

# High-performance liquid chromatography analysis of plant saponins: An update 2005-2010

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## ABSTRACT

Saponins are widely distributed in plant kingdom. In view of their wide range of biological activities and occurrence as complex mixtures, saponins have been purified and separated by high-performance liquid chromatography using reverse-phase columns at lower wavelength. Mostly, saponins are not detected by ultraviolet detector due to lack of chromophores. Electrospray ionization mass spectrometry, diode array detector, evaporative light scattering detection, and charged aerosols have been used for overcoming the detection problem of saponins.

**Key words:** Charged aerosol, diode array detector, evaporative light scattering detection, ESI, high-performance liquid chromatography, mass spectrometry, mobile phase, photodiode array detectors, saponins, sapogenins, ultraviolet

## INTRODUCTION

Saponins are plant glycosides which are recognized by their ability to produce a soapy lather when shaken with water. They are widely distributed in the nature and have been reported to be present in at least 500 genera of plants. All saponins are polar in nature, thus they are freely soluble in water but insoluble in nonpolar solvents. They are glycosides of triterpenoid or steroid aglycones with a varying number of sugar side chains. A wide variety of plants belonging to family Liliaceae are major source of saponins. The saponins isolated from *Asparagus* species, referred as Shatavarins such as shatavarin I, II, III, and IV, derived from steroidal aglycones, have been identified and characterized by spectroscopic method.<sup>[1]</sup> Saponins on hydrolysis yield an aglycone known as "sapogenin." The applications of saponins stretch over several areas such as additives in food and cosmetics, as wetting agents for the agriculture and photographic industry, and as adjuvants in the pharmaceutical industry.<sup>[2]</sup>

The structural complexity of saponins results in a number of physical, chemical, and biological properties. Saponins are usually amorphous substances having high molecular weight. Due to the presence of a lipid-soluble aglycone and water-soluble sugar chain in their structure (amphiphilic nature), saponins are surface active compounds with detergent, wetting, emulsifying, and foaming properties. In aqueous solutions, surfactants form micelles above a critical concentration called critical micelle concentration (CMC). Saponins, including soybean saponins, saponins from *Saponaria officinalis* and *Quillaja saponaria*, form micelles in aqueous solutions, the size and structure of which are dependent on the type of saponin.<sup>[3]</sup> The micelle-forming properties such as CMC and the aggregation number (number of monomers in a micelle) of quillaja saponins were affected by temperature, salt concentration, and pH of the aqueous phase.<sup>[4]</sup>

Saponins possess a variety of biological activities, viz. antioxidant, immunostimulant, antihepatotoxic, antibacterial, anticarcinogenic, antidiarrheal, antiulcerogenic, antioxytotic, hypocholesterolemic, anticoagulant, hepatoprotective, hypoglycemic, neuroprotective, anti-inflammatory activity, useful in diabetic retinopathy, inhibition of dental caries, and platelet aggregation.<sup>[5,6]</sup> Many saponins are known to be antimicrobial to inhibit mould and to protect plants from insects. They may be considered forming the defense system and have been included in a large group of protective molecules found in plants named phytoanticipins or phytoprotectants. Saponin-rich plant has been found to improve growth, feed efficiency, and health in ruminants.<sup>[7]</sup> This article briefly reviews the high-performance liquid chromatography (HPLC) methods, columns, detectors, mobile and stationary phases used for saponins and sapogenin.

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**Table 1: Separation of saponins by HPLC using different stationary and mobile phases**

Name of plants/ saponins/ sapogenins	Column (stationary phase)	Solvent system (mobile phase)	Detector	Mode of elution/ flow rate	References
Sarsasapogenin as benzoyl chloride	Alltima C <sub>18</sub> (150 x 4.6 mm, 5 µm)	MeOH-H <sub>2</sub> O (99 : 1)	UV λ = 230 nm	Gradient, 1.1 ml/min	[15]
<i>Asparagus racemosus</i>	Dynamax (250 x 10 mm) OmniSpher 5 C <sub>18</sub>	MeOH-H <sub>2</sub> O	ELSD	Gradient	[16]
<i>A. racemosus</i>	OmniSpher C <sub>18</sub> (150 x 4.6 mm)	CH <sub>3</sub> CN- H <sub>2</sub> O	ELSD-LT	2 ml/min	[17]
<i>A. racemosus</i>	OmniSpher C <sub>18</sub> (150 x 4.6 mm)	CH <sub>3</sub> CN- H <sub>2</sub> O	ELSD-LT	Gradient, 2 ml/min	[18]
<i>A. officinalis</i>	Luna C <sub>18</sub> (150 x 4.6 mm, 5 µm)	CH <sub>3</sub> CN-H <sub>2</sub> O and CH <sub>3</sub> CN- CH <sub>3</sub> COOH	MS	Gradient 1 ml/min	[11]
<i>Platycodi radix</i>	Zorbex SB-Aq C <sub>18</sub> (150 x 4.6 mm, 5 µm)	CH <sub>3</sub> CN-H <sub>2</sub> O	ELSD/MS	Gradient 1 ml/min	[19]
<i>Ilex paraguariensis</i>	Novapack C <sub>18</sub> , 4 µm, Aq C <sub>18</sub> (150 x 3.9 mm)	CH <sub>3</sub> CN-H <sub>2</sub> O (70 : 30)	UV, λ = 230 nm	1 ml/min	[12]
<i>Medicago truncatula</i>	C <sub>18</sub> (250 × 4.6 mm, 5 µm)	H <sub>2</sub> O-CH <sub>3</sub> CN 95 : 5-5 : 95 with 1% acetic acid	MS	Gradient, 0.8 ml/min	[20]
<i>Medicago truncatula</i>	LiChroprep C <sub>18</sub>	MeOH-H <sub>2</sub> O		Gradient	[21]
Triterpenoid saponins	Silica gel	EtOAc-Hexane			[14]
Soya saponins			ELSD		[22]
<i>Bacopa monnieri</i>		0.2% phosphoric acid and acetonitrile (65:35 v/v)	PDA	Isocratic	[23]
Flos saponins		(A) Acetonitrile-acetic acid (95 : 0.5) and (B) 0.5% aqueous acetic acid	ELSD	Gradient	[24]
<i>Ilex paraguariensis</i>	C <sub>18</sub>		UV		[25]
<i>Phytolacca bogotensis</i>			ESI/MS		[26]
<i>Paris polyphylla</i>	C <sub>18</sub>	0.1% Aqueous formic acid- CH <sub>3</sub> CN	ESI-MS	Gradient	[27]
Steroidal saponins			DAD, MS		[28]
Soyasaponins			ESI-MS		[29]
<i>Medicago truncatula</i>			PDA/ESI/MS/MS		[30]
<i>Ziziphus jujuba</i>	C <sub>18</sub>	(A) 0.1% Aqueous acetic acid and (B) methanol with 0.1% acetic acid	ELSD	1.8 ml/min Gradient	[31]
<i>Tupistra chinensis</i>	YMC-Pack ODS-AQ (4.6 x 250 mm, 5 µm)	CH <sub>3</sub> CN-H <sub>2</sub> O		Gradient	[32]
<i>Panax notoginseng</i>	C <sub>18</sub>	Acetonitrile and 0.01% aqueous formic acid	UV	Gradient	[33]
Saponins			Charged aerosol		[34]
Folium Ginseng and Radix Ginseng	C <sub>18</sub>		DAD-MS		[35]
<i>Panax notoginseng</i>	C <sub>18</sub>				[36]
<i>Tahini Halva</i>	C <sub>18</sub>	MeOH-H <sub>2</sub> O-acetic acid 60/34/6 (v/v/v)	PDA	1.5 ml/min	[37]
<i>Pulsatilla koreana</i>			ELSD		[38]
<i>Aesculus chinensis</i>		MeOH-H <sub>2</sub> O-acetic acid			[39]
Saponins			ELSD		[40]
Steroidal saponins	C <sub>18</sub>				[41]
Triterpene saponins			ELSD		[42]
Triterpene saponins		Chloroform-methanol-water (4 : 4 : 2, v/v)		1.5 ml/min	[43]
Steroidal glycosides	C <sub>18</sub>		MS/MS		[44]
Saponins					[45]

UV: Ultra violet, ELSD: Light-scattering detector

## HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF SAPONINS

The normal- and reverse-phase HPLCs are commonly used for separation, identification, and purification of saponins. But for the best separation of saponins, RP-HPLC is normally used. HPLC is increasingly used in the separation of various compounds including saponins. This technique is rapid, selective, and highly sensitive. Separation of saponins can be affected by HPLC using variety of stationary and mobile phases. The separation of major saponin components in Liquorice, *Bupleuri radix* on a column of octadecylsilylated silica (ODS) gel LS-40 with a mixture of methanol, water, acetic acid, and triethylamine as mobile phase have been achieved using HPLC.<sup>[8]</sup> Saponins of *Platycodi radix* have been separated and quantitated by HPLC with light-scattering detector (ELSD) using C<sub>18</sub> column and gradient aqueous acetonitrile mobile phase.<sup>[9]</sup> Most studies are concerned with triterpene saponins and have generally adopted the chromatographic conditions preferred for HPLC-ultraviolet (UV), i.e., a C<sub>18</sub> column and mobile phase gradient of aqueous acetonitrile.<sup>[10]</sup> The chromatographic behaviors of steroidal saponins of *Anemarrhena asphodeloides*, *Asparagus officinalis*, *Convallaria majalis*, *Digitalis purpurea*, and *Ruscus aculeatus* were studied by HPLC-MS on C<sub>18</sub> reverse-phase column and aqueous acetonitrile or aqueous methanol as mobile phase in gradient manner with or without the addition of 1% acetic acid.<sup>[11]</sup> The hydrolyzed saponins of *Ilex paraguariensis* were separated and quantified by HPLC method with UV detection. The total concentrations of saponins in aqueous extract were found to be 352 µg/ml.<sup>[12]</sup> HPLC analyses on octadecylsilyl porous glass were investigated for acidic saponins in ginseng, bupleurum root, and senega. These acidic saponins as well as neutral saponins were separated rapidly with aqueous acetonitrile containing K<sub>2</sub>HPO<sub>4</sub> mobile phase at room temperature.<sup>[13]</sup> Only conjugated soyasaponins which have UV absorption maximum at 295 nm, glycyrrhetic acid glycosides, and cucurbitacins could be successfully detected with UV-Vis detector. Saponins have also been successfully separated on Spherisorb ODS, C<sub>18</sub>, and C<sub>8</sub> column using MeCN-H<sub>2</sub>O. Similar validated method using ELSD was developed for saponin determination in *Flos Lonicerae*, a herb used in traditional Chinese medicine.<sup>[14]</sup>

The benzoate derivatives of the major saponin (hecogenin, dehydrohecogenin, and tigogenin) occurring in the *Agave* species have been analyzed by reversed phase HPLC on LiChrosorb RP-8 column using MeOH-H<sub>2</sub>O (80 : 20) mobile phase. Different stationary phases and mobile phases used in the separation of saponins are listed in Table 1.

## CONCLUSION

Most of the naturally occurring saponins are separated by C<sub>18</sub> and ODS column using MeOH-H<sub>2</sub>O and CH<sub>3</sub>CN-H<sub>2</sub>O with or without addition of acids as mobile phase. HPLC-ELSD (evaporative light scattering detection), HPLC-MS (mass

spectrometry), and HPLC-RI (refractive index) methods have been developed for overcoming the detection problem of saponins by UV detector.

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