Talanta 81 (2010) 1258–1263

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Rapid quantification of 14 saponins of *Maesa lanceolata* by UPLC–MS/MS

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ARTICLE INFO

Article history:
Received 5 October 2009
Received in revised form 2 February 2010
Accepted 7 February 2010
Available online 13 February 2010

Keywords:
MS quantification
Saponins
Relative response factors
UPLC–MS/MS
Sodium adduct

ABSTRACT

Saponins are high molecular weight glycosides which are known for their broad range of biological activities. In case of *Maesa lanceolata*, a tree growing in African countries, the maesasaponins showed virucidal, haemolytic, molluscicidal and anti-angiogenic activity. Since the different activities are dependent on the structure of the saponins, a method was developed and validated for the analysis of the individual saponins in this plant.

Since the saponins were only present in small amounts, it was necessary to develop a very sensitive analytical method. For the fast and sensitive analysis of the extracted and purified plant samples ultra-performance liquid chromatography was coupled to a triple quadrupole mass spectrometer for MS/MS detection. A method in positive ESI mode, using sodium acetate in the mobile phase, was developed. The sodium adduct ion was selected as the precursor ion since it provided better sensitivity and a better, more stable fragmentation compared to the deprotonated and protonated ions. The intensity of the signal obtained by fragmentation of the sodium adducts of the saponins, was optimized by the addition of different concentrations of sodium acetate to the mobile phase. Reference standards were not available for all 14 saponins. Therefore, a relative MS/UV response was calculated allowing the estimation of the saponins in real samples. α-Hederin was used as external standard.

The method was linear over the investigated concentration range with a good correlation coefficient (>0.99). The intra- and inter-day precisions were below 15% for most maesasaponins with the exception of maesasaponin II, which showed a precision within 20%. The recoveries of the spiked pure compounds maesasaponin IV.1 and VII.1 were 96.6% and 85.5%, respectively.

The validated method can be applied in the investigation of the content of 14 saponins in transgenic and non-transgenic plant material of *M. lanceolata*.

1. Introduction

Maesa lanceolata Forsskal var. *gonlungensis* Welw. is a shrub or small tree growing in many African countries. The plant is used in Rwandan traditional medicine against various diseases including infectious hepatitis, bacillary dysentery and some types of dermatoses and neuropathies, while it is also used in East African folk medicine to prevent cholera [1,2]. A series of triterpenoid ester saponins from this plant were isolated and characterised. These maesasaponins contain the same glycosyl moiety and have aglycons with an oleanane skeleton that contain a 13,28-epoxy bridge, and are hydroxylated at position C-16, C-21 and C-22. One, two or all three hydroxyl groups are esterified with short-chain fatty acids (Fig. 1) [3–5]. Structure–activity relationships for the antiviral, haemolytic and molluscicidal properties revealed an important influence of the esterification pattern. A free 16-OH and acylation of the 22-OH appeared to be essential for the virucidal activity, while a high molluscicidal effect was associated with a 21,22-diangeloyl acylation pattern. Furthermore, the mixture of maesasaponins showed a moderate anti-angiogenic activity in the CAM (chick embryo chorioallantoic membrane) assay. The diester maesasaponin II showed to be one of the most promising anti-angiogenic compounds with no membrane-irritating or haemolytic effect [6,7], but was only present in very small amounts in the plant. To increase the amount, a platform of combinatorial biosynthesis in the plant was developed. By introducing genes involved in saponin biosynthesis we are attempting to identify new active compounds, and a higher production of the known compounds. In the first phase of the project, only small amounts of transgenic plant material are available. Therefore a very sensitive analytical method was developed.

A HPLC–UV method has been previously developed to analyse maesasaponins in transgenic and non-transgenic plants of *M. lanceolata* [8]. Since the HPLC–UV method does not allow to quantify the individual maesasaponins and due to the very small amounts of

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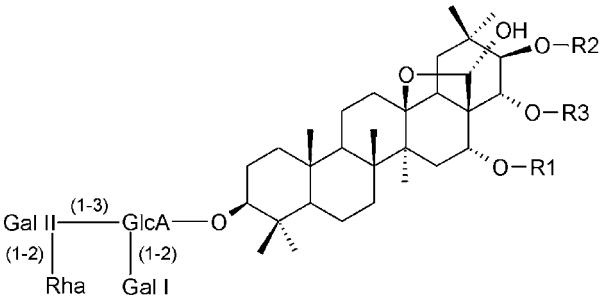
doi:10.1016/j.talanta.2010.02.018



	m/z	R1	R2	R3
Maesasaponin I	1234	H	Angeloyl	H
Maesasaponin II	1276	Acetyl	Angeloyl	H
Maesasaponin III.1	1306			
Maesasaponin III.2	1276	H	Angeloyl	Acetyl
Maesasaponin IV.1	1248			
Maesasaponin IV.2	1318	Acetyl	Angeloyl	Acetyl
Maesasaponin IV.3	1290	H	Angeloyl	Propanoyl
Maesasaponin V.1	1260			
Maesasaponin V.2	1332	Acetyl	Angeloyl	Propanoyl
Maesasaponin V.3	1304	H	Angeloyl	Butanoyl
Maesasaponin V.4	1316			
Maesasaponin VI.1	1338			
Maesasaponin VI.2	1316	H	Angeloyl	Angeloyl
Maesasaponin VI.3	1346	Acetyl	Angeloyl	Butanoyl
Maesasaponin VII.1	1358	Acetyl	Angeloyl	Angeloyl
Maesasaponin VII.2	1314			

Fig. 1. The molecular structure of the maesasaponins.

saponins present in the samples, the development and validation of a hyphenated UPLC–MS/MS method was necessary.

2. Experimental

2.1. Reagents and solutions

Formic acid, methanol and acetonitrile of HPLC-grade were purchased from Acros Organics (Morris Plains, NJ, USA). Deionised water for UPLC analysis was prepared with a Millipore water purification system (Millipore, Bedford, MA, USA). Sodium acetate was obtained from Sigma (St. Louis, MO, USA). A mixture of compounds of *M. lanceolata* (Maesasaponin I, II, III.1, III.2, IV.1, IV.2, IV.3, V.1, V.2, V.3, V.4, VI.1, VI.2, VI.3, VII.1 and VII.2) and a sample of both maesasaponin IV.1 and maesasaponin VII.1 were available in the lab from previous investigations [4]. α-Hederin used as the external standard was obtained from extrasynthese (Genay, France).

2.2. Plant material

The leaves of *M. lanceolata* Forsskal var. *golungensis* Welw. were collected in Butare, Rwanda and identified by Dr J. Mvukiyumwami of the botanical department of the IRST (Institut de la Recherche Scientifique et Technique) in August 1989. A voucher specimen is kept within the institute. The plant material was dried and powdered.

2.3. Instrumentation

2.3.1. Chromatographic conditions

An acquity ultra-performance liquid chromatography (UPLC) system, consisting of an autosampler and a binary pump (Waters, Milford, MA) equipped with a 10 μL loop, was used. Compounds were separated on an Acquity HSS T3 C18 column (2.1 mm × 100 mm, 1.8 μm; Waters, Milford, MA). The column and autosampler were maintained at a temperature of 30 and 15 °C, respectively. A flow rate of 0.5 mL/min was chosen and the following gradient was used: solvent A = water + 0.05% formic acid + 0.01 mM sodium acetate; solvent B = acetonitrile + 0.05% formic acid; gradient: from 10 to 25% B in 0.3 min – from 25 to 55% B in 5.7 min – from 55 to 90% B in 2 min – from 90 to 98% B in 0.1–0.9 min on 98% B – from 98 to 10% B in 0.1–0.9 min on 10% B. 10 μL was injected using full loop injection.

2.3.2. Mass spectrometer conditions

The UPLC system was coupled to a TQD triple quadrupole mass spectrometer (Waters, Milford, MA) equipped with an electrospray ionization (ESI) source operated in the positive ion mode. The experimental conditions for the operation of the instrument were optimized by direct infusion of a solution of maesasaponins and α-hederin. The optimal conditions were as follows: capillary voltage 4000 V, extractor voltage 4 V, cone voltage 90 V, source temperature 150 °C, desolvation temperature 450 °C, RF lens 0.1 V, desolvation gas flow 1000 L/h, cone gas flow 50 L/h. The quadrupole was set for maximum resolution. The quantification of the sodium adducts of all compounds was performed using the SRM mode to increase selectivity. All data were recorded and processed using Masslynx software, version 4.1 (Waters).

2.4. Sample preparation

The amount of saponins present in the leaves of *M. lanceolata* was quantified according to the following procedure, which is an adaptation and faster compared to the preparation procedure described earlier [8]. About 100 mg of dried plant material was sonicated in 5 mL 50% methanol (v/v) for 1 h. After filtration, this procedure was repeated on the residue. Both extracts were combined and dried under reduced pressure. The dried sample was reconstituted in 6 mL water and the solution was brought on a Chromabond® SPE C_{18} cartridge (500 mg, Machery-Nagel, Düren, Germany) which was preconditioned with methanol and

water. The column was successively rinsed with 6 mL water and 12 mL methanol 30% (v/v). Finally the saponins were eluted with 12 mL methanol. The resulting fraction was dried under vacuum and redissolved in 10.0 mL methanol 80% (v/v). The samples were 10-fold diluted before analysis.

2.5. Validation

The method was validated according to the ICH guidelines [9,10].

2.5.1. Response function—calibration model

The calibration models of α-hederin and maesasaponins IV.1 and VII.1 were investigated. Seven concentration levels of α-hederin were prepared, ranging from 10 to 700 ng/mL. For maesasaponin IV.1 and maesasaponin VII.1, respectively seven and eight concentration levels were prepared, ranging from 16 to 630 ng/mL and from 10 to 994 ng/mL. To check the concentration range in which linearity was obtained for all maesasaponins, solutions of the mixture of maesasaponins at 13 different concentration levels (10–1 × 10^6 ng/mL) were prepared. All solutions were analyzed in duplicate. The ratio between the calibration curves obtained with the UPLC–MS/MS method and those obtained with HPLC/UV according the method of Theunis et al. [8] were used to calculate a correction factor for the MS response of each individual maesasaponin. The repeatability of these correction factors was investigated by the analysis of two solutions, namely one of the external standard α-hederin (700 ng/mL) and one of maesasaponin VII.1 (250 ng/mL) in duplicate on two different days. The correction factor found for both days was compared to the ones found based on the calibration lines.

2.5.2. Matrix effect

To evaluate the influence of the matrix on the quantification of the compounds, two calibration curves of α-hederin were acquired in duplicate within the concentration range 10–700 ng/mL. One calibration curve was made of a solution of α-hederin in methanol 80%, the other of a sample prepared as described above spiked with α-hederin.

2.5.3. Precision

The intermediate precision was determined by analyzing six independently prepared samples (100%; 100 mg) according to the above described method. The procedure was repeated on three different days. In order to evaluate the repeatability on different concentration levels, six samples weighing 50% of the normal mass (50 mg) and six samples weighing 150% of the normal mass (150%; 150 mg) were analysed. For the analysis of the samples at a different concentration range slight adaptations were made of the dilution procedure performed at the end of the sample preparation procedure. More specifically, instead of a tenfold dilution, the samples containing 50% were diluted 1/5 and the samples containing 150% were diluted 1/15. Four concentration levels (ranging from 100 to 700 ng/mL) of the external standard α-hederin were prepared daily. The methanol 80% standard solutions were injected twice before the samples for calibration and once at the end of the sequence in order to investigate the stability of the MS signal.

2.5.4. Accuracy

To investigate the accuracy of the method, a recovery experiment was performed. To 50% of the plant material (50 mg) a known amount of either maesasaponin IV.1 or maesasaponin VII.1 was added until a total concentration of 100% of either one of the maesasaponins was obtained. For both saponins the samples were prepared in triplicate according to the described procedure.

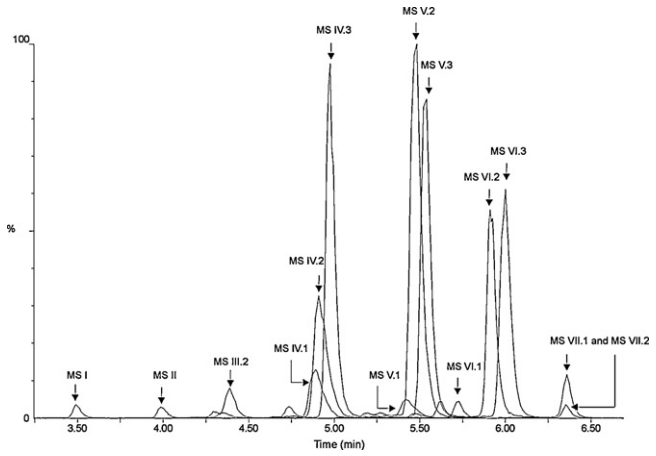


Fig. 2. UPLC–MS/MS chromatogram of the sodium adducts of the maesasaponin mixture.

3. Results and discussion

3.1. Method development

Previously, an HPLC–UV method was developed to measure the saponin content in dried *Maesa* leaves [8]. Although this method gave an acceptable accuracy, precision and specificity, the low UV sensitivity of maesasaponins and the lack of a baseline separation of the peaks gave rise to the need of a more sensitive and selective method. Especially, because of the low amounts of saponins in cell cultures and transgenic *Maesa* lines. Therefore a UPLC–MS method was preferred above the HPLC–UV method. The application of UPLC instead of HPLC allows a drastic reduction of analysis time with higher chromatographic resolution and sensitivity, while the selectivity of SRM transition in the triple quadrupole makes it possible to quantify the maesasaponins separately (Fig. 2) [11].

The previously developed extraction method [8] was slightly changed to decrease the sample preparation time. Furthermore, the extraction starts from less plant material, which was necessary for the expected amounts of available material.

Within the method development one of the major issues was the lack of an appropriate, cheap and single component, commercially available standard for the saponins to be quantified. The saponins of *M. lanceolata* are not commercially available and their isolation would be very time consuming and expensive, which would make the method not useful for other research groups. Previously oleanolic acid was used as external standard [8], but due to insufficient fragmentation and the chemical deviation from the maesasaponins with the lack of the presence of a glycan part which leads to a chromatographic elution, far later then the elution of the compounds of interest. Therefore, this compound could not be used. To overcome this problem, α-hederin was chosen as a secondary standard and relative UV/MS response factors were used whenever possible to quantify the maesasaponins in plant samples.

3.2. LC–MS/MS analysis

All maesasaponins showed a MS response in both positive and negative ESI mode. The intensity of the signal obtained for the $[M-H]^-$ ions and $[M+Na]^+$ ions was superior to that of the $[M+H]^+$ ions. The fragmentation of the $[M+Na]^+$ ions provided a much more stable signal and the intensity of the fragments was higher compared to those obtained by fragmentation of the deprotonated molecules. No optimal collision energy could be obtained to fragment the deprotonated molecules since either no fragmentation was observed or complete destruction of the molecules

Table 1
Influence of the concentration of sodium acetate on the signal intensity and stability of α-hederin.

Concentration sodium acetate (mM)	Concentration α-hederin			
	100 ng/mL		700 ng/mL	
	Mean area	RSD%	Mean area	RSD%
0	23	26	156	7
0.01	281	9	1555	4
0.5	108	8	705	6
1	66	52	305	11

occurred. The fragmentation of the sodiated molecules predominantly occurred on the glycan part [12]. All maesasaponins showed a transition to the same fragment at *m*/*z* 349, with an optimal intensity at a collision energy of 60 eV. After collision-induced dissociation, the most abundant ion in the product ion spectrum of α-hederin was at *m*/*z* 301 at a collision energy of 49 eV. Sodium acetate was added to solvent A to make sure that all remaining protonated molecules were replaced by their sodiated adduct. In order to use the optimal concentration of sodium acetate in the mobile phase, two different concentrations of α-hederin (100 and 700 ng/mL) were injected 5 times at different concentrations of sodium acetate (0, 0.01, 0.5 and 1 mM). The addition of 0.01 mM of sodium acetate to solvent A gave the most intense signal with an acceptable RSD% (Table 1). The conditions of the final method are described in the experimental section.

3.3. Validation

3.3.1. Response function—calibration model

For α-hederin, maesasaponin IV.1 and maesasaponin VII.1 the calibration model was investigated by evaluation of the least square lines by means of a Student's *t*-test and correlation coefficients were calculated. A log–log transformation of the peak area and concentration was used in all calculations to stabilize the variances. The results of the calibration curves are shown in Table 2. For all compounds of which the linearity was investigated, the log–log plot showed a linear relationship with a correlation coefficient $R^2 > 0.99$, the slopes of the curves differed from zero and residuals were scattered randomly. Since the regression lines did not contain the point (0, 0) a single point calibration could not be used and a calibration curve of the external standard α-hederin should be made every analysis. To obtain the relative linear ranges of all maesasaponins, the limit of quantification was taken into account by analysing the signal/noise ratio (S/N > 10), which was carried out displaying the peak-to-peak values by Masslynx software. Based on the same conditions an absolute limit of quantification could be calculated for α-hederin, maesasaponin IV.1 and VII.1, that was 10.0, 6.6 and 2.6 ng/mL, respectively.

Analysis of the solutions with a different concentration of the maesasaponin mixture proved that the linear dynamic ranges of the calibration curves exceeded three orders of magnitude for all maesasaponins. The linear range of the maesasaponins clearly exceeded the range of the external standard, which was linear for two orders of magnitude. However since no better secondary standard could be obtained and this method was used for relative quantification only, this was considered to be acceptable.

To calculate the relative response factors of maesasaponin IV.1 and VII.1 the calibration curves of these two individual compounds were used. For both maesasaponin IV.1 and VII.1 a correction factor for the ESI-MS response was calculated on different concentration levels based on the calibration curves. The lowest concentration was excluded to avoid large deviations. For maesasaponin IV.1 the mean correction factor was 1.14 while maesasaponin VII.1 showed a correction factor of 1.25 relative to the response of α-hederin. Using this mean correction factor in the calculations of the different concentration levels, based on the external calibration curve of α-hederin, led to a concentration that deviated less than 3% from the true concentrations of maesasaponin IV.1 above 25 ng/mL. In case of maesasaponin VII.1 deviations lower than 6.6% and 12.2% for the lowest concentration (9.94 ng/mL) could be found. For the other maesasaponins present in the leaves the correction factor could not be calculated based on the calibration curves, since these saponins could not be obtained in sufficient amount and purity. For the saponins present in the maesasaponin mixture that showed baseline separation in the HPLC–UV chromatogram obtained by the method of Theunis et al., a correction factor for the relative MS response could be calculated based on the areas of the compounds, since all compounds had the same chromophoric group and showed the same UV absorption. Based on the MS response versus maesasaponin VII.1 (100 ng/mL) and the calibration curve for the latter saponin, a MS response factor could be calculated for the different saponins relative to α-hederin. The relative MS response factors that were obtained for maesasaponins I, II, VI.1 and VII.2 were 1.30, 1.19, 1.15 and 1.26, respectively. For the maesasaponins that showed no baseline separation in the HPLC–UV chromatogram no specific factor could be obtained. Therefore, a mean overall correction factor of 1.21, calculated based on the response factors found for all other maesasaponins, was used instead in all calculations. Since this mean correction factor is not specific for each compound, the method can only be used for relative quantification. The correction factor for maesasaponin VII.1 was recalculated on two different days by comparing its response with that of α-hederin within the linear range of the ESI-MS. A response factor of 1.25 for maesasaponin VII.1 was obtained on both days. This factor was the same as found using the calibration lines and proves the stability of the factors.

3.3.2. Matrix effect

The effect of the plant sample matrix on the intensity of the signal of the compounds was evaluated by measuring on the one hand a calibration curve of α-hederin dissolved in methanol 80% and on the other hand by spiking different concentrations of α-hederin to a prepared sample. Regression analysis of both curves showed that the 95% confidence interval for both the slope and the intercept overlapped. Furthermore statistical analysis by means of a Student's *t*-test indicated no differences. No matrix effect could be found since the responses of the two sets of calibration solutions were the same.

Table 2
Data of the linear regression of the secondary standard α-hederin and two maesasaponin standards (maesasaponin IV.1 and VII.1) in a limited concentration range.

	α-Hederin	Maesasaponin IV.1	Maesasaponin VII.1
Correlation coefficient	0.9930	0.9952	0.9992
Slope ± standard error	1.0125 ± 0.0246	1.0717 ± 0.0214	1.0355 ± 0.0077
Intercept ± standard error	0.4038 ± 0.0517	0.6239 ± 0.0464	0.9805 ± 0.0171
Confidence interval (95%)	0.2911–0.5166	0.5319–0.7339	0.9439 ± 1.0172
Range (ng/mL)	10–700	16–630	10–994
Number of standards (in duplicate)	7	7	8

Table 3
The data on the precision on different days and different concentration levels.

	MS I	MS II	MS III.2	MS IV.1	MS IV.2	MS IV.3	MS V.1	MS V.2	MS V.3	MS VI.1	MS VI.2	MS VI.3	MS VII.1	MS VII.2	Total saponin
Precision on different days ($n = 3$)															
Repeatability															
Number of replicates									6						
Mean content day 1 (%)	0.0040	0.0016	0.0413	0.2262	0.0727	0.2640	0.0367	0.1315	0.2335	0.0947	0.2421	0.0602	0.0259	0.0073	1.4415
RSD% day 1	9.0	18.0	5.0	2.9	2.7	3.6	3.6	2.9	4.4	9.0	6.1	4.5	5.0	5.3	3.3
Mean content day 2 (%)	0.0040	0.0012	0.0444	0.2847	0.0797	0.3357	0.0398	0.1549	0.2862	0.1145	0.3255	0.0667	0.0272	0.0070	1.7715
RSD% day 2	7.3	15.2	4.0	6.0	3.7	4.3	6.5	5.1	4.3	7.2	7.3	3.2	5.9	6.8	3.4
Mean content day 3 (%)	0.0032	0.0011	0.0418	0.2378	0.0790	0.3456	0.0354	0.1566	0.2958	0.1150	0.3102	0.0664	0.0274	0.0071	1.7224
RSD% day 3	5.8	15.6	4.8	3.9	3.8	4.5	4.3	3.9	4.4	5.7	4.4	4.8	5.6	8.1	3.0
Intermediate precision															
Number of days									3						
Number of replicates									6						
RSD% between groups	13.3	23.4	5.7	13.2	5.9	14.7	7.7	10.2	13.0	12.6	16.2	6.9	5.9	6.7	11.2
Horwitz	9.28	10.89	6.43	4.93	5.88	4.76	6.56	5.33	4.87	5.59	4.81	6.04	6.90	8.42	3.71
F_{calc} (F_{crit}: 3.68)	12.95	6.57	4.25	41.25	12.54	66.08	8.67	30.55	47.38	13.16	36.47	11.13	1.85	0.80	66.5
Mean RT (min)	3.62	4.10	4.51	5.02	5.03	5.10	5.56	5.61	5.66	5.85	6.05	6.13	6.49	6.48	
RT RSD% between groups	1.71	1.62	1.55	1.76	1.17	1.35	1.33	1.33	1.32	1.17	1.23	1.22	1.20	1.11	
Precision on different concentration levels															
Number of replicates									6						
Mean content 50% (%)	0.0045	0.0015	0.0439	0.2422	0.0823	0.2971	0.0397	0.1534	0.2614	0.1089	0.2834	0.0718	0.0302	0.0086	1.6290
RSD% 50%	8.9	16.4	13.4	7.6	5.1	6.8	7.8	7.4	8.4	10.3	9.0	5.1	4.2	4.7	6.4
Mean content 150% (%)	0.0032	0.0010	0.0389	0.2149	0.0700	0.3032	0.0328	0.1439	0.2724	0.1057	0.2991	0.0605	0.0259	0.0065	1.5780
RSD% 150%	6.8	21.3	10.4	5.2	7.8	7.8	6.5	8.3	10.6	11.0	7.3	9.1	9.1	11.2	7.8
Intermediate precision															
Number of days									5						
Number of replicates									6						
RSD% between groups	16.7	27.7	9.9	13.0	9.1	11.6	11.9	9.3	10.8	10.9	12.4	9.7	10.1	14.4	9.2
Horwitz	9.27	10.86	6.44	4.95	5.88	4.77	6.56	5.33	4.86	5.59	4.81	6.03	6.87	8.38	3.72
F_{calc} (F_{crit}: 2.76)	18.12	7.94	2.43	14.12	10.46	18.38	9.37	7.79	9.25	5.51	15.15	10.52	7.36	13.46	14.44

3.3.3. Precision

The repeatability and the intermediate precision of the method was evaluated by analysing six independent samples on three different days and on three different concentration levels (50%, 100% and 150%). The mean content of each maesasaponin and the overall mean percentage of saponins, together with the RSD% within day, between days and between concentrations were calculated. The calibration curve of α-hederin was injected in triplicate: two injections before the samples used for calibration and one at the end of the sequence as control samples to check the MS stability during analysis. For the evaluation of the results an ANOVA single factor was used, while the RSD% between the groups was compared to the limits obtained by Horwitz [13]. The homogeneity of the variances between the different groups (days and concentrations) was confirmed with a Cochran's test. The repeatability of the retention times with the RSD% between the groups was investigated. All results are summarized in Table 3.

The results ($F_{calc} < F_{crit}$; $RSD_{between} < RSD_{Horwitz}$) showed a good precision between the days for maesasaponin III.2, IV.2, VII.1 and VII.2. For most of the compounds differences between the groups were indicated. Nevertheless, the RSD% within and between obtained for most saponins was acceptable since they were below 15%, which is generally accepted for the technique used. Only maesasaponin VI.2 and maesasaponin I had a RSD% of respectively 16.2 and 16.7 for the repeatability on different days and on different concentrations. The low amounts of maesasaponin II in the leaves of *M. lanceolata* close to the LOQ could explain the higher RSD% found. However since the method was developed for the analysis of plants that produce higher amounts of the latter saponin the method could be used for the quantification of the compound.

3.3.4. Accuracy

The accuracy of the method was determined by spiking 3 samples with a known amount of either maesasaponin IV.1 or VII.1. Due to the low amounts of those standard compounds available, the recovery was determined at one concentration level only. For maesasaponin IV.1 a mean recovery of 96.6% was found with a RSD% of 1.2% while for maesasaponin VII.1 85.5% was recovered with a RSD% of 5.4%.

4. Conclusion

A rapid and sensitive UPLC–MS/MS method was developed to relatively quantify the amount of 14 individual maesasaponins present in the plant material of *M. lanceolata*. When compared to the previous method which used HPLC–UV, the proposed method presents faster analysis, with better sensitivity and a similar accuracy. Since the analytical method uses the hyphenated techniques UPLC and a triple quadrupole detection, both techniques will prove the specificity of the method by means of the retention time and fragmentation of the different compounds to their selected ion.

This method can be used in the selection of transgenic modified plants producing the highest amount of biological valuable saponins.

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