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Optimization of a Method for the Profiling and Quantification of Saponins in Different Green Asparagus Genotypes

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ABSTRACT: The main goal of this study was the optimization of a HPLC−MS method for the qualitative and quantitative analysis of asparagus saponins. The method includes extraction with aqueous ethanol, cleanup by solid phase extraction, separation by reverse phase chromatography, electrospray ionization, and detection in a single quadrupole mass analyzer. The method was used for the comparison of selected genotypes of Huétor-Tájar asparagus landrace and selected varieties of commercial diploid hybrids of green asparagus. The results showed that while protodioscin was almost the only saponin detected in the commercial hybrids, eight different saponins were detected in the Huétor-Tájar asparagus genotypes. The mass spectra indicated that HT saponins are derived from a furostan type steroidal genin having a single bond between carbons 5 and 6 of the B ring. The total concentration of saponins was found to be higher in *triguero* asparagus than in commercial hybrids.

KEYWORDS: *steroidal saponins, extraction, purification, triguero asparagus*

INTRODUCTION

Spain is one of the main asparagus producers in Europe, and Andalusia, a southern region of the country, accounts for more than 50% of the total Spanish production. Traditionally, Andalusia has devoted large country areas to the farming of white asparagus. However, during the past few years, the percentage of green asparagus has increased from 10 to 30% of the total production.[1,2] In addition to the common commercial hybrids, most of them derived from a single population of diploid cultivars,[3,4] Spain also produces the so-called *triguero* asparagus. Wild *triguero* asparagus is a traditional product in Spain and other Mediterranean countries, which has arisen from different species of plants from the genus *Asparagus* that are part of the wild vegetation and not farmed. Five edible species can be found in Spain, which are not farmed: *Asparagus acutifolius*, *Asparagus albus* L., *Asparagus aphylus* L., *Asparagus maritimus* L., and *Asparagus horridus* L. Due to their similarities with wild species, among the *triguero* asparagus is also included the native asparagus from Huétor-Tájar (HT), which is a farmed tetraploid landrace from Granada. Both types of asparagus differ from the commercial hybrids: *triguero* asparagus types are thinner, their shoots have darker colors (bronze and purple), and, organoleptically, they are characterized by a more bitter flavor, a strong aroma, and a flexible and fleshy texture. *Triguero* asparagus is highly prized in the regions where it is grown, primarily because of its distinctive organoleptic properties.[5] Recent studies showed that the functional properties of these asparagus types are different, e.g., it was shown that the antioxidant activity of ethanol extracts of HT asparagus was higher than that of the commercial hybrids.[6] It was also shown that the *triguero* asparagus has higher contents of flavonoids[7] and a different flavonoid profile.[8,9]

Apart from flavonoids, the other most important group of bioactive components of asparagus is saponins. The existing literature suggests that saponin in the diet might have beneficial health effects. Research conducted over the past fifteen years revealed that the saponins in different vegetables reduce plasma cholesterol levels in both experimental animals and humans.[10,11] Because of this, saponins are beginning to be considered as potential nutritional supplements in the control of dislipidemias and obesity.[12,13]

Additionally, steroidal saponins from different species of asparagus showed cytotoxic properties against various human tumor cell lines such as hepatoma, lung carcinoma, adenocarcinoma, and melanoma.[14,15] Different steroidal saponins, similar to those found in asparagus, also demonstrated both cytostatic and cytotoxic effects in the human leukemia, colon carcinoma, and fibrosarcoma cell lines.[16−19]

The saponins present in the plants of the genus *Asparagus* are responsible not only for their biological activity but, in those species that are edible, they might be determinant for their characteristic bitter-sweet flavor and therefore for the organoleptic properties of asparagus.[20−22]

Several analytical techniques have been used for the quantification of saponins in asparagus spears; the two most commonly used are TLC[23] or HPTLC[24] and HPLC.[25,26] In the first case, the saponins are quantified by a colorimetric reaction usually with anisaldehyde or Erlich's reagent. When these reagents are used in parallel, they can distinguish between spirostanol and furostanol

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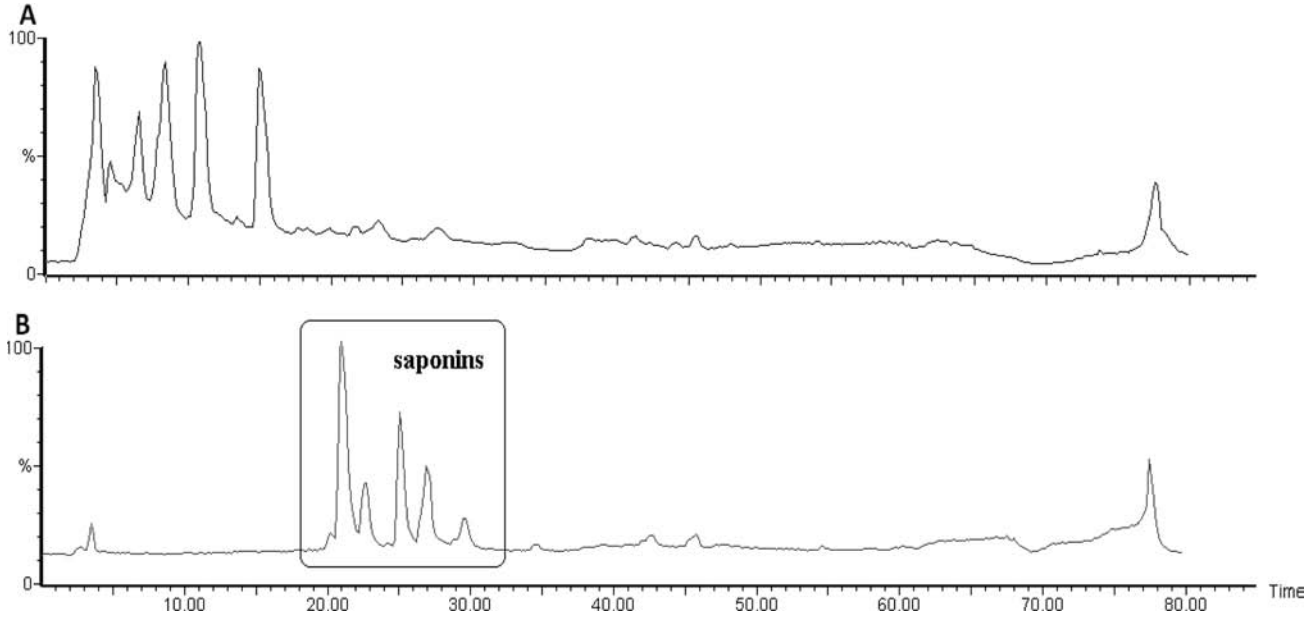

Figure 1. Fractions obtained in the purification process with C-18 SPE column: A, 20% ethanol fraction rich in flavonoids, and B, 40% ethanol fraction containing total saponins.

saponins. When saponin separation is accomplished by HPLC, the detection can be done by UV[25] or by ELS or MS.[26]

The objective of this research was the development of an analytical method suitable for the profiling and quantification of saponins in different asparagus varieties and genotypes by means of HPLC−MS. We applied the method for the study of selected genotypes of Huétor-Tájar asparagus and compared them with selected varieties of commercial diploid hybrids (CH) of green asparagus.

MATERIALS AND METHODS

Plant Material. The samples evaluated consisted of spears from nine native lines of *triguero* asparagus from the HT landrace and the green asparagus shoots of nine outstanding commercial hybrids from an existing collection at Las Torres Agricultural Research Center, Alcalá del Rio, Sevilla, Spain.

The spears were harvested from experimental fields and immediately transported to the laboratory, where they were trimmed to a final length of 21 cm, weighed, and frozen at −20 °C. Afterward they were freeze-dried, ground to fine powder, and stored at −20 °C until analyzed.

Chemicals and Reagents. Protodioscin (97%) and shatavarin (98.6), purity checked by NMR, were purchased from Chromadex Chemical Co. (Barcelona, Spain). Ethanol, formic acid (96%), and acetonitrile, HPLC grade, were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The C-18 cartridges (500 mg) were purchased from Varian Incorporated (Lake Forest, CA, USA). Pure deionized water was obtained from a Milli-Q 50 system (Millipore Corporation, Bedford, MA, USA).

Saponin Extraction. Each sample, consisting of 2.5 g of lyophilized material, was extracted with 100 mL of 80% ethanol in water in an Ultraturrax (T25) (Staufen, Germany) for 1 min at maximum speed and filtered. The residue was extracted again in the same conditions. The ethanol extracts were pooled and evaporated to dryness at reduced pressure. All extractions were made in triplicate.

Sample Purification. The dried ethanol extract was dissolved in 10 mL of Milli-Q water and loaded into a C-18 cartridge previously activated with 10 mL of 96% EtOH and washed with 10 mL of Milli-Q water. The cartridge was eluted with increasing percentages of ethanol in water: water (20 mL), 20% EtOH (60 mL), 40% EtOH (20 mL), and 96% EtOH (20 mL). The 40% EtOH fraction, which contained the saponins, was evaporated, dissolved in 1 mL of 80% EtOH, centrifuged at 12,000 rpm for 3 min, and injected (20 μL) into the HPLC−MS system.

Saponin Analysis by HPLC-DAD−MS. An HPLC Waters Alliance (Manchester, U.K.) system fitted to a mediterranea sea_{18} reverse-phase analytical column (25 cm length × 4.6 mm i.d., 5 μm particle size; Teknokroma, Barcelona) was used. An elution gradient was used with solvents A (water with 1% formic acid) and B (acetonitrile with 1% formic acid): 0−30 min, 20% B; 30−60 min, linear gradient to 30% B; 60 to 70 min, linear gradient to 100% B; and 70−80 min, linear gradient to 20% B. The column end was connected directly with a DAD (diode array detector) (Waters 996, Millipore, Manchester, U.K.), and subsequently, the flow in the MS was regulated using a split (flow: 0.3 mL/min).

The saponins were detected using an online connected quadrupole mass analyzer (ZMD4, Micromass, Waters, Inc., Manchester, U.K.). Electrospray ionization (ESI) mass spectra were obtained at ionization energies of 50 and 100 V (negative mode) and 50 V (positive mode) with scans from m/z 200 to 1200. Capillary voltage was 3 kV; the desolvation temperature was 200 °C; source temperature 100 °C and extractor voltage 12 V.

Quantitative Analysis. The external standard method was used for the quantification of asparagus saponins. Two different external standards were used: protodioscin, a furostanol saponin derived from diosgenin, and shatavarin IV, a spirostanol saponin derived from sarsasapogenin. For each standard, 10 dilutions from 0 to 500 μg/mL were prepared and injected into the LC−MS system. For each standard, the selected ion chromatogram corresponding to its molecular ion in negative mode at 100 V was integrated and the peak area plotted against concentration and subjected to regression analysis.

The limit of detection (LOD) of the saponins was defined as the lowest sample concentration that can be detected (signal-to-noise ratio = 3). The limit of quantification (LOQ) was defined as the lowest sample concentration that can be quantitatively determined with suitable precision and accuracy (signal-to-noise ratio = 10).

Repeatability was evaluated from three sequential replicates of injections while reproducibility was determined by comparing the analyses of four different days.

RESULTS AND DISCUSSION

Optimization of Sample Cleanup. In some cases we observed that crude asparagus extracts showed a low saponin concentration and contained many additional compounds that might interfere with saponin quantification. Therefore, we have

Table 1. Saponins Identified by HPLC−MS (+/−) of *Triguero* Asparagus from Huétor-Tájar

			molecular ion (m/z)		ion fragmentation	
saponin	t_R[a] (min)	MW[b]	neg ion	pos ion	neg mode	pos mode
HTSAP-1	21.00	1052	1051 $[M-H]^-$	1075 $[M+Na]^+$	-Pen[c]919-Hex[d]757-Hex595-Hex433[e]	$[-Na-H_2O]$1035-Hex873-Hex711-Pen579-Hex417[e]
HTSAP-2	22.55	920	919 $[M-H]^-$	943 $[M+Na]^+$	-Hex757-Hex595-Hex433	$[-Na-H_2O]$903-Hex741-Hex579-Hex417
HTSAP-3	25.03	1022	1021 $[M-H]^-$	1045 $[M+Na]^+$	-Pen889-Pen757-Hex595-Hex433	$[-Na-H_2O]$1005-Pen873-Hex711-Pen579-Hex417
HTSAP-4	26.12	1046	1045 $[M-H]^-$	1069 $[M+Na]^+$	-DoHex[f]889-DoHex753-Unk[g]595-Hex433	$[-Na-H_2O]$1029-Hex867-DoHex721-DoHex575-Unk417
HTSAP-5	26.74	890	889 $[M-H]^-$	913 $[M+Na]^+$	-Pen757-Hex595-Hex433	$[-Na-H_2O]$873-Hex711-Pen579-Hex417
protodioscin	27.05	1048	1047 $[M-H]^-$	1071 $[M+Na]^+$	-DoHex901-DoHex755-Hex593-Hex431[e]	$[-Na-H_2O]$1031-Hex869-DoHex723-DoHex577-Hex415[e]
HTSAP-6	27.70	1036	1035 $[M-H]^-$	1059 $[M+Na]^+$	-Pen903-DoHex757-Hex595-Hex433	$[-Na-H_2O]$1019-DoHex873-Pen741-Hex579-Hex417
HTSAP-7	29.84	890	890 $[M-H]^-$	913 $[M+Na]^+$	-Pen757-Hex595-Hex433	$[-Na-H_2O]$873-Hex711-Pen579-Hex417
HTSAP-8	29.53	904	903 $[M-H]^-$	927 $[M+Na]^+$	-DoHex757-Hex595-Hex433	$[-Na-H_2O]$887-Hex725-DoHex579-Hex417

[a]t_R: retention time. [b]Molecular weight. [c]Pen: Pentose. [d]Hex: Hexose. [e]433/431/417/415: Genins. [f]DoHex: Deoxyhexose. [g]Unk: Unknown.

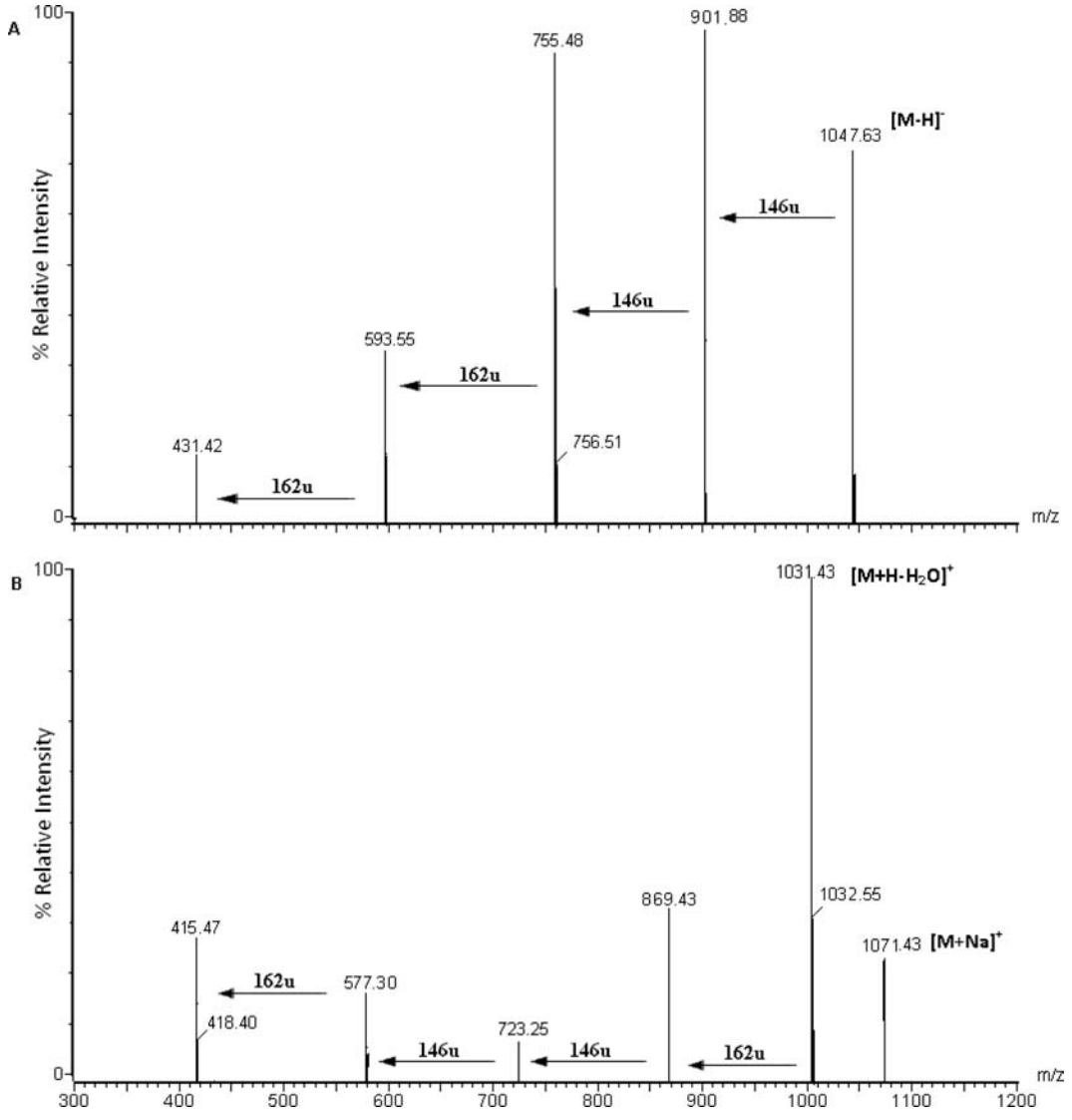

Figure 2. ESI mass spectra of protodioscin in negative (A, 100 V−) and positive (B, 50 V+) modes. Arrows indicate the loss of single monosaccharide moieties.

developed a C-18 SPE saponin purification method to increase their concentration and remove interfering compounds, thus obtaining a partially purified saponin fraction.

The process was optimized in terms of the different solvent volumes. The C-18 SPE eluates were treated as described in Materials and Methods, and aliquots were injected into the

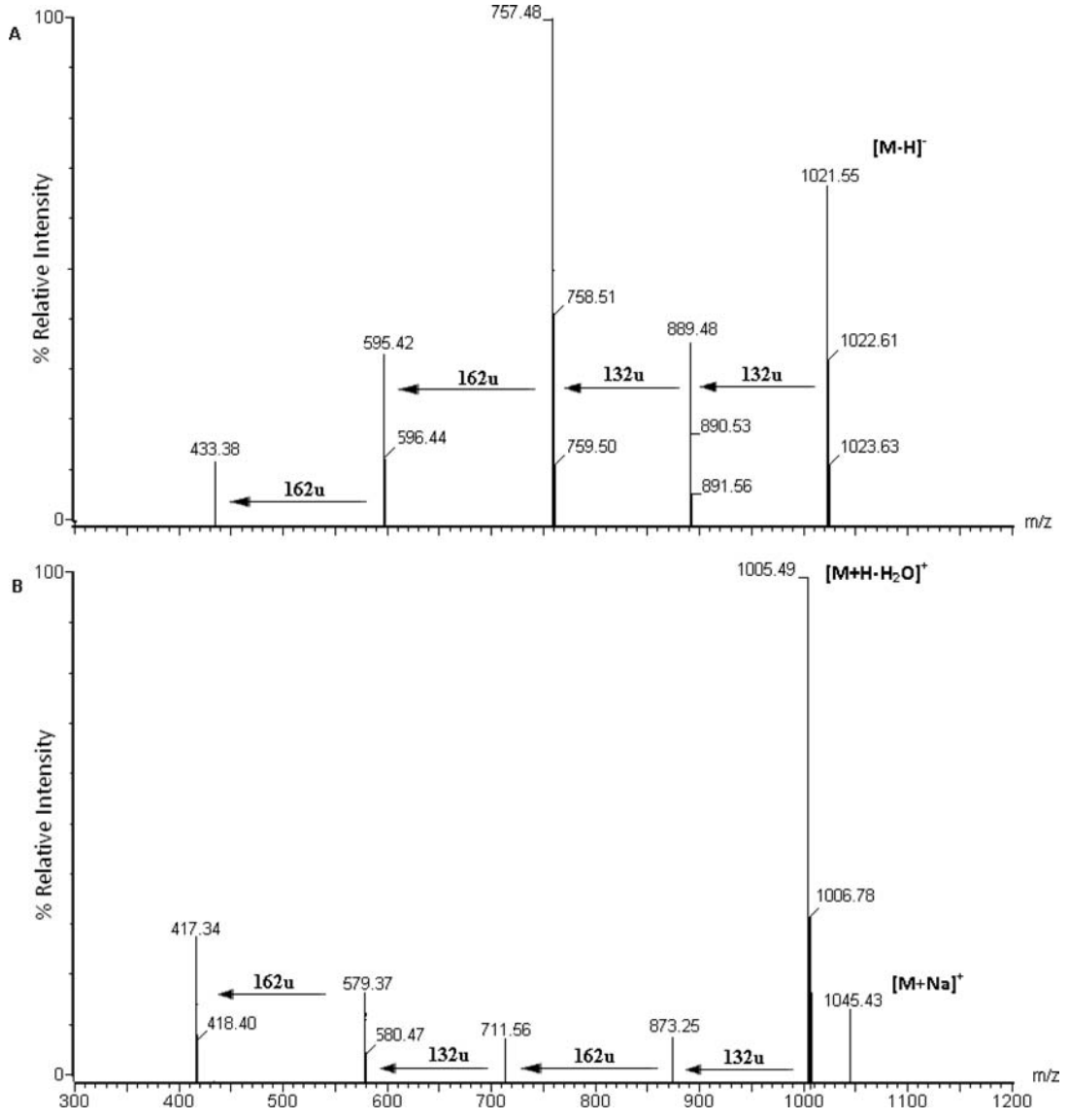

Figure 3. ESI mass spectra of HTSAP-3 in negative (A, 100 V−) and positive (B, 50 V+) modes. Arrows indicate the loss of single monosaccharide moieties.

HPLC−MS system. The water eluate contained mostly sugars as determined colorimetrically by the anthrone method (data not shown). The compounds eluted by 20% EtOH were identified as flavonoids (Figure 1A). Their mass and UV-absorbance spectra (data not shown) correspond to flavonoids previously identified by our group as glycosides of quercetin, kaempferol, and isorhamnetin.[9] Saponins eluted in the 40% EtOH fraction free from other asparagus components (Figure 1B). Finally, the remaining components were eluted with 96% EtOH and their mass spectrum could be compatible with sterols.

Characterization of Saponins in *Triguero* HT Asparagus. Once the extraction and purification of saponins were completed, a study was conducted with nine genotypes of HT asparagus and nine CH. Nine distinct peaks were identified in the HT asparagus, eight of them corresponding to new saponins (HTSAP1 to HTSAP8) and another one for protodioscin (Table 1).

In the case of protodioscin, at low V in the negative mode only the molecular ion $[M - 1]^-$ (m/z 1047) was observed. However at 100 V, together with the molecular ion, all the peaks corresponding to the consecutive neutral losses of rhamnose and glucose were detected at m/z 901 corresponding to the loss of rhamnose (146 u), m/z 755 resulting from the loss of the second rhamnose, m/z 593 from the loss of a glucose (162 u), and m/z 431 from the loss of a second glucose (162 u), which corresponds to deprotonated protodiosgenin (Figure 2A). The most prominent peaks observed in positive mode (Figure 2B) were m/z 1071, corresponding to the adduct that forms with sodium $[M + Na]^+$, and m/z 1031, corresponding to $[M + H - H_2O]^+$. In addition, the peaks corresponding to sugar losses were detected: m/z 869, resulting from the loss of one glucose; m/z 723, resulting from the loss of one rhamnose; m/z 577, resulting from the loss of the second rhamnose; m/z 415, resulting from the loss of the second glucose.

Based on the mass spectra of each new saponin a tentative structure can be deduced. For example, HTSAP-3 mass spectrum in negative mode (Figure 3A) showed a molecular ion at m/z 1021 $[M - 1]^-$, m/z 889 corresponding to the loss of a pentose (132 u), m/z 757 resulting from the loss of a second pentose (132 u), m/z 595 resulting from the loss of a hexose (162 u), and m/z 433 resulting from the loss of a second hexose (162 u).

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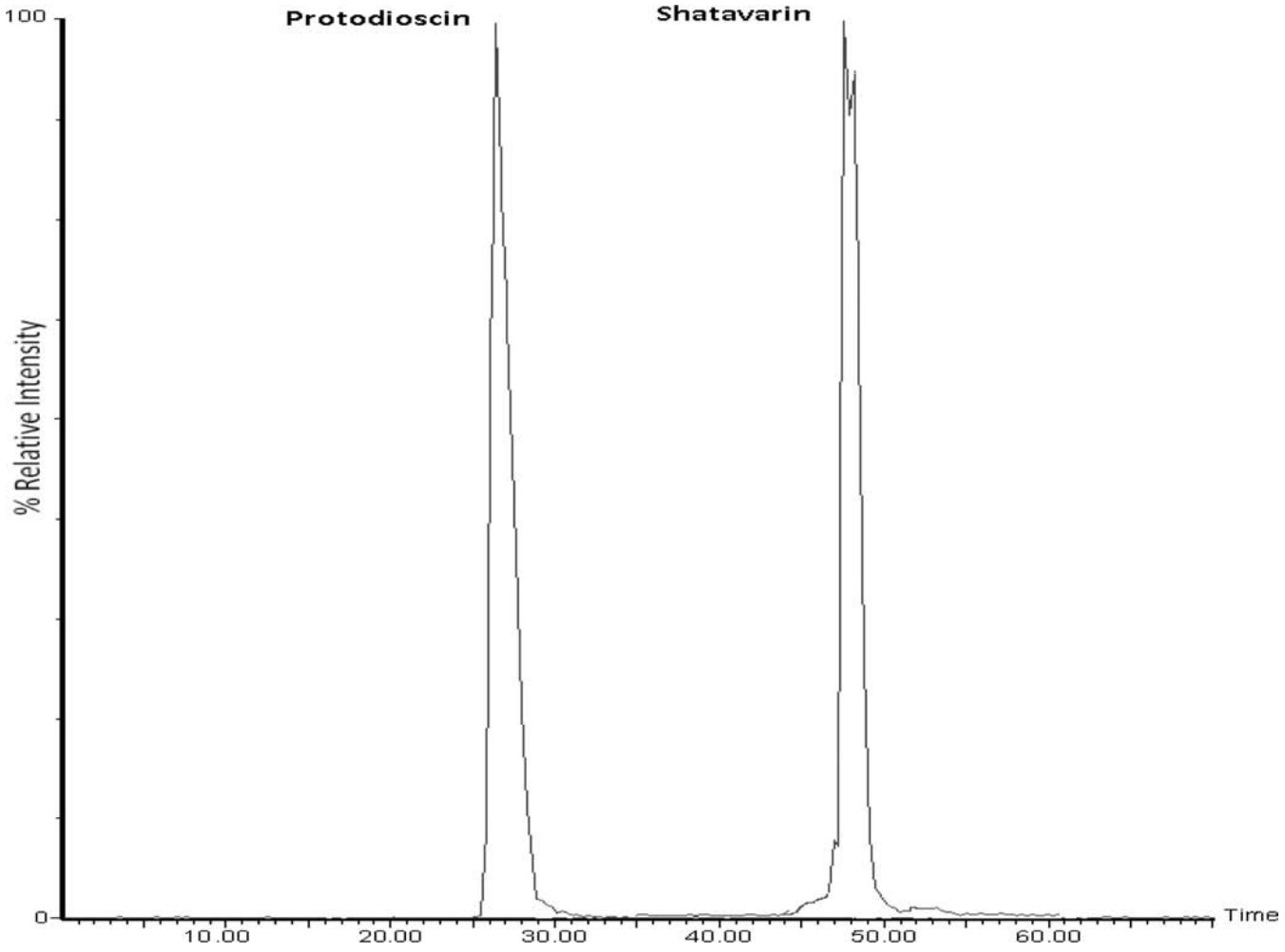

Figure 4. Single ion $[M - H]^-$ chromatogram of the two saponin standards, protodioscin (1047) and shatavarin (885), obtained at 100 V−.

Comparing this spectrum with that of protodioscin we can see that the smallest fragmentation ion is 433, which is compatible with the presence of a furostanol genin with a single bond between carbons 5 and 6 in the B-ring. The positive mode spectrum showed ions at m/z 1045 $[M + Na]^+$ corresponding to the adduct that the molecule forms with sodium, m/z 1005 $[M + H - H_2O]^+$ typical dehydratation product of a furostanol type glycoside,[27] m/z 873 resulting from the loss of a pentose (132 u), m/z 711 resulting from the loss of a hexose (162 u), m/z 579 resulting from the loss of a pentose (132 u), and m/z 417 resulting from the loss of a hexose (162 u) (Figure 3B). The other new saponins found in the Huétor asparagus follow a similar fragmentation model (Table 1).

To determine the exact structure of these saponins further studies are necessary including purificacition of each individual saponin and 1D and 2D NMR experiments. It is reasonable to think that the eight saponins (HTSAP) from *triguero* HT asparagus are all structurally related to protodioscin, i.e., they are furostanol glycosides, but differ from it by having a single rather than a double bond between C5 and C6 of the B-ring and by the different saccharides attached to C3.

Design and Validation of HPLC−MS Quantitative Method. For quantification the external standard method was used. Among the different saponins that have been described in asparagus, the only two standards that we could find commercially were protodioscin and shatavarin IV. Their purity was higher than 98% and was checked by HPLC−ELSD, HPLC−UV, and NMR according to the seller's certificate of analysis. A mixture of both standards was injected into the HPLC−MS system (Figure 4), and the mass spectra were collected at 50 and 100 V in the negative mode and at 50 V in the positive mode.

For shatavarin, at low V in the negative mode only the molecular ion $[M - 1]^-$ was observed (m/z 885). Unlike protodioscin where the whole fragmentation was observed (see above), in the case of shatavarin at 100 V, together with the molecular ion, only two ions were detected, one corresponding to a deoxyhexose loss and the other to an additional loss of a hexose (Figure 5A). The major peak observed in positive mode for shatavarin (Figure 5B) was the sodium adduct $[M + Na]^+$ m/z 909. In addition, there was one ion at m/z 579, probably resulting from the loss of two deoxyhexoses, and an ion representing the protonated genin, m/z 417.

For calibration, the different modes were compared and, for each mode, the peak areas in either the total ion chromatogram or the molecular ion selected chromatogram were plotted against concentration and subjected to linear regression analysis. The results of the tests performed in triplicate are listed in Table 2. According to the results, the best correlation coefficient found for protodioscin corresponds to the selected molecular ion chromatogram at either 100 V− or 50 V+, although all the other correlations were acceptable. However, for shatavarin not all the correlations were suitable and only the one corresponding to the

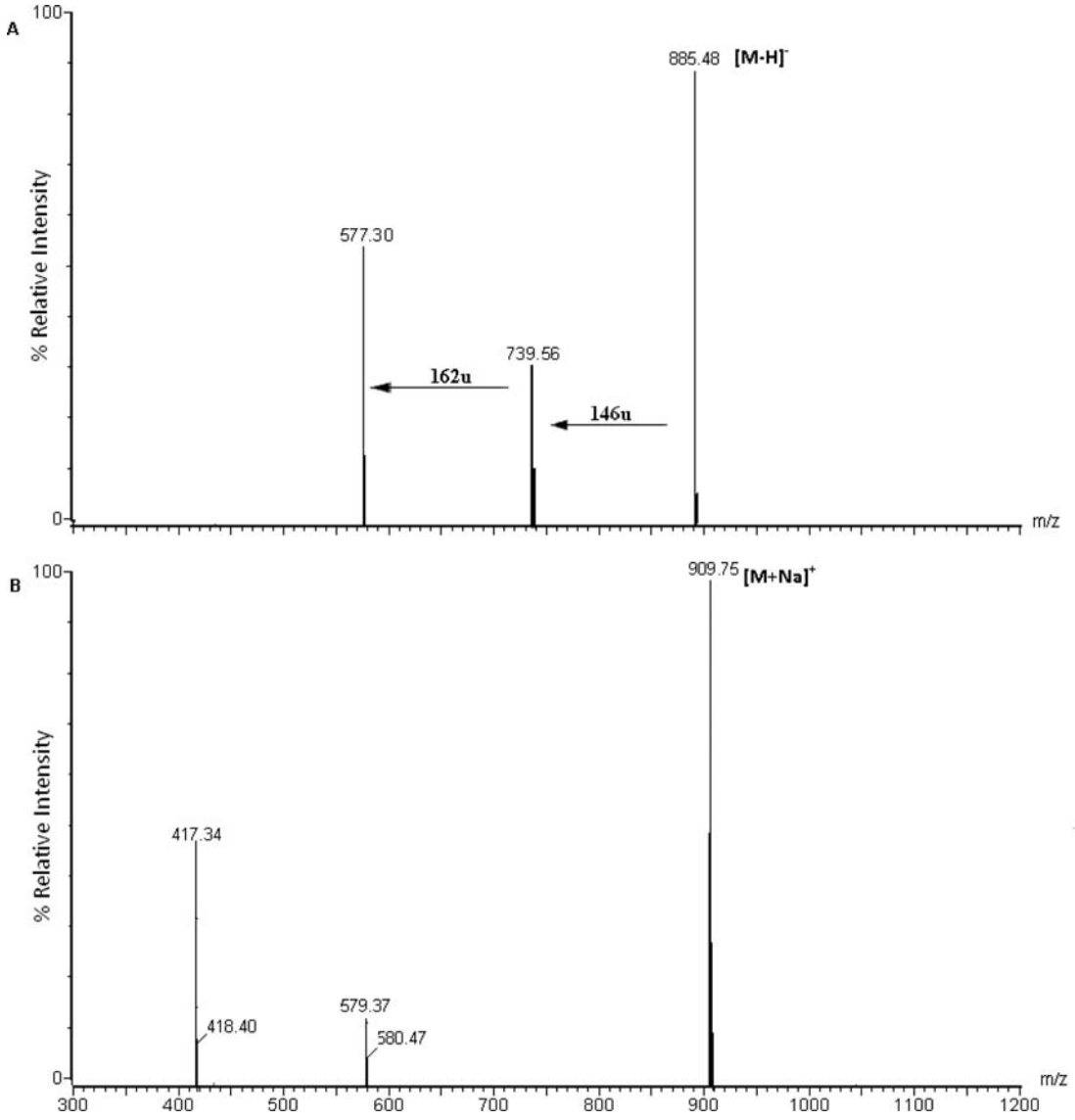

Figure 5. ESI mass spectra of shatavarin in negative (A, 100 V−) and positive (B, 50 V+) modes. Arrows indicate the loss of single monosaccharide moieties.

Table 2. Correlation Lines for Protodioscin and Shatavarin (mg/mL)

mode	molecular ion	total ion
	Protodioscin	
100 V−	$y = 0.01x$ ($R^2 = 0.994$)	$y = 0.25x$ ($R^2 = 0.976$)
50 V−	$y = 0.005x$ ($R^2 = 0.932$)	$y = 0.3x$ ($R^2 = 0.923$)
50 V+	$y = 0.005x$ ($R^2 = 0.995$)	$y = 0.05x$ ($R^2 = 0.946$)
	Shatavarin	
100 V−	$y = 0.01x$ ($R^2 = 0.992$)	$y = 0.45x$ ($R^2 = 0.922$)
50 V−	$y = 0.005x$ ($R^2 = 0.922$)	$y = 0.3x$ ($R^2 = 0.868$)
50 V+	$y = 0.4x$ ($R^2 = 0.797$)	$y = 0.1x$ ($R^2 = 0.841$)

selected ion chromatogram at 100 V− gave a correlation coefficient close to 0.99, therefore this mode was chosen for the quantification of both saponins. Identical straight lines were found for both standards, $C = 0.01A$ ($R^2 = 0.994$), where C is the saponin concentration and A is the area of the peak. Protodioscin and shatavarin represent two structurally different compounds: the former is a furostanoid saponin with a double bond between carbons 5 and 6 in the B-ring, and the latter is a spirostanoid saponin with a single bond between carbons 5 and 6 of the B-ring. However it is remarkable that in the working conditions they have the same response factor (Figure 6). Since the new HT saponins are structurally related to both standards, they are furostanoid saponins with a single bond between carbons 5 and 6 of the B-ring, it seems reasonable to expect the same response factor for all the saponin peaks found in asparagus extracts. Therefore the quantitative data provided in this study can be considered a good approximation to the real values and, in any case, useful for the purpose of comparison between different genotypes.

To ensure the accurate assessment of the saponin contents found in wild asparagus, the HPLC−MS method was validated according to the ICH guidelines on the validation of analytical methods[28] prior to its application for the quantitative analysis of different asparagus samples.

The LOD was 0.1784 μg/mL, which indicated high sensitivity under these HPLC conditions, and the LOQ was 0.5944 μg/mL for both standards. The method provided good precision and stability because the overall intra- and interday variations were less than 10% for all saponins. The analytical characteristics of the

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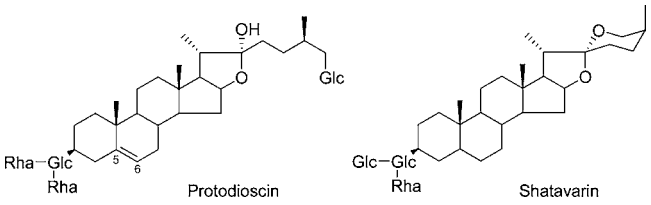

Figure 6. Structures of the two standards: protodioscin and shatavarin.

calibration graphs and the precision (0.87 and 7.01 for intraday and interday, respectively) and accuracy (109% and 94% respectively) of the method were satisfactorily comparable to those reported by other authors that developed HPLC−MS analytical methods for the determination of saponin compounds from several plant materials.[25,29] It can be concluded that the proposed method is reliable and accurate for the qualitative and quantitative determination of steroidal saponins from asparagus.

Quantification of Saponins in Nine Varieties of HT Asparagus and Nine CH. According to the literature, the major saponin found in asparagus spears is protodioscin, a saponin derived from diosgenin. Green spears contain from 0.024 mg/100 g fw at the top of the spear to 2.5 mg/100 g fw at the bottom cuts,[26] while the white spears content ranged from 1.4 to 5 mg/100 g fw depending on the cultivar and portion of the spear.[24,25]

The total content of saponin detected in Huétor asparagus (Table 3A) ranged from 1.09 to 2.73 mg/100 g fw. This value is in the range described in the literature,[26] but it is interesting to highlight that the Huétor samples (90%) have a saponin concentration higher than 1 mg/100 g fw. By contrast, commercial hybrids showed saponin concentrations (Table 3B) between 0.03 and 1.22 mg/100 g fw, and 90% of the samples have saponin contents lower than 1 mg/100 g fw. The results suggest that HT asparagus varieties have a higher saponin concentration than commercial hybrids. In addition, another important difference observed is that while commercial hybrids showed protodioscin as the primary saponin, HT asparagus showed a saponin profile constituted by eight saponins derived from a furostan type steroidal genin having a single bond between carbons 5 and 6 of the B ring, and only three genotypes contained protodioscin. To our knowledge this is the first time that these saponins have been described in the edible part of asparagus as the major components, on the contrary most studies have shown that the main saponin in asparagus is protodioscin. Many different steroidal saponins have been described from different asparagus cultivars and species.[30] The main species studied are *Asparagus racemosus,*[31] *Asparagus filicinius,*[32] *A. acutifolius*[33] and *Asparagus officinalis*.[34] In most cases the saponins were characterized and isolated from the roots and in some cases from the fruits. However, in the edible part of asparagus (spears) only a few saponins were detected apart from protodioscin. Held et al.[35] reported the presence in the etiolated shoots of *A. officinalis* of the sapogenins diosgenin, yamogenin. and sarsapogenin. Kawano et al.[36] isolated two minor saponins ASP I and ASPII from the button cuts of white asparagus, both of them derived from yamogenin and diosgenin. ASPI has a trisaccharide Rha-Glc-Rha and a strong bitter taste, while ASPII has a disaccharide Rha-Glc and is tasteless. Shimoyamada et al.[37] isolated a minor saponin from the bottom cuts of white asparagus, which resulted in a sarsasapogenin glycoside with antifungal activity. The same authors[38] isolated a second saponin with antifungal activity derived from yamogenin. Wang et al.[26] found, in green asparagus, besides protodioscin, two minor saponins derived from diosgenin

Table 3. Saponins Identified in Samples of Asparagus, Calculated from the Molecular Ion of the Peak Area Obtained by HPLC−MS

			A. Triguero HT Genotypes								
saponin	Rt	MW	HT-1	HT-2	HT-3	HT-4	HT-5	HT-6	HT-7	HT-8	HT-9
HTSAP-1	21.00	1052	1.39[a] (55.95)[b]	0.65 (39.97)	0.48 (17.56)	0.31 (28.60)	0.04 (1.79)	0.33 (28.28)	0.27 (18.88)	0.82 (43.06)	0.40 (20.33)
HTSAP-2	22.55	920	0.71 (28.57)	ND[c]	0.20 (7.36)	ND	ND	ND	ND	ND	ND
HTSAP-3	25.03	1022	0.09 (3.59)	0.19 (11.69)	0.98 (35.76)	ND	1.63 (66.02)	0.21 (17.72)	ND	ND	1.2 (66.40)
HTSAP-4	26.12	1046	ND	ND	ND	ND	ND	ND	ND	0.08 (4.32)	ND
HTSAP-5	26.74	890	ND	ND	0.22 (8.17)	ND	0.45 (18.30)	ND	ND	ND	ND
protodioscin	27.05	1048	ND	ND	ND	0.61 (55.82)	ND	ND	1.04 (72.86)	0.30 (16.81)	ND
HTSAP-6	27.70	1036	0.14 (5.61)	0.60 (36.87)	0.10 (3.72)	0.17 (15.58)	0.07 (2.83)	0.49 (41.59)	ND	0.10 (5.25)	0.05 (2,98)
HTSAP-7	29.84	890	ND	ND	ND	ND	0.27 (11.05)	0.15 (12.42)	ND	ND	0.12 (6,33)
HTSAP-8	29.53	904	0.16 (6.29)	0.19 (11.47)	0.75 (27.45)	ND	ND	ND	0.12 (8.26)	0.60 (31.57)	0.07 (3,97)
total saponins			2.49 (100)	1.63 (100)	2.73 (100)	1.09 (100)	2.47 (100)	1.18 (100)	1.42 (100)	1.90 (100)	1.83 (100)
			B. Commercial Hybrids								
saponin	Rt	MW	CH-1	CH-2	CH-3	CH-4	CH-5	CH-6	CH-7	CH-8	CH-9
protodioscin	27.05	1048	0.22 (100)	0.03 (100)	0.03 (100)	1.22 (100)	0.03 (100)	0.26 (100)	0.80 (100)	1.22 (100)	0.04 (100)

[a]Data mg/100 g fresh asparagus are the mean of three replicates. [b]Percentage (%). [c]Not detected. VC < 10%.

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and sarsasapogenin. Schwarzbach et al.[24] found six different saponins in white asparagus spears. Except for protodioscin and methylprotodioscin, the only saponin that was partially characterized is one similar to ASPII isolated by Kawano et al.[36] Sun et al.[39] characterized another saponin from the stems of *A. officinalis* with a structure similar to protodioscin but with an OH group at C21.

These results show clearly that the saponin profiles of HT asparagus are very different from those of commercial hybrids. This could explain the different organoleptic characteristics of these two types of asparagus. Recently the importance of saponins in the sensory characteristics of asparagus[21,22] has been pointed out. The purification and sensory characterization of these saponins could help to clear up this relationship.

Morever, the saponin profile could have an important influence on the functional properties of asparagus. According to some authors, the bioactive properties of saponins are dependent on the saponin structure. Small structural variations lead to substantial variations in physical, chemical, and biological properties.[40] For instance, Mimaki et al.[17] concluded that the structure of the oligosaccharide moieties in steroidal saponins plays an important role in the specific cytotoxicity against tumor cells. Therefore, conducting more in-depth studies on the isolation and structural characterization of asparagus saponins may establish relationships between these compounds and the beneficial health effects associated with asparagus consumption.

The method described in this manuscript is straightforward, requiring fairly simple equipment and yielding a clean mixture of saponins that are easily separated and quantified. The results showed that the proposed method can be useful for the profiling of saponin in different asparagus genotypes and, therefore, for the differentiation of asparagus types.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

HT, Huétor-Tájar; TLC, thin layer chromatography; HPTLC, high-performance thin layer chromatography; HPLC, high-performance liquid chromatographic; LC, liquid chromatography; DAD, diode-array detection; MS, mass spectrometry; UV, ultraviolet; ELS, evaporative light scattering; ESI, electrospray ionization; LOD, limit of detection; LOQ, limit of quantification

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