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

Jagmohan S. Negi[1,2], Pramod Singh[1], Geeta Joshi Nee Pant[1], M. S. M. Rawat[1]

[1]Department of Chemistry, HNB Garhwal University, Srinagar (Garhwal), [2]Herbal Research and Development Institute, Gopeshwar (Chamoli), Uttarakhand, India

Submitted: 17-07-2010 Revised: 04-04-2011 Published: 23-12-2011

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, potodiode 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 Liliacae 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.[1] 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.[2]

Address for correspondence:
Dr. Jagmohan S. Negi
Herbal Research and Development Institute,
Gopeshwar (Chamoli) - 246 401, Uttarakhand, India
E-mail: negi_js1981@yahoo.co.in

Access this article online	
Quick Response Code:	Website: www.phcogrev.com
	DOI: 10.4103/0973-7847.91109

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.[3] 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.[4]

Saponins possess a variety of biological activities, viz. antioxidant, immunostimulant, antihepatotoxic, antibacterial, anticarcinogenic, antidiarrheal, antiulcerogenic, antioxytocic, hypocholesterolemic, anticoagulant, hepatoprotective, hypoglycemic, neuroprotective, anti-inflammatory activity, useful in diabetic retinopathy, inhibition of dental caries, and platelet aggregation.[5,6] 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.[7] This article briefly reviews the high-performance liquid chromatography (HPLC) methods, columns, detectors, mobile and stationary phases used for saponins and sapogenin.

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	Alltimal C_{18} (150 x 4.6 mm, 5 µm)	MeOH-H_2O (99 : 1)	UV λ = 230 nm	Gradient, 1.1 ml/min	[15]
Asparagus racemosus	Dynamax (250 x 10 mm) OmniSpher 5 C_{18}	MeOH-H_2O	ELSD	Gradient	[16]
A. racemosus	OmniSpher C_{18} (150 x 4.6 mm)	CH_3CN- H_2O	ELSD-LT	2 ml/min	[17]
A. racemosus	OmniSpher C_{18} (150 x 4.6 mm)	CH_3CN- H_2O	ELSD-LT	Gradient, 2 ml/min	[18]
A. officinalis	Luna C_{18} (150 x 4.6 mm, 5 µm)	CH_3CN-H_2O and CH_3 CN- CH_3COOH	MS	Gradient 1 ml/min	[11]
Platycodi radix	Zorbex SB-Aq C_{18} (150 x 4.6 mm, 5 µm)	CH_3CN-H_2O	ELSD/MS	Gradient 1 ml/min	[19]
Ilex paraguariensis	Novapack C_{18}, 4 µm, Aq C_{18} (150 x 3.9 mm)	CH_3CN-H_2O (70 : 30)	UV, λ = 230 nm	1 ml/min	[12]
Medicago truncatula	C_{18} (250 × 4.6 mm, 5 µm)	H_2O-CH_3CN 95 : 5-5 : 95 with 1% acetic acid	MS	Gradient, 0.8 ml/min	[20]
Medicago truncatula	LiChroprep C_{18}	MeOH-H_2O		Gradient	[21]
Triterpenoid saponins	Silica gel	EtOAc-Hexane			[14]
Soya saponins			ELSD		[22]
Bacopa monnieri		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]
Ilex paraguariensis	C_{18}		UV		[25]
Phytolacca bogotensis			ESI/MS		[26]
Paris polyphylla	C_{18}	0.1% Aqueous formic acid- CH_3CN	ESI-MS	Gradient	[27]
Steroidal saponins			DAD, MS		[28]
Soyasaponins			ESI-MS		[29]
Medicago truncatula			PDA/ESI/MS/MS		[30]
Ziziphus jujuba	C_{18}	(A) 0.1% Aqueous acetic acid and (B) methanol with 0.1% acetic acid	ELSD	1.8 ml/min Gradient	[31]
Tupistra chinensis	YMC-Pack ODS-AQ (4.6 x 250 mm, 5 µm)	CH_3CN-H_2O		Gradient	[32]
Panax notoginseng	C_{18}	Acetonitrile and 0.01% aqueous formic acid	UV	Gradient	[33]
Saponins			Charged aerosol		[34]
Folium Ginseng and Radix Ginseng	C_{18}		DAD–MS		[35]
Panax notoginseng	C_{18}				[36]
Tahini Halva	C_{18}	MeOH-H_2O-acetic acid 60/34/6 (v/v/v)	PDA	1.5 ml/min	[37]
Pulsatilla koreana			ELSD		[38]
Aesculus chinensis		MeOH-H_2O-acetic acid			[39]
Saponins			ELSD		[40]
Steroidal saponins	C_{18}				[41]
Triterpene saponins			ELSD		[42]
Triterpene saponins		Chloroform-methanol-water (4 : 4 : 2, v/v)		1.5 ml/min	[43]
Steroidal glycosides	C_{18}		MS/MS		[44]
Saponins					[45]

UV: Ultra voilet, 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.[8] Saponins of *Platycodi radix* have been separated and quantitated by HPLC with light-scattering detector (ELSD) using C_{18} column and gradient aqueous acetonitrile mobile phase.[9] Most studies are concerned with triterpene saponins and have generally adopted the chromatographic conditions preferred for HPLC-ultraviolet (UV), i.e., a C_{18} column and mobile phase gradient of aqueous acetonitrile.[10] 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_{18} reverse-phase column and aqueous acetonitrile or aqueous methanol as mobile phase in gradient manner with or without the addition of 1% acetic acid.[11] 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.[12] 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_2HPO_4 mobile phase at room temperature.[13] Only conjugated soyasaponins which have UV absorption maximum at 295 nm, glycyrrhetinic acid glycosides, and cucurbitacins could be successfully detected with UV-Vis detector. Saponins have also been successfully separated on Spherisorb ODS, C_{18}, and C_8 column using MeCN-H_2O. Similar validated method using ELSD was developed for saponin determination in *Flos lonicerae,* a herb used in traditional Chinese medicine.[14]

The benzoate derivatives of the major sapogenins (hecogenin, dehydrohecogenin, and tigogenin) occurring in the *Agave* species have been analyzed by reversed phase HPLC on LiChrosorb RP-8 column using MeOH-H_2O (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_{18} and ODS column using MeOH-H_2O and CH_3CN-H_2O 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.

ACKNOWLEDGEMENT

The authors are thankful to Dr. Asha Budakoti, NCL, Pune, for providing some references.

REFERENCES

1. Ravikumar PR, Soman R, Chetty GL, Pandey RC, Sukhdev. Chemistry of Ayurvedic crude drugs: Part VIa-(Shatavari-I): Structure of Shatavarin-IV. Ind J Chem 1987;26:1012-7.
2. San Martin R, Briones R. Industrial uses and sustainable supply of *Quillaja saponaria* (Roseaceae). *Econ Bot 1999;53:302-11.*
3. Sidhu GS, Oekenfull DG. A mechanism for the hypocholesterolaemic activity of saponins. Br J Nutr 1986;55:643-9.
4. Mitra S, Dungan SR. Micellar Properties of Quillaja Saponin. Effects of Temperature, Salt, and pH on Solution Properties. J Agric Food Chem 1997;*45*:1587-95.
5. Rao AV, Gurfinkel DM. The bioactivity of saponins: Triterpenoid and steroidal glycosides. Drug Metabol Drug Interact 2000;17:211-35.
6. Guclu-Ustundag O, Mazza G. Saponins: Properties, applications and processing. Crit Rev Food Sci Nutr *2007;47:231-58.*
7. Mader TL, Brumm MC. Effect of feeding sarsaponin in cattle and swine diets. J Anim Sci 1987;65:9-15.
8. Kimata H, Sumida N, Matsufuji N, Orita T, Ito K, Yata N, *et al*. Interaction of saponin of *Bupleuri radix* with ginseng saponin: Solubilization of saikosaponin-a with chikusetsu saponin V (=ginsenoside-Ro). Chem Pharm Bull 1985;33:2849-53.
9. Ha YW, Na YC, Seo JJ, Kim SN, Linhardt RJ, Kim YS. Qualitative and Quantitative determination of ten major saponins in *Platycodi radix* by High performance liquid chromatography with evaporative light scattering detection and mass spectrometry. J Chromatogr A 2006;1335:27-35.
10. Oleszek W, Bialy Z. Chromatographic determination of plant saponins--an update (2002-2005). J Chromatogr A 2006;1112:78-91.
11. Kite GC, Porter EA, Simmonds MS. Chromatographic behaviour of steroidal saponins studied by high-performance liquid chromatography–mass spectrometry. J Chromatogr A 2007;1148:177-83.
12. Gnoatto SC, Schenkel EP, Bassani VL. HPLC Method to assay total saponins in *Ilex paraguariensis* aqueous extract. J Braz Chem Soc 2005;16:723-6.
13. Kanazawa H, Nagata Y, Matsushima Y, Tomota M, Takai N. Determination of acidic saponins in crude drugs by high-performance liquid chromatography on octadecylsilyl porous glass. J Chromatogr 1993;630:408-14.
14. Chai XY, Li SL, Li P. Quality evaluation of *Flos Lonicerae* through a simultaneous determination of seven saponins by HPLC with ELSD. J Chromatogr A 2005;1070:43-8.
15. Peng Y, Luo F, Wang S, LI L, Sun Y, Pan W. Determination of sarsasapogenin in *Rhizoma Anemarrhenae* with precolumn derivatization by HPLC. J Shenyng Pharm University 2008;25:372-5.
16. Patricia YH, Aisyah HJ, Reg L, Kerry P, William K, James JD. Steroidal saponins from the roots of *Asparagus racemosus*. Phytochemistry 2008;69:796-804.

17. Patricia YH, Aisyah HJ, Reg L, Kerry P, William K, James JD. Structural revision of shatavarins I and IV, the major components from the roots of *Asparagus racemosus*. Tetrahedron Lett 2006;47:6965-9.
18. Patricia YH, Aisyah HJ, Reg L, Kerry P, William K, James JD. Asparinins, asparosides, curillins, curillosides and shavatarins: Structural clarification with the isolation of shatavarin V, a new steroidal saponin from the root of *Asparagus racemosus.* Tetrahedron Lett 2006;47:8683-7.
19. Ha YW, Na YC, Seo JJ, Kim SN, Linhardt RJ, Kim YS. Qualitative and quantitative determination of ten major saponins in Platycodi Radix by high performance liquid chromatography with evaporative light scattering detection and mass spectrometry. J Chromatogr A 2006;1135:27-35.
20. Huhman DV, Berhow MA, Sumner LW. Quantification of Saponins in aerial and subterranean tissues of Medicago truncatula. J Agric Food Chem 2005;53:1914-20.
21. Kapusta I, Stochmal A, Perrone A, Piacente S, Pizza C, Oleszek W. Triterpene saponins from Barrel Medic (*Medicago truncatula*) aerial parts. J Agric Food Chem 2005;53:2164-70.
22. Lin J, Wang C. An analytical method for soy saponins by HPLC/ELSD. J Food Sci 2006;69**:**C456-62.
23. Phrompittayarat W, Wittaya-Areekul S, Jetiyanon K, Putalun W, Tanaka H, Wittaya-Areekul S, *et al*. Determination of saponin glycosides in *Bacopa monnieri* by reversed phase high performance liquid chromatography. Thai Pharm Health Sci J 2007;2:26-32.
24. Chai XY, Li SL, Li P. Quality evaluation of *Flos Lonicerae* through a simultaneous determination of seven saponins by HPLC with ELSD. J Chromatogr A 2005;1070:43-8.
25. Cabral P, Paula G, Petrovick PR, Gosmann G, Ortega G, George. Development and validation of an HPLC method for the characterization and assay of the saponins from *Ilex paraguariensis* Fruits**.** J Liq Chromatogr Relat Technol 2007;30:87-95.
26. Montoya G, Arango GJ, Unger M, Holzgrabe U. O-glycoside sequence of pentacyclic triterpene saponins from *Phytolacca bogotensis* using HPLC-ESI/multi-stage tandem mass spectrometry. Phytochem Anal 2009;20:465-74.
27. Zhang T, Liu H, Liu XT, Xu DR, Chen XQ, Wang Q. Qualitative and quantitative analysis of steroidal saponins in crude extracts from *Paris polyphylla* var. *yunnanensis* and *P. polyphylla* var. *chinensis* by high performance liquid chromatography coupled with mass spectrometry. J Pharm Biomed Anal 2010;51:114-24.
28. Ma C, Fan M, Tang Y, Li Z, Sun Z, Ye G*, et al*. Identification of major alkaloids and steroidal saponins in rat serum by HPLC-diode array detection-MS/MS following oral administration of Huangbai-Zhimu herb-pair Extract**.** Biomed Chromatogr 2008;22:835-50**.**
29. Jin M, Yang Y, Su B, Ren Q. Rapid quantification and characterization of soyasaponins by high-performance liquid chromatography coupled with electrospray mass spectrometry. J Chromatogr A 2006;1108:31-7.
30. Kapusta I, Janda B, Stochmal A, Oleszek W. Determination of saponins in aerial parts of Barrel Medic (*Medicago truncatula*) by Liquid Chromatography-Electrospray Ionization/Mass Spectrometry. J Agric Food Chem 2005;53:7654-60.
31. Zhao J, Li SP, Yang FQ, Li P, Wang YT. Simultaneous determination of saponins and fatty acids in *Ziziphus jujuba* (Suanzaoren) by high performance liquid chromatography-evaporative light scattering detection and pressurized liquid extraction. J Chromatogr A 2006;1108:188-94.
32. Zhou Y, Zou K, Yu LL, Qin SJ, Xu LL, Liu C. Determination of two saponins in *Tupistra chinensis* rhizomes by RP-HPLC. Zhongguo Zhong Yao Za Zhi 2008;33:2647-9.
33. Gao X, Dan M, Zhao A, Xie G, Jia W. Simultaneous determination of saponins in flower buds of Panax notoginseng using high performance liquid chromatography. Biomed Chromatogr 2008;22:244-9.
34. Bai C, Han S, Chai X, Jiang Y, Li P, Tu P. Sensitive Determination of saponins in *Radix et Rhizoma Notoginseng* by charged aerosol detector coupled with HPLC. J Liq Chromatogr Relat Technol 2009;32:242-60.
35. Qian ZM, Lu J, Gao QP, Li SP. Rapid method for simultaneous determination of flavonoid, saponins and polyacetylenes in *Folium Ginseng* and *Radix Ginseng* by pressurized liquid extraction and high-performance liquid chromatography coupled with diode array detection and mass spectrometry. J Chromatogr A 2009;1216:3825-30.
36. Chen W, Dang Y, Zhu C. Simultaneous determination of three major bioactive saponins of *Panax notoginseng* using liquid chromatography-tandem mass spectrometry and a pharmacokinetic study. Chin Med 2010;5**:**12.
37. Ceyhun Sezgin AE, Artik N. Determination of saponin content in turkish *Tahini halvah* by using HPLC. Advance J Food Sci Technol 2010;2:109-15.
38. Lee KY, Cho YW, Park J, Lee DY, Kim SH, Kim YC*, et al.* Quality control of *Pulsatilla koreana* based on the simultaneous determination of triterpenoidal saponins by HPLC-ELSD and principal component analysis. Phytochemical analysis 2010;314-21.
39. Wei F, Ma L, Cheng X, Lin R, Jin W, Khan IA, *et al*. Preparative HPLC for Purification of Four Isomeric Bioactive Saponins from the Seeds of *Aesculus chinensis*. J Liq Chromatogr Relat Technol 2005;28:763-73.
40. Niiho Y, Nakajima Y, Yamazaki T, Okamoto M, Tsuchihashi R, Kodera M, *et al.* Simultaneous analysis of isoflavones and saponins in Pueraria flowers using HPLC coupled to an evaporative light scattering detector and isolation of a new isoflavone diglucoside. J Nat Med 2010;64:313-20.
41. Zhang T, Liu H, Liu XT, Xu DR, Chen XQ, Wang Q. Qualitative and quantitative analysis of steroidal saponins in crude extracts from *Paris polyphylla* var. yunnanensis and *P. polyphylla* var. chinensis by high performance liquid chromatography coupled with mass spectrometry. J Pharm Biomed Anal 2010;51:114-24.
42. Eom HY, Park SY, Kim MK, Suh JH, Yeom H, Min JW, *et al.* Comparison between evaporative light scattering detection and charged aerosol detection for the analysis of saikosaponins. J Chromatogr A 2010;1217:4347-54.
43. Chen Q, Lai D, Sun W, Zhang T, Ito Y. Separation and purification of triterpene saponins from roots of *Radix phytolaccae* by high-speed countercurrent chromatography coupled with evaporative light scattering detection. J Liq Chromatogr Relat Technol 2010;33:563-71.
44. Montoro P, Skhirtladze A, Perrone A, Benidze M, Kemertelidze E, Piacente S. Determination of steroidal glycosides in *Yucca gloriosa* flowers by LC/MS/MS. J Pharm Biomed Anal 2010;52:791-5.
45. Niiho Y, Nakajima Y, Yamazaki T, Okamoto M, Tsuchihashi R, Kodera M, *et al.* Simultaneous analysis of isoflavones and saponins in Pueraria flowers using HPLC coupled to an evaporative light scattering detector and isolation of a new isoflavone diglucoside. J Nat Med 2010;64:313-20.

How to cite this Article: Negi JS, Singh P, Pant GN, Rawat MM. High-performance liquid chromatography analysis of plant saponins: An update 2005-2010. Phcog Rev 2011;5:155-8.

Source of Support: Nil, **Conflict of Interest:** None declared