ELSEVIER

Journal of Chromatography A, 777 (1997) 233–238

JOURNAL OF CHROMATOGRAPHY A

Short communication

Identification of soyasaponins by liquid chromatography–thermospray mass spectrometry

N. Fuzzati*, R. Pace, G. Papeo, F. Peterlongo

Indena SpA, Laboratori Ricerca e Sviluppo, Via Don Minzoni 6, 20090 Settala (Milan), Italy

Abstract

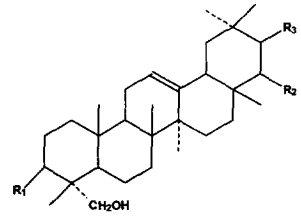
Saponins are important bioactive molecules widespread in the plant kingdom. Soyasaponins, isolated from *Glicine max* (Leguminosae), have been shown to exhibit various biological activities, e.g., an inhibitory effect on lipid-oxidation and liver-lesion generation and an improving effect on hyper-cholesteremia. Mass spectral investigation of these metabolites requires soft ionization techniques such as desorption chemical ionization, fast atom bombardment and thermospray mass spectrometry. A high-performance liquid chromatographic method was developed for thermospray mass spectrometric analysis of saponins contained in soybean flour extracts. The analyses were performed using a ternary eluent (water–acetonitrile–methanol) in gradient conditions with post-column addition of aqueous ammonium acetate. Six saponin components could be separated and identified. The mass spectra obtained provided information concerning both molecular masses and aglycone composition. © 1997 Elsevier Science B.V.

Keywords: Soybean; Saponins

1. Introduction

Saponins are natural amphiphilic compounds present in many food of plant origin. Legumes, in particular, are rich sources of dietary saponins. Populations consuming diets high in legumes are therefore exposed to a high level of saponins. In recent years there has been an increasing interest in compounds present in vegetables which are potentially useful in the prevention of chronic diseases such as cardiovascular disease and cancer. Saponins have been found to possess important biological properties, including hypocholesterolemic, immune-stimulatory and anti-tumorigenic activity [1].

Soybean seeds [*Glicine max* (L.) Merril, Leguminosae] contain about 5–6% of saponins [2]. The triterpene glycosides isolated from soybeans have been divided in the so-called 'group A', 'group B' and 'group E' saponins on the basis of their aglycone structure [3,4] (Fig. 1). The structures of



Soyasapogenols	R_1	R_2	R_3
A	OH	OH	OH
B	OH	OH	H
E	OH	=O	H

Fig. 1. Aglycones isolated from soybean seed.

*Corresponding author.

PII S0021-9673(97)00157-X

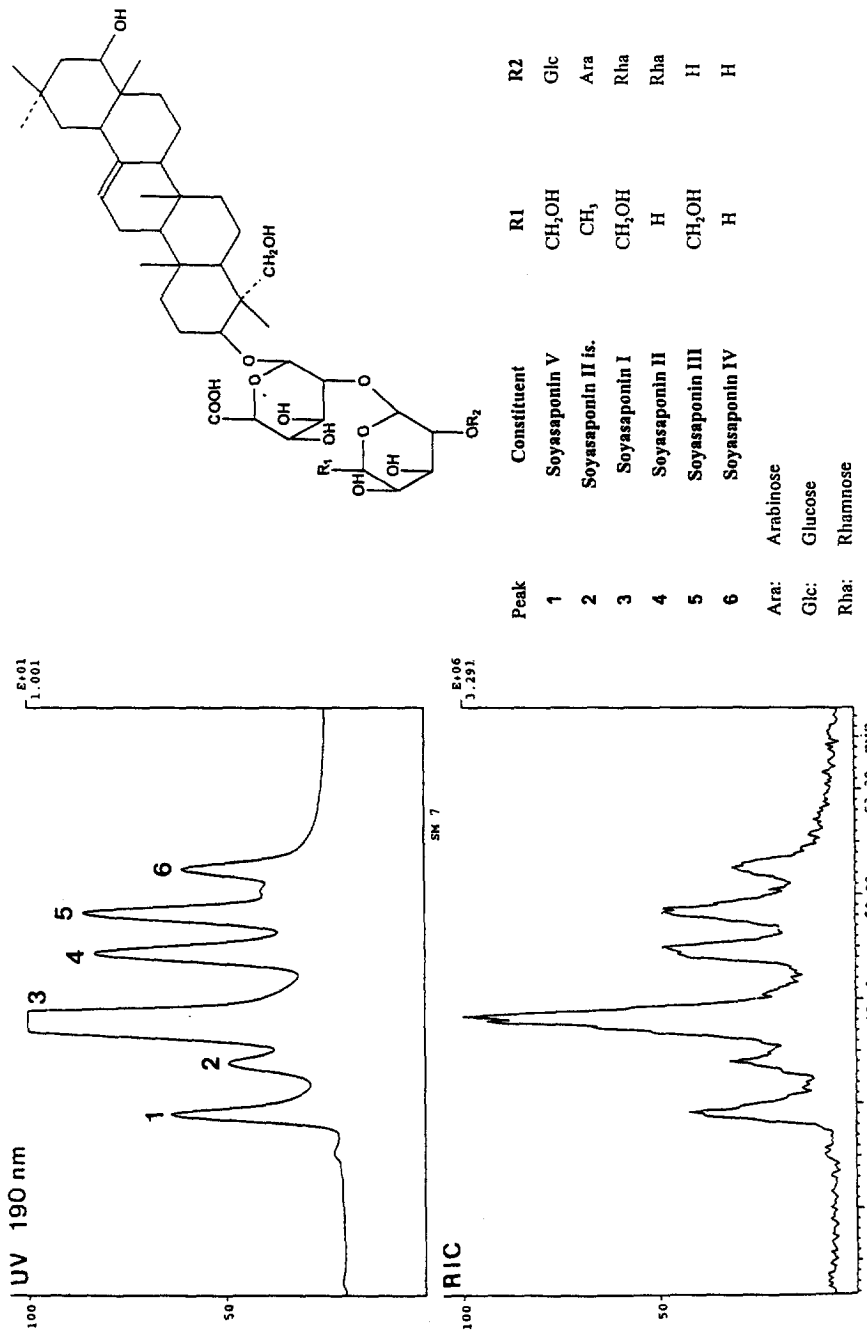


Fig. 2. Total ion current (RIC) and UV trace of the HPLC–MS analysis of the soyasaponins crystallized fraction together with the structures of the peaks 1–6.

Table 1
Solvent composition of the step gradient (linear curve) of the HPLC analysis

Time (min)	Water (%)	Acetonitrile (%)	Methanol (%)
0	80	10	10
15	72	18	10
20	72	18	10
25	50	40	10
55	40	50	10
60	10	80	10

soyasaponins have been elucidated using chemical derivatization and spectroscopic techniques including nuclear magnetic resonance (NMR) and mass spectrometry (MS) [3–5]. Soft ionization techniques, such as fast atom bombardment (FAB) [3,4] and field desorption (FD) [6], have been employed in MS to obtain molecular mass and sugar sequence information for soyasaponins.

Analyses of soyasaponins have been performed using thin-layer chromatography (TLC) [7,8] and high-performance liquid chromatography (HPLC) techniques [8,9]. HPLC, because of its speed and sensitivity is ideal for the analysis of saponins. The single largest difficulty is the lack of suitable chromophore for UV detection in most soyasaponins. Detection is often undertaken at short wavelengths limiting the choice of solvent and gradient. Different ways of overcoming this problem have been employed such as derivatization [8] or evaporative light-scattering detection [9]. Recently, some authors have described the determination of saponins in crude plant extracts using thermospray liquid chromatography–mass spectrometry (LC–TSP-MS) [10]. This technique has been shown to be suitable for the separation, identification and structural determination of saponins directly from the biological matrix.

In the frame of our research concerning the characterization and standardization of soybean extracts we have employed LC–TSP-MS analysis for the detection of soyasaponins in a crystallized fraction of the methanolic soybean extract.

2. Experimental

2.1. Extraction and fractionation

Soybean flour (50 kg) was extracted with cold MeOH (500 l). The extract was concentrated up to 50 l under vacuum and defatted with *n*-hexane. The alcoholic layer was concentrated, diluted with water and extracted with *n*-BuOH. Evaporation to dryness of the organic layer afforded about 500 g of residue. The residue was dissolved in a hot mixture of 2.25 l of aqueous 80% MeOH and 4.5 l of EtOAc. The solution was left overnight at room temperature and the precipitate was collected by filtration and treated with 460 ml of hot 95% ethanol. The suspension was

Table 2
Major ions (*m/z*) observed in the TSP mass spectra of peak 1–6 together with their relative intensities (%)

Ion	Peak					
	1	2	3	4	5	6
$[Aglycone+H]^+$	459 (10)	459 (27)	459 (21)	459 (24)	459 (15)	459 (43)
$[(Aglycone+GlcA+H)2H_2O]^+$	599 (63)	599 (83)	599 (82)	599 (100)	599 (69)	599 (100)
$[(Aglycone+GlcA+H)H_2O]^+$	617 (17)	617 (35)	617 (26)	617 (43)	617 (12)	617 (13)
$[Aglycone+GlcA+H]^+$	635 (19)	635 (52)	635 (21)	635 (30)	635 (10)	635 (24)
$[(M+H)Glc]^+$	797 (18)	–	–	–	–	–
$[(M+H)Rha]^+$	–	–	797 (100)	767 (26)	–	–
$[(M+H)Ara]^+$	–	781 (43)	–	–	–	–
$[M+H]^+$	959 (83)	913 (8)	943 (84)	913 (17)	797 (100)	767 (41)
$[M+Na]^+$	981 (45)	935 (81)	965 (22)	935 (19)	819 (16)	789 (20)
$[M+CH_3CN+NH_4]^+$	1017 (100)	971 (100)	1001 (40)	971 (80)	855 (22)	825 (53)

Ara: Arabinose.
Rha: Rhamnose.
Glc: Glucose.
GlcA: Glucuronic acid.

diluted with 200 ml of water and refluxed until complete solubilization. The clear solution was then left at room temperature overnight. The precipitate was filtered, suspended in 5 l of mixture MeCN–water (3:2, v/v) and refluxed until complete solubilization. The clear solution was then left at room temperature overnight and the precipitate was collected and dried 12 h in vacuo at 60°C. Yields: 43 g.

2.2. LC–TSP-MS analysis

A Finnigan MAT (San Jose, CA, USA) TSQ-700 triple quadrupole mass spectrometer equipped with a 5100 DEC station and ICIS data system and a TSP-2 interface was used for data acquisition and processing. Mass spectrometer conditions were optimized in order to achieve maximum sensitivity. TSP values were: source block temperature 270°C, vaporizer temperature 100°C and repeller voltage 30 V. The electron multiplier voltage was 1500 V, dynode 20 kV and the filament and discharge were off-mode. Full scan spectra m/z 400–1300 u in the positive ion mode were obtained (scan time 1 s). The HPLC system included a Waters 600-MS pump (Bedford,

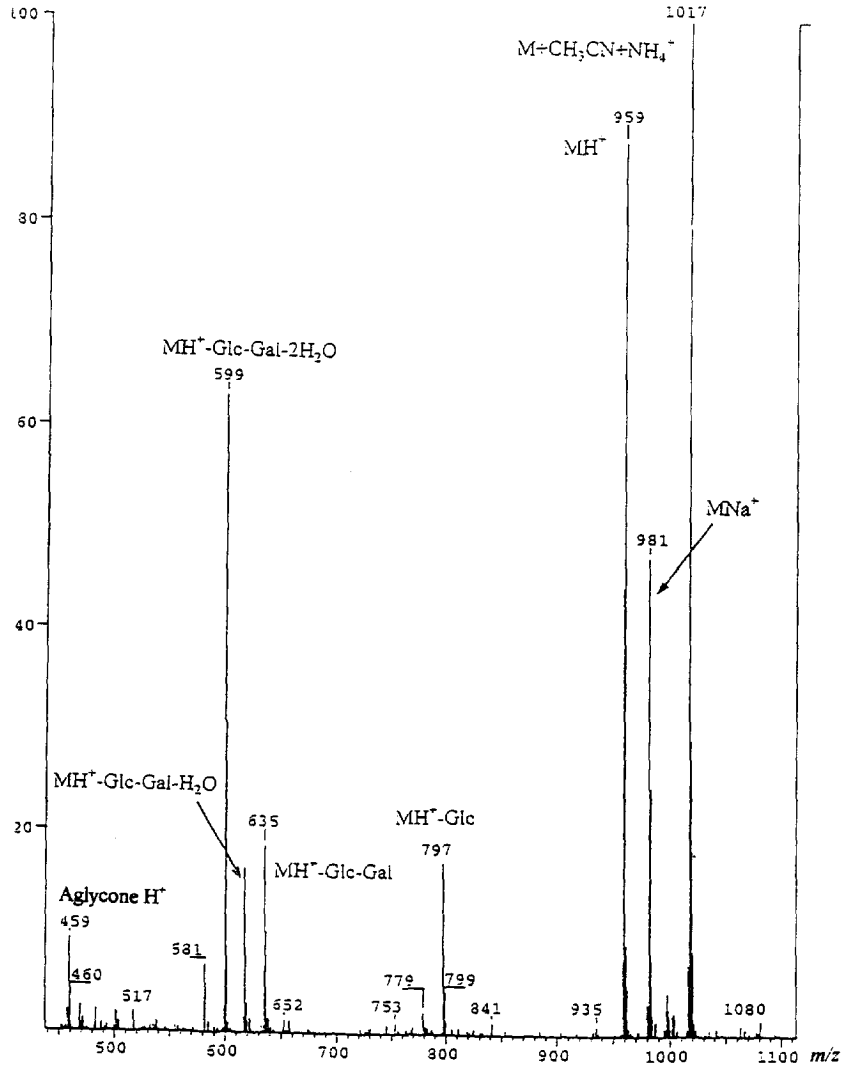


Fig. 3. TSP-MS spectrum of peak 1.

MA, USA) equipped with a gradient controller. The UV trace was recorded at 190 nm with a tunable UV–Vis Waters 486 detector. Post-column addition of buffer (0.5 *M* NH_4OAc) was achieved with a Waters 590-MS pump (0.2 ml min^{-1}) using a simple T-junction. The solution of the sample (30 mg ml^{-1} in MeOH) was injected (20 μl) with a Waters 717 automatic sample injection module.

Separation was performed on a Supelco-Sil LC-ABZ (5 μm) column (250×4.6 mm I.D.) from Supelco (Bellefonte, PA, USA). A step gradient of water–MeCN–MeOH with 0.01% trifluoroacetic acid was used (1 ml min^{-1}). The steps are presented in Table 1.

3. Results and discussion

A good chromatographic separation of soyasaponins was achieved on reversed-phase using a step gradient of water–MeCN–MeOH (Table 1). The expanded region of the HPLC chromatogram corresponding to the soyasaponins elution is shown in Fig. 2. The UV trace recorded at 190 nm exhibited

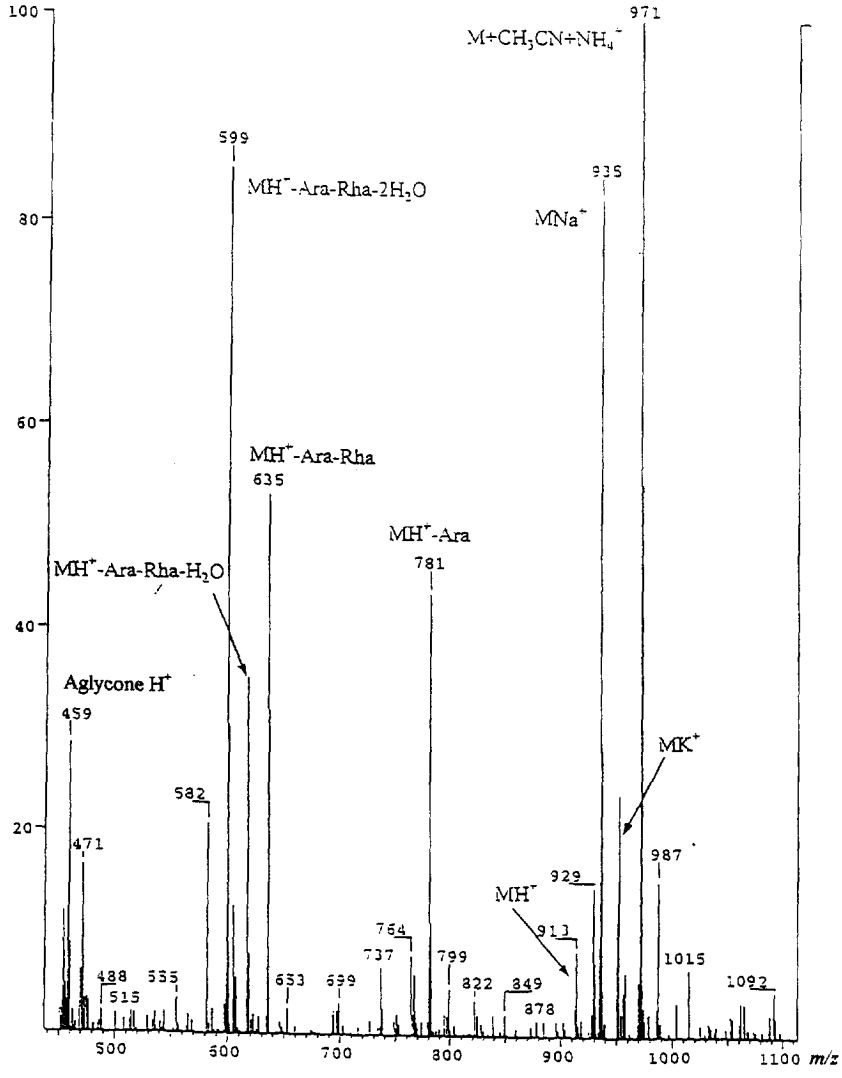


Fig. 4. TSP-MS spectrum of peak 2.

the presence of six main peaks (1–6). MS detection was operated in the scan mode (m/z 400–1300). A good TSP–MS response using post-column addition of a buffer (0.5 M NH_4OAc) was obtained. In these conditions, the LC–MS total ion current corresponded well with the UV trace at 190 nm (Fig. 2).

All TSP mass spectra of peaks 1–6 (Table 2 and in Figs. 3 and 4) display the $[M+H]^+$ protonated molecule, together with strong adduct species such as $[M+CH_3CN+NH_4]^+$ and $[M+Na]^+$, easily allowing the determination of the molecular weight. Peaks at m/z 635 [aglycone+glucuronic acid+H]$^+$, m/z 617 [aglycone+glucuronic acid$-H_2O$+H]$^+$ and m/z 599 [aglycone+glucuronic acid$-2H_2O$+H]$^+$ were present in all spectra. In addition, the same [aglycone+H]$^+$ ion at m/z 459, corresponding to the soyasapogenol B protonated molecule, was visible indicating that peaks 1–6 belong to the soyasaponins 'group B'. Peaks 1–6 exhibited the same fragmentation pattern: peaks corresponding to the successive loss of mono- and diglycoside units were observed showing the presence of a linear glycosidic chain (Table 2). Comparison of the TSP-MS spectra and literature data concerning soyasaponins allows the identification of peaks 1, 3, 4, 5 and 6 as soyasaponins V, I, II, III and IV, respectively.

TSP mass spectrum of peak 2 (Fig. 4) exhibited a $[M+H]^+$ protonated molecule at m/z 913 together with adduct species $[M+CH_3CN+NH_4]^+$ and [M+Na]$^+$ at m/z 971 and m/z 935 respectively, showing that peak 2 is an isomer of soyasaponin II (peak 4). Furthermore, the ion at m/z 781 $[M+H-132]^+$, indicating the cleavage of an arabinosyl moiety, suggests the presence of the arabinose in terminal position. Further studies are necessary in order to confirm the structure of peak 2.

4. Conclusion

Solvent precipitation and crystallization of the butanolic fraction of a defatted soybean flour methanol extract provide a selected mixture of 'group B' soyasaponins.

Soyasaponins, which are polar and non-volatile compounds can be easily analysed with LC–TSP-MS. Addition of ammonium acetate as buffer provides an ionization similar to that obtained with desorption/chemical ionization (D/CI) using NH_3 in the positive-ion mode. However, salt and solvent adducts which do not appear in the D/CI mass spectra occur in the TSP mass spectra.

Important structural information such as molecular mass, sugar units sequence and aglycone moiety are produced allowing the identification of known soyasaponins without complex isolation procedures or derivatizations.

Moreover, from the analysis, the presence of an unknown constituent, an isomer of soyasaponin II was detected. Further isolation is in progress in order to elucidate the structure of this compound.

References

[1] K. Hostettmann and A. Marston, Saponins—Chemistry and Pharmacology of Natural Product, Cambridge University Press, Cambridge, 1995.
[2] D. Oakenfull, Food Chem. 6 (1981) 19.
[3] M. Shiraiwa, S. Kudo, M. Shimoyamada, K. Harada, K. Okubo, Agric. Biol. Chem. 55 (1991) 315.
[4] S. Kudou, M. Tonomura, C. Tsukamoto, M. Shimoyamada, T. Uchida, K. Okubo, Biosci. Biotech. Biochem. 56 (1992) 142.
[5] I. Kitagawa, H.K. Wang, T. Taniyama, M. Yoshikawa, Chem. Pharm. Bull. 36 (1988) 153.
[6] S. Tsurumi, T. Takagi, T. Hashimoto, Phytochemistry 31 (1992) 2435.
[7] K.R. Price, C.L. Curl, G.R. Fenwick, J. Sci. Food Agric. 42 (1988) 183.
[8] T. Tani, T. Katsuki, M. Kubo, S. Arichi, I. Kitagawa, Chem Pharm. Bull. 33 (1985) 3829.
[9] P.A. Ireland, S.Z. Dziedzic, J. Chromatogr. 361 (1986) 410.
[10] M.P. Maillard, K. Hostettmann, J. Chromatogr. 647 (1993) 137.