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Non-aqueous capillary electrophoresis with diode array and electrospray mass spectrometric detection for the analysis of selected steroidal alkaloids in plant extracts

Samir Cherkaoui[a], Khalid Bekkouche[a], Philippe Christen[a], Jean-Luc Veuthey[b,*]

[a]*Laboratory of Pharmaceutical Analytical Chemistry, University of Geneva, Bd d'Yvoy 20,1211 Geneva 4, Switzerland*

[b]*Laboratory of Medicinal Plants and Phytochemistry, Department of Biology, Faculty of Sciences-Semlalia, P.O. Box 2390, Marrakesh, Morocco*

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Abstract

Nonaqueous capillary electrophoresis coupled to UV detection is described for the separation and determination of steroidal alkaloids. After optimization of electrophoretic parameters, including the electrolyte nature and the organic solvent composition, a reliable separation of solasodine and solanidine was achieved in a methanol–acetonitrile (20:80, v/v) mixture containing 25 m*M* ammonium acetate and 1 *M* acetic acid. For quantitative purposes, a fused-silica capillary with a bubble cell was used and detection was performed at low wavelength (195 nm). Method performances, including migration time and peak area reproducibility, linearity, sensitivity and accuracy, were also evaluated. The method was applied to determine solasodine in *Solanum elaeagnifolium* berries and *Solanum sodomaeum* leaves and seeds. To further improve sensitivity in the analysis of solasodine-related compounds, solanidine, demissidine and tomatidine, the developed method was interfaced with electrospray ionization mass spectrometry. In the case of solasodine, the detection limit was estimated at 3 μg/ml for NACE–UV and at 0.05 μg/ml for NACE–MS, in the selected ion-monitoring mode.

Keywords: Non-aqueous capillary electrophoresis; Plant materials; *Solanum* spp.; Alkaloids; Steroids; Solasodine; Solanidine

1. Introduction

Steroidal glycoalkaloids found in the genus *Solanum* (Solanaceae) form an important group of secondary metabolites. These natural compounds are of interest as the starting material for the synthesis of steroidal drugs. Natural steroidal glycoalkaloids contain three structural portions: a polar, water-soluble glycosidic moiety with three or four monosaccharides linked to the 3-position of the aglycone, a non-polar steroid unit and either indolizidine or oxa-azaspirodecane basic portion [1,2]. In probably the majority of plants which elaborate glycoalkaloids, the main aglycone is solasodine (Fig. 1) [3,4], a nitrogen analogue to sapogenins. In *Solanum* plants, solasodine occurs as solasonine and solamargine triglycosides, and can be readily converted to 16-

*Corresponding author. Tel.: +41-22-702-6336; fax: +41-22-702-6808.

E-mail address: jean-luc.veuthey@pharm.unige.ch (J.-L. Veuthey).

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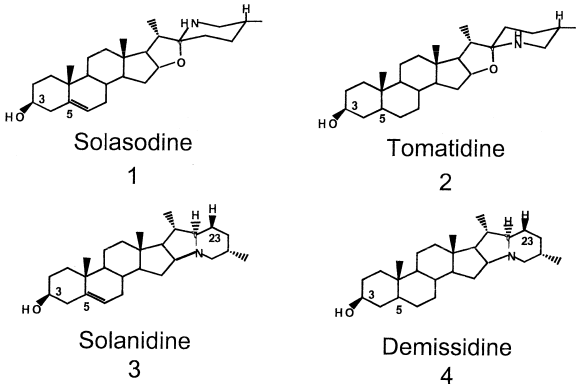

Fig. 1. Structure of the investigated alkaloids, with their identification numbers.

dehydropregnenolone, a key intermediate in the synthesis of steroid drugs, e.g. progesterone, cortisone [5]. Another steroidal alkaloid, often encountered in plants of the *Solanum* genus, is solanidine which generally appears in the form of α-solanine and α-chaconine glycosides. Beside solasodine and solanidine, other steroidal glycoalkaloid aglycones, namely demissidine and tomatidine, are also considered in this study (Fig. 1).

Because of the great pharmaceutical potential of solasodine and its related compounds, their separation and determination have been achieved through various analytical methods such as colorimetry [6,7], immunoassays [8,9], thin-layer chromatography [10,11], gas chromatography [12–14] and high-performance liquid chromatography [15,16].

Capillary electrophoresis (CE) represents an interesting alternative to chromatographic techniques. It has revealed an enormous separation potential for the analysis of plant secondary metabolites [17,18]. There has been a growing interest in CE using non-aqueous buffers [19–21] and several applications have been reported [22–28]. Compared to those of water, the different physical and chemical properties (dielectric constant, viscosity, auto-protolysis constant, polarity, volatility etc.) of organic solvents are particularly interesting in terms of selectivity manipulation and reduction of separation time. In particular, organic solvents improve the solubility of hydrophobic compounds and in some cases may reduce the degradation rate of some labile substances. In this context, an NACE–UV method has been recently described for the analysis of steroidal alkaloids [29]. This method mainly focused on the analysis of solasodine and its glycoside, solasonine, in plant samples, and no quantitative determination was reported. Moreover, because of the lack of chromophore groups, these compounds exhibited low UV sensitivity.

This paper focuses on the analysis and determination of solasodine in plant extracts, using non-aqueous (NA) CE coupled to UV detection. The influence of the background electrolyte on the separation of a standard mixture was investigated. Because steroidal alkaloid aglycones such as solasodine possess a very low molar absorptivity, on-line coupling with electrospray ionization mass spectrometry was also evaluated. Finally, the developed NACE–UV–MS method was applied to the analysis of steroidal alkaloids in plant extracts.

2. Experimental

2.1. Chemicals

Solasodine, solanidine, demissidine and tomatidine hydrochloride were purchased from Sigma (St. Louis, MO, USA). Homatropine hydrobromide was obtained from Merck (Darmstadt, Germany). Analytical reagent-grade ammonium acetate, acetic acid and formic acid were obtained from Fluka (Buchs, Switzerland). HPLC-grade methanol, acetonitrile and isopropanol were supplied from Romil (Kölliken, Switzerland). All other reagents and solvents were analytical grade reagents from Fluka. Ultra-pure water was supplied by a Milli-Q RG unit from Millipore (Bedford, MA, USA).

2.2. Instrumentation

2.2.1. NACE–UV

NACE data were generated in a HP^{3D} CE system (Hewlett-Packard, Waldbronn, Germany) equipped with an on-column diode-array detection system, an autosampler and a power supply able to deliver up to 30 kV. A CE CHEMSTATION (Hewlett-Packard) was used for CE and mass spectrometry control, data acquisition and data analysis. The extended path-length capillary (Hewlett-Packard) was 80.5 cm

long, while the length to the detector was 72 cm, with a 50 μm I.D. (bubble factor 3). An alignment interface containing an optical slit matched to the internal diameter was used and the detection wavelength was set at 195 nm with a bandwidth of 10 nm.

All experiments were carried out in cationic mode (anode at the inlet and cathode at the outlet). A constant voltage of 30 kV was applied during analysis and the capillary was thermostated at 20°C. Samples were kept at ambient temperature in the autosampler and injected by applying a pressure of 50 mbar for 5 s (6 nl).

The capillary was rinsed daily with 0.1 *M* sodium hydroxide, Millipore water, acetonitrile and separation buffer for 10 min each. This flushing procedure is expected to remove any trace of water from the capillary. Between analyses, the capillary was flushed with the running buffer for 3.5 min. As electrolysis of the buffer solution can occur and subsequently change the electroosmotic flow (EOF), the separation buffer was replaced every ten injections. When not in use, the capillary was washed with 0.1 *M* sodium hydroxide and water and then dry stored.

Unless otherwise specified, the non-aqueous buffer was prepared by mixing the appropriate amount of ammonium acetate and acetic acid in a methanol–acetonitrile mixture (20:80, v/v). Before use, electrolyte solutions were filtered through 0.20-μm microfilters (Supelco, Bellefonte, PA, USA) and degassed in an ultrasonic bath for 10 min.

2.2.2. Mass spectrometry

On-line coupling of the CE instrument to the mass spectrometer was achieved with a commercial coaxial sheath liquid interface (Hewlett-Packard, Palo Alto, CA, USA) via the electrospray ionisation (ESI) mode. The ESI-MS measurements were carried out in the positive ionization mode and performed in a single quadrupole HP Series 1100 MSD (Hewlett-Packard), with an upper mass limit of 3000 u. NACE–MS experiments were performed in a fused-silica capillary (Composite Metal Service, Worcestershire, UK) with 85 cm total length×50 μm I.D. To maintain a stable electrospray, a 20-mm portion of the polyimide coating was removed from the outlet edge of the capillary. This procedure was found effective in providing better mixing characteristics at the probe tip. The drying and nebulizing gases were, in both cases, nitrogen. ESI capillary was set at +4.0 kV. The nebulizing pressure and drying gas flow-rate were set at 4 p.s.i. and 4 l/min, respectively. The gas temperature was set at 150°C and fragmentor voltage at 50 V. Coaxial sheath liquid, consisting of isopropanol–water (50:50, v/v) in presence of 0.5% formic acid was delivered by a Harvard Model 22 syringe pump (South Natick, MA, USA) at 3 $\mu l\,min^{-1}$. In the scan mode, masses were scanned from 300 to 450 u at the rate of 1.0 scan/s. In the single ion monitoring (SIM) mode, four selected masses were acquired, respectively 398, 400, 414 and 416 for solanidine, demissidine, solasodine and tomatidine; with a dwell time of 50 ms each.

2.3. Standard and sample preparation

2.3.1. Standard solutions

Stock standard solutions of solasodine and related species were prepared by dissolving each compound in methanol (1.0 mg/ml). Further dilutions were performed in methanol containing homatropine used as internal standard at a concentration of 50 μg/ml.

2.3.2. Sample preparation

Solanum sodomaeum extracts (leaves and seeds) and *Solanum elaeagnifolium* extract (berries) were obtained according to the procedure described elsewhere [12]. The extracts were dissolved in methanol and filtered through a 0.2-μm filter prior to injection.

3. Results and discussion

3.1. NACE–UV

NACE was found appropriate for the analysis of the studied steroidal alkaloids because of their very low solubility in water. Moreover, because some of them (e.g. tomatidine and demissidine) do not possess a chromophore agent, our first investigations using NACE coupled to UV detection were limited to solasodine and solanidine. According to previous papers [26,28], a methanol–acetonitrile mixture proved to be efficient in analyzing hydrophobic

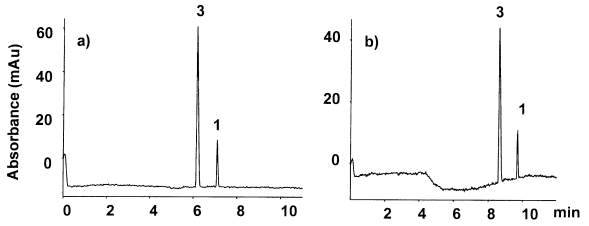

Fig. 2. Separation of solasodine and solanidine by NACE–UV with (a) 20% methanol and (b) 80% methanol in acetonitrile. Electrophoretic conditions: a methanol–acetonitrile mixture containing 25 m*M* ammonium acetate and 1 *M* acetic acid. Uncoated fused-silica capillary: 80.5 cm (effective length 72 cm)×50 μm I.D. (bubble factor 3). Applied voltage; 30 kV, sample injection; 50 mbar for 5 s, temperature; 20°C. Detection at 195 nm. Peak assignment as in Fig. 1.

compounds as well as drugs and metabolites that are difficult to separate in aqueous buffers. Thus, a methanol–acetonitrile mixture containing ammonium acetate and acetic acid was selected to separate solasodine and solanidine. By keeping the electrolyte concentration constant (25 m*M* ammonium acetate and 1 *M* acetic acid), different methanol–acetonitrile mixtures were investigated between 0 and 100% methanol with a step of 20%. As expected [20], varying the methanol percentage in acetonitrile induced changes in terms of electrophoretic mobility (Fig. 2a and b), efficiency and resolution due to concomitant modifications of viscosity (η) and dielectric constant (ϵ). Indeed, since electrophoretic mobility and efficiency are directly proportional to ϵ/η and ϵ^2/η ratio, respectively [19], a MeOH–MeCN (20:80, v/v) mixture results in an efficient and rapid separation (Fig. 2a). Therefore, methanol–acetonitrile (20:80, v/v) containing 25 m*M* ammonium acetate and 1 *M* acetic acid was selected for further investigations.

3.2. Quantitative determination of solasodine in plant extracts

The optimized method for solasodine determination in plant extracts was validated in a similar way to that generally adopted for HPLC and now employed to validate CE methods [30,31]. Migration time and peak area ratio (solasodine peak area divided by homatropine used as internal standard), reproducibility, detector response linearity with sample concentration, sensitivity and recovery were systematically assessed. Relative standard deviation (RSD) values calculated for the migration time and peak area ratio of solasodine were 0.5 and 1.7%, respectively. Intermediate precision was also evaluated over 3 days by performing six successive injections daily. RSD values were 1.3 and 2.7% for migration time and peak area ratio, respectively. These results show that the method precision is satisfactory.

The limit of detection (LOD), defined as the lowest analyte concentration that can be clearly detected, is estimated as three times the signal-to-noise ratio. The estimated limit of detection was 3 μg/ml giving a limit of quantification (LOQ) value of 9 μg/ml for solasodine. Method accuracy was determined by performing the dosage of solasodine in *S. elaeagnifolium* extract. Three different amounts of solasodine were added to the berries extract of *Solanum elaeagnifolium.* Each experiment was repeated thrice. A mean recovery of 101.27% was observed for solasodine with RSD values $<1.5\%$, indicating good precision of the described method (Table 1).

Finally, the validated method was applied to the quantitative determination of solasodine in *Solanum elaeagnifolium* berries and *Solanum sodomaeum*

Table 1
Accuracy data

Dosage (n=3)	Component	Amount of solasodine (μg/ml)		Recovery (%)	RSD (%)
		Added	Recovered		
S. elaeagnifolium extract	Solasodine	100	102.14	102.14	0.72
		200	201.01	100.50	1.01
		300	303.53	101.17	1.28
Mean				101.27	1.003

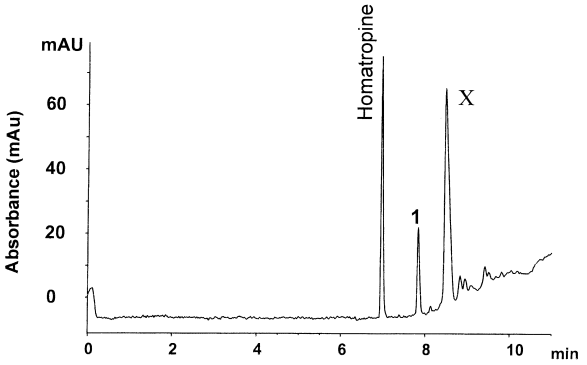

Fig. 3. NACE–UV separation of *Solanum elaeagnifolium* berry extracts. Electrophoretic conditions: MeOH–MeCN (20:80, v/v) containing 25 m*M* ammonium acetate and 1 *M* acetic acid. X, Unknown compound; other conditions as in Fig. 2.

Table 2
Solasodine content of *Solanum elaeagnifolium* berry extracts and *Solanum sodomaeum* leaf and seed extracts

Source	Solasodine content (mg/g of dry mass)[a]
Solanum elaeagnifolium	
Berries	4.72±0.11
Solanum sodomaeum	
Leaves	2.36±0.28
Seeds	3.39±0.10

[a] Results expressed as mean±standard deviation.

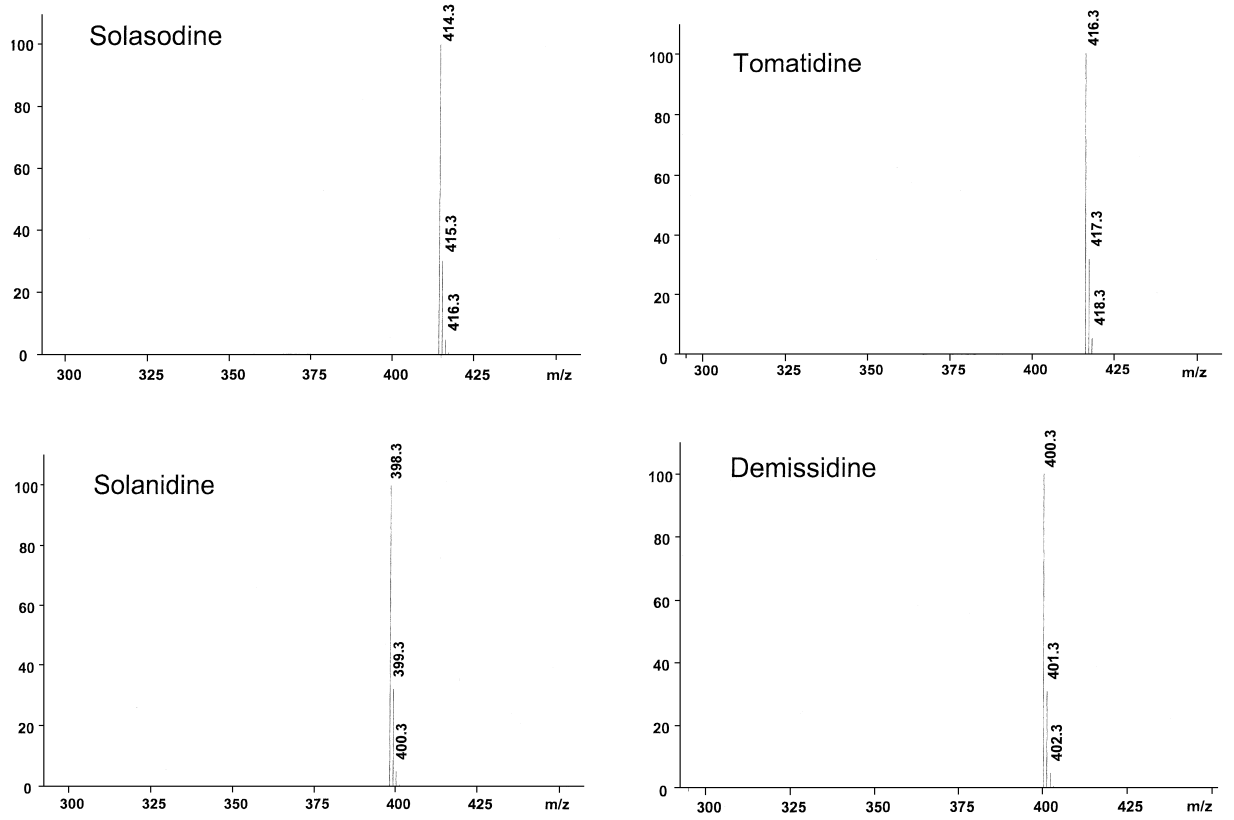

Fig. 4. Positive ESI-MS spectra of solasodine, tomatidine, solanidine and demissidine.

leaves and seeds. A typical electropherogram is shown in Fig. 3. Since solasodine mainly occurs in the plants in its glycoside form, the extraction of solasodine from the samples involved an acid hydrolysis. Identification was carried out by spiking extracts with standard solasodine and solanidine. In all the studied extracts, solanidine was not detected and solasodine was present in varying amounts (Table 2). Between the two investigated species, *S. elaeagnifolium* is the best source of solasodine, because the concentration in berries is significantly higher.

3.3. NACE–MS

UV spectrophotometry is the detector of choice for the on-line detection of compounds in CE. However, this detector suffers from its relatively low sensitivity and is limited to compounds which have a chromophore. Furthermore, peak identity can only be confirmed with migration times, which is often insufficient to unequivocally identify compounds of interest since plants often contain numerous unknown interfering compounds. In this context, MS is becoming an interesting alternative detector in CE since it allows highly selective and sensitive analyses as well as information about the mass and, potentially, the structure of the separated compounds.

Successful CE–ESI-MS coupling can be limited by the non-volatility of commonly used aqueous buffers. Thus, because of their physicochemical properties, organic solvents containing volatile electrolytes present a valuable alternative to extend the application range of CE–MS [32–35]. Several steroidal alkaloids do not possess a chromophore; therefore the on-line coupling of NACE with ESI-MS was

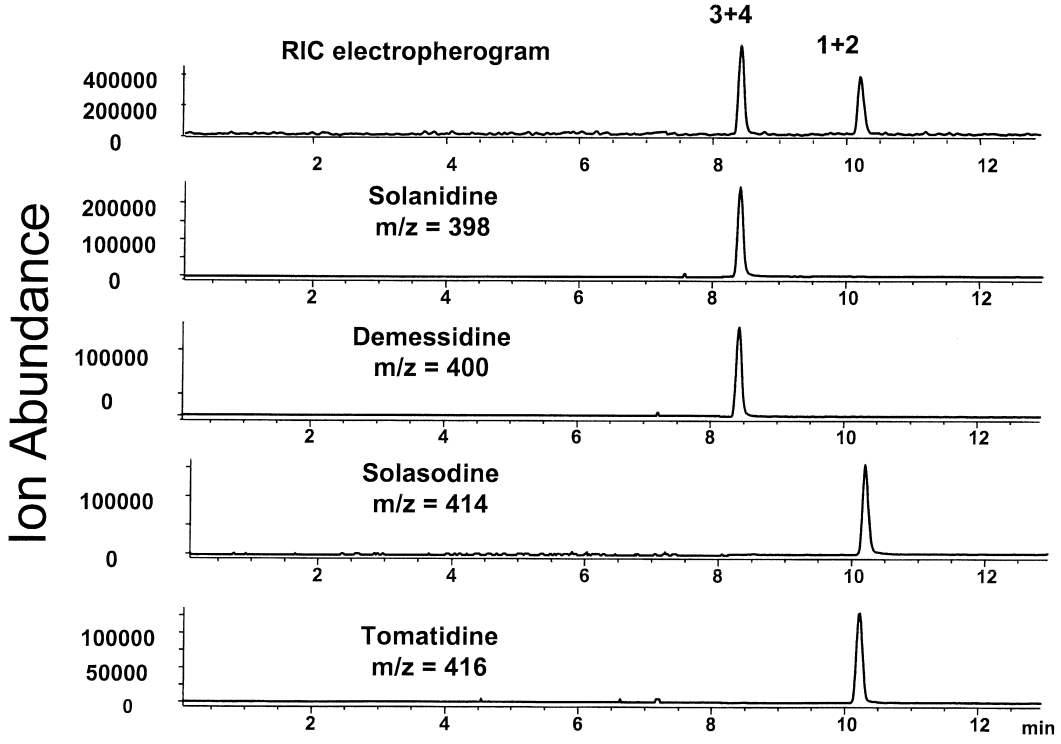

Fig. 5. Typical NACE–ESI-MS electropherogram of a standard mixture of investigated alkaloids. NACE conditions: running buffer, MeOH–MeCN (20:80, v/v) containing 25 m*M* ammonium acetate and 1 *M* acetic acid; uncoated fused-silica capillary dimensions, 85 cm total length×50 μm I.D.×360 μm O.D.; pressure injection, 50 mbar for 6 s; applied voltage, 25 kV; temperature, 20°C. ESI-MS conditions: SIM positive ion mode (four ions); capillary voltage, 4 kV; fragmentor, 50 V; drying gas N_2 flow and temperature, 6 l/min and 150°C; nebulizer pressure 3 p.s.i (1 p.s.i.=6894.76 Pa); sheath liquid, 0.5% formic acid in water–isopropanol (50:50, v/v); sheath flow-rate, 3 μl/min.

investigated. Under the selected ESI-MS conditions, the predominant ion was the protonated molecular ion ($[M+H]^+$), as shown for the mass spectrum of each compound (Fig. 4). Nevertheless, two minor peaks at $[M+H+1]^+$ and $[M+H+2]^+$ of 30 and 5% of the major peak, respectively, are also present. These percentages correspond to the isotopic abundance. A standard mixture of solasodine, solanidine, tomatidine and demissidine was analyzed using the NACE conditions described above. Because of their structural similarity, solasodine and tomatidine as well as solanidine and demissidine were not resolved (Fig. 5). However, the SIM traces of masses corresponding to their respective protonated molecular ions allowed efficient identification and quantification of the studied alkaloids (Fig. 5). Demissidine and tomatidine, which do not possess a chromophore, are clearly detected under NACE–ESI-MS conditions.

In order to evaluate the sensitivity of NACE–ESI-MS in terms of concentration, several dilutions of solasodine standard solution were analyzed. As a result, in the SIM mode, the limit of detection was estimated at 0.05 μg/ml for solasodine. Compared to UV detection, sensitivity enhancement represents a gain of 10^2.

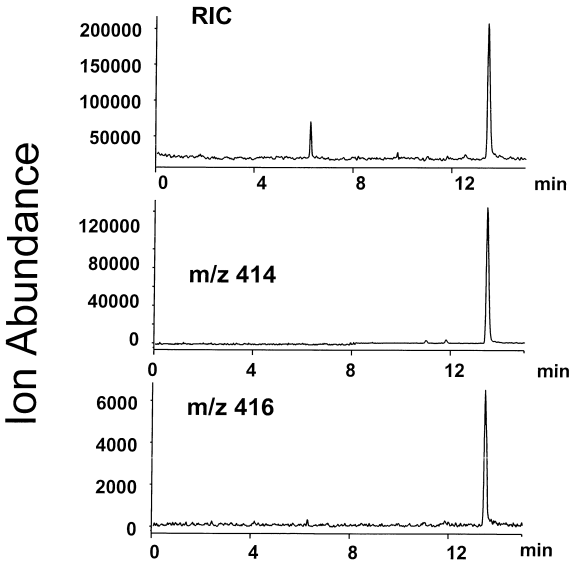

Fig. 6. NACE–ESI-MS analysis of *Solanum elaeagnifolium* berry extracts. NACE and ESI-MS conditions as in Fig. 5. RIC, reconstructed ion chromatogram.

3.3.1. Application

To demonstrate the potential of the NACE–ESI-MS method in the analysis of steroidal alkaloids, two plant extracts were selected. In this study, a qualitative analysis only is presented. Solasodine, tomatidine, solanidine and demissidine were analyzed in *S. elaeagnifolium* berry extracts and *S. sodomaeum* leaf and seed extracts. As shown in Fig. 6, solasodine (m/z 414) was found in a high amount, which is in accordance with the NACE–UV data already reported. An additional peak was also detected at m/z 416. The ratio observed between peaks 414 and 416 in the extracts was identical to that measured in the solasodine standard. This result confirms that solasodine is the only steroidal alkaloid to be found in the studied extracts. Thus, the presence of tomatidine, which has a protonated molecular ion at m/z 416, is invalidated. Furthermore, solanidine and demissidine were not detected.

4. Conclusion

This paper presents the potential of nonaqueous capillary electrophoresis for the analysis of water insoluble steroidal alkaloids. A successful NACE–UV method was developed using a running buffer consisting of 25 m*M* ammonium acetate and 1 *M* acetic acid in methanol–acetonitrile (20:80, v/v). This method was applied to determine in a reliable manner the solasodine content of three plant extracts. Organic solvents allow an easy coupling of NACE with ESI-MS. Under optimized NACE–ESI-MS conditions, solasodine is determined without ambiguity, while other investigated compounds are not detected. Moreover, the SIM mode allows to improve method sensitivity by a factor of 10^2 compared with NACE coupled to UV detection. Taking its sensitivity and specificity into account, NACE–ESI-MS is of great value for the analysis of natural products in complex matrices, especially compounds lacking a chromophore. Hence, the described method represents a valuable tool for the analysis of steroidal alkaloids in plant extracts.

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