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Qualitative and Quantitative Analysis of Steroidal Saponins in Crude Extract and Bark Powder of *Yucca schidigera* Roezl.

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Supporting Information

ABSTRACT: Steroidal saponins in commercial stem syrup and in extract of a bark of *Yucca schidigera* were identified with high-performance liquid chromatography ion trap mass spectrometry and quantitated using ultraperformance liquid chromatography with quadrupole mass spectrometric detection. Fragmentation patterns of yucca saponins were generated using collision-induced dissociation and compared with fragmentation of authentic standards as well as with published spectrometric information. In addition to detection of twelve saponins known to occur in *Y. schidigera*, collected fragmentation data led to tentative identifications of seven new saponins. A quantitation method for all 19 detected compounds was developed and validated. Samples derived from the syrup and the bark of yucca were quantitatively measured and compared. Obtained results indicate that yucca bark accumulates polar, bidesmosidic saponins, while in the stem steroidal glycosides with middle- and short-length saccharide chains are predominant. The newly developed method provides an opportunity to evaluate the composition of yucca products available on the market.

KEYWORDS: *Yucca schidigera* Roezl., LC−MS, steroidal saponins, quantitation

INTRODUCTION

Yucca schidigera Roezl. ex Ortgies is a desert plant from the family Agavaceae that grows natively in the Southwestern region of North America. Condensed juice pressed out from the stem of *Y. schidigera* (yucca syrup) is one of the major commercial sources of steroidal saponins[1] and finds uses in cosmetic, pharmaceutical and beverage industries as well as in animal nutrition.[2,3]

Primary saponins from *Y. schidigera* were reported as glycosides of three C-25 epimeric pairs of sapogenins: sarsasapogenin and smilagenin, markogenin and samogenin, gitogenin and neogitogenin. Derivatives of other sapogenins were also reported in smaller quantities.[4,5] While structure and distribution of individual sapogenins is well documented in different species belonging to the Agavaceae family and in different plant organs, the variety and variability of their glycosides is not very extensively investigated. Several, usually the most abundant, steroidal saponins were to date isolated and identified from *Y. schidigera*.[5,6] According to the structure of their aglycon, they can be classified as either spirostane- or furostane-type derivatives. Their C-3 carbohydrate chains are typically branched oligosaccharides with pentapyranosyl and/or hexopyranosyl terminal units. In the case of furostane bidesmosides, C-26 linked carbohydrate is usually also a hexopyranose.[6]

Using conventional procedures, identification of additional saponins, especially these occurring in smaller quantities, is likely to be not only very tedious and time-consuming but also very difficult due to the remarkable complexity of yucca extracts. Instead, an alternative approach that employs liquid chromatography−mass spectrometry can be used to obtain information on structures of investigated compounds. Combined with the ability to quantitate individual saponins in crude extracts, such a methodology is suitable for monitoring the composition of yucca saponin products used in animal nutrition. Standardization of these products is essential for correlation of their observed biological effects with the saponin composition and contents. So far, the only tool for this purpose was a procedure based on the gravimetric determination of a butanol fraction (obtained by partitioning of yucca extract between 1-butanol and water). Aided by the GC−MS analysis of sapogenins, this method was successfully used in some studies.[7,8] However, it does not provide any information on concentrations of individual saponins in the mixture. Furthermore, yucca-derived preparations are known to contain large amounts of phenolic compounds,[9] which are easily extractable by butanol and can cause inaccurate estimation of saponin contents in the sample.

Detailed analysis of yucca products is important. Even though they possess a GRAS (Generally Regarded as Safe) label from the Flavor and Extracts Manufacturer's Association in the USA, some of their components were suspected to exhibit adverse effects on livestock.[10] Consequently, our primary goal was to develop a method for quick and reliable determination of steroidal saponins in yucca using LC−MS. We then aimed to investigate saponins composition in typical crude yucca products, such as yucca stem syrup and bark powder.

MATERIALS AND METHODS

Reagents and Plant Material. The *Y. schidigera* syrup was purchased from Desert King International (San Diego, CA). Yucca bark powder was prepared from dried yucca bark samples collected from the

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Table 1. Saponins Identified in *Yucca schidigera* Extracts

no.	*m/z*	primary MS^2 ions: *m/z* (intensity)	notes
1	1067	935 (100), 905 (80), 773 (30), 611 (5), 449 (2)	
2	1095	933 (100), 771 (12), 609 (5), 447 (2)	
3	1063	931 (95), 901 (100), 769 (15), 751 (10), 607 (2), 445 (1)	
4	1065	933 (100), 903 (90), 771 (20), 753 (15) 609 (3), 447 (2)	
5	935	917 (10), 773 (100), 611 (7), 449 (2)	
6	1079	917 (100), 755 (15), 593 (4), 431 (2)	
7	1081	919 (100), 757 (20), 595 (4), 433 (1)	
8	1051	919 (90), 889 (100), 757 (30), 595 (4), 433 (2)	presumably identical to saponin 6[6]
9	1049	917 (100), 887 (95), 755 (30), 593 (2), 431 (1)	corresponding by the accurate mass, but not by the aglycon structure, to saponin 6[6]
10	883	751 (70), 721 (100), 589 (30), 427 (5)	schidigera-saponin B1[5]
11	915	753 (100), 591 (20), 429 (3)	saponin 3[6]
12	885	753 (60), 723 (100), 591 (10), 429 (2)	schidigera-saponin C1/E1[5] saponin 1[6]
13	887	755 (100), 725 (85), 593 (30), 431 (2)	schidigera-saponin F1[5]
14	755	711 (20), 593 (100), 431 (1)	schidigera-saponin F2[5]
15	899	737 (100), 575 (30), 413 (2)	schidigera-saponin A3[5]
16	869	737 (80), 707 (100), 575 (20), 413 (2)	schidigera-saponin A1/A2[5]
17	901	739 (100), 577 (40), 415 (2)	schidigera-saponin D3/D4[5]
18	871	739 (65), 709 (100), 577 (15), 415 (2)	schidigera-saponin D1/D2[5] saponin 2b[6]
19	739	577 (100), 415 (2)	schidigera-saponin D5[5] saponin 2a[6]

plants growing in the wild. Standards of saponins **11**, **12** and **18** (Table 1) were previously prepared from yucca extract.[6] Internal standard, avenacoside B in bidesmosidic form, was prepared from oat seeds using a previously reported protocol.[11] HPLC-grade solvents were from J.T. Baker (Phillipsburg, NJ); all other chemicals were from Sigma-Aldrich (St. Louis, MO).

Preparation of Standards. Yucca saponin reference standards and avenacoside B internal standard were weighed to 10 mg in a volumetric flask using an analytical balance. For each standard, the volume was brought to 10 mL with 95% EtOH to make a 1 mg/mL stock solution, which was subsequently stored at −20 °C and used to make dilutions for calibration curves.

Preparation of Samples. Crude, semiliquid yucca syrup (5−7 mg) was diluted with 0.1% formic acid to a final volume of 500 μL, and 10 μL of internal standard solution (65 pmol/μL avenacoside B) was added. The samples were purified by either liquid−liquid extraction (LLE) or solid phase extraction (SPE) procedures.

The samples purified by liquid−liquid extraction were first partitioned against three volumes of ethyl acetate and then partitioned against three volumes of water-saturated 1-butanol. Organic phases from each type of solvent were combined and dried in vacuum.

For SPE purification, samples were applied to a 30 mg/1 mL Waters Oasis HLB SPE cartridge (Milford, MA) equilibrated with distilled water. After a wash with 2 mL of 0.1% formic acid, saponin fractions were eluted first with 2 mL of 80% methanol containing 0.1% formic acid. Then, an additional elution with 100% methanol was performed to release residual material bound to the cartridge.

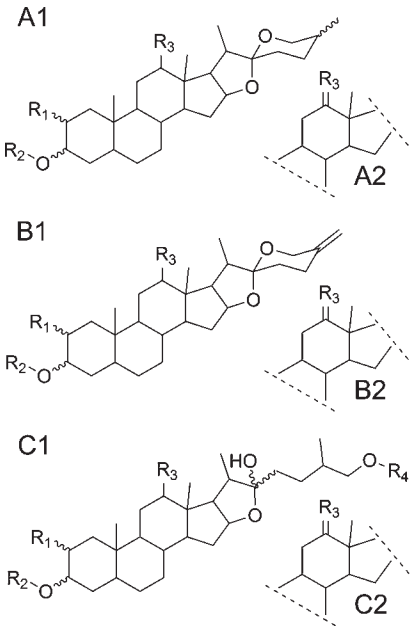
Bark powder samples were room temperature extracted with 80% methanol, centrifuged for 4 min at approximately 18000*g*, diluted with 0.1% formic acid and purified on the Oasis HLB cartridges as above.

Purified samples were dissolved in 500 μL of 5% methanol, filtered using Mini-UniPrep 0.45 μm filter (Whatman, Kent, U.K.) and analyzed by LC−MS. For the evaluation of internal standard recovery, fractions from each step of the purification were dried and processed in the same way as other samples.

Liquid Chromatography−Mass Spectrometry. Structural information and general saponin profiles were gathered using a Thermo LCQ Advantage Max ion-trap mass spectrometer coupled with a Surveyor HPLC system. Separation was performed on a 150 mm × 2.1 mm i.d., 3.5 μm Symmetry C18 column (Waters) using a linear, 25 min gradient from 2.5 to 60% of solvent B (acetonitrile containing 0.1% formic acid) in solvent A (water containing 0.1% formic acid) with a flow of 0.4 mL/min. The mass spectrometer was operated in the negative electrospray mode with the following ion source parameters: spray voltage 3.9 kV, capillary voltage −47 V, tube lens offset −60 V, capillary temperature 240 °C. Nitrogen sheath and auxiliary gas flows were of 65 and 10 arbitrary units, respectively. The acquisition was performed in a data-dependent manner. Following a general scan from 150 to 2000 mass units (3 microscans, max. inject time of 150 ms), peaks with signals higher than 4×10^5 counts were subjected to MS/MS analysis. In each scan, the peak with the highest intensity was fragmented using collision-induced dissociation (CID) and product ions in the range from the cutoff to the parent mass were detected (3 microscans, max. inject time of 250 ms).

Quantitations were done using a Waters UPLC system with Waters triple quadrupole detector (TQD) operating in the negative electrospray mode. Ion source parameters were as follows: cone voltage 35 V, capillary voltage 3 kV, extractor 3 V, RF lens 100 mV, source temperature 140 °C, desolvation temperature 350 °C, desolvation gas flow 600 L/h, cone gas flow 50 L/h, and collision gas flow 100 μL/min. Collision cell parameters were as follows: entrance −2, exit 0.5, and collision energy 22 eV. Parameters of quadrupole 1 were set to achieve maximal mass resolution: both LM and HM resolutions were set to 15, and ion energy was set to 0.8.

Saponins were separated on a 100 mm × 1 mm i.d., 1.8 μm Acquity HSS column (Waters) using the same solvents as for the structural analysis; however, due to differences in stationary phase properties and



No.	Aglycon	R_1	R_2	R_3	R_4
1	C1	OH	Pent-Hex$_2$	H	Hex
2	C2	H	Hex$_3$	O	Hex
3	A2	OH	Pent-Hex$_3$	O	
4	C2	H	Pent-Hex$_2$	O	Hex
5	C1	OH	Hex$_3$	H	Hex
6	A1	OH	Hex$_4$	H	
7	C1	H	Hex$_3$	H	Hex
8	C1	H	Pent-Hex$_2$	H	Hex
9	A1	OH	Pent-Hex$_3$	H	
10	B2	OH	Pent-Hex$_2$	O	
11	A2	OH	Glc(1→2)[Glc(1→3)]Glc	O	
12	A2	OH	Glc(1→2)[Xyl(1→3)]Glc	O	
13	A1	OH	Pent-Hex$_2$	H	
14	A1	OH	Hex$_3$	H	
15	B1	H	Hex$_3$	H	
16	B1	H	Pent-Hex$_2$	H	
17	A1	H	Hex$_3$	H	
18	A1	H	Glc(1→2)[Xyl(1→3)]Glc	H	
19	A1	H	Hex$_2$	H	

Figure 1. Structures of steroidal saponins from *Yucca schidigera* Roezl. For compounds identified tentatively, groups R_2 and R_4 represent carbohydrate chains composed of hexoses (Hex) and pentoses (Pent).

column dimensions, a different, 17 min long linear gradient from 15 to 65% of solvent B in solvent A with a mobile phase flow of 100 μL/min was employed. In each case, 1 μL of the sample was injected using the "partial loop with needle overfill" mode. Quantitations in the single ion-monitoring mode were performed using saponins **11** and **12** as concentration references and avenacoside B as an internal standard. The internal standard recovery study was performed in the conditions described above, except that external calibration curve for quantitation was prepared from avenacoside B standard in the concentration range between 5 and 70 pmol/μL.

Method Validation. Intraday reproducibility was estimated by analyzing 1 μL injection of a yucca extract sample six times during the day. For interday reproducibility, a fresh aliquot of the same sample was analyzed nine times over three days. For all injections, the percent relative standard deviation was calculated from the peak area of each analyzed compound.

Table 2. Recovery of the Internal Standard with Different Purification Methods

phase	LLE meth, stem [%]	fraction	SPE meth, stem [%]	SPE meth, bark [%]
water	11.7	flow-through and wash	20.9	nd[a]
1-BuOH	81.5	80% MeOH	80.3	74.6
EtOAc	4.3	100% MeOH	1.1	nd
total	97.4		102.3	

[a] Not determined.

Precision of the instrument was determined by calculating percent relative standard deviation of the internal standard peak area from all the sample injections over a period of three days.

RESULTS AND DISCUSSION

Structural Characterization. During the collision-induced dissociation, quasi-molecular ions of steroidal saponins repeatedly lose carbohydrate units from their side chains. In addition to providing information on the number, the type and the carbohydrate sequence in the molecule, this process frequently leads to diagnostic ions of sapogenins, hence allowing for tentative identifications. Using data-dependent full scan analysis we identified 19 unique masses related, based on their fragmentation patterns, to the known and unknown steroidal saponins (Table 1, Figure 1). As this technique cannot distinguish between isobaric epimers, it is possible that more than one saponin contributed to each recorded signal. Nevertheless, a classification of detected compounds could be made based on the general type of aglycon and the nature of a glycan part.

Tentative identifications of observed saponins listed in Table 1 were made based on published fragmentation data and their nominal masses calculated from known structures.[5,6] Saponins matching compounds **1** through **7** were not reported before in *Y. schidigera* and related species. Saponin **3** gave an aglycon fragment ion at *m*/*z* 445, possibly indicating a derivative of manogenin or neomanogenin ((25*R*)- or (25*S*)-2α,3β-dihydroxy-5α-spirostan-12-one), reported earlier in *Y. schidigera* as a trace component.[4] Fragmentation patterns of saponins **7** and **8** included an ion at *m*/*z* 433, suggesting a furostane-type aglycon identified previously.[6] Aglycon fragment ions at *m*/*z* 449 and 447 for saponins **1**, **5** and **2**, **4** belong to yet unidentified sapogenins. Based on the preliminary data from MS^3 experiments, they may represent, respectively, 2,3,12,26-tetrahydroxyfurostane and 2,3,26-trihydroxyfurostanone, but we were thus far unable to confirm these assignments using other analytical techniques.

Tentative identifications of saponins **8** and **9** are complicated because Oleszek et al.[6] report for **8** the accurate mass and formula which do not match the proposed furostane-derived structure. Compound **9** is likely to be a new spirostane-type saponin with nominal mass and formula the same as ones published before for **8**.

Among the other detected saponins, those with epimeric sapogenins were most frequently found. Sarsapogenin or smilagenin-derived saponins had fragmentation patterns with ions at *m*/*z* 415, while derivatives of markogenin, samogenin, gitogenin or neogitogenin produced ions at *m*/*z* 431. Additional

sapogenins described previously in *Y. schidigera*, schidigeragenins A, B and C,[5] were also observed by their fragment ions at m/z 413, 427 and 429, respectively.

Oligosaccharides at C-3 in analyzed saponins showed very small variability; they consisted of two groups distinguishable by mass spectrometry with chain sequence containing hexoses and pentose, and chains composed entirely of hexoses. The latter class can be further divided using other analytical techniques.[5] In the case of unknown compounds with side chains containing pentose (compounds **1**, **3**, **4** and **8**), two prominent peaks could be observed, matching independent losses of pentose and hexose. In analogy to fragmentation of structure-characterized saponins **9**, **10**, **12**, **13**, **16** and **18**, such a pattern indicates a branching point from which terminal sugars were lost. This type of carbohydrate chain was reported earlier in yucca.[5,6]

In the cases of saponins **11**, **12**, and **18**, extracts were spiked with authentic standards[6] and separated in the same chromatographic conditions to confirm their identity.

Method Development and Validation. With the exception of saponins **11**, **12** (analogous to schidigera-saponin E1 and schidigera-saponin D1[5]) and **18**, which were purified from the yucca extract during our previous investigation,[6] standards of individual yucca saponins are not available commercially or otherwise. Making a true external calibration for all 19 detected steroidal glycosides would then require a substantial, time-consuming effort to obtain good quality reference compounds from the plant material. Since this was not feasible, we developed a method in which saponins without proper reference standards are quantitated relatively, based on the response curves for saponins **11** and **12**. This type of approach is frequently used in metabolome analysis where, due to the lack of appropriate standards for a large number of analytes, the use of direct calibration is prohibitively complicated, if not impossible. Relative quantitation was also successfully employed for LC−MS determination of over 30 saponins in *Medicago truncatula*.[12,13]

The surrogate internal standard (IS), oat avenacoside B, was used to compensate for losses during purification and sensitivity fluctuations during measurements. Avenacoside B is steroidal bidesmoside of medium polarity, which is suitable enough as an internal reference for a range of yucca saponins with different polarities. No traces of compounds producing similar quasi-molecular ion ($[M - H]^-$ at m/z 1223.6) were detected during the analysis of yucca extracts, although high-level background signal was observed in some samples.

Initially, we attempted to use the single reaction monitoring (SRM) MS/MS technique for quantitation, but it produced results unsatisfactory in terms of both accuracy and sensitivity. The response in SRM mode can be vastly different for different compounds and depends on a number of factors, including both structural features and chemical properties. Thus, while usually superior in terms of selectivity, SRM could not be reliably used for the relative quantitation purpose. This was mostly because of difficulty with selecting a suitable transition reaction. Additionally, in the case of overlapping acquisition functions of poorly separated compounds with similar product ions, SRM can generate cross-channel interferences, which are difficult or impossible to distinguish from the real signal. On the other hand, the signal in single ion monitoring (SIM) mode depends mostly on the ionization efficiency of the analyzed substance, which is rather comparable between the compounds of similar chemical properties. Table 2 shows details of SIM response curves obtained for saponins **11** and **12** we selected as concentration reference standards. These saponins have identical aglycon and differ only in one saccharide of their C-3 side chains: in saponin **12**, terminal glucose is replaced by xylose. Their response curves are remarkably similar and could be used for cross-quantitation with tolerable errors. An almost identical response curve was obtained in SIM mode for saponin **18**, which shares C-3 trisaccharide sequence with saponin **12**, but has a different aglycon. Based on these results, we concluded that unless excellent levels of accuracy are required, quantitation using two available standards could produce acceptable results.

However, the separation we obtained for our target compounds was still far from optimal and some cross-channel interferences were impossible to avoid (Figure 2B−D). To improve "selectivity" of the SIM-based measurements, a secondary ion corresponding to deprotonated adduct between the analyte and the formic acid was measured for every quantitated compound. This additional signal was used as a qualifier to aid the identification of a proper $[M - H]^-$ peak on the chromatogram.

Linearity of response was tested for both standards in the range between 0.20 and 40 pmol/μL, and linearity of the response was confirmed up to 28 pmol/μL. Therefore, after a preliminary estimate of saponins concentrations in the analyzed material, an appropriate dilution level was selected and subsequently used for all the samples. Limits of detection (LOD) estimated from calibration curves were close to 0.20 pmol/μL, thus placing calculated limits of quantitation (LOQ) at 0.60 pmol/μL (Table S1, Supporting Information).

For method precision, the percent relative standard deviation (RSD) from areas of the internal standard peak in 25 independently prepared and analyzed in sequence samples was investigated. Relatively low RSD value for avenacoside B (4.2%), indicates that during the analytical period of 72 h, the method was adequately precise in terms of injection volume and mass spectrometer response (Table S2, Supporting Information).

Intraday and interday reproducibility of the method was estimated from peak area RSDs for individual saponins. Intraday RSDs did not exceed 7% for each of the 19 saponins. Interday RSDs were at a similar level, indicating the method was sufficiently reproducible for the routine analysis of yucca saponins.

Quantitation of Saponins in Yucca Samples. As a testing procedure, we performed measurements of saponins in three different types of samples: in the methanol extract from powdered yucca bark, yucca juice pressed out from the stem and purified using solid phase extraction and the same extract purified using liquid-to-liquid extraction.

To evaluate the sample purification efficiency, recoveries of the internal standard were determined for each type of sample (Table 2). Typically, more than 80% of added avenacoside B was recovered from the samples. The best recoveries were observed for stem samples purified by LLE and SPE, whereas for bark samples purified by SPE, recovery was slightly lower. During the SPE purification procedure, the greatest loss of internal standard occurred in the sample application phase; up to 21% of added avenacoside B was recovered from the "flow-through and wash" fraction. In comparison, the water phase from LLE contained on average only 11% of added internal standard. Trace amounts of IS were also recovered after washing SPE cartridges with 100% methanol and in the ethyl acetate phase from LLE.

Regardless of purification method and material type, general qualitative profiles of yucca samples were similar. All 19 measured saponins were detected in each sample although, in some

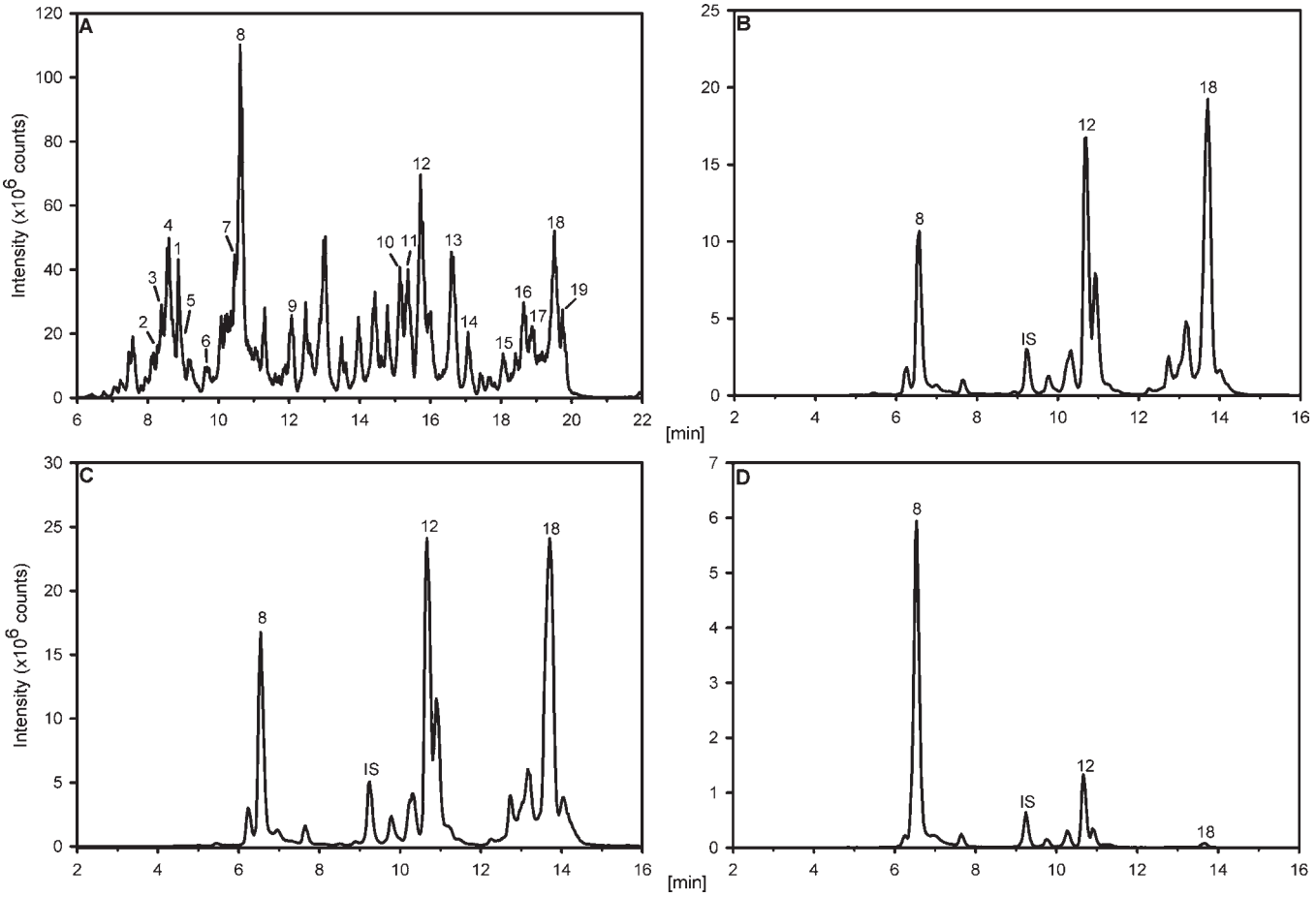


Figure 2. LC−MS chromatograms of saponins from yucca samples. A: Base peak chromatogram from data-dependent acquisition experiment on the crude yucca syrup. B−D: Sums of SIM channels for internal standard m/z 1223, saponin **8**, saponin **11** and saponin **12** recorded in LLE- (B) and SPE-purified (C) syrup samples as well as in the SPE-purified bark extract sample (D).

cases, at or only slightly above the established detection limit (Table 3).

Quantitatively, differences between the samples were much larger. The same starting material with two different purification methods afforded slightly different composition of the samples. Compared to SPE-purified samples, those purified with LLE were enriched with medium polarity saponins. This is in agreement with the observed high recoveries of the internal standard, also a medium polarity saponin. The breakdown of more complex and polar saponins is unlikely to be a source of this enrichment, because similar levels of polar analytes were observed in the SPE-purified samples. The other possibility is that water-saturated butanol is more efficient in solubilization of the yucca extract "sludge", thus releasing more of the medium and low polarity compounds into the organic phase of the liquid. A similar effect could be expected for SPE-purified samples when cartridges were eluted with 100% methanol, causing partial solubilization of particles adsorbed on the cartridge frits. This however was not observed. Alternatively, butanol may be inefficient as a solvent for very polar saponins, which remain in the water phase after the extraction. This was observed with alfalfa extracts,[14] where the most polar saponins, zahnic acid glycosides, did not partition into the butanol phase during the standard sample purification protocols and for a long time remained undetected and unknown. Only application of SPE extraction allowed for their identification. Overall, the differences between samples obtained with two different purification methods were minor. Consistent with our previous findings, in both types of stem juice samples the highest concentrations were recorded for saponins **12** and **18** (Table 3, Figures 2B and 2C), with saponins **13**, **8** and **10** at approximately half of that level.

The bark powder samples were dominated by high polarity, bidesmosidic saponins but were almost completely deficient of saponins identified as primary components of stem juice samples. The main compound in the bark was saponin **8** (Table 3, Figure 2D), followed by saponins **4**, **3** and **1** at much lower concentrations. Concentrations of **12** and other medium and low polarity saponins were considerably lower in the bark.

It could be argued that differences between yucca bark and stem juice samples are largely due to incompatible concentration units, referenced by the "fresh" weight for the juice and dry weight for the bark. If so, simple scaling down by the factor of extract moisture contents would account for them, and this does not seem to be the case. Our hypothesis is that these differences may reflect a more fundamental phenomenon, namely, the deposition of highly glycosylated saponins in the bark. Traditionally, bidesmosidic saponins are regarded as transport and storage forms that lack activity;[15,16] little, however, is known about their actual tissue distribution. Perhaps, in analogy to other plant chemical defense systems, these saponins represent metabolic product

Table 3. Analysis of *Y. schidigera* Saponins

no.	*m/z*	LLE meth, stem [nmol/mg FW] (±SD, *n* = 3)	SPE meth, stem [nmol/mg FW] (±SD, *n* = 3)	SPE meth, bark [nmol/mg DW] (±SD, *n* = 3)
1	1067	10.66 ± 0.79	9.68 ± 1.40	39.59 ± 3.02
2	1095	4.85 ± 0.30	6.82 ± 1.17	9.70 ± 0.91
3	1063	5.06 ± 0.25	5.38 ± 0.77	49.81 ± 5.13
4	1065	17.64 ± 0.60	17.60 ± 2.49	82.30 ± 7.99
5	935	3.09 ± 0.24	2.66 ± 0.45	6.08 ± 0.39
6	1079	1.26 ± 0.14	0.91 ± 0.11	11.48 ± 0.74
7	1081	4.30 ± 0.36	3.16 ± 0.42	15.16 ± 1.26
8	1051	30.43 ± 2.70	21.95 ± 2.58	146.97 ± 9.75
9	1049	14.64 ± 0.97	11.93 ± 1.95	19.75 ± 1.03
10	883	28.52 ± 2.85	23.87 ± 1.75	32.17 ± 1.16
11	915	23.73 ± 1.83	16.01 ± 1.41	4.61 ± 0.26
12	885	77.17 ± 5.49	58.15 ± 6.82	29.44 ± 1.87
13	887	42.14 ± 5.30	25.73 ± 2.37	2.76 ± 0.41
14	755	11.92 ± 0.72	19.21 ± 2.56	1.35 ± 0.08
15	899	9.62 ± 1.51	8.56 ± 0.38	0.18 ± 0.02
16	869	24.94 ± 3.03	26.54 ± 3.28	1.58 ± 0.18
17	901	19.19 ± 3.41	12.05 ± 0.60	0.19 ± 0.03
18	871	72.59 ± 8.91	49.16 ± 1.44	1.65 ± 0.20
19	739	24.60 ± 5.06	30.81 ± 3.35	0.87 ± 0.11
total		426.37 ± 44.47	350.19 ± 35.32	455.67 ± 34.56

accumulating in the specific target tissues. Deglucosylation occurring by the action of specific or nonspecific glycosidases upon the tissue breakage may convert these bidesmosides into compounds highly active against the native yucca pests.

With the exception of *Yucca gloriosa* flowers, where six steroidal saponins were quantitated,[17] nothing is known about the concentrations of individual saponins in both *Yucca* species and yucca products. Typical quality control procedures for the products usually involve acid hydrolysis of the butanol phase followed by GC-FID analysis of resulting sapogenins. Initially, the GC-FID method was developed for analysis of spirostanol-derived saponins in yucca plant organs,[4] but later was adapted for analysis of yucca extracts.[8] Only six primary epimeric sapogenins are typically analyzed,[7,8,10] and in the published works, the total spirostanol content was estimated between 5.2 and 42 mg/g.[8,10] Our method gave slightly higher results. Depending on the sample, a total content of equivalent compounds, sapogenins of **13**, **14** and **17**−**19**, would be between 29 and 73 mg/g. However, taking into the account all schidigeragenin- and 12-keto-spirostanol-derived sapogenins, the total spirostanol content would rise to the 300 mg/g level. Considering that activity and toxicity of some of these compounds is in many cases untested, this illustrates the need for supplementary analysis of intact glycosides.

We believe that the method presented, while not free from caveats and constraints discussed above, gives a possibility for better assessment of yucca products in terms of their potentially active ingredients. This is especially important when yucca extracts are evaluated as feed additives in animal models, where correlation between the observed effects and the composition and concentrations of active constituents should be carefully scrutinized.

■ ASSOCIATED CONTENT

Supporting Information. Table S1, containing calibration curve details including limits of detection and limits of quantitation and Table S2 with validation results. This material is available free of charge via the Internet at http://pubs.acs.org.

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