm in all experiments. When only UV detection was used, the distance to the detector was 71.5 cm. In the PF-MECC–ESI-MS experiments, UV detection was made at 20 cm to monitor the repeatability of the analysis. The temperature of the capillary cassette was 25 °C in all experiments.

The MECC runs were performed at a +20 kV separation voltage. The sample was injected at 50 mbar pressure for 5 s. The analytes were detected at 197, 235, 239 and 283 nm, which are their absorbance maxima or as close to them as possible.

The PF-MECC–ESI-MS runs were performed at a +20 kV separation voltage. The sample was injected at 50 mbar pressure for 25 s. The longer injection time was necessary in order to increase the amount of ions reaching the MS system. The micellar plug was also introduced to the capillary with 50 mbar pressure and the injection time was optimised. Even though the injection of the micellar solution would have been faster at high pressure, the low pressure gave better repeatability.

The mass spectrometer was a Bruker ESQUIRE (Bruker Daltonics, Bremen, Germany) equipped with an electrospray ionisation source (Analytica of Branford, Branford, CT, USA). The spectrometer comprised a hexapole and an ion trap mass analyser. Nitrogen was used as drying gas. It was heated to 200 °C and the flow-rate was 100 l/h. The analytes were detected as alkali adducts in the positive ion mode. The alkali ions were introduced in the sheath

liquid (flow-rate 200 μl/h) consisting of 1.0 m*M* lithium acetate in a water–methanol (50:50, v/v) mixture. The composition of the sheath liquid was optimised in a previous study [6]. The MS glass capillary exit was maintained at 95 V and the skimmer at 32 V. Ions were scanned in the range 100–550 *m*/*z* and cut-off was set at 80 *m*/*z*. The tip of the silica capillary was 0.5 mm outside the metal capillary. Several different electrospray voltages were tested and the voltage was finally set at −4.0 kV for the capillary, −1.8 kV for the cylinder and −3.5 kV for the end plate.

3. Results and discussion

3.1. Effect of dodecyl sulfate counterion on the separation of iridoids

Before attempting the separation of all 11 iridoid glycosides we studied the effect of the dodecyl sulfate counterion on the separation and MS detection of five neutral iridoid glycosides, the separation of which was optimised in our previous study [6]. Sodium dodecyl sulfate, ammonium dodecyl sulfate and lithium dodecyl sulfate were tested. The concentration of the surfactant was 100 m*M* in all the experiments, and both unbuffered solutions (surfactant dissolved in purified water) and buffered solutions (surfactant dissolved in running buffer) were studied. The resolution of the analytes was not clearly better with buffered SDS than with unbuffered SDS, but the buffering diminished the noise at the MS system and the signal-to-noise ratio increased slightly.

Some previous studies on the separation properties of different surfactants have been reported. In a comparison of the retention factors of several compounds, Fuguet et al. [22] have found that the *k* values obtained with SDS and LDS were mostly similar. In the analysis of pesticides by PF-MECC–ESI-MS [23], the repeatability and sensitivity of the analysis were found to be better with SDS than with ADS.

In the present study the resolution of the analytes was better when SDS was used. ADS was not suitable for MS work, because it contained unidentified polymeric substances. These substances prevented the detection of the most hydrophilic iridoids as they overlapped with the analyte zones. In addition, some of the *m*/*z* ratios of the interfering compounds coincided with those of iridoid glycosides. We also noticed that the baseline in the total ion electropherogram was much higher with ADS than with SDS or LDS because of the many background peaks in the spectrum. The resolution of the analytes was only slightly lower with buffered LDS than with SDS, while the noise at the MS was about the same with the two surfactants.

In considering these results it is necessary to remember that a part of the counterions in the buffered micellar solution is ammonium even when SDS or LDS are used, since in the pH value used the ammonium of the buffering agent functioned as the buffer. The contribution of the ammonium ions originating from the buffer can be approximated to be the same in all buffered micellar solutions, however.

The effect of the injection time of the micellar plug on the peak widths of KET and VERB was studied with all micellar solutions. The injection time was increased from 200 to 300 s. With SDS solution the peak widths increased by 15% as the injection time was increased, but with LDS the peak widths decreased to about 80% of the original width. Table 2 compares the plate numbers for KET and VERB with the three buffered micellar solutions and different injection times.

By taking into consideration the low price of SDS it was selected for further studies.

3.2. Separation of 11 iridoid glycosides by MECC

A reliable capillary electrophoretic method was developed for the analysis of 11 iridoid glycosides: CAT, UNE, HARP, MEC, MOR, ASP, GRIS, KET, VERB, LOG, and CIN. Ammonium acetate, a volatile substance, was chosen as the buffer in order to make the system compatible with MS. Since the iridoid glycosides are neutral, MECC was used, with SDS as the surfactant.

It was experimentally shown that increasing the ammonium acetate concentration did not increase the resolution of the analytes. Increasing the SDS concentration, however, increased the resolution noticeably. The peak pairs most difficult to separate

Table 2
Comparison of SDS, LDS and ADS

	LDS, 200 s	LDS, 300 s	SDS, 200 s	SDS, 300 s	ADS, 200 s	ADS, 300 s
N(KET)	64 400	93 300	115 600	95 400	51 900	8300
N(VERB)	51 400	69 000	92 100	69 200	48 500	5600
I (μA)	11	11	11	11	11	17

The surfactants were dissolved in 20 m*M* ammonium acetate, pH 9.2, and the concentration of each surfactant was 100 m*M*. The analyses were performed as PF-MECC runs at +15 kV in a 80 cm capillary (71.5 cm to UV detector). The sample was injected for 5 s at 50 mbar pressure, and the same pressure was used for the injection of the micellar solutions.

were CAT and UNE, and also ASP and GRIS. Separation of CAT and UNE, which are highly polar compounds, was impossible with reasonable concentrations of SDS, but ASP and GRIS were separated when the SDS concentration was raised to 100 m*M*. The resolution of the analytes was best at pH 9.5. Under the analysis conditions used, pH in the range 8.7–10.0 affected the resolution less than increasing the SDS concentration from 80 m*M* to 100 m*M*. An electropherogram showing the separation of the analytes is presented in Fig. 2.

The linearity of the absorbance versus concentration curve was studied separately for each iridoid glycoside. The linear ranges were as follows: 25–400 mg/l for CAT and ASP, 25–300 mg/l for HARP, 10–1000 mg/l for UNE, MOR and CIN, 10–400 mg/l for KET and VERB, 12–500 mg/l for MEC, 25–500 mg/l for GRIS, and 50–700 mg/l for LOG. The limits of detection (LODs) were 25 mg/l for CAT and HARP, 10 mg/l for UNE, 12 mg/l for MEC, 9 mg/l for ASP, 8 mg/l for MOR and GRIS, 5 mg/l for KET, 4 mg/l for VERB and LOG and 3 mg/l for CIN. Where the linear range did not extend to the LOD, the LOD value was determined based on the signal-to-noise ratio of 2.

The partition coefficients of the compounds between the water and micelle phases were also determined. For the partition coefficient we used the equation:

$$k = \frac{(t_m - t_{eo})}{t_{eo}\left(1 - \frac{t_m}{t_{mc}}\right)} = P\left(\frac{V_{mc}}{V_{aq}}\right)$$

in which P is the partition coefficient, k is the partition factor of the analyte corresponding to

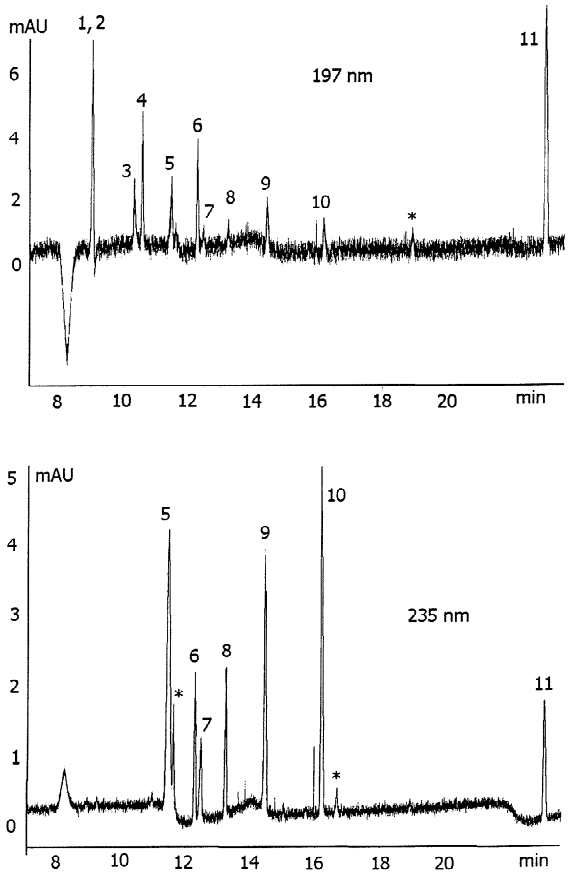

Fig. 2. An electropherogram showing the separation of the 11 iridoid glycosides. The electrolyte solution contained 100 m*M* SDS in 20 m*M* ammonium acetate, pH 9.5. The capillary was 80 cm long (71.5 cm to the UV detector), and the applied voltage was +20 kV, producing a current of ca. 25 μA. The compounds are 1, unedoside; 2, catalpol; 3, harpagide; 4, methyl catalpol; 5, morroniside; 6, asperuloside; 7, griselinoside; 8, 7-ketologanin; 9, verbenalin; 10, loganin; 11, 10-cinnamoyl catalpol. Impurities are marked with an asterisk (*). The concentration of the analytes is 50 mg/l and the sample was injected for 5 s at 50 mbar pressure.

retention factor in chromatography, t_m is the migration time of the analyte, t_{eo} is the electroosmotic flow marker (methanol) and t_{mc} is the micelle marker (timepidium bromide). V_{aq} is the volume of the aqueous phase and V_{mc} is the volume of the micelles. V_{mc} is determined as:

$$V_{mc} = V_{tot} - V_{free}$$

where V_{tot} is the total micellar volume and V_{free} is the volume of the free, non-micellised, surfactant in the solution [which can be obtained from the critical micelle concentration (CMC)]. The total micellar volume was calculated from the volumes of the groups in the surfactant [24]. The CMC was determined by conductometric titration. In 20 m*M* ammonium acetate at pH 9.5 the CMC of SDS is 3.4–3.5 m*M*.

The partition coefficient was calculated for the conditions used in the analysis of the 11 iridoid glycosides, viz. an electrolyte solution containing 20 m*M* ammonium acetate and 100 m*M* SDS at pH 9.5 according to Ref. [25]. The log *P* values of the iridoids are given in Table 1. No values of log P_{ow} for the iridoid glycosides were found in the literature.

3.3. PF-MECC–ESI-MS in screening of plant samples

Since we had screened plant extracts for CAT, aucubin (AUC), KET, VERB, LOG and CIN in earlier work [6], we concentrated here on the six new iridoid glycosides.

The six iridoid glycosides, UNE, HARP, MEC, MOR, ASP and GRIS, which were investigated with PF-MECC–ESI-MS, have different mass-to-charge ratios. In a standard substance study, they could have been analysed by directly infusing them into the MS system. However, plant sample extracts contain hundreds of substances in addition to the iridoid glycosides, and these would have hampered the direct MS analysis. In addition, the extracts are dirty and would have fouled the MS system if they had been infused directly. Capillary electrophoretic separation was therefore necessary. On the other hand, plant extracts cannot be analysed using only UV detection because the unknown compounds present in the extract would overlap with the iridoid glycosides at the wavelength used.

The effect of sample pretreatment was also studied. Even though the most hydrophobic of the analytes, CIN with a log *P* value of 2.47, easily dissolves in water even at 1000 mg/l concentrations, we thought it worthwhile to check if it could be extracted more effectively with organic solvents or after matrix hydrolyzation with sodium hydroxide. The hydrolyzation was performed in 0.1 *M* NaOH at 60 °C for 60 min, and afterwards the sample was extracted with hot water as in other experiments. The hydrolyzation increased the extraction efficiency for CIN, but it decreased the amounts of the more polar iridoids extracted. Some CIN was found in *V. spicata*, but none was found in *P. lanceolata* and *V. chamaedrys*. CIN was not visible in methanolic extracts of *V. spicata*.

The iridoids found in the plant samples, particularly ASP, exhibited the same sort of extraction behaviour as CAT and AUC [4]. In other words, all the iridoid glycosides in this study were extracted more effectively with hot methanol (2 h) than by methanol at room temperature (24 h), and they were extracted most efficiently with hot water.

Using hot water as the extraction medium, the extraction time for the iridoid glycosides was optimised. It was seen that increasing the extraction time increased the amounts of iridoid glycosides extracted. However, the results for extracts pretreated by keeping the sample in water at room temperature for 40 min and then heating at 100 °C for 60 min were on average as good as, or even better than, the results for extracts pretreated by heating at 100 °C for 120 min without the room temperature stage. Thus, the best pretreatment method was the same as in our previous study [4], in which we concentrated only on the extraction behaviour of CAT and AUC.

In the PF-MECC–ESI-MS experiments we used 300 s injection of buffered SDS (100 m*M* SDS in 20 m*M* ammonium acetate, pH 9.5) since the repeatability of the analysis was better with 300 s injection than with 200 s injection. The micellar plug at 300 s injection was 19.0 cm long; the velocity of the micellar solution in the capillary was determined by injecting the micellar solution into a capillary filled with the background electrolyte at 50 mbar pressure until a signal was seen at the detector.

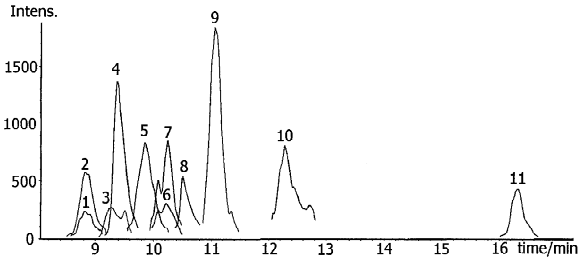

Fig. 3. A reconstructed ion electropherogram of the 11 iridoid glycosides, numbered as in Fig. 2. The concentration of the analytes is 50 mg/l and the sample was injected for 25 s at 50 mbar pressure. The background electrolyte was 20 m*M* ammonium acetate at pH 9.5, and the micellar solution (100 m*M* SDS in the BGE) was injected for 300 s. The capillary was 80 cm long.

To increase the amount of ions (or lithium adducts) at the MS system we used 25 s injection of sample; shorter injection times (5 and 10 s) gave poorer results. Owing to peak broadening the peaks of the analytes were overlapped at the MS system, but they could still be detected since they had different m/z values. Fig. 3 shows a reconstructed ion electropherogram of the 11 iridoid glycosides and Fig. 4 a reconstructed ion electropherogram of a *P. lanceolata* extract. It is seen that the resolution is worse in Fig. 3 using much longer injection time and partial filling technique than in Fig. 2.

The limit of quantitation (LOQ) for the analytes was determined by adding standard compounds to an extract of *Valeriana sambucifolia*, that had previously been screened to ensure that it did not contain any of these iridoid glycosides. The LOQs of the analytes ($S/N=3$) were UNE 40 mg/l, HARP 25 mg/l, MEC 15 mg/l, MOR 20 mg/l, GRIS and ASP 30 mg/l. The LOQs of the other iridoids had previously been determined to be CAT 50 mg/l, AUC 50 mg/l, KET 25 mg/l, VERB 15 mg/l, LOG 15 mg/l and CIN 50 mg/l.

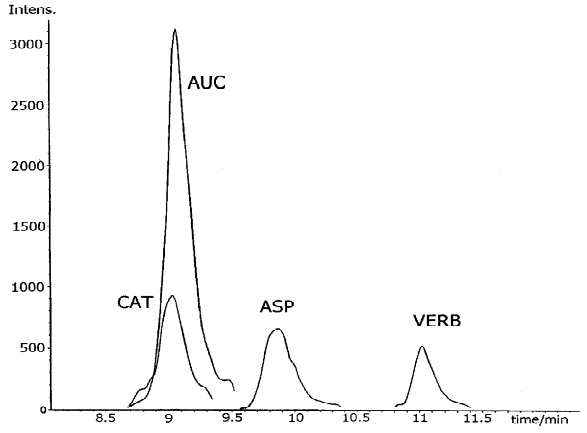

Fig. 4. A reconstructed ion electropherogram of a hot water extract of *Plantago lanceolata*. Conditions as in Fig. 3. Catalpol, aucubin, asperuloside and verbenalin are marked in the figure.

The day-to-day repeatability of migration times and peak heights was determined. The RSDs of peak heights ($n=6$) were 10.3% for UNE, 10.0% for HARP, 10.8% for MEC, 7.4% for ASP, 13.4% for MOR and 14.0% for GRIS, as determined from a 100 mg/l calibration standard sample in *Valeriana sambucifolia* extract. The RSDs of migration times measured at the MS system were 3.6% for UNE, 4.7% for HARP, 4.7% for MEC, 5.0% for ASP, 5.5% for MOR and 6.0% for GRIS ($n=6$). Since the MS run was started manually after sample injection at CE, the analysis times were not as repeatable as with automatic system. In MECC runs the RSDs were less than 1% for all the compounds except MOR, for which the RSD was 1.6% in nine analyses made on different days.

The calibration curves based on peak heights of the analytes showed some linearity, but the range was more limited than with UV detection. For most of the iridoids, the signal intensity vs. concentration curve was linear in the range 25 to 300 mg/l, but GRIS and ASP were only linear from 50 to 300 and 50–400 mg/l, respectively.

Seven plant species were screened for the iridoids. Our results confirmed the presence of ASP in *P. lanceolata*, which was first reported by Bianco et al. [21]. The amount of ASP in the plant is significant, even though it is less than the amount of the main iridoid glycosides CAT and AUC. We could not confirm Handjieva et al.'s [12] finding of CIN in *P. lanceolata*, perhaps because the iridoid glycoside concentrations of *P. lanceolata* individuals vary in different countries, as we have observed earlier [7]. In regard to its iridoid glycosides, *V. spicata* is a somewhat neglected species. We identified the compound MEC in it for the first time, and also found CIN, confirming our earlier tentative finding [6]. Likewise, information on the iridoid content of *V. chamaedrys* is scarce. GRIS has not previously

Table 3
Results from screening plant samples for the iridoid glycosides

Iridoid	Concentration (mg/l)					
	Plantago lanceolata	*Veronica spicata*	*Veronica chamaedrys*	*Melampyrum pratense*	*Melampyrum sylvaticum*	*Succisa pratensis* *Valeriana sambucifolia*
CAT	200–500	200–500	100–200	Trace (ca. 10)	Trace (ca. 10)	
AUC	200–500	200–500	100–200	100–300	100–300	
UNE						
HARP						
MEC		25–50				
MOR						
ASP	50–100					
GRIS			50–100			
KET						
VERB	20–40		10–30			
LOG					20–40	
CIN		Trace (ca. 10)				

Screening results from Ref. [6] are included. The iridoid glycosides are CAT, catalpol; AUC, aucubin; UNE, unedoside; HARP, harpagide; MEC, methyl catalpol; MOR, morroniside; ASP, asperuloside; GRIS, griselinoside; KET, 7-ketologanin; VERB, verbenalin; LOG, loganin; CIN, 10-cinnamoyl catalpol. In some cases, larger samples (up to 500 mg) were needed for quantitation of the analytes. The amounts of iridoid glycosides are calculated for a 100 mg sample and extract dissolved in 2 ml of water.

been reported for this plant. We also found VERB, which was not identified in the samples studied in our previous article [6].

The results for the plant samples are collected in Table 3.

4. Conclusions

We developed a reliable MECC separation method for eleven neutral iridoid glycosides and used the method, modified for partial filling technique, in MECC–ESI-MS. We screened seven plant species for the iridoids using PF-MECC–ESI-MS and found some of the compounds in the samples. The CE–MS system operated well for determination of the presence and amount of the iridoid glycosides in the plant samples, which contained hundreds of different compounds.

We compared the properties of ammonium dodecyl sulfate, lithium dodecyl sulfate and sodium dodecyl sulfate in the MECC separation and MS analysis of iridoid glycosides. SDS gave the best results in MECC, and the noise at the MS was diminished when the SDS was dissolved in the background electrolyte (BGE) solution instead of in pure water.

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