Extraction and Isolation of Saponins

Article *in* Methods in molecular biology (Clifton, N.J.) · February 2012

DOI: 10.1007/978-1-61779-624-1_16 · Source: PubMed

1 author:

Runner R T Majinda
University of Botswana
97 PUBLICATIONS **1,721** CITATIONS

SEE PROFILE

Chapter 16

Extraction and Isolation of Saponins

Runner R.T. Majinda

Abstract

Due to their special structural features, extraction and isolation of saponins poses a serious challenge. Conventional methods have been explored as well as the recent, relatively greener, efficient, solvent-economic, time-saving, newer methods of extraction. Both traditional and recent methods of isolation are also discussed. Finally, examples are given involving both conventional and newer methods of extraction and isolation. Though in general it is difficult to use a single technique for isolation of saponins, recent literature work seems to point to the fact that high speed counter-current separation coupled to evaporative light scattering detector (ELSD) gives superior separation. The ELSD appears to have circumvented the long-standing problem of saponin detection as most of these do not have a chromophore, and hence making UV detection only nonspecific and at range 200–210 nm.

Key words: Saponins, Soap-like, (mono, bi, tri)-Desmosidic, Microwave-assisted extraction, Ultrasound-assisted extractions, HPLC, HSCCC, ELSD

1. Introduction

Saponins are phytochemicals, found mainly but not exclusively in plants, which exhibit foaming characteristics, and consist of polycyclic aglycones attached to one or more sugar side chains. The aglycone part, which is also called a sapogenin, is either a steroid (C27) or a triterpene (C30). The foaming ability of saponins is caused by the combination of a hydrophobic (fat soluble) sapogenin and a hydrophilic (water soluble) sugar part. Saponins characteristically have a bitter taste and some are known to be toxic.

The number of saccharide chains attached to the sapogenins (aglycone) can vary as can the length of each chain. The saccharide chain length, so far, varies from 1 to 11 sugar residues, with the numbers 2–5 being most frequently encountered with both linear and branched chains being represented (1). All saponins have attachment of at least one sugar chain to the aglycone and can be described as mono, di, or tridesmosidic depending on the number

Satyajit D. Sarker and Lutfun Nahar (eds.), *Natural Products Isolation*, Methods in Molecular Biology, vol. 864,
DOI 10.1007/978-1-61779-624-1_16,

of saccharide chains attached to the aglycone. When a single sugar chain, normally attached at C-3 of aglycone, we have a monodesmosidic saponin, while two sugar chains, often attached through ether linkage at C-3, and one through ester linkage (acyl glycoside) at C-28 (triterpene saponins) or ether linkage at C-26 (furostanol saponins), define bidesmosidic saponins. Tridesmosidic saponins have three sugar chains and are usually rare, though a number of these have recently been reported in, among others, *Astralagus* species (Fabaceae) (2–4), *Chenopodium quinoa* (Amaranthaceae) (5) and *Solidago virga-aurea* (Compositae) (6).

The saccharide moiety forming a chain may be linear or branched and to date the highest number of monosaccharide units found in saponins is eleven and was found in the species *Clematis mansuriea* (Ranunculaceae). Generally, most saponins tend to have short and often unbranched sugar chains containing 2–5 monosaccharide residues. The most common monosaccharide moieties found in plants are D-glucose (Gluc), D-galactose (Gal), D-glucoronic acid (GlucA), D-galactronic acid (GalA), L-rhamnose (Rha), L-arabinose (Ara), D-xylose (Xyl), and D-fucose (Fuc), while saponins from marine organisms often contain D-quinovose (Qui), glucose, Ara, GlucA, and Xyl attached to the aglycone. The configuration of the interglycosidic linkages is usually α and β, and the monosaccharide can either be in pyranose or furanose forms (1). Saponins can be acidic depending on the presence of carboxylic acid group in aglycone or sugar parts. The aglycone part of saponins can either be triterpene and steroidal. The steroidal saponins are sometimes further subdivided into furostanol or spirostanol (1) while some authors suggested a further subdivision of the triterpene and steroidal saponins into sixteen subclasses (7).

1.1. Sources of Saponins

Saponins are found in most vegetables, beans, and herbs. The most well-known sources are soybeans, peas, and some herbs with names that indicate foaming properties, such as soapwort (*Saponaria officinalis*, Caryophyllaceae), soapberry (*Sapindus saponaria*, Sapindaceae), soapbark (*Quillaja saponaria*, Quillajaceae), soaproot (*Chlorogalum pomeridianum*, Agavaceae), soapnut (*Sapindus mukurossi*), and soapwood (*Clethra occidentalis*, Clethraceae) (1, 8). Commercial saponins are extracted from *Quillaja saponaria* and *Yucca schidigera* (Agavaceae).

1.2. Problems Associated with Extraction and Isolation of Saponins

Extraction and isolation of saponins poses a challenge to researchers due to structural variety arising from different substituents, e.g., OH, CH_3, or COOH, in the aglycone moiety. This is further complicated by the number, arrangement, and orientation of the sugar units, as well as the number and types of sugar chains attached to the aglycone moiety. In general, saponins have high polarity, are chemically and thermally labile, are nonvolatile, and are usually found in low concentrations in plants (9). Thus, extra care needs to be taken in performing extractions (and preliminary treatment)

as relatively mild conditions should be applied since some saponins can undergo enzymic hydrolysis during water extractions while esterification of acidic saponins may also occur during an alcoholic extraction. Furthermore, transacylation (see Note 1) reaction may also take place (1).

In the time past, work on saponins involved hot extraction of the plant material using aqueous alcoholic solutions followed by evaporation of the alcohol and then extraction of saponins into butanol by liquid–liquid extraction. The problem with hot extractions is that labile functionalities (e.g., acylated forms) may disintegrate to produce artifacts rather than genuine saponins. Furthermore, extraction with methanol (MeOH) especially for steroidal saponins may result in the formation of methyl derivatives not originally found in the plant (8). Thus, to obtain real composition of saponins, cold extractions with ethanol–water solutions would be better. It should be noted that in liquid–liquid extractions, some highly polar saponins, such as bidesmosides and tridesmosides, may all remain in the aqueous layer or the extraction may not be quantitative.

The fact that saponins occur in plants as a mixture of structurally similar compounds of similar polarity renders a challenge when it comes to separating them. It comes as no surprise therefore that in the isolation of these compounds, a number of different separation techniques, e.g., thin layer chromatography (TLC), column chromatography (CC), low pressure liquid chromatography (LPLC), medium pressure liquid chromatography (MPLC) and high performance liquid chromatography (HPLC), are usually used to effect complete separation and isolation of pure individual components.

TLC is becoming a rather supporting technique for the analysis of saponins fractions from CC. It has been used for confirmation of purity and identity of isolated compounds. HPLC is one of the most versatile of the separation techniques. However, the absence of a chromophore (see Note 2) in most saponins hampers their detection under ultraviolet light and only allows nonspecific detection at 200–210 nm. The problem is that at this wavelength, components other than saponins may also absorb in this region thus making saponin determination difficult. This problem has recently been overcome by evaporative light scattering detection (ELSD) which allows the detection that measures the scattered light generated by the nonvolatile particles of analytes produced by nebulization into droplets of the effluent. ELSD is a universal, nonspecific detection method that can provide a stable baseline even with gradient elution (8, 10–12). High speed counter-current chromatography (HSCCC) is an all-liquid chromatographic system (13) (see Chapter 9). Irreversible adsorbing effects and artifact formation is minimized. Application of HSCCC in natural products chemistry is increasing steadily because of its superior separation abilities and excellent recovery rates (14–17).

2. Materials

The plant material (root, stem-bark, leaves, flowers, fruits, bulbs, etc.) is generally air-dried and then milled into powder before extractions. The water used is either distilled or de-ionized and organic solvents used are either of analytical grades or distilled in case of general purpose reagents. Extraction devices, as appropriate, e.g., maceration, microwave extraction or ultrasonic bath, are used for extraction. Standard chromatographic equipment and devices, required for performing TLC, CC, HSCCC, or HPLC, are essential. LC–ELSD, LC–MS, GC–MS, LC–NMR, or other hyphenated techniques can be used (see Chapter 12). For structure elucidation, standard spectroscopic devices are used. Often, saponins are hydrolyzed to free aglycone and component sugars, to facilitate structure elucidation, and thus, require acid or bases or various enzymes to effect hydrolysis.

3. Methods

3.1. Extraction of Saponins

The first step in the processing of saponins involves their extraction from the plant matrix. The extraction solvent, extraction conditions (e.g., temperature, pH, and solvent to feed ratio), and the properties of the feed material (e.g., composition and particle size) are the main factors that determine process efficiency.

3.1.1. Conventional Methods of Extraction

Pretreatment steps, which are carried out to increase the efficiency of the extraction include drying, particle size reduction, and defatting (use lipophilic solvent, such as ethyl acetate or *n*-hexane). Defatting can also be carried out after extraction of the saponins. Particle size reduction (grinding) is usually carried out to increase the mass transfer efficiency of the extraction. The efficiency of the separation is improved by using part of the plant with the highest saponin concentration.

Although this may not be a norm, most saponins extractions are performed on powdered plant material (various parts) using MeOH, ethanol (EtOH), water, or aqueous alcohol as extracting solvents. This is followed by a defatting (to remove lipophilic substances) step (generally with petroleum ether or *n*-hexane) which is carried out before the extraction step or on the extract itself. The extracts are then dissolved or suspended in water and shaken with *n*-butanol saturated with water. The *n*-butanol aliquots are then combined and the liquid removed to give crude saponin extract to work with. Some workers go yet a step further and opt for a precipitation stage using diethyl ether or acetone. Others go further still and include a dialysis stage to remove small water-soluble molecules, such as sugars (1, 18).

3.1.2. Recent Methods of Extraction

Conventional methods of extraction and purification of chemical constituents from plant tissues present some disadvantages. They require mainly longer extraction time, larger solvent amounts, and are sometimes lower in efficiency. Moreover, many natural products are thermally unstable and may degrade during extraction. Compared to traditional methods recent methods (see below) have many advantages, such as shorter extraction time, less solvent used, and higher extraction rate (19). While traditional extraction methods are commonly used for the production of saponin extracts, recent research focuses on technologies that improve the extraction efficiency by reducing extraction time and solvent consumption/waste without compromising sample quality. Microwave (20–23) (see Chapter 5) and ultrasound (24)-assisted extractions (MAE and UAE, respectively) are relatively inexpensive, simple, and efficient. These processes involve disruption of the internal cell structure and release of intracellular product to facilitate mass transfer, which is achieved by rapid and selective heating of raw material in a solvent which is (partially) transparent to microwave energy (in microwave extractions) (20–23) and the mechanical effects of acoustic cavitations (in ultrasonic extractions) (24).

Lab scale MAE and UAE were investigated for the extraction of ginsenosides from ginseng (21, 23), and saponins from chickpeas (25). The ginsenoside yield and composition of a 80% MeOH (50 mL) extract obtained from ginseng powder (5 g) using MAE for 30 s were (at 72.2°C) comparable to those of 12 h conventional reflux extraction carried out under similar conditions (21). Similarly, a maximum saponin yield of 7.4 mg/100 mg DW could be obtained in 6 min by MAE of ginseng (100 mg of sample: 15 mL of water-saturated *n*-butanol, 50°C) compared to 8 h for Soxhlet extraction (7.7 mg/100 mg DW; 100 mg of sample: 80 mL of MeOH, 70°C), 6 h for heat reflux extraction (6.7 mg/100 mg DW; 100 mg of sample: 15 mL of MeOH, 70°C), and 2 h for ultrasonic extraction (7.6 mg/100 mg DW; 100 mg of sample: 15 mL of water-saturated *n*-butanol) (23). Following are some examples of available modern extraction methods.

(a) Ultrasonic extraction: In this method, extractions are carried out in an ultrasonic bath that allows for variation of amplitude and temperature. Working frequency is set at a certain value, say 33 kHz. A given amount of material (e.g., 4 g) is extracted with a given volume (say, 100 mL) of 95% (*v*/*v*) EtOH in a conical flask and sonicated for a given time, say 15 min, at room temperature. After extraction the contents are filtered and evaporated to dryness.

(b) Supercritical fluid extraction: A supercritical fluid extractor (e.g., Hua'an SFE Instrument Company) is used (see Chapter 3). Samples (80 g) are extracted with carbon dioxide and

EtOH (e.g., 60 mL) as entrainer under the working pressure of, say, 25 MPa and temperature of 55°C. The flow rate (e.g., 30 L/h) and the separation temperature (e.g., 37°C) are set.

(c) Microwave-assisted extraction: The use of microwave energy enables fast dissolution, drying, acidic digestion, and extraction of organic compounds from complex matrices (see Chapter 5). The microwave heats the solvent or solvent mixture directly and the direct interaction of microwaves with the free water molecules present in the glands and vascular systems results in subsequent rupture of the plant tissue and release of components into the organic solvent. Its main advantages are reduced solvent volume and time consumption and increased sample throughput (20, 26). Thus, MAE provides an alternative method to conventional extraction methods in plants. MAE is performed in a closed vessel unit (see Note 3). The unit is equipped with a temperature sensor and a maximum oven power of 800 W.

Defatted powder (e.g., 4 g) is mixed with a solvent of choice (MeOH, EtOH, or EtOH–H_2O 7:3, *n*-butanol or *n*-butanol–water 1:1, 16 mL) in 20-mL closed vials and irradiated at 2,450 MHz for 10 or 20 min. The solvent temperature is kept constant at 60°C using an automatic temperature device (ATC-FO, Milestone, Sorisole, Italy) submerged into a solvent containing vessel. Twelve sample vessels are used at a time, with pressure and temperature monitoring capabilities, in an MPR-600/12S rotor (Milestone, Sorisole, Italy). The microwave power is limited to 300 W and after cooling to room temperature the extract is collected and stored until used.

3.2. Isolation of Saponins

Methods for isolation of saponins have been made available elsewhere (5, 27, 28). The conventional methods of solvent extraction, CC, and preparative TLC (see Chapter 6) may sometimes yield pure substances, but in many cases it is difficult to isolate individual saponins. Generally, the following method has been employed by various researchers. The defatted powdered material is treated with petroleum ether and extracted with MeOH in a Soxhlet for 72 h (29) or maceration with MeOH at room temperature. The extract is concentrated under reduced pressure and partitioned successively using *n*-hexane, ethyl acetate (or $CHCl_3$), and *n*-BuOH. The *n*-BuOH soluble fraction and the aqueous part afford the major saponin triterpene fraction (30). The crude extracts are applied separately to columns of Diaion HP-20 which are washed with water–MeOH in various ratios (0, 50, 85, and 100) and finally with acetone. The fractions found to have the same pattern are mixed together and separated further by silica gel column chromatography with $CHCl_3$–MeOH–H_2O (40:10:1

v/*v*/*v*). Finally, the saponin compounds could be separated by HPLC on ODS column using MeOH–water as eluent. Saponin compounds could be detected on TLC after spraying with 10% (*v*/*v*) H_2SO_4 in EtOH solution and the Lieberman–Burchard reagent (see Note 4) or a mixture of *p*-anisaldehyde-sulfuric acid-glacial acetic acid (1:2:100 *v*/*v*/*v*) and triterpene saponins produce blue–violet spots on heating.

3.3. Specific Examples

Specific examples of extraction and isolation of saponins are presented in Table 1.

Table 1
Examples of saponin extraction and isolation

Source	Method	Reference
Bellium bellidioides (Asteraceae)	*Extraction*: Plant material (495 g) was refluxed 1 h with 3.6 L of 80% MeOH. Residue was diluted with H_2O to 750 mL. Extract was defatted with $CHCl_3$ and extracted four times with *n*-BuOH. The dried *n*-BuOH extract was dissolved in MeOH and dropped into an excess of Et_2O giving 30.7 g of a brown, powdery crude glycosidic mixture *Isolation*: Part of crude glycoside mixture (9.7 g) was subjected to a Sephadex LH-20 CC (solvent MeOH) and a saponin containing fraction was obtained (yield 5.4 g). Portion (2.15 g) of that fraction was separated by CC on silica gel using $CHCl_3$–MeOH–H_2O (7:3:1, lower layer) giving 546 mg of bellidioside A (**1**). This was further purified by HPLC (LiChrosorb® RP-18, 7 µm, 250 × 10 mm, MeOH–H_2O 66:34, 4 mL/min). For the isolation of Bellissaponin BA_1 (**2**) and deacylbellidioside B4 (**3**) another 2.1 g of the saponin fraction was separated 2× on silica gel using $CHCl_3$–MeOH–H_2O mixtures (10:3:1, lower layer, and 9:3:1, lower layer) followed by HPLC (LiChrosorb® RP-18, 7 µm, 250 × 10 mm, MeOH–H_2O 51:49, 4 mL/min) giving 73 mg of **2** and 114 mg of **3**. Another part (700 mg) of crude mixture was put on prep-centrifugal accelerated radial TLC (chromatotron) on silica gel using $CHCl_3$–MeOH–H_2O [13:3:1, lower layer giving fractions A (33 mg), B (430 mg) and C (33 mg). HPLC of each of these fractions on LiChrosorb® RP-18 (7 µm, 250 mm × 10 mm id, MeOH–H_2O, 32:18, 6 mL/min] giving **3** (3 mg) and deacylbellidioside B3 (**4**, 12 mg). Detection was at 206 nm	(31, 32)

(continued)

Table 1 (continued)

Source	Method	Reference
Aralia decaisneana (Araliaceae)	*Extraction*: Dried roots (5 kg) were extracted three times with EtOH–H_2O (7:3) under reflux. The extract was concentrated under reduced pressure to give a reddish brown gum (360 g) *Isolation*: Gum (150 g) CC on silica gel (900 g) column, eluted with $CHCl_3$–MeOH–H_2O (13:7:0.16) increasing MeOH and H_2O proportions to give fractions A–M; Fraction D (15.2 g) was separated into 17 fractions (a–q) by prep-HPLC (Develosil Lop-ODS, 5 μm × 50 cm × 2 cm; MeOH–H_2O (7: 3 → 4: 1) linear gradient). Compounds obtained: araliasaponin VI (**12**, 375 mg) from fraction d, araliasaponin V (**11**, 20 mg) from j and ur-3-gluc(1 → 3)ara (**14**, 32 mg) from fraction o. Fraction g (1.9 g) was further separated on HPLC [Develosil ODS 10/20, 5 μm × 50 cm × 2 cm; CH_3CN–H_2O (13:7) recycle] to give elatoside F (tarasaponin VI) (**6**, 300 mg), araliasaponin I (**7**, 132 mg), matesaponin 1 (**15**, 135 mg), and araliasaponin VIII (**16**, 422 mg). Fraction e (184 mg) on HPLC (Develosil PhA-7, 2 cm × 25 cm; CH_3CN–H_2O (3:7) yielded fractions 1 (70 mg) and 2 (75 mg). Fraction 1 on HPLC [YMC Pack SH-843-5 C_4, 2 cm × 25 cm; CH_3CN–H_2O (13:27) recycle] gave araliasaponin II (**8**, 30 mg) and araliasaponin X (**18**, 32 mg). Fraction 2 on HPLC [YMC Pack SH-843-5 C_4, 2 cm × 25 cm; CH_3CN–H_2O (3:7) recycle] gave araliasaponin IV (**10**, 40 mg) and araliasaponin XI (**19**, 18 mg). Fraction m (250 mg) was on HPLC [YMC Pack SH-843-5 C_4, 2 cm × 25 cm; CH_3CN–H_2O (2:3) + 0.05% TFA recycle] gave elatoside E (**5**, 112 mg) and araliasaponin IX (**17**, 90 mg). Fraction I (2.0 g) on HPLC (Develosil Lop-ODS, 5 cm × 50 cm × 2 μm; MeOH–H_2O (13:7 → 7:3) linear gradient) yielded araliasaponin III (**9**, 160 mg) and araliasaponin VII (**13**, 435 mg). Detection was at 205 nm	(33)
Panax notoginseng (Araliaceae)	*Extraction*: Five tablets of *P. notoginseng* extract were powdered and extracted with MeOH (3 × 50 mL). Extracts were combined and evaporated to dryness under reduced pressure to yield 283 mg of yellow powder which was directly used for high speed counter-current chromatography (HSCCC) separation *Isolation*: HSCCC-multilayer coil counter current chromatograph, manufacturer—PC (Potomac, MD, USA), equipped with a 385-mL coil column made of polytetrafluroethylene tubing (2.6 mm ID). The mobile phase was delivered by a Biotronik HPLC pump BT 3020 (Jasco, Gross-Umstadt, Germany). A two phase solvent system, *n*-hexane-*n*-butanol-	(34)

(continued)

Table 1 (continued)

Source	Method	Reference
	water (3:4:7, *v/v/v*), was equilibrated on a separating funnel and the two resulting layers separated shortly before use. The multilayer coil column was entirely filled with the upper organic phase as the stationary phase (SP) and the apparatus was rotated at 43 × *g*. Two hundred and eighty-three milligram of extract was dissolved in 50 mL of mobile phase, injected into the HSCCC system using a PTFE sample loop, followed by pumping of mobile phase at a flow rate of 2.5 mL/min. Twenty milliliter fractions were collected using a Superfrac fraction collector (Pharmacia, Uppsala, Sweden). The separation yielded ginsenoside-Rb_1 (**20**, 157 mg), ginsenoside-Re (**21**, 13 mg), ginsenoside-Rg_1 (**22**, 56 mg), and notoginsenoside-R_1 (**23**, 17 mg). Detected on TLC sprayed with 5% conc. H_2SO_4 in EtOH	
Panax ginseng (Araliaceae)	*Extraction*: Dried Korean red ginseng (steam-treated *Panax ginseng*) (700 g) was extracted twice with 70% EtOH by sonication for 3 h, followed by rotary evaporation (40°C) under reduced pressure. The extract was loaded onto reverse-phase C18 open column (50 cm × 3 cm, column volume 250 mL), and subsequently eluted with water, 30% MeOH and 90% MeOH. The enriched ginsenoside fraction was eluted with 90% MeOH. This fraction was evaporated, lyophilized to give 350 mg of saponin enriched extract *Isolation*: Preparative HSCCC was carried out using model CCC-1000 multilayer coil of polytetrafluoroethylene (PTFE) tubing, inner diameter 1.6 mm, total capacity 325 mL (Pharma-Tech-Research, Baltimore, MD, USA). Methylene chloride–methanol–water–isopropanol (6:6:4:1, *v/v*) was used as a two-phase solvent system. The solvent mixture was thoroughly equilibrated in a separation funnel at room temperature, and the two phases were separated shortly before use. Three hundred and fifty milligram of ginsenoside enriched fraction was dissolved in 20 mL mixture of upper and lower layer (1:1, *v/v*). The multilayer coiled column filled entirely with the upper phase as the SP. The apparatus is then rotated at 54 × *g* and the lower organic mobile phase was pumped into the head end of the column at a flow rate of 1.3 mL/min. After hydrodynamic equilibrium was reached, indicated by clear mobile phase elution at the tail end, the sample solution was injected through the sample port. Monitoring of eluent was by connecting tail end of column to ELSD (T = 55°C, gain = 2, P_N = 1.7 bar)) system through spit valve. The eluted fractions yielded ginsenoside-Rg3 (**24**, 28.8 mg), ginsenoside-Rk1 (**25**, 26.6 mg), ginsenoside-Rg5 (**26**, 32.2 mg), and ginsenoside-F4 (**27**, 8.1 mg). Detection by ELSD detector	(10)

(continued)

Table 1 (continued)

Source	Method	Reference
Panax ginseng (Araliaceae)	*Extraction*: The dried roots of *P. ginseng* (0.9 kg) were ground to a course powder and extracted three times with 7.2 L of 90% aqueous ethanol for 45 min. The extract solution was filtered and concentrated. The concentrated solution was diluted with deionized water and separated using 0.6 L macroporous resin column. Seven bed volumes of deionized water were flushed through the column to remove contaminants. Four bed volumes of 70% aq. EtOH were then used to elute ginsenosides in isocratic mode with a flow rate of 10 mL/min. The fractions were concentrated under vacuum at 60°C to give crude saponin sample *Isolation*: Preparative HPCCC was carried out using midi-CCC from Dynamic Extractions (Slough, UK), having a coil of 204 mL and 4.0 mm bore tubing, working at a speed of 192 × *g*. This was coupled to evaporative light scattering detector (ELSD) PL-ELS-1000 (Polymer Laboratories, USA). Methylene chloride–MeOH–5 mM aqueous ammonium acetate-isopropanol (6:2:4:3, *v*/*v*) was used as a two-phase solvent system. The solvent mixture was thoroughly equilibrated in a separation funnel at room temperature, and the two phases were separated shortly before use. 480 mg crude sample was dissolved in 20 mL of SP (upper layer). The coil was filled with SP in head to tail mode. The mobile phase was pumped into the coil at a flow rate of 20 mL/min and centrifuge rotational speed of 1,250 rpm at 25°C. At hydrodynamic equilibrium, the sample was injected into the coil through a 20 mL sample loop and monitored by connecting tail outlet to ELSD through a split valve, yielding ginsenoside-Rf (**28**, 10.7 mg), ginsenoside-Rd (**29**, 11.0 mg), ginsenoside-Re (**30**, 13.4 mg), and ginsenoside-Rb1 (**31**, 13.9 mg) with percentage purities of 99.2%, 88.2%, 93.7%, and 91.8%, respectively	(11)

4. Notes

1. Transacylation is a chemical reaction involving the reversible transfer of an acyl radical.
2. A chromophore is the part of a molecule responsible for its color, which arises when a molecule absorbs certain wavelengths of visible light and transmits or reflects others. The chromophore is a region in the molecule, where the energy difference between two different molecular orbitals falls within the range of the visible spectrum.

3. An example of closed vessel MAE is MDS202, Xin'yi Microwave Extraction Unit Instrument Company, Shanghai or a mechanically modified oven ETHOS1600, Milestone, Sorisole, Italy.
4. The Lieberman–Burchard or acetic anhydride test is used for the detection of cholesterol and other sterols. The formation of a green or green–blue color after a few minutes indicates the presence of cholesterol or sterols.

References

1. Hostettmann K, Marston A (1995) Saponins. Cambridge University Press, Cambridge
2. Çalis I, Yusofoglu H, Zerbe O, Sticher O (1999) Cephalotoside A: a tridesmosidic cycloartane type glycoside from *Astralagus cephalotes var. brevecalys*. Phytochemistry 50: 843–847
3. Yesilada E, Bedir E, Çalis I, Takaishi Y, Ohmoto Y (2005) Effects of triterpene saponins from *Astralagus* species on in vitro cytokine release. J Ethnopharmacol 96:71–77
4. Semmar N, Tomofumi M, Mrabet Y, Lacaille-Dubois M-A (2010) Two new acylated tridesmosidic saponins from *Astralagus armatus*. Helv Acta Chim 93:871–876
5. Kuljanabhagavad T, Wink M (2009) Biological activities and chemistry of saponins from *Chenopodium quinoa* Willd. Phytochem Rev 8:473–490
6. Inose Y, Miyase T, Ueno A (1992) Studies on constituents of *Solidago virga-aurea* L. II. Structures of solidagosaponins. Chem Pharm Bull 40:946–953
7. Vincken J-P, Heng L, de Groot A, Gruppen H (2007) Saponins, classification and occurrence in the plant kingdom. Phytochemistry 68: 275–276
8. Oleszek W, Bialy Z (2006) Chromatographic determination of plant saponins – an update (2002–2005). J Chromatogr A 1112:78–91
9. Li B, Abliz Z, Tang M, Fu G, Yu S (2006) Rapid structural characterization of triterpenoid saponins in crude extract from *Symplocos chinensis* using liquid chromatography combined with electrospray ionization tandem mass spectrometry. J Chromatogr A 1101:53–62
10. Ha YW, Lim SS, Ha IN, Na Y-C, Seo J-J, Shin H, Son SH, Kim YS (2007) Preparative isolation of four ginsenosides from Korean red gingseng (steam-treated *Panax gingseng* C. A. Meyer), by high-speed counter-current chromatography coupled with evaporative light scattering detection. J Chromatogr A 1151: 37–44
11. Qi X, Ignatova S, Luo G, Liang Q, Jun FW, Wang Y, Sutherland I (2010) Preparative isolation and purification of Rf, Re, Rd and Rb1 from the roots of *Panax gingseng* with a salt containing solvent system and flow step-gradient by high performance counter-current chromatography with evaporative light scattering detector. J Chromatogr A 1217:1995–2001
12. Müller A, Ganzera M, Stuppner H (2005) Analysis of phenolic glycosides and saponins in *Primula elatior* and *Primula veris* (primula root) by liquid chromatography, evaporative light scattering detection and mass spectrometry. J Chromatogr A 1112:218–223
13. Ito Y, Conway WD (1996) High speed countercurrent chromatography. Wiley-Interscience, New York
14. Du Q, Cheng H, Ito Y (2001) Separation of radioactive metabolites in cultured tea cells fed with [^{14}C]phenylalanine using high-speed counter-current chromatography. J Chromatogr A 921:331–334
15. Du QZ, Li ZH, Ito Y (2001) Preparative separation of isoflavone components in soybeans using high-speed counter-current chromatography. J Chromatogr A 921:271–274
16. Du Q, Xia M, Ito Y (2002) Purification of icariin from the extract of *Epimedium segittatum* using high-speed counter-current chromatography. J Chromatogr A 962:239–241
17. Du QZ, Chai WJ, Ito Y (2002) Preparative separation of fruit extract of *Silybum marianum* using a high-speed countercurrent chromatograph with scale-up columns. J Liq Chromatogr Rel Technol 25:2515–2520
18. Weng A, Jenett-Siems K, Schmieder P, Bachran D, Bachran C, Görick C, Thakur M, Fuchs H, Melzig MF (2010) A convenient method for saponin isolation in tumor therapy. J Chromatogr B 878:713–718
19. Chen Y, Xie M-Y, Gong X-F (2007) Microwave-assisted extraction used for the isolation of total triterpenoid saponins form *Ganoderma atrum*. J Food Eng 81:162–170

20. Kwon JH, Bélanger JMR, Páre JRJ (2003) Optimisation of microwave-assisted extraction (MAE) for gingseng components by response surface methodology. J Agric Food Chem 51:1807–1810
21. Kwon JH, Bélanger JMR, Páre JRJ, Yaylayan VA (2003) Applications of microwave-assisted process (MAP™) to the fast extraction of ginseng saponins. Food Res Int 36:491–498
22. Kwon JH, Lee GD, Bélanger JMR, Páre JRJ (2003) Effects of ethanol concentration on the efficiency of extraction of ginseng saponins when using when using a microwave-assisted process (MAP™). Int J Food Sci Tech 38:615–622
23. Vongsangnak W, Gua J, Chauvatcharin S, Zhong JJ (2004) Towards efficient extraction of notoginseng saponins from cultured cells of *Panax notoginseng*. Biochem Eng J 18:115–120
24. Wu J, Lin L, Chau F (2001) Ultrasound-assisted extraction of ginseng spaonins cultured in ginseng cells. Sonochem 8:347–352
25. Kerem Z, German-Shashoua H, Yaden O (2005) Microwave-assisted extraction of bioactive saponins from chickpea (*Cicer arietinum* L). J Sci Food Agric 85:406–412
26. Eskilsson CS, Bjorkland E (2000) Analytical-scale microwave-assisted extraction. J Chromatogr A 902:227–250
27. Marston A, Wolfender JL, Hostettmann K (2000) Analysis and isolation of saponins from plant material. In: Oleszek WA, Marston A (eds) Saponins in foodstuffs and medicinal plants. Annual proceedings of the phytochemical society, Clarendon, pp 1–12
28. Oleszek WA (2002) Chromatographic determination of plant saponins. J Chromatogr A 967:147–162
29. Woldemichael GM, Wink M (2001) Identification and biological activities of triterpenoid saponins from *Chenopodium quinoa*. J Agric Food Chem 49:2327–2332
30. Kuljanabhagavad T, Thongphasuk P, Chamulitrat W, Wink M (2008) Triterpene saponins from *Chenopodium quinoa* Willd. Phytochemistry 69:1919–1926
31. Schöpke T, Agha MIH, Wray V, Hiller K (1994) Triterpenoid saponins from *Bellium bellidioides*. Phytochemistry 36:449–453
32. Schöpke T, Wray V, Nimtz M, Hiller K (1994) Triterpenoid saponins from *Bellium bellidioides*. Structure of the minor deacylsaponins. Phytochemistry 41:1399–1403
33. Miyase T, Shiokawa K-I, Zhang DM, Ueno A (1996) Araliasaponin I–XI, triterpene saponins from the roots of *Aralia decaisneana*. Phytochemistry 41:1411–1418
34. Du Q, Jerz G, Waibel R, Winterhalter P (2003) Isolation of dammarane saponins from *Panax ginseng* by high speed counter-current chromatography. J Chromatogr A 1008:173–180