Comparison of electrospray ionization and atmospheric pressure chemical ionization techniques in the analysis of the main constituents from *Rhodiola rosea* extracts by liquid chromatography/mass spectrometry

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**INTRODUCTION**

*Rhodiola rosea* L. (Golden Root) has been used for a long time as an adaptogen in Chinese traditional medicine and is reported to have many pharmacological properties. A liquid chromatographic (LC) method with mass spectrometric (MS) detection based on selected ion monitoring (SIM) was developed for determining salidroside, sachaliside 1, rosin, 4-methoxycinnamyl-O-β-glucopyranoside, rosarin, rosavin, cinnamyl-(6′-O-β-xylopyranosyl)-O-β-glucopyranoside, 4-methoxy-cinnamyl-(6′-O-α-arabinopyranosyl)-O-β-glucopyranoside, rosiridin and benzyl-O-β-glucopyranoside from the callus and plant extracts in one chromatographic run. Good linearity over the range 0.5–500 ng ml\(^{-1}\) for salidroside, 2–2000 ng ml\(^{-1}\) for rosavin and 2–500 ng ml\(^{-1}\) for benzyl-O-β-glucopyranoside was observed. The intra-assay accuracy and precision within quantitation ranges varied between −10.0 and +13.2% and between 0.7 and 9.0%, respectively. Optimization of the ionization process was performed with electrospray and atmospheric pressure chemical ionization techniques using four different additive compositions for eluents in the LC/MS scan mode, using both positive and negative ion modes. The best ionization sensitivity for the compounds studied was obtained with electrospray ionization when using pure water without any additives as the aqueous phase. Copyright © 2003 John Wiley & Sons, Ltd.

**KEYWORDS:** Rhodiola rosea L.; phenylpropanoids; salidroside; rosavin; liquid chromatography/mass spectrometry; selected ion monitoring; electrospray; atmospheric pressure chemical ionization

*Rhodiola rosea* L. (Golden Root) has been known as a medicinal plant for a long time and has been known as a medicinal plant for a long time and has been used in Chinese traditional medicine to enhance the body’s resistance against fatigue and to extend human life.\(^1\) The plant is distributed globally in Arctic regions, including northern Asia, Alaska and northern parts of Europe. The plant has been connected with a number of biological activities, such as effects on prolyl endopeptidase inhibition,\(^2\) antiallergenic effects,\(^3\) antimicrobial effects,\(^4\) effects on memory and learning,\(^5\) antidepressant and anti-inflammatory effects,\(^6,7\) effects on liver regeneration and cancer therapy\(^8–12\) and prophylactic effects on ischemic cerebral circulation disorders.\(^13\)

Bioactivity in the plant is believed to be due to a number of glycosidic compounds isolated from the plant and its callus culture extracts, such as hydroxyphenylethyl glucoside salidroside (1) and the phenylpropanoids rosin (2), rosarin (3), rosavin (4), 4-hydroxycinnamyl-O-β-glucopyranoside (sachaliside 1, triandrin) (5) and 4-methoxycinnamyl-O-β-glucopyranoside (vimalin) (6) (Fig. 1). Compounds 1–4 are reported to be pharmacologically active as antioxidants and neurostimulants,\(^14,15\) and the monoterpenoid glycoside rosiridin (7) has been found to have stimulant properties.\(^16\) In addition to these long-known metabolites, also benzyl-O-β-glucopyranoside (8) and the phenylpropanoid cinnamyl-(6′-O-β-xylopyranosyl)-O-β-glucopyranoside (9) and 4-methoxycinnamyl-(6′-O-α-arabinopyranosyl)-O-β-glucopyranoside (10) were recently isolated from the plant (Fig. 1).\(^17\)

A few earlier papers have been published concerning the analysis of *R. rosea* extracts by liquid chromatography (LC) with UV\(^{18–21}\) and atmospheric pressure chemical ionization (APCI) mass spectrometric (MS)\(^22\) detection. However, the methods were focused on only a few of the compounds mentioned above, and also require a long analysis time of from 30 to 45 min per analysis. Recently, we reported the first LC method capable of determining all of the mentioned compounds simultaneously in one chromatographic run,\(^23\) but owing to the use of UV detection the separation required over 30 min and the sensitivity was not good enough for measuring low-level concentrations from cell culture extracts.

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Here a sensitive LC/MS method was developed for the determination of compounds 1–10 from callus or plant extracts of *R. rosea* simultaneously in one run. The MS detection was based on selected ion monitoring (SIM) of sodium adducts of the compounds, allowing the use of a faster chromatographic method owing to the selectivity and an increase in sensitivity. In order to find the maximum sensitivity, both common atmospheric pressure ionization sources, electro-spray ionization (ESI) and APCI, were utilized and tested for the compounds with full-scan mode LC/MS runs in both positive and negative ion modes. The effect of different eluent modifiers on the sensitivity was also studied.

**EXPERIMENTAL**

**Reagents and materials**

Salidroside and rosavin standards were purchased from Anti Aging Center (Budapest, Hungary) and benzyl-O-β-glucopyranoside standard from Toronto Research Chemicals (North York, Canada). Internal standard salicin (11), HPLC-grade acetonitrile and methanol (LiChrosolv GG) were purchased from Merck (Darmstadt, Germany). Ammonium acetate and formic acid were purchased from BDH (Poole, UK). Laboratory water was distilled and purified with a Simplicity 185 water purifier (Millipore, Molsheim, France). Purities of the standards were determined by LC with photodiode-array detection using a maximum plot in the range 190–800 nm. Results for salidroside and rosavin were 97% and 96%, respectively.

**Sample preparation and extraction**

The cell culture and plant material used were grown in the Department of Biology and at the Botanical Gardens at the University of Oulu. For quantitative analyses 30 mg of freeze-dried and powdered cell or plant material from *R. rosea* were extracted for 60 min in an ultrasonic bath with 1.5 ml of 60% aqueous methanol containing 400 ng ml⁻¹ of internal standard salicin. The extracts were centrifuged for
5 min at 10 000 rpm with a Minispin centrifuge (Eppendorf, Hamburg, Germany) and filtered with 13 mm GHP Acrodisc 0.45 µm syringe filters (Gelman Sciences, Ann Arbor, MI, USA). In the case of the concentrated plant samples, the extracts were diluted 1:99 with 6% methanol containing 400 ng ml\(^{-1}\) of internal standard to avoid exceeding the linear range. The samples used for optimizing and comparing the ionization methods were similarly prepared plant extracts, in to which small amounts of isolated analyte compounds were spiked to ensure their presence. All the compounds examined were isolated from the plant extract and identified with UV, mass and one- and two-dimensional NMR spectrometry, as described earlier.\(^{17}\)

**Chromatography**

A model 2690 Alliance LC system (Waters, Milford, MA, USA) was used. The LC separation was performed using a Waters Polarity 2.1 × 50 mm column with 3 µm particle size together with a Synergi Max-RP 2.0 × 4.0 mm precolumn (Phenomenex, Torrance, CA, USA). The temperature of the column oven was 35 °C and the injection volume was 5 µL. The eluent flow-rate was 0.3 ml min\(^{-1}\). The gradient conditions were initially 90% A (aqueous phase)–5% B (acetonitrile)–5% C (methanol), changed linearly to 76% A–12% B–12% C in 16 min. Different modifier compositions for the aqueous phase were tested when optimizing the ionization process (see Table 1). After gradient elution the column was washed for 1 min with acetonitrile and equilibrated for 5 min under the initial conditions, leading to a total time of 22 min for one analysis.

**Mass spectrometry**

**Ionization optimization**

All LC/ESI- and APCI-MS experiments were performed with a Micromass (Altrincham, UK) Quattro II triple-quadrupole instrument equipped with a Z-spray ionization source, using MS1 in the full-scan mode over the m/z range 80–530 with a 0.9 s scan time. In ESI experiments, capillary voltages of 3.4 and −3.2 kV were used in the positive and negative ion mode, respectively. The sample cone voltages were 22 V in the positive ion mode and −20 V in the negative ion mode, and the extraction cone voltages were 4 and −3 V, respectively. The sample cone was adjusted to produce a maximum amount of molecular ion. Nitrogen was used as both drying and nebulizing gas at flow-rates of 400 and 20 l h\(^{-1}\), respectively. The desolvation temperature was 320 °C and the source temperature 150 °C. Post-column splitting of the flow to source with a ratio of 1:4 was tested but did not give any improvement in sensitivity, so direct inlet from the column at a flow-rate of 0.3 ml min\(^{-1}\) was used.

In APCI experiments, a corona discharge needle voltage of 2.1 kV was used in the positive ion mode and −2.3 kV in the negative ion mode. The cone voltages were 22 and −20 V and the extraction cone voltages were 5 and −4 V, respectively. Nitrogen was used as the drying gas at a flow-rate of 300 l h\(^{-1}\). The probe temperature was set to 420 °C and the source temperature to 150 °C. The flow was delivered into the ionization source directly at a flow-rate of 0.3 ml min\(^{-1}\).

**Table 1. Aqueous eluent phases used in solvent optimization studies**

<table>
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<tr>
<th>Eluent system</th>
<th>Composition of aqueous phase A</th>
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<tr>
<td>I</td>
<td>Water</td>
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<td>II</td>
<td>0.1% (v/v) formic acid</td>
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<td>III</td>
<td>0.02% formic acid + 10 mM ammonium acetate</td>
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<tr>
<td>IV</td>
<td>10 mM ammonium acetate</td>
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\(^{a}\)The gradient system with all eluent systems contained same percentage of organic solvents (acetonitrile (B) and methanol (C), see text).

**Analytical method**

In analytical method testing with eluent system 1, the experiments were performed with positive ion electrospray ionization and SIM mode acquisition. The capillary voltage was 3.3 kV, and the extraction cone voltage 4 V for all compounds.

The sample cone voltages were 22 V for internal standard salicin (II) (Fig. 1) and analyte 1, 20 V for phenylpropanoids 2–6, 9 and 10, 22 V for 7 and 26 V for 8. The desolvation temperature was 320 °C and the source temperature 150 °C. Nitrogen was used as both drying and nebulizing gas at flow-rates of 400 and 20 l h\(^{-1}\), respectively. SIM data were acquired by focusing the sodiated molecules [M + Na]\(^{+}\) via the first quadrupole mass filter (Q1) to the first detector. The dwell times used were 0.2 s. The time windows for monitoring each ion were 0–3.8 min for 11 (m/z 309), 1.0–4.2 min for 1 (m/z 323), 2.8–5.8 min for 8 (m/z 293), 10.0–14.2 min for 2 (m/z 319), 13.0–17.5 min for 3, 4 and 9 (m/z 451), 14.5–18.0 min for 10 (m/z 481) and 14.2–18.0 min for 7 (m/z 355), and compounds 5 (m/z 335) and 6 (m/z 349) were monitored simultaneously in the time window 4.0–18.0 min.

**Calibration**

For the calibration of the LC/MS (SIM) method with ESI and eluent system 1, eight concentration levels of standards were prepared for obtaining calibration curves. Owing to the lack of availability of commercial compounds, salidroside (I) was used as an external standard for 1 and 7, benzyl-O-β-D-glucopyranoside (8) for 8 and rosavin (4) for 2–6, 9 and 10. Compound 11 was chosen as the internal standard owing to its suitable LC retention behavior and its structural similarity to the compounds studied. Standard solutions were prepared in 6% methanol containing 400 ng ml\(^{-1}\) of internal standard. For all standards the concentration levels prepared were 0.5, 2, 5, 20, 50, 200, 500 and 2000 ng ml\(^{-1}\). Calibration curves were constructed by plotting the response of the standard compounds relative to the response of the internal standard (measured in triplicate for each concentration level) against the concentration of standard compounds. A linear fit with 1/x weighting was used.
RESULTS AND DISCUSSION

Comparison of ionization methods and conditions

A number of papers concerning the effect of different mobile phase additives on ionization in LC/ESI- and APCI-MS studies have been reported, but to our knowledge, no optimization for similar types of phenylpropanoid glycosides to those here have been published. By using different additives in LC/MS applications, not only the sensitivity can be optimized by affecting the ionization process, but also the amount of structural information gained from the mass spectra can be adjusted. In natural product analysis, the most common additives used with application of both ESI and APCI are acetic acid, formic acid, ammonium acetate, and ammonium formate. Trifluoroacetic acid has also been used in some studies, despite the fact that in many cases it is found to suppress the ionization owing to ion pairing and surface tension effects. The acid concentrations added to the aqueous mobile phase are usually between 0.05 and 2%, and the ammonium buffer concentrations are usually in the range 0.5–100 mM. Some less common additives, such as ammonia and ammonium-containing compounds, e.g. triethylammonium acetate, have also been used. However, in some applications the best sensitivity was obtained without any additives in the LC mobile phase.

In order to find the most sensitive ionization method for the compounds studied, ESI and APCI were tested with the same LC gradient using four different additive compositions for the aqueous mobile phase (Table 1).

The sample used was a plant extract, to which small amounts of isolated analyte compounds were spiked to ensure their presence. The pseudo-molecular ions formed and the most abundant fragment ions were identified from the mass spectra obtained (Table 2), and ion chromatograms corresponding to these main peaks for each analyte were extracted to minimize the background noise. The intensity of these extracted chromatographic peaks based on different eluent systems and ionization methods were compared using signal-to-noise ratios (calculated from peak to peak), and are presented in Fig. 2. An example of the extracted ion chromatograms from these full-scan LC/MS experiments is presented in Fig. 3, where the eluent system used was I. Retention times were 2.1 min for internal standard 1, 2.6 min for 2, 4.4 min for 8, 5.6 min for 5, 12.7 min for 2, 13.7 min for 3, 14.9 min for 4, 15.7 min for 9, 15.7 min for 7, 15.8 min for 10 and 16.0 min for 6.

The positive ion ESI mode was found to be clearly the most sensitive ionization method for the analyte compounds studied, generally resulting two to five times higher signal-to-noise ratios for the extracted ion chromatograms than the negative ion ESI mode and positive ion APCI mode, which resulted in about the same abundances. The negative ion APCI mode was clearly the most unsuitable ionization technique for the analytes studied, resulting in 10–100 times lower signal-to-noise ratios compared with the positive ion ESI mode.

Positive ESI

In positive ion ESI experiments, the compounds studied showed a very high tendency to form alkali metal adducts, mainly with sodium, but in some cases small peaks for potassium adducts were also detected. The sodium adduct [M + Na]+ was the main peak in the spectra of all compounds when using eluent systems I–III and present also in spectra with eluent system IV, where the main peak was the ammonium adduct [M + NH4]+. The ammonium adduct was also present in spectra obtained using eluent system III.
Table 2. The ions detected (m/z) in spectra with different ionization modes at constant cone voltages

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<tr>
<th>Mode</th>
<th>Compound</th>
<th>[M + Na]^+</th>
<th>[M + K]^+</th>
<th>[M – glu]^+</th>
<th>[M – gluO]^+</th>
<th>[M – ara/ xyl – glu]^+</th>
<th>[M + NH4]^+</th>
<th>[M – OgluO]^+</th>
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<td>ESI+</td>
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<td>121</td>
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where also the potassium adduct [M + K]^+ was seen. The high tendency for adduct formation replaced the formation of protonated molecules completely, so that peaks for [M + H]^+ ions were not observed in any spectra. With all eluent systems mainly only one fragment ion was present, which was formed by loss of sugar units, i.e. the charged aglycone part of the molecule. For 1 this loss of glucose was seen as an ion [M – 163]^+, for 2 and 5–8 the glucose ring cleaved from the other side of the bonding glucose oxygen forming ion [M – 179]^+, whereas for 3, 4, 9 and 10 the corresponding ions due to loss of both sugar units were [M – (149 + 162)]^+. The relative abundance of these fragment ions was lowest in spectra obtained using eluent systems I and II, being about half that of the main peaks, whereas on using eluent systems with ammonium acetate (III and IV) the abundance of fragment ions were as high as those of [M + NH4]^+ and [M + Na]^+ or higher. This is probably due to the lower stability of [M + NH4]^+ ion compared with [M + Na]^+. The signal-to-noise ratios of the extracted ion chromatogram peaks were clearly highest for all compounds.
with eluent system I, which is as expected when the dominant ionization process is the formation of stable sodium adducts in the liquid phase. The addition of any mobile phase modifiers decreased the efficiency of sodium adduct formation and increased substantially the amount of background noise, the latter especially with formic acid with eluent system I. Ions used for extraction are marked in each experiment (full-scan mode) with positive ESI and eluent systems I, II, and IV.

The pKₐ values taken from the SciFinder database are 10.2 ± 0.15 and 9.9 ± 0.15 for hydroxy-substituted analytes I and 5, respectively, and 12.9 ± 0.70 for the rest of the compounds. As these phenols and their glycosides are acidic compounds, they should be generally neutral in solutions with the conditions used. Therefore the weak protonation and effective ionization via adduct formation is quite expected. The suppression of adduct formation by formic acid addition may be partly due to the capability of [HCOO]⁻ ions to neutralize positive sodium cations. The ammonium adduct formation with eluent systems III and IV is, however, able to replace the sodium adduct formation, as is typical for glycosides and phenolics.

**Negative ESI**

In negative ion ESI mode experiments, the peak due to the deprotonated molecule [M − H]⁻ was seen in all spectra and was clearly the main peak for all compounds with eluent system I, and a small peak for [M + Na − 2H]⁻ was also detected for some compounds. With eluent systems II and III, the most intense peak was due to formate adduct [M + HCOO]⁻ formation and was seen at m/z [M + 45]⁻. With eluent system III, the acetate adduct [M + OAc]⁻ at m/z [M + 59]⁻ and [M − H]⁻ were also fairly abundant, whereas with eluent system IV the most intense peak was clearly due to [M + OAc]⁻. The amount of fragmentation varied, generally being lower than when using the positive ion mode, greatest with eluent system I and very low with eluent systems II and III. The usual fragment ions were formed owing to loss of aglycones [M − H–AgOH]⁺, so that the sugar units retained the negative charge, leading to peaks at m/z 161 for glucosides and at m/z 293 for 3, 4, 9 and 10 with two sugar units. The fragment ions of one sugar unit retaining the charge were also detected, at m/z 179 for glucosides and m/z 149 for 3, 4, 9 and 10.

In contrast to the positive ion mode, none of the eluent systems was clearly better than others in producing the maximum sensitivity for all compounds. However, eluent system I without any mobile phase additives seemed to be the most sensitive for almost all the compounds studied, whereas the acidic eluent system II resulted in the highest signal-to-noise ratio for 7 and also generally gave slightly better sensitivity than eluent systems III and IV. For 2, the highest signal-to-noise ratio was obtained with eluent systems III and IV.

The formation of [HCOO]⁻ adducts suggest that the protons present are transferred to weaker acids, water, methanol and acetonitrile with high proton affinities, in either liquid- or gas-phase reactions. The small amount of [M − H]⁻ ions suggests that no effective proton transfer reaction between analyte compounds and [HCOO]⁻ occurred at pH 2.6, as opposed to earlier study with flavonoids.

The negative ion ESI mode was shown to result in about 2–10 times lower signal-to-noise ratios than the positive ion mode, when the most sensitive eluent systems for each compounds were compared (eluent systems I and II for negative ESI and I for positive ESI). The only exception to this was 1, for which eluent system I resulted in similar intensities using both the positive and negative ion modes. Owing to their acidic nature, the analyte compounds should be ionized more effectively at negative ion mode at high pH conditions. However, as their pKₐ values are high, the pH conditions used here were apparently too low to effectively deprotonate the analyte ions in liquid phase. The best ionization efficiency gained with ESI with eluent system I (at highest pH) when compared with the other eluent systems, was as expected.

**Positive APCI**

The phenomenon of intense adduct formation occurred also in the positive ion APCI mode. The same sodium and potassium adducts as formed with ESI were stable also during the chemical ionization process, so that mainly the same peaks and fragment ion peaks as in ESI experiments were detected. With eluent systems I and II, [M + Na]⁺ was the dominant ion, and weak [M + K]⁺ also was present. A charged aglycone fragment ion due to loss sugar units was present with eluent system II at a similar abundance to [M + Na]⁺, whereas with eluent system I the extent of fragmentation was about halved. With eluent systems III

**Figure 3.** Extracted ion chromatograms from LC/MS experiment (full-scan mode) with positive ESI and eluent system I. Ions used for extraction are marked in each chromatogram ([M + Na]⁺/fragment ion). The concentrations of the compounds in the sample were 200–1200 ng ml⁻¹.

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and IV, the [M + NH₄]⁺ and [M + Na]⁺ ions were seen for all compounds; with eluent III their abundance were similar and with eluent IV the ammonium adduct was much more abundant. The fragmentation with eluent systems III and IV was not as great as in ESI experiments under similar conditions, which suggests that the APCI conditions are milder for labile [M + NH₄]⁺ ions.

The signal-to-noise ratios obtained with the positive ion APCI mode were generally of the same magnitude as in the negative ion ESI, mode i.e. 2–10 times lower than with the positive ion ESI mode. The effect of different eluent systems was similar when compared with the positive ion ESI mode, so that eluent I gave the highest signal-to-noise ratios and eluents II–IV gave about the same abundances. Also similarly to the positive ion ESI mode, addition of mobile phase modifiers led to strongly increased background noise. As the liquid-phase adduct formation seemed to be the dominant ionization process, the effect of mobile phase modifiers on general gas-phase reactions in APCI ionization cannot be directly seen from the results. However, owing to the different probe properties, adduct formation with APCI was much more weaker than in with ESI and the sensitivity therefore lower.

The sensitivity in the positive ion APCI mode was decreased further when the probe temperature was decreased from 420°C, probably owing to the high vaporization temperature of the glycosides. However, an increase in temperature did not improve the sensitivity, but led to increased background noise and greater fragmentation.

**Negative APCI**

In the negative ion APCI mode, the ions formed were mainly the same as with the negative ion ESI mode. The only exception was with eluent system I, where the presence of the [M + 32]⁻ ion was observed for all compounds. The origin of this ion could be reaction with methanol, producing a negatively charged complex of analyte and methanol molecules. The abundance of these ions was close to that of [M – H]⁻, the main peak with this eluent system. With eluent system II the main ions seen were [M – H]⁻ and [M + HCOO]⁻, of which the latter was more abundant. With eluent system III, [M – H]⁻ and [M + HCOO]⁻ were seen, the most abundant being [M + HCOO]⁻. No [M + OAc]⁻ ions were detected with eluent system III, in contrast to negative ESI experiments with the same eluent system. With eluent system IV the main peaks in spectra were due to [M – H]⁻ and [M + OAc]⁻ ions, with similar abundances.

The fragmentation detected was close to that observed in negative ion ESI mode experiments, i.e. only ions due to loss of sugar units with low abundance.

The negative ion APCI mode was clearly the most ineffective ionization process for the analyte compounds studied. The signal-to-noise ratios obtained were very low compared with the other three methods, so that for six out of 10 compounds studied all the signal-to-noise ratios were at the detection limit or below, i.e. ≤3. For those compounds (1, 4, 7 and 8) for which real peaks were observed, the highest signal-to-noise ratios were obtained with eluent system II. As in the negative ESI case, the formation of [M + HCOO]⁻ ion at low pH suggests the presence of another process that transfers the available protons to other mobile phase components present with higher proton affinities.

**LC/MS with SIM detection**

In LC/MS experiments, ESI with eluent system I was found to be the most suitable for the compounds studied, producing the most intense LC/MS peaks when ion chromatograms were extracted using molecular ions and fragment ions. However, when a more sensitive detection method was developed for analytical use, the usual multiple reaction monitoring (MRM) with fragment ion detection was not possible with this eluent system. The molecular ions formed were sodium adducts [M + Na]⁺, which in the case of analyte compounds with only one sugar unit were found to be very stable and produced only negligible amounts of product ions in collision-induced dissociation (CID) experiments (with argon gas; data not shown), so that MRM detection was not possible with 1, 2 and 5–8. Interestingly, this unfavorable phenomenon did not occur with compounds with two sugar moieties (3, 4, 9 and 10), but instead [M + Na – 334]⁻ and [M + Na – Agl]⁻ fragment ions were observed. A reason for this different behavior may be different coordination sites for sodium cations in molecules with one and two sugar units. Of the possible detection modes, SIM for sodium adducts, MRM for ammonium adducts (with eluent system IV) and ‘surviving ion MRM’ for sodium adducts of the analyte compounds were optimized and tested. In the last case, the detection was changed from typical MRM, the quadrupole after the collision cell being set to detect the precursor ion surviving from the collision with argon gas in the collision cell. When these detection modes were adjusted to their maximum sensitivity, SIM with sodium adducts was found to be only slightly more sensitive than surviving ion MRM, being about 10–20 times more sensitive than MRM with ammonium adducts in eluent system IV.

With SIM detection for 1, good linearity of response with a correlation coefficient of 0.994 was obtained in the range 0.5–500 ng ml⁻¹. The accuracy for 1 determined with the same standard solutions varied within the linear range from −9.1 to +13.2% and the intra-assay precision for different concentrations was between 1.0 and 8.9% (relative standard deviation (RSD)) (Table 3). A 15% deviation was considered to be the limit of acceptance for quantitation, so the −22.2% deviation with a 2000 ng ml⁻¹ standard was not acceptable.

For 4 a correlation coefficient of 0.999 was obtained for the range 2.0–2000 ng ml⁻¹. The accuracy within quantitation limits ranged from −2.9 to +11.7% and the intra-assay RSD for different concentrations was between 1.0 and 4.3%. For 8 a correlation coefficient of 0.999 was obtained for the range 2.0–500 ng ml⁻¹. The accuracy within quantitation limits ranged from −10.0 to +11.0% and the intra-assay RSD for different concentrations was between 0.8 and 8.2%.

**CONCLUSIONS**

A sensitive and reproducible LC method with selected ion MS detection was developed for the determination of bioactive constituents from *Rhodiola rosea* extracts. Positive ion
ESI without any mobile phase additives was found to be the most sensitive ionization method for the analyte compounds studied, producing the highest abundances. This method is recommended when using LC/MS for these compounds. The compounds studied showed a very strong tendency to ionize via sodium adduct formation, which was replaced by ammonium adduct formation when ammonium acetate was added to the eluents. Ionization via protonation was not observed, but adduct formation was the main ionization process even under APCI conditions. In the negative ion mode the ionization occurred through deprotonation or formate and acetate adduct formation. Under the experimental conditions used, the in-source fragmentation was not very abundant the main fragment ions being due to the loss of sugar units with abundances usually lower than those of the molecular ions. Owing to the high stability of sodium adducts of the analytes, the collision cell CID was very weak, preventing the use of traditional MRM detection. Using LC/MS with SIM, high sensitivity and good a linear range of detection for the analyte compounds were obtained.

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Table 3. Intra-assay accuracy and precision results for the LC/MS method using the SIM mode with eluent system I

<table>
<thead>
<tr>
<th>[1] (ng ml⁻¹)</th>
<th>Accuracy (% deviation)</th>
<th>Precision (RSD, %)</th>
<th>[4] (ng ml⁻¹)</th>
<th>Accuracy (% deviation)</th>
<th>Precision (RSD, %)</th>
<th>[8] (ng ml⁻¹)</th>
<th>Accuracy (% deviation)</th>
<th>Precision (RSD, %)</th>
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<td>−10.0</td>
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