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Short communication

Determination of the content and the composition of the main saponins from *Solidago gigantea* AIT. using high-performance liquid chromatography

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Abstract

A method is presented that allows the determination of the content and the composition of the saponins from *Solidago gigantea*. A cleanup procedure using C_{18} cartridges yields saponin extracts that can be analysed by HPLC using an internal standard. The combination of HPLC on RP-8 and silica results in the complete separation of the main saponins and thus the composition can be determined. The good correlation between saponin content and hemolytic activity from different samples is shown.

Keywords: *Solidago gigantea*; Saponins

1. Introduction

Early golden-rod herb (*Herba Solidaginis giganteae*) is used as a substitute for the golden rod (*Herba Virgaureae*), favoured by its higher saponin and flavonoid content [1]. The higher content of saponins is reported several times in the literature [2–6], but in fact, no serious quantification of the saponins has been carried out so far. These statements are based on a publication from 1949 [7] where *Solidago gigantea* AIT. was found to possess higher hemolytic activity in comparison to *Solidago virgaurea*. However, the hemolytic indices cannot be compared because of the different structures of the saponins [8] and thus an estimation of the saponin content is not possible.

Therefore we worked out a method that allows the absolute and relative quantification of the main saponins from *S. gigantea*. The availability of sufficient amounts of the pure main saponins giganteasaponin 1–4 from former isolation procedures [9,10] enabled the development of this method.

2. Experimental

2.1. Plant material

Above-ground parts of *S. gigantea* AIT. were collected in august 1995 from different locations in Vienna and Lower Austria: Auhof, Kogl, Au (two

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locations) and Zwettl. Voucher specimens are deposited in the herbarium of the Institute of Pharmacognosy, University of Vienna.

2.2. Sample preparation

Amounts of 0.5 g of above-ground, air-dried and ground parts of *S. gigantea* were extracted exhaustively with methanol–water (80:20, v/v) by percolation (yield approx. 170 mg crude saponin mixture, depending on the sample). A 150-mg quantity of this extract was suspended in 5 ml water in a centrifuge vial for 5 min in an ultrasonic bath. After centrifugation 3 ml of the supernatant was applied to a cartridge (Inchrom Clean-Up C_{18}, 5000 mg, preconditioned with 40 ml methanol). After 5 min the cartridge was rinsed with 60 ml chloroform and 90 ml methanol–water (40:60, v/v). Then the internal standard was applied to the cartridge (15 ml of stock solution) and eluted together with the saponins using 70 ml methanol. The solvent was removed in a tared vial under reduced pressure and the residue was dissolved in methanol–water (adjusted to pH 5 with TFA) (53:47, v/v) (30 μl/mg). After further centrifugation the supernatant was analysed by reversed-phase HPLC.

2.3. Stock solution internal standard

Digitoxin (Sigma, No. D 5878), 6.6 mg/500 ml methanol–water (40:60, v/v).

2.4. Apparatus and conditions

The liquid chromatograph consisted of a Perkin-Elmer (Norwalk, USA) series 200 pump, an LC 235C diode array detector and Turbochrom software for data processing. For reversed-phase HPLC we used LiChrospher RP-8, 5 μm, 250×4 mm I.D. (Fa. SRD Pannosch, Vienna, Austria) and methanol–water (adjusted to pH 5 with TFA) (53:47, v/v) as mobile phase. For HPLC on silica we used LiChrosorb Si 60, 5 μm, 250×4 mm I.D. (Fa. SRD Pannosch) and a gradient from *n*-butanol–water (adjusted to pH 5 with TFA)–methanol (85:15:0, v/v) to *n*-butanol–water (adjusted to pH 5 with TFA)–methanol (68:12:20, v/v) in 35 min as solvent. In both cases the flow-rate was 1 ml/min and the saponins were detected at 210 nm. The detection limit was determined as 0.25 μg for the saponins.

2.5. Internal standard method

Digitoxin turned out to be a good internal standard for the determination of the saponins. Using the HPLC on RP-8 the standard is eluted between the saponins at $t_R = 39.6$ min (Fig. 1, top).

The correction factor of the standard (f_{St}) was determined using pure giganteasaponin 3 and digitoxin with different concentrations in the expected range as 7.93. Due to the equation that the quotient f_{St}/M_r (molecular mass) is constant, it can be calculated as 0.00408 and the f_{St} can be deduced for all main saponins with known molecular mass.

The percentage recovery of the saponins was determined by spiking the extract three times in advance of the cleanup procedure with pure giganteasaponins 2 and 3 as 99.8% (±3%). The percentage recovery of the internal standard was determined as 99.3% (±4%).

To determine the reproducibility of the method, one of the samples (location Auhof) was analysed five times and the relative standard deviation was calculated as ±7.2%.

For quantification three analyses were carried out for each sample and the mean value was calculated.

The hemolytic indices (HI) of the drugs were determined according the Austrian Pharmacopoeia (ÖAB).

3. Results and discussion

The quantification of the main saponins of *S. gigantea* was carried out by HPLC analysing plant extracts that were purified by solid-phase extraction.

The introduced cleanup procedure requires small amounts of sample and yields quite pure saponin extracts in reasonable time, avoiding impurities on the HPLC column head. The given amounts of sorbents and solvents should be considered to achieve the required purity without loss of saponins and standard.

Unfortunately our investigations on a large scale showed that a complete separation of all four main saponins cannot be achieved on silica or on RP

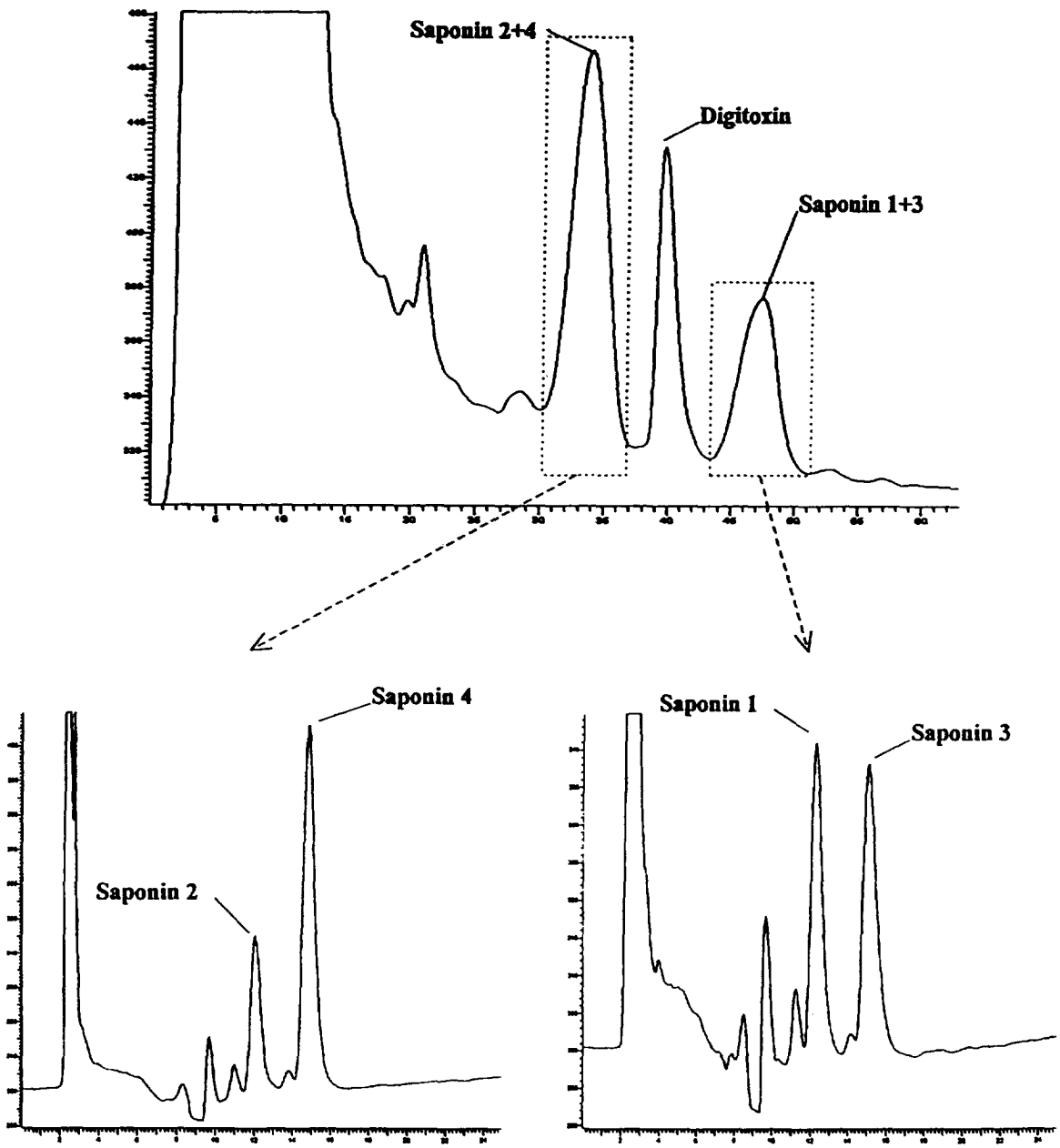

Fig. 1. Analysis of the saponins from *Solidago gigantea*, location AU 1. Top: analysis on LiChrospher RP-8 using digitoxin as internal standard. Bottom: separation of saponins 1,3 and 2,4 on LiChrosorb Si 60, collected during the preceding analysis on RP-8.

phases alone: HPLC on RP-8 shows two peaks, because giganteasaponins 1 and 3 (t_R=47.7 min) and 2 and 4 (t_R=33.8 min) elute together. After the analysis on silica also only two peaks are obtained, but this time giganteasaponins 1 and 2 (t_R=12.4 min) and 3 and 4 (t_R=15.0 min) cannot not be separated. Therefore a combination of HPLC on silica and on RP-8 is necessary to get single peaks for each of the four main saponins.

First the analysis of the saponin extract containing

the internal standard is performed by HPLC on RP-8 giving the absolute content of saponin (Fig. 1, top). To achieve good separation some RP-8 phases (LiChrosorb, LiChrospher, Nucleosil 100) with different particle sizes were tested using mixtures of acetonitrile–water and methanol–water as mobile phases. The best HPLC was obtained with LiChrospher RP-8 (5 μm) and methanol–water (53:47, v/v). Moreover it turned out that it is necessary to adjust the water to pH 5 with trifluoracetic acid (TFA) in order to achieve good peak shapes. During this analysis on RP-8 the two saponin peaks (the first containing giganteasaponins 2 and 4, the second giganteasaponins 1 and 3) are collected separately and the fractions are evaporated. The residues are dissolved in 60 μl water (adjusted to pH 5 with TFA)–*n*-propanol (50:50, v/v) and each saponin mixture is analysed by HPLC on silica (Fig. 1, bottom).

The separation of the saponins on silica is quite difficult. We tested many stationary phases such as Polygosil 60 (7 μm), Spherisorb S (5 μm), Nucleosil 100 (10 μm), Supersphere Si 60 (4 μm) and LiChrosorb Si 60 (5 μm); the latter was found to give the best results. To enable detection at 210 nm we tried numerous mixtures of butanol, propanol, methanol and water as mobile phases. The best separation was achieved with *n*-butanol–water and a gradient of methanol. The high purity of the saponin peaks was demonstrated using a diode array detector. The Turbochrom software calculated purity values between 1.1 and 1.8, whereas peaks with purities of 1.5 or below are considered to be pure.

This procedure combining HPLC on reversed-phase as well as on silica yielded the complete separation of the single saponins, and thus the content and composition of giganteasaponins 1–4 from *S. gigantea* from different locations could be determined. The results are summarized in Table 1.

4. Conclusions

As shown in Table 1 the content of saponins of the investigated *S. gigantea* varies from 0.8 to 1.9%. These results are confirmed by determining the hemolytic activities of the different samples. Considering the high inaccuracy of biological assays (±30%) the hemolytic indices are in good accordance with the saponin content. In the literature the hemolytic index of *Solidago gigantea* is given as 1200–1250 [7].

Some variations are found in the composition of the four main saponins from different locations, but giganteasaponin 4 is the main compound in all cases.

Considering the content of saponins from *Herba Virgaureae* described as 0.2–0.3% [11] with a hemolytic index of 250 – 1000 [7], *Solidago gigantea* contains significantly more saponins, the content was determined by HPLC to be approximately four- to eight-fold.

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Table 1
Absolute content, composition (relative percentage) of the main saponins and hemolytic indices (HI) from *Solidago gigantea*

Location	Absolute content (%)	Gigantea saponin 1	Gigantea saponin 2	Gigantea saponin 3	Gigantea saponin 4	HI
Au 1	1.9±8	16%	18%	17%	49%	2400
Au 2	0.8±7	21%	17%	19%	43%	1200
Auhof	1.7±7	19%	16%	25%	40%	2100
Kogl	0.9±3	9%	20%	7%	64%	1300
Zwettl	1.5±5	13%	11%	25%	51%	1400

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