ELSEVIER

Journal of Chromatography A, 854 (1999) 69–79

JOURNAL OF CHROMATOGRAPHY A

Liquid chromatography–electrospray mass spectrometric identification of ginsenosides in *Panax ginseng* roots

N. Fuzzati*, B. Gabetta, K. Jayakar, R. Pace, F. Peterlongo

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

Abstract

A high-performance liquid chromatographic method was developed for electrospray mass spectrometric analysis of ginsenosides in *Panax ginseng* roots. The analyses were performed on a reversed-phase C_{18} column using a binary eluent (aqueous 8 m*M* NH_4OAc, buffered to pH 7 with NH_4OH–acetonitrile) under gradient conditions. Twenty-five ginsenosides could be separated and detected. The mass spectra obtained provided information on their molecular masses. A MS–MS experiment was undertaken in order to determine the sugar unit sequences and the aglycone moieties.

Keywords: *Panax ginseng*; Plant materials; Ginsenosides

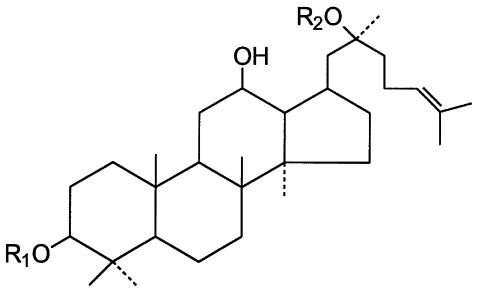
1. Introduction

Ginseng is a very old Chinese medicine which belongs to the group of products which stimulate the non-specific resistance of an organism and build up general vitality (*adaptogens*) [1]. The drug has been obtained for over 2000 years from the roots of *Panax ginseng* C.A. Meyer (Araliaceae). The pharmacological properties of Ginseng are generally attributed to its triterpene glycosides, called ginsenosides [1]. Ginsenosides are mainly dammarane triterpenes with (20*S*)-protopanaxadiol and (20*S*)-protopanaxatriol aglycon moieties [2] (Fig. 1). The only oleanolic acid-type saponin identified in the roots of *P. ginseng* is ginsenoside Ro. Four malonyl-ginsenosides (acidic saponins) were also isolated and characterized [3]. These compounds are unstable and are readily demalonylated by heating. Yamaguchi et al. [4] showed that heating a solution of the saponin fraction of ginseng in 70% methanol at 80°C for 7 h resulted in a decrease of malonyl-ginsenosides and a consequent increase of the corresponding neutral saponins.

High-performance liquid chromatography (HPLC) has been in the last 20 years the method of choice for the analysis of ginsenosides [1]. However, methods which employed heat for the sample treatment failed to detect the malonyl-ginsenoside because of the thermal conversion of these compounds to the corresponding neutral ginsenosides [5,6]. More recently, several HPLC methods have been developed for the simultaneous determination of both the acidic and neutral saponins using reversed-phase columns with K_2HPO_4 in the mobile phase [7–10]. All these methods employ UV for the detection of ginsenosides. Since these compounds have a weak UV absorption, their detection is performed at short wavelengths. Consequently, the sensitivity and the choice of solvent and gradient are limited. Different ways of overcoming this problem, such as photo-

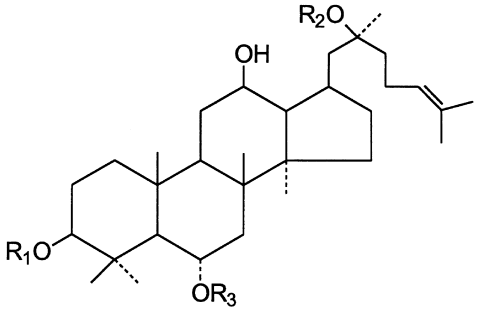
*Corresponding author.

0021-9673/99/$ – see front matter
PII: S0021-9673(99)00463-X



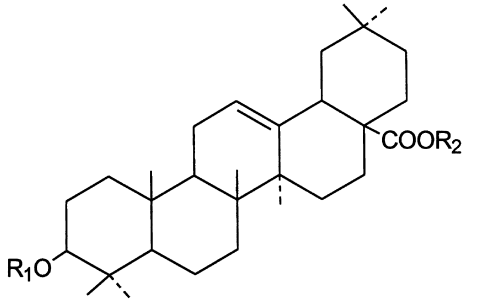
(20S)-Protopanaxadiol
(R_1=R_2=H)

Ginsenoside	R_1	R_2
Rh_2	Glc	H
Rg_3	Glc^2-Glc	H
Rd	Glc^2-Glc	Glc
Malonyl-Rd	Glc^2-Glc^6-Ma	Glc
Rb_2	Glc^2-Glc	Glc^6-Ara*p*
Malonyl-Rb_2	Glc^2-Glc^6-Ma	Glc^6-Ara*p*
Rc	Glc^2-Glc	Glc^6-Ara*f*
Malonyl-Rc	Glc^2-Glc^6-Ma	Glc^6-Ara*f*
Rb_1	Glc^2-Glc	Glc^6-Glc
Malonyl-Rb_1	Glc^2-Glc^6-Ma	Glc^6-Glc
Rb_3	Glc^2-Glc	Glc^6-Xyl
Ra_1	Glc^2-Glc	Glc^6-Ara*p*^{4}-Xyl
Ra_2	Glc^2-Glc	Glc^6-Ara*f*^{2}-Xyl
Ra_3	Glc^2-Glc	Glc^6-Glc^3-Xyl



(20S)-Protopanaxatriol
(R_1=R_2=R_3=H)

Ginsenoside	R_1	R_2	R_3
Rh_1	H	H	Glc
Rg_1	H	Glc	Glc
Rf	H	H	Glc^2-Glc
20-Glc-Rf	H	Glc	Glc^2-Glc
Rg_2	H	H	Glc^2-Rha
Re	H	Glc	Glc^2-Rha



Ginsenoside Ro

R_1	R_2
Glc^2-Glc	Glc

Glc= β-D-glucose Rha= α-L-rhamnose
Ara*p*= α-L-arabinose (pyranose)
Ara*f*= α-L-arabinose (furanose)
Xyl= β-D-xylose Ma= malonyl

Fig. 1. Structures of ginsenosides isolated from *Panax ginseng*.

reduction fluorescence detection [11] or evaporative light-scattering detection (ELSD) have been employed [12]. All the previously developed methods employed isolated standards of ginsenosides for the identification of the extract constituents and for the evaluation of the specificity of the analytical method. Thus several minor peaks occurring in the chromatogram were not identified.

In the last few years HPLC–MS techniques have been successfully applied to the on-line analysis of non-volatile molecules such as saponins [1]. HPLC combined with a frit-fast atom bombardment (Frit-FAB) [13,14] and thermospray (TSP) [15] interfaces were used for the qualitative analysis of *P. ginseng*. However, these works did not deal with the detection and the identification of the thermally unstable malonyl-ginsenoside. Electrospray ionization (ESI) interface was recently, introduced as a highly sensitive and soft ionization technique for the HPLC–MS analysis of thermolabile molecules [16].

The aim of the present study was to develop a specific HPLC method to be employed for the assay of ginsenosides in *P. ginseng* root extracts using ELSD. HPLC–ESI-MS analyses were undertaken to identify both neutral and acidic ginsenosides and to verify the specificity of the method.

2. Experimental

2.1. Standards and samples

Ginsenosides Rg_1, Re, Ro, Rb_1 and Rb_2 were isolated and characterized at the Indena Chemical Labs. [1,2]. Rg_2, Rc, Rd and Rf were kindly furnished by Professor K. Hostettmann, Institute de Pharmacognosie et Phytochimie, Universitè de Lausanne, Lausanne, Switzerland. These compounds were used as reference compounds

About 2 g of the powdered *P. ginseng* plant material was extracted at room temperature with 50 ml of 40% aqueous ethanol for 12 h using an automatic shaker. The solution, filtered with a 0.45 μm filter unit, was used as is for the HPLC–ELSD and HPLC–ESI-MS analyses.

2.2. Solvents and reagents

Acetonitrile was HPLC grade from J.T. Baker (Deventer, The Netherlands); ammonium acetate for analysis from Carlo Erba (Milan, Italy), ammonia solution 32% extra pure from Merck (Darmstadt, Germany). Water was purified by a Milli-Q_{plus} system from Millipore (Milford, MA, USA).

2.3. Instrumentation

2.3.1. HPLC–ELSD analysis

The HPLC system included a Waters 600 pump (Bedford, MA, USA) equipped with a gradient controller and an automatic sample injector module Waters 717 plus. ELSD was achieved with a Sedex Model 55 from S.E.D.E.R.E. (Alfortville, France). ELSD conditions were optimized in order to achieve maximum sensitivity: temperature of the nebulizer 70°C, N_2 was used as nebulizing gas at a pressure of 2.5 bar. Separation was performed on a Hypersil BDS C_{18} (5 μm) column (250×4.6 mm I.D.) from Hypersil (Astmoor Runcorn, UK). A step gradient of (A) 8 m*M* ammonium acetate, pH 7 with ammonium hydroxide and (B) MeCN was used (0.7 ml/min). The gradient is presented in Table 1. Column temperature, controlled with a column heater–cooler HP Series 1100 from Hewlett-Packard (Waldbronn, Germany), was set to 25°C.

2.3.2. HPLC–ESI-MS analyses

The HPLC conditions for the HPLC–MS analysis were the same as those used for the HPLC–ELSD analysis.

(a) A Finnigan MAT (San Jose, CA, USA) LCQ ion-trap mass spectrometer equipped with a Mi-

Table 1
Solvent composition of the gradient of the HPLC analysis

Time (min)	A[a] (%)	B[b] (%)	Curve
0.0	80	20	Linear
15.0	72	28	Linear
35.0	68	32	Linear
55.0	60	40	–
60.0	60	40	–

[a] A=Aqueous 8 m*M* NH_4OAc, buffered to pH 7 with NH_4OH.
[b] B=MeCN.

crosoft Windows NT data system and an ESI interface was used. The HPLC system included a Thermo Separation Products P4000 pump (San Jose, CA, USA) and a tunable Thermo Separation Products UV1000 detector. Mass spectrometer conditions were optimized in order to achieve maximum sensitivity. ESI conditions: source voltage 4.78 kV, sheath gas flow-rate 5.3 bar, auxiliary gas flow 1.2 bar, capillary voltage −31 V and capillary temperature 290°C. Full scan spectra from 200 to 1400 u in the negative ion mode were obtained (scan time 1 s). For the MS–MS analysis a collision energy of 30 eV was used.

(b) An API III+ triple quadrupole mass spectrometer (PE-Sciex, Thornill, Canada) equipped with an articulated ion spray interface was employed. Zero grade compressed air was used as nebulizer gas at 4 l/min. A curtain gas (99.999% UHP nitrogen) flow 1.2 l/min was employed. The interface heater was set at 60°C. The ion spray tip was held at a potential of 4.6 kV. Mass spectra were obtained at a dwell time of 1 ms (Q1 scan range 700÷1500 u 10 scan averaged and a step size of 0.2 u) in the negative ion mode. The HPLC system included two Gilson 505 pumps (Melville, France).

3. Results and discussion

A good chromatographic separation of ginseng constituents was achieved on reversed-phase using a linear gradient (Table 1) of 8 m*M* aqueous ammonium acetate, buffered to pH 7 with ammonium hydroxide and MeCN. The HPLC–ELSD chromatogram (Fig. 2) showed the presence of 25 peaks. The reference compounds Rg_1 and Rb_1 were analyzed in loop injection in order to optimize the MS conditions. These trials showed that the negative ion mode was more sensitive than the positive ion mode. MS detection was then operated in negative ion mode in the scan range from m/z 200 to 1400. The HPLC–ESI-MS chromatogram exhibited good agreement with the HPLC–ELSD chromatogram.

The ESI-MS spectra of peaks 1–25 using conditions (a) described in Section 2.3.2 displayed two clearly different behaviors. The MS analysis allows the attribution of peaks 1–3, 11, 13, 15–25 to the neutral ginsenosides and peaks 4–10, 12, 14–16 to the malonyl conjugates. Examples of MS spectra of a protopanaxadiol (Rb_1, peak 17), a protopanaxatriol (Rg_2, peak 18) and a malonyl-conjugate (malonyl-Rb_1, peak 5) are reported in Figs. 3–5, respectively. The MS spectra of neutral saponins exhibited the quasi-molecular ion $[M-H]^-$, adduct ions $[M+AcO]^-$ and $[M-CH_2O+AcO]^-$ and strong double-charged adduct species such as $[M-H+AcO]^{2-}$ and $[M+2AcO]^{2-}$ (Table 2 and Figs. 3 and 4). The MS spectra of the malonyl conjugates exhibited the quasi-molecular ion $[M-H]^-$ (Table 2 and Fig. 5). Since these compounds belongs to the class of malonyl-ginsenosides which are thermally unstable [4], they decompose in the interface capillary (290°C) losing CO_2 from the malonic acid unit. The decomposition products gave rise to quasi-molecular ions $[M-CO_2\text{-}H]^-$, adduct ions $[M-CO_2+AcO]^-$ and strong double-charged adduct species such as $[M-CO_2\text{-}H+AcO]^{2-}$ and $[M-CO_2+2AcO]^{2-}$. In order to confirm the molecular mass of these thermolabile constituents HPLC–ESI-MS analysis using conditions (b) in Section 2.3.2 has been undertaken. Since the instrumentation employed in this experiment allows to use low temperature for the interface (60°C) no decomposition products are observed. The MS spectra of all the constituents showed only the quasi-molecular ion $[M-H]^-$ confirming the molecular mass assignment for all the detected peaks.

The MS–MS spectra of neutral ginsenosides exhibited a fragmentation pattern corresponding to the successive loss of the glycosidic units (Table 3, Figs. 3 and 4). In addition an [aglycone-H]$^-$ ion at m/z 475 corresponding to the (20*S*)-protopanaxatriol aglycon moiety was visible for peaks 1, 2, 13, 18. Peaks 17, 19–25 showed an [aglycone-H]$^-$ ion at m/z 459 corresponding to the (20*S*)-protopanaxadiol aglycon moiety. The MS–MS spectra of malonyl-ginsenosides exhibited intense signals due to the loss of malonyl unit together with signals due to successive losses of glycosidic units (Table 3, Fig. 5).

All the peaks detected were tentatively identified by careful studies of the MS and MS–MS spectra and by comparison with literature data. The identification of peaks 1–3, 13, 17–19, 22, 23, 25 as ginsenosides; Rg_1, Re, Ro, Rf, Rb_1, Rg_2, Rc, Rb_2, Rb_3 and Rd, respectively; was confirmed by spiked injection of the reference compounds. Several iso-

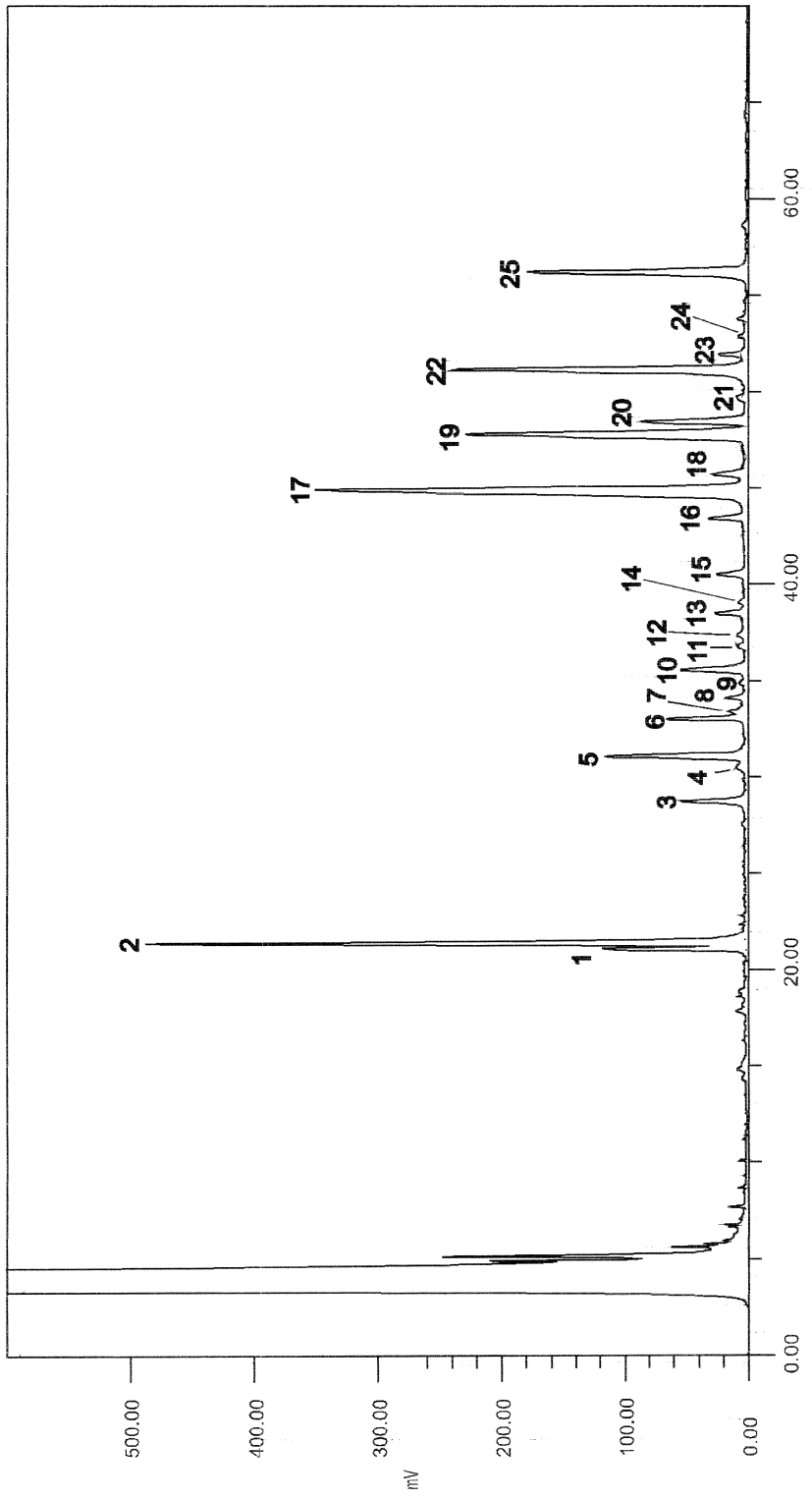


Fig. 2. HPLC–ELSD profile of *P. ginseng* root extract.

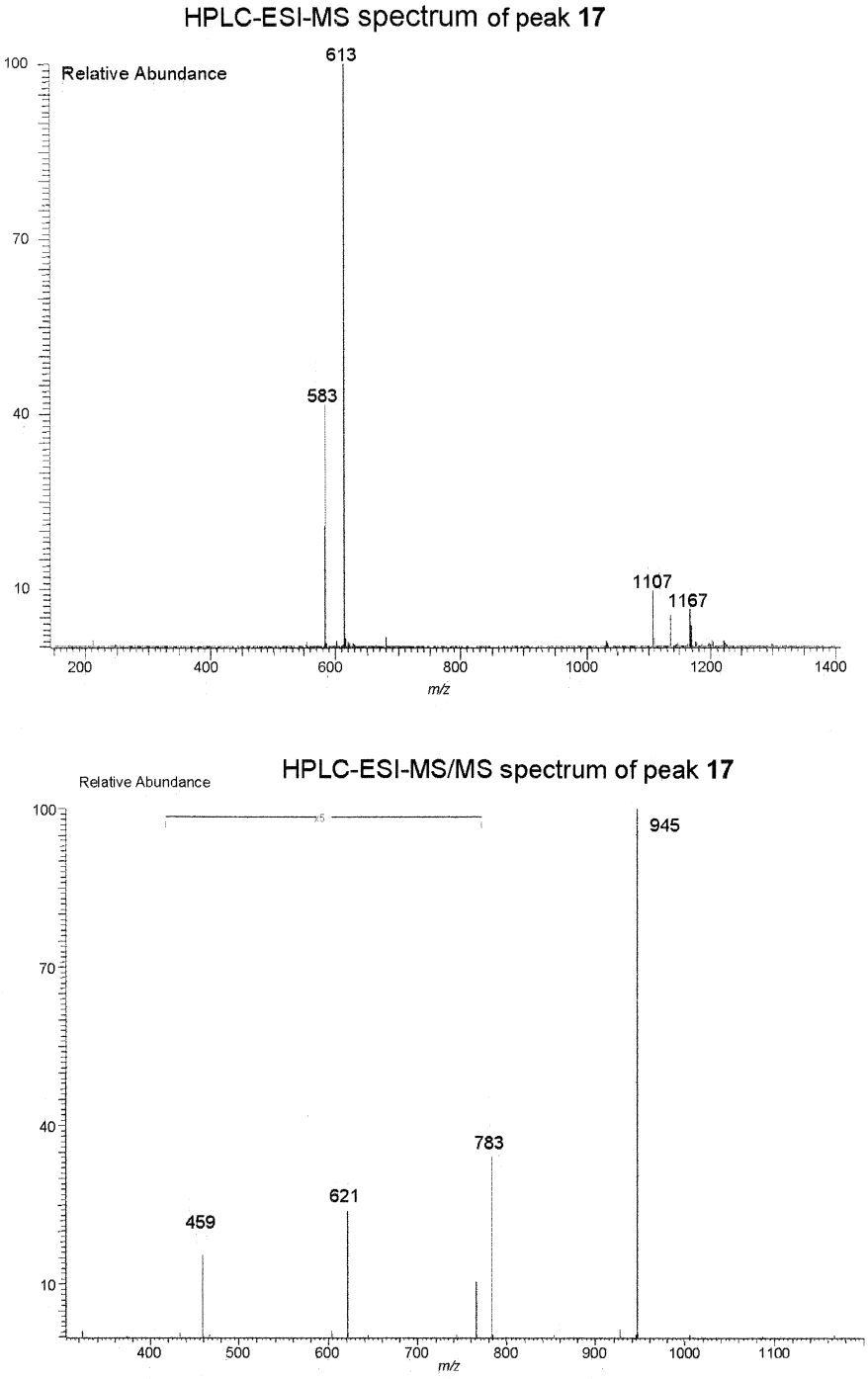


Fig. 3. HPLC–ESI-MS and MS–MS of peak 17.

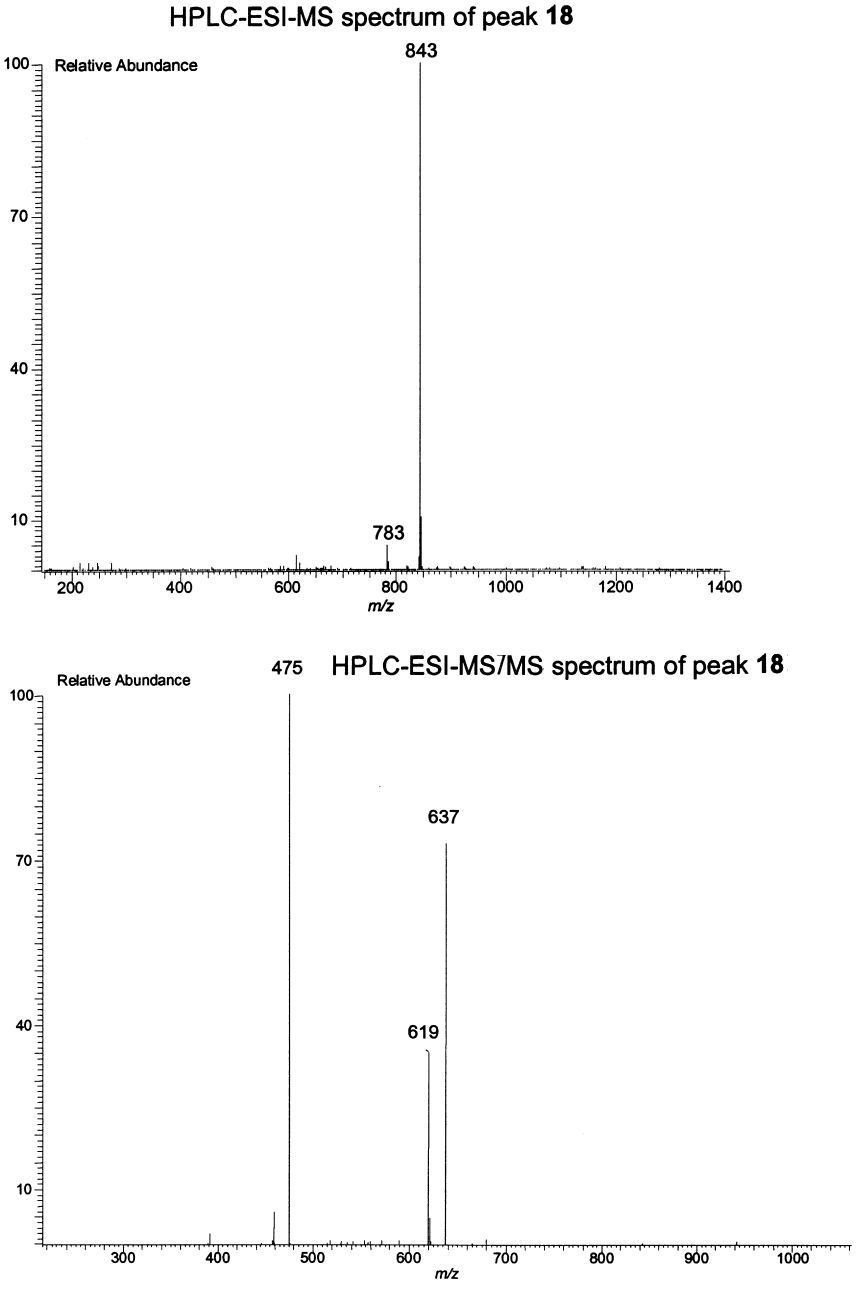


Fig. 4. HPLC–ESI-MS and MS–MS of peak 18.

mers which exhibited the same MS and MS–MS spectra were detected. In particular peaks 16, 20, 21 and 24 were attributed to ginsenoside Ra_1 and Ra_2 and to two isomers never described before. Until now, only four malonyl ginsenosides have been described [1]. This analysis allowed to detect seven more malonyl conjugates: peaks 4 and 8 were tentatively identified as malonyl conjugates of gin-

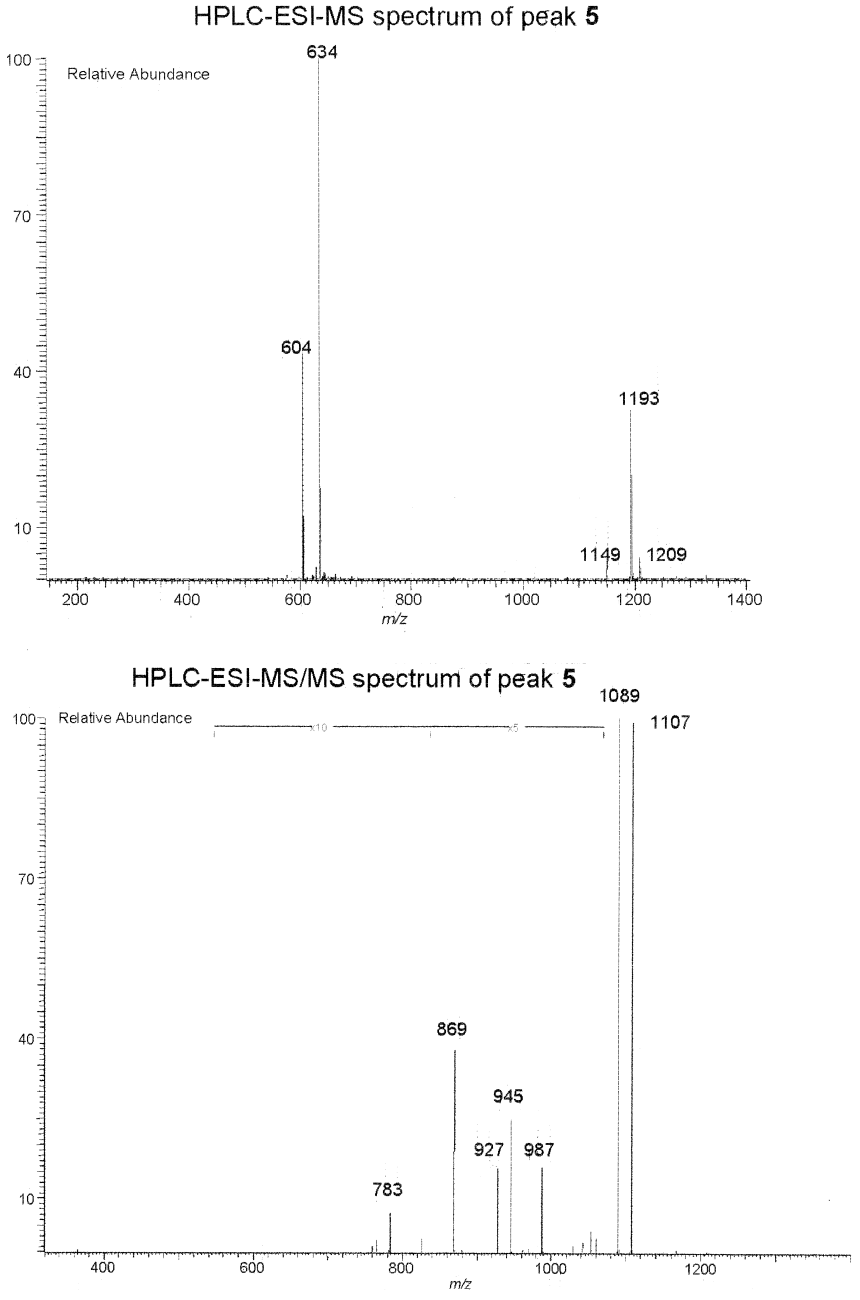


Fig. 5. HPLC–ESI-MS and MS–MS of peak 5.

senosides Ra_1 and Ra_2, peaks 9, 12 and 14 as isomers of malonyl-ginsenosides Rb_2 and Rc and peaks 7 and 16 as isomers of malonyl-ginsenosides Rb_1 and Rd, respectively.

Peaks 15 and 16 showed signals belonging to two co-eluting compounds, tentatively identified as reported in Tables 2 and 3. All the other peaks did not display signals of co-eluting compounds.

Table 2
HPLC–ESI-MS ions of neutral and malonyl ginsenosides in *P. ginseng* [*m*/*z* with relative abundance (%) in parentheses]

Peak	Identification	t_R (min)	$[M-H]^-$, *m*/*z* (%)	$[M-CO_2-H]^-$, *m*/*z* (%)	$[M+AcO]^-$, *m*/*z* (%)	$[M-CO_2+AcO]^-$, *m*/*z* (%)	$[M-CO_2+2AcO]^{2-}$, *m*/*z* (%)	$[M-CO_2-H+AcO]^{2-}$, *m*/*z* (%)	$[M-CH_2O+AcO]^-$, *m*/*z* (%)	$[M+2AcO]^{2-}$, *m*/*z* (%)	$[M-H+AcO]^{2-}$, *m*/*z* (%)
1	Rg_1	20.9	799 (5)	–	859 (100)	–	–	–	–	–	–
2	Re	21.2	945 (39)	–	1005 (45)	–	–	–	975 (8)	532 (100)	502 (9)
3	Ro	28.5	955 (100)	–	–	–	–	–	–	–	507 (52)
4	Malonyl-Ra_1/Ra_2	30.3	1295 (45)	1251 (11)	–	–	685 (100)	655 (54)	–	–	–
5	Malonyl-Rb_1	30.8	1193 (34)	1149 (5)	–	1209 (5)	634 (100)	604 (42)	–	–	–
6	Malonyl-Rb_2/Rb_3/Rc	32.8	1163 (40)	1119 (5)	–	1179 (6)	619 (100)	589 (27)	–	–	–
7	Malonyl-Rb_1 is.	33.1	1193 (37)	1149 (8)	–	1209 (7)	634 (100)	604 (82)	–	–	–
8	Malonyl-Ra_1/Ra_2	33.9	1295 (40)	1251 (6)	–	–	685 (100)	655 (51)	–	–	–
9	Malonyl-Rb_2/Rb_3/Rc	35.0	1163 (44)	1119 (9)	-	1179 (15)	619 (100)	589 (67)	–	–	–
10	Malonyl-Rb_2/Rb_3/Rc	35.3	1163 (34)	1119 (4)	–	1179 (5)	619 (100)	589 (35)	–	–	–
11	Ra_3	36.5	1239 (4)	–	1299 (3)	–	–	–	–	679 (100)	649 (33)
12	Malonyl-Rb_2/Rb_3/Rc	37.1	1163 (18)	1119 (2)	–	1179 (5)	619 (100)	589 (34)	–	–	
13	Rf	38.3	799 (22)	–	859 (100)	–	–	–	–	–	–
14	Malonyl-Rb_2/Rb_3/Rc	38.8	1163 (47)	1119 (10)	–	1179 (17)	619 (100)	589 (72)	–	–	–
15	Malonyl-Rd	40.3	1031 (100)	987 (40)	–	1047 (18)	553 (12)	–			
	Notoginsenoside R_2/F3		769 (100)	–	829 (25)	–	–	–	–	–	–
16	Malonyl-Rd is.	43.2	1031 (20)	987 (18)	–	1047 (100)	553 (10)	–	–	–	–
	Ra_1/Ra_2/isomer		1209 (4)	–	1269 (3)	–	–	–	1239 (2)	664 (100)	634 (41)
17	Rb_1	44.7	1107 (10)	–	1167 (6)	–	–	–	1137 (5)	613 (100)	583 (42)
18	Rg_2	45.6	783 (5)	–	843 (100)	–	–	–	–	–	–
19	Rc	47.6	1077 (6)	–	1137 (9)	–	–	–	1107 (8)	598 (100)	568 (21)
20	Ra_1/Ra_2/isomer	48.2	1209 (12)	–	1269 (1)	–	–	–	1239 (1)	664 (100)	634 (33)
21	Ra_1/Ra_2/isomer	49.7	1209 (4)	–	1269 (1)	–	–	–	1239 (1)	664 (100)	634 (37)
22	Rb_2	50.9	1077 (8)	–	1137 (10)	–	–	–	1107 (6)	598 (100)	568 (34)
23	Rb_3	51.7	1077 (2)	–	1137 (4)	–	–	–	1107 (1)	598 (100)	568 (32)
24	Ra_1/Ra_2/isomer	52.7	1209 (6)	–	1269 (3)	–	–	–	1239 (1)	664 (100)	634 (65)
25	Rd	56.3	945 (18)	–	1005 (100)	–	–	–	975 (5)	532 (35)	502 (5)

Table 3
HPLC–ESI-MS–MS ions of neutral and malonyl-ginsenosides in *P. ginseng* [*m*/*z* with relative abundance (%) in parentheses]

Peak	Identification	t_R (min)	MS–MS, *m*/*z* (%)
1	Rg_1	20.9	637 (100) $[M-H-Glc]^-$; 475 (18) Agl.
2	Re	21.2	799 (58) $[M-H-Rha]^-$; 783 (100) $[M-H-Glc]^-$; 765 (24) $[M-H-Glc-H_2O]^-$; 637 (79) $[M-H-Glc-Rha]^-$; 619 (25) $[M-H-Glc-Rha-H_2O]^-$; 475 (44) Agl.
3	Ro	28.5	793 (100) $[M-H-Glc]^-$; 775 (4) $[M-H-Glc-H_2O]^-$; 731 (3) $[M-H-Glc-H_2O-CO_2]^-$; 613 (1) $[M-H-2Glc-H_2O]^-$; 595 (2) $[M-H-2Glc-2H_2O]^-$; 569 (3) $[M-H-2Glc-H_2O-CO_2]^-$; 523 (4)
4	Malonyl-Ra_1/Ra_2	30.3	1209 (47) $[M-H-Mal]^-$; 1191(100) $[M-H-Mal-H_2O]^-$; 1089 (9) $[M-H-CO_2-Glc]^-$; 1059 (6) $[M-H-Mal-H_2O-Xyl]^-$; 1077 (5) $[M-H-Mal-Xyl]^-$; 1029 (4) $[M-H-Mal-H_2O-Glc]^-$
5	Malonyl-Rb_1	30.8	1107 (98) $[M-H-Mal]^-$; 1089 (100) $[M-H-Mal-H_2O]^-$; 987 (3) $[M-H-CO_2-Glc]^-$; 945 (4) $[M-H-Mal-Glc]^-$; 927 (3) $[M-H-Mal-H_2O-Glc]^-$; 869 (8) $[M-H-2Glc]^-$; 783 (1) $[M-H-Mal-2Glc]^-$
6	Malonyl-Rb_2/Rb_3/Rc	32.8	1077 (95) $[M-H-Mal]^-$; 1059 (100) $[M-H-Mal-H_2O]^-$;987(20) $[M-H-CO_2$- Ara$]^-$; 927 (3) $[M-H-Mal-H_2O-Ara]^-$; 915 (2) $[M-H-Mal-Glc]^-$; 897 (1) $[M-H-Mal-H_2O-Glc]^-$; 783 (1) $[M-H-Mal-Glc-Ara]^-$; 765 (1) $[M-H-Mal-H_2O-Ara-Glc]^-$
7	Malonyl-Rb_1 is.	33.1	1107 (100) $[M-H-Mal]^-$; 1089 (90) $[M-H-Mal-H_2O]^-$;987 (2) $[M-H-CO_2$- Glc$]^-$; 945 (5) $[M-H-Mal-Glc]^-$; 927 (2) $[M-H-Mal-H_2O-Glc]^-$; 783 (1) $[M-H-Mal-2Glc]^-$
8	Malonyl-Ra_1/Ra_2	33.9	1209 (80) $[M-H-Mal]^-$; 1191 (100) $[M-H-Mal-H_2O]^-$; 1077 (30) $[M-H-Mal-Xyl]^-$; 1059 (5) $[M-H-Mal-H_2O-Xyl]^-$; 1047 (2) $[M-H-Mal-Glc]^-$; 1029 (2) $[M-H-Mal-H_2O-Glc]^-$
9	Malonyl-Rb_2/Rb_3/Rc	35.0	1077 (94) $[M-H-Mal]^-$; 1059 (100) $[M-H-Mal-H_2O]^-$; 987 (4) $[M-H-CO_2$- Ara$]^-$; 927 (3) $[M-H-Mal-H_2O-Ara]^-$; 915 (2) $[M-H-Mal-Glc]^-$; 897 (1) $[M-H-Mal-H_2O-Glc]^-$; 783 (1) $[M-H-Mal-Glc-Ara]^-$; 765 (1) $[M-H-Mal-H_2O-Ara-Glc]^-$
10	Malonyl-Rb_2/Rb_3/Rc	35.3	1077 (90) $[M-H-Mal]^-$; 1059 (100) $[M-H-Mal-H_2O]^-$; 987 (4) $[M-H-CO_2$- Ara$]^-$; 927 (2) $[M-H-Mal-H_2O-Ara]^-$; 915 (2) $[M-H-Mal-Glc]^-$; 897 (1) $[M-H-Mal-H_2O-Glc]^-$; 783 (2) $[M-H-Mal-Glc-Ara]^-$; 765 (1) $[M-H-Mal-H_2O-Ara-Glc]^-$
11	Ra_3	36.5	1107 (100) $[M-H-Xyl]^-$; 1077 (37) $[M-H-Glc]^-$; 945 (20) $[M-H-Glc-Xyl]^-$; 783 (7) $[M-H-2Glc-Xyl]^-$
12	Malonyl-Rb_2/Rb_3/Rc	37.1	1077 (92) $[M-H-Mal]^-$; 1059 (100) $[M-H-Mal-H_2O]^-$; 987 (2) $[M-H-CO_2$- Ara$]^-$; 927 (2) $[M-H-Mal-H_2O-Ara]^-$; 915 (2) $[M-H-Mal-Glc]^-$; 897 (1) $[M-H-Mal-H_2O-Glc]^-$; 783 (1) $[M-H-Mal-Glc-Ara]^-$; 765 (1) $[M-H-Mal-H_2O-Ara-Glc]^-$
13	Rf	38.3	637(66) $[M-H-Glc]^-$; 475 (100) Agl.
14	Malonyl-Rb_2/Rb_3/Rc	38.8	1077 (91) $[M-H-Mal]^-$; 1059 (100) $[M-H-Mal-H_2O]^-$; 987 (3) $[M-H-CO_2$- Ara$]^-$; 927 (2) $[M-H-Mal-H_2O-Ara]^-$; 915 (2) $[M-H-Mal-Glc]^-$; 897 (1) $[M-H-Mal-H_2O-Glc]^-$; 783 (1) $[M-H-Mal-Glc-Ara]^-$; 765 (1) $[M-H-Mal-H_2O-Ara-Glc]^-$
15	Malonyl-Rd	40.3	945 (100) $[M-H-Mal]^-$; 927 (55) $[M-H-Mal-H_2O]^-$; 825 (1) $[M-H-CO_2-Glc]^-$; 783 (4) $[M-H-Mal-Glc]^-$; 765 (5)$[M-H-Mal-H_2O-Glc]^-$; 621(1) $[M-H-Mal-2Glc]^-$
	Notoginsenoside R_2/F3		637 (100) $[M-H-Xyl]^-$; 475 (50) Agl.
16	Malonyl-Rd is.	43.2	945 (100) $[M-H-Mal]^-$; 927 (52) $[M-H-Mal-H_2O]^-$; 825 (2) $[M-H-CO_2-Glc]^-$; 783 (3) $[M-H-Mal-Glc]^-$; 765 (5) $[M-H-Mal-H_2O-Glc]^-$; 621 (1) $[M-H-Mal-2Glc]^-$
	Ra_1/Ra_2/isomer		1077 (100) $[M-H-Xyl]^-$; 1047 (18) $[M-H-Glc]^-$; 945 (4) $[M-H-Ara-Xyl]^-$; 915 (7) $[M-H-Xyl-Glc]^-$;783 (4) $[M-H-Xyl-Ara-Glc]^-$; 621 (1) $[M-H-Xyl-Ara-2Glc]^-$; 459 (1) Agl.
17	Rb_1	44.7	945 (100) $[M-H-Glc]^-$; 783 (7) $[M-H-2Glc]^-$; 621 (5) $[M-H-3Glc]^-$; 459 (3) Agl.
18	Rg_2	45.6	637 (70) $[M-H-Rha]^-$; 621 (34) $[M-H-Glc]^-$; 475 (100) Agl.
19	Rc	47.6	945 (100) $[M-H-Ara]^-$; 915 (20) $[M-H-Glc]^-$;783 (45) $[M-H-Ara-Glc]^-$; 621 (6) $[M-H-Ara-2Glc]^-$; 459 (2) Agl.
20	Ra_1/Ra_2/isomer	48.2	1077 (100) $[M-H-Xyl]^-$; 1047 (28) $[M-H-Glc]^-$; 945 (8) $[M-H-Ara-Xyl]^-$; 915 (8) $[M-H-Xyl-Glc]^-$;783 (4) $[M-H-Xyl-Ara-Glc]^-$; 621 (1) $[M-H-Xyl-Ara-2Glc]^-$; 459 (1) Agl.
21	Ra_1/Ra_2/isomer	49.7	1077 (100) $[M-H-Xyl]^-$; 1047 (30) $[M-H-Glc]^-$; 945 (7) $[M-H-Ara-Xyl]^-$; 915 (3) $[M-H-Xyl-Glc]^-$;783 (2) $[M-H-Xyl-Ara-Glc]^-$; 621(1) $[M-H-Xyl-Ara-2Glc]^-$; 459 (1) Agl.
22	Rb_2	50.9	945 (100) $[M-H-Ara]^-$; 915 (18)$[M-H-$ Glc$]^-$; 783 (40) $[M-H-$ Ara-Glc$]^-$; 621(3) $[M-H-Ara-2Glc]^-$; 459 (1) Agl.
23	Rb_3	51.7	945 (100) $[M-H-Xyl]^-$; 915 (30) $[M-H-Glc]^-$; 783 (60) $[M-H-$ Xyl-Glc$]^-$; 621 (10) $[M-H-$ Xyl-2Glc$]^-$; 459 (4) Agl.
24	Ra_1/Ra_2/isomer	52.7	1077 (100) $[M-H-Xyl]^-$; 1047 31) $[M-H-Glc]^-$; 945 (11) $[M-H-Ara-Xyl]^-$; 915 (7) $[M-H-Xyl-Glc]^-$;783 (5) $[M-H-Xyl-Ara-Glc]^-$; 621 (3) $[M-H-Xyl-Ara-2Glc]^-$; 459 (1) Agl.
25	Rd	56.3	783 (100) $[M-H-Glc]^-$; 621 (25) $[M-H-2Glc]^-$; 459 (10) Agl.

4. Conclusions

The HPLC–ELSD analysis of *P. ginseng* roots extract showed the presence of 25 constituents. Ginsenosides, which are polar and non-volatile compounds, can be easily identified by HPLC–ESI-MS and MS–MS analysis. Important structural information such as molecular mass, sugar unit sequence and aglycone moiety are produced allowing the identification of the constituents. Peaks 1–3, 13, 17–19, 22, 23, 25 were identified as ginsenosides Rg_1, Re, Ro, Rf, Rb_1, Rg_2, Rc, Rb_2, Rb_3 and Rd, respectively by spiked injection of the reference compounds. Several minor ginsenosides were detected which were not previously described. In particular, two suspect isomers of ginsenosides Ra_1 and Ra_2 and seven malonyl-ginsenosides. However, this technique does not readily allow the structural identification of the isomers. Furthermore, the HPLC–ESI-MS analysis showed that the present HPLC method is specific.

HPLC–ESI-MS demonstrated to be a higly sensitive analytical method for the detection and on-line identification of both neutral and acidic ginsenosides. However, nowadays this technique is still too expensive to be used in routine analyses. The HPLC–ELSD analysis presented showed to be a sensitive method suitable for the routine control of ginsenoside in ginseng extracts. The validation of this method, including linearity, precision and accuracy, for the quantification of the major constituents Rg_1, Re, Ro, Rf, Rb_1, Rg_2, Rc, Rb_2, Rb_3 and Rd in *P. ginseng* root extracts is in progress.

References

[1] K. Hostettmann, A. Marston, Saponins – Chemistry and Pharmacology of Natural Products, Cambridge University Press, Cambridge, 1995.
[2] O. Tanaka, R. Kasai, in: W. Herz, H. Grisebach, G.W. Kirby, Ch. Tamm (Eds.), Progress in the Chemistry of Organic Natural Products, Vol. 46, Springer, New York, 1984, p. 1.
[3] I. Kitagawa, T. Taniyama, M. Yoshikawa, Y. Ikenishi, Y. Nakagawa, Chem. Pharm. Bull. 37 (1989) 2961.
[4] H. Yamaguchi, R. Kasai, H. Matsuura, O. Tanaka, T. Fuwa, Chem. Pharm. Bull. 36 (1988) 3468.
[5] F. Soldati, O. Sticher, Planta Med. 38 (1980) 348.
[6] I. Asaka, I. Li, M. Hirotani, Y. Asada, T. Furuya, Phytochemistry 36 (1994) 61.
[7] H. Yamaguchi, H. Matsuura, R. Kasai, O. Tanaka, M. Satake, H. Kohda, H. Izumi, M. Nuno, S. Katsuki, S. Isoda, J. Shoji, K. Goto, Chem. Pharm. Bull. 10 (1988) 4177.
[8] H. Kanazawa, Y. Nagata, E. Kurosaki, Y. Matsushima, J. Chromatogr. 632 (1993) 79.
[9] W.C. Chuang, S.J. Sheu, J. Chromatogr. A 685 (1994) 243.
[10] W.C. Chuang, H.K. Wu, S.J. Sheu, S.H. Chiou, H.C. Chang, Y.P. Chen, Planta Med. 61 (1995) 459.
[11] M.K. Park, B.K. Kim, J.H. Park, Y.G. Shin, K.H. Cho, J. Liq. Chromatogr. 18 (1995) 2077.
[12] M.K. Park, J.H. Park, S.B. Han, Y.G. Shin, I.H. Park, J. Chromatogr. A 736 (1996) 77.
[13] M. Hattori, Y. Kawata, N. Kakiuchi, K. Matsuura, T. Tomimori, T. Namba, Chem. Pharm. Bull. 36 (1988) 4467.
[14] M. Hattori, Y. Kawata, N. Kakiuchi, K. Matsuura, T. Namba, Shoyakugaku Zasshi 42 (1988) 228.
[15] M.K. Park, J.H. Park, S.B. Han, Y.G. Shin, I.H. Park, Korio Insam Hakhoechi 19 (1995) 134.
[16] R.B. Cole (Ed.), Electrospray Ionization Mass Spectrometry – Fundamentals, Instrumentation and Applications, Wiley-Interscience, New York, 1997.