BIBLID: ISSN 0253-9756 中国药理学报 *Acta Pharmacologica Sinica* 1993 Mar; **14** (2) : 97-100

Combination of HPLC and 252-Cf plasma desorption mass spectrometry for identifying composition of ginseng tinctures

Yuri N ELKIN, Vecheslav V MAKHANKOV, Nina L UVAROVA
(*Pacific Institute of Bioorganic Chemistry, Far East Division, Russian Academy of Sciences, Vladivostok 690022, Russia*)
Pavel V BONDARENKO, Roman A ZUBAREV, Anatoly N KNYSH
(*PA Electron, Sumy 244030, Ukraine*)

ABSTRACT The 252-Cf plasma desorption mass spectrometry (252-Cf PDMS) determination or confirmation of the ginsenoside saponins has been proposed to investigate the composition of high performance liquid chromatography (HPLC) peaks of ginseng tinctures and galenic preparations. That ionization technique is well suitable for the analysis of natural mixtures of these saponins. The 252-Cf PD mass spectra of standard ginsenosides Rb_1, Rb_2, Rc, Re, Rg_1, Rd, NG-R_2, Z-R_1 contain the peaks of two types of ions, namely, molecular adduct ions (MAI) and aglycone ions. By mass the latter may be referred to either protopanaxadiol or protopanaxatriol. The masses of MAI and aglycone ions are determined by the carbohydrate chains.

The collected HPLC fractions of *P ginseng* tincture can be tested for content of ginsenosides. After studying two MAI peaks from the 252-Cf PD mass spectra of the basic ginsenosides, an example of distinction between two galenic preparations from different *Panax* has been shown.

KEY WORDS ginseng; saponins; high pressure liquid chromatography; mass spectrum analysis

Panax ginseng C A Meyer is the perennial herb indigenous to China and Russia. Its pharmacological properties are connected with the presence of saponins. HPLC was developed for separation of ginsenosides[1,2], which prompted the investigation of the possibilities and limitations of peak identification with on-line and off-line methods[3]. Hence field desorption mass spectrometry (FDMS) was developed. The determination of the molecular weight is followed from base peak of spectrum due to molecular adduct ions (MAI) with alkali cations.

The fast atom bombardment (FAB) ionization technique for polar and non-volatile compounds appeared to be more fruitful. Advantage of FAB MS for studying saponins[4,5], including ginsenosides[6], has been shown. For digitionin, the combination of the 252-Cf plasma desorption mass spectrometry (PDMS) and counter current chromatography were fruitful to test purity of commercial preparation[7]. The 252-Cf PD mass spectrum of ginsenoside Rd was reported too[8]. Supposing that MAI and aglycone ions are better than MAI and sequence ions to verify molecular nature of saponins, in this paper we tried to show potential abilities of 252-Cf PDMS together with HPLC to examine the quality of the ginseng tincture. For this purpose, 252-Cf PD mass spectra of nine ginsenosides were studied and the data obtained were employed to verify ginsenosides in HPLC fractions of the ginseng tincture.

MATERIALS AND METHODS

Ginseng The wild 15-year-old ginseng was collected in Chuguevka region (August). The 5-year-old planted ginseng was bought from farmer Nikolai KOVALCHUK, Melniki, Shuchan region (September). The ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, NG-R_2, and Z-R_1 were confirmed by ^1H- and ^{13}C-nuclear magnetic resonance.

HPLC The tincture of the methanol solution of a summary glycoside fraction[9] 10 μl were loaded into a column (ODS 5 μm, 64× 2 mm) of a HPLC MILICHROM (PO Nauchpribor, Orel) and eluted with acetonitrile-water system in a gradient from 20 : 80 to 60 : 40. The gradient was made by

Received 1992-09-29 Accepted 1993-01-04

successive accumulating specific volums of acetonitrile–water mixture into syringe–pump. Eluate feed rate was 100 $\mu l \cdot min^{-1}$. Spectrophotometric detection of the eluate was performed at 204 nm (The sensitivity decreased at wavelengths >204 nm). The fractions were collected into glass micro test tubes and estimated[9]. Increasing concentration and fully removing the solution were performed with argon stream.

252–Cf PDMS The specimen of Biochemical Mass Spectrometer (MSBX) (PO Electron, Sumy) was made by off–axis disposition scheme of the source of the fission fragments, sample and detectors. the sample was applied by microsyringe onto the gilded disk of 60 mm diameter covered with nitrocellulose (80 $\mu g / cm^2$) by electrospraying with Sample–Spraying Unit (PA Electron, Sumy). Activity of the 252–Cf source was 370 kBq. Acceleration voltage was 25 kV. Ions were counted for 200 000 fission events.

RESULTS AND DISCUSSION

Reverse–phase HPLC carriers were satisfactory for separation of ginsenosides (Rb_1, Rb_2, Rc, Rd, Rg_1, Rf)[1,2]. Similar results were obained repeatedly using microcolumn HPLC[9]. The internal standard (IS): 3,20–di–*O*–β, *D*–glucopyranoside dammar–24–en–3β,12β,17α,20(*S*)–tetraol differs from natural ginsenoside F_2 by additional hydroxyl at C_{17} that increases its retention index.

Chromatograms of the tincture of the wild and planted ginseng are shown in Fig 1. That the wild roots contained ginsenoside Rd several times more than the planted roots puts a question if this Rd is the only one determining a peak on the chromatogram of the wild root tincture. To answer this question, mass spectrometry is necessary for testing HPLC fraction composition.

252–Cf PD mass spectra of the 9 ginsenosides (Rb_1, Rb_2, Rc, Rd, Rf, Re, Rg_1, NG–R_2, and Z–R) were studied. Fission fragments desorbed only two types of ions: MAI and aglycone ions. The spectra of Rd and NG–R_2 ginsenosides glycosylated at C_3 and C_6, respectively, are given as examples in Fig 2. No alkaline salts were added to ginsenosides solutions. The ratio of Na^+ and K^+ in MAI shows their presence in the samples and a nitrocellulose matrix. Each aglycone in the spectrum is represented by 2 ions. The main of them for C_6–glycosylated ginsenoside showed m/z 424, whereas analogous peak from C_3–glycosylated ginsenoside showed m/z 408. Fragmentation of Rd ginsenoside, mentioned in Ref 7, involving the cleavage of monosaccharide residue and hexacarbon fragment of side chain was not confirmed.

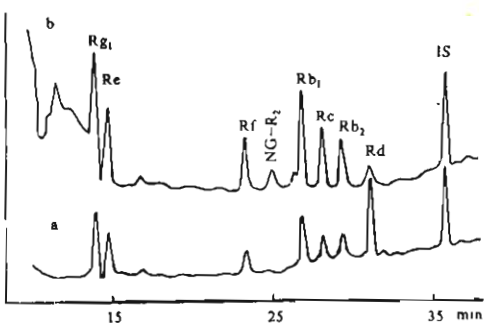

Fig 1. HPLC of tinctures from *Panax ginseng* root, wild (a) and planted (b).

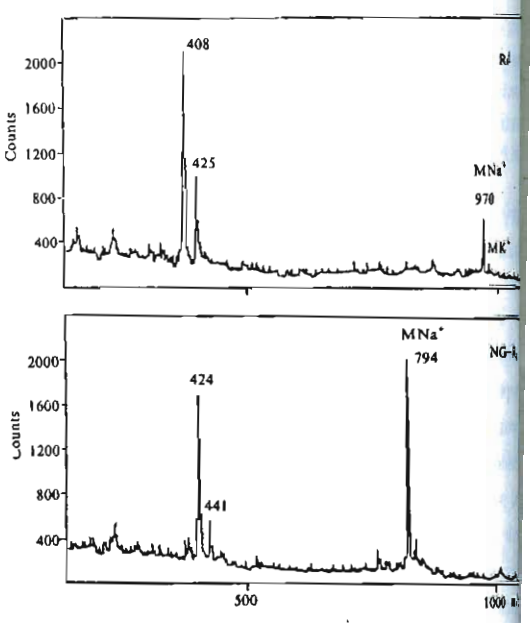

Fig 2. 252–Cf PD mass spectra of standard ginsenosides Rd and NG–R_2 obtained from nitrocelullose matrix.

These differences in the spectra are probably due to peculiarities of design of the ion sources that appear while transferring excess internal energy at formation of the molecular ion. It is clear than using the nitrocellulose matrix reduces the possiblity to obtain excess energy. However, desorption spectra from alumina and gilded surfaces did not contain these fragment ions either. Besides, spectra obtained by desorption from metal surfaces showed the formation of a new ion $M-3^+$ (Fig 3). 252–Cf spectrum of ginsenoside Rf applied onto gilded surface shows the peak $M-3^+$ with m/z 797 which is as high as the peak of MAI (m/z 824) (Fig 3). Desorption from alumina surface increased the yield of $M-3^+$ ion as regards to MAI yield. The target material affected ion energy transfer to molecules of ginsenoside when the instrument with off–axis of source fission fragment was used.

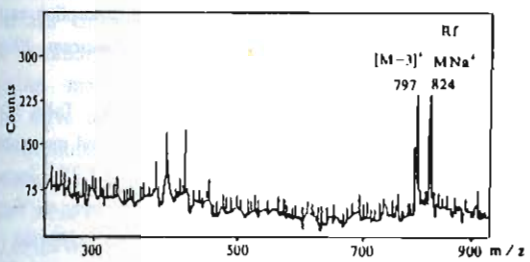

Fig 3. 252–Cf PD mass spectrum of standard ginsenoside Rf obtained from gilded surface.

It is still unknown if ion $M-3^+$ is resulted from the loss of 3 hydrogen atoms or from the attachment of CH_3 residue to dehydrated M^+. Anyway desorption of ginsenosides from NC matrix yielded unequivocal results: molecular adduct ions and aglycone fragment ion were formed. The negative ions of 252–Cf PD mass spectra of ginsenosides were studied, too. They were characterized by peaks of ions MCl^- and $M-H^-$ only.

To take account of the first utility of 252–Cf PDMS for confirmation of ginsenoside composition preparations, we studied negative ion fast atom bombardment mass spectra of the ginsenosides and HPLC fractions. Six ginseosides (Rg_1, Re, Rd, Rc, Rb_1, Rb_2) were studied earlier[5] and new data obtained are in a complete agreement. The overall view of FAB MS of ginsenosides NG–R_2, Rf, and Z–R_1 shows the same types of ions: $M-H^-$ and its deglycosylated fragments (sequence ions).

To verify ginsenoside Rd in a HPLC fraction of the tincture from wild root (Fig 1a), which was determined by its retention index, 70 μl eluent of the fraction was collected. The spectrophotometry showed 15 μg of the substance. The 3rd part of the fraction was electrosprayed onto a target. Really, mass spectrum of the collected fraction were identical to standard of Rd (Fig 2).

On the next stage, we tried to verify tinctures of *Panax ginseng* root and medicinal form of VINA–PANAX (Viet–nam) at once (Fig 4). Since

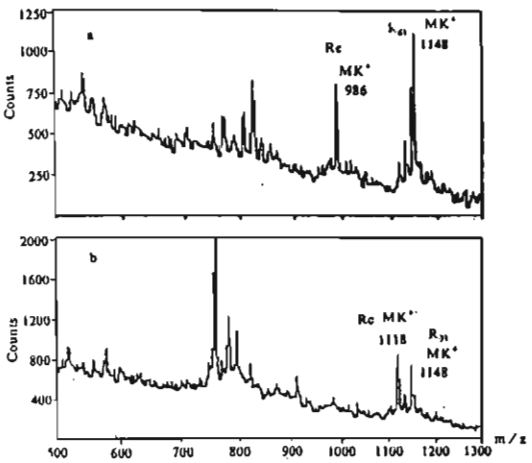

Fig 4. 252–Cf PD mass spectra of tinctures from: (a) *Panax ginseng* root and (b) medicinal form VINI–PANAX.

content of the ginsenosides into the tincture amounted to 0.1–1%, we expected <30 μg of each from the main ginsenosides in a drop (3 μl), applied onto a target. Due to assumption of a small amount of ginsenosides, the ions were accumulated for 500 000 fission events. The picture of the spectra unequivocally distinguishes the tinctures. The regions higher than m/z 500 are shown in Fig 4. The nature

of substances causing the group of peaks in the region of 750–900 a.e.m. has not been established. At the same time, heavier ions of m/z 1118, 1148, and 986, 1148 should be related to MK^+ of the main ginsenosides from the tinctures of the root (Re, Rb_1) and the medicinal form VINA-PANAX (Rc, Rg_1), respectively. In addition, there were peaks of the dihexosides with m/z 381 (MK^+) not shown in Fig 4. They may be related to disaccharides, which may be products of autohydrolysis. It was found in 252-Cf PDMS of methanolic solution of standard ginsenosides Rb_1 and Rc which were stored at 0℃ for a year. Considerable peaks of m/z 365 and 335 (MNa^+) were found, caused by hexosylhexoside and pentosylhexoside, respectively.

Thus, 252-Cf PDMS with HPLC was proved to be suitable to identify a ginseng tincture and galenic preparations.

REFERENCES

1 Meier B, Meier-Bratschi A, Dallenbach-Tolke K, Sticher O. Quantitative analysis of ginseng by HPLC. In: Chang HM, Yeung HW, Tso W-W, Koo A, editors. *Advances in Chinese medicinal materials research.* Singapore: World Scientific Publ Co, 1985 : 471–84.

2 Zhou Zh, Zhang GD. Analysis of ginseng. IV. HPLC determination of ginsenosides in *Panax ginseng*. *Acta Pharm Sin* 1988; **23** : 137–41.

3 Schulten H-R, Soldati F. Identification of ginsenosides from *Panax ginseng* in fractions obtained by high-performance liquid chromatography by field desorption mass spectrometry, multiple internal reflection infrared spectroscopy and thin-layer chromatography. *Chromatogr* 1981; **213** : 37–49.

4 Facino RM, Carini M, Traldi P, Pelli B, Gioia Arlandini E. Confirmative assay and quantitative det mination of *Hedera helix* L. saponins (α-hederi hederacosides B and C) in raw plant extracts and in co metic formulations by EI and CAD MIKE spectrometry *Biomed Environ Mass Spectrom* 1987; **14** : 187–94.

5 Chen Y, Chen N, Li H, Zhao F, Chen N. Fast ato bombardment and collisional activation ma spectorometry in structural analysis of steroid oligoglycosides. *Biomed Environ Mass Spectrom* 198 **14** : 9–15.

6 Fraisse D, Tabet JC, Becchi M, Raynaud J. Fast ato bombardment mass spectrometry of triterpenic saponins *Biomed Environ Mass Specirom* 1986; **13** : 1–19.

7 Yang YM, Lloyd HA, Pannell LK, Fales HM MacFarlane RD, McNeal CJ, Ito Y. Separation of th components of commercial digitonin using high performance liquid chromatography and centrifuga countercurrent chrmatography. Identifcation of th products by californum-252 plasma desorption mas spectrometry. *Biomed Environ Mass Spectrom* 1986; **13** : 439–45.

8 Della Negra S, Fraisse D, Lebeyec Y, Tabet JC. Mass measurements of natural saponins and metastable decomposition analysis with a new 252-Cf TOF instrument. In: Todd JFJ, editor. *Advances in mass spectrometry; part B.* NY: Wiley, 1986: 1599–560.

9 Makhankov VV, Samoshina NF, Malinovskaya GV, Atopkina LN, Denisenko VA, Isakov VV, *et al.* Analysis of the mixture of synthetic and natural dammarane glycosides by HPLC. *Khim Prir Soedin* 1990; **57** : 57–61.