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Isolation and analysis of ginseng: advances and challenges

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Abstract

Ginseng occupies a prominent position in the list of best-selling natural products in the world. Because of its complex constituents, multidisciplinary techniques are needed to validate the analytical methods that support ginseng's use worldwide. In the past decade, rapid development of technology has advanced many aspects of ginseng research. The aim of this review is to illustrate the recent advances in the isolation and analysis of ginseng, and to highlight their new applications and challenges. Emphasis is placed on recent trends and emerging techniques. The current article reviews the literature between January 2000 and September 2010.

1 Introduction

Plants have been the basis of traditional medicines for thousands of years and continue to be considered valuable materials in medicines. Natural products isolated from plants provide an unparalleled source of chemical diversity for discovery of biologically active molecules. For various medical conditions, especially chronic diseases, use of medicinal plants has expanded globally and gained considerable popularity.¹ Among these plants, ginseng has a long history and is today one of the world's most widely used medicinal plants. Ginseng products are commercially available in roots, tablets and capsules, liquid extracts, carbonated drinks and teas.²

The name ginseng is derived from a Chinese term referring to the "man-like" shape of the root. The genus is *Panax*, meaning "cure all" in Greek. *Panax ginseng* C. A. Meyer (commonly referred to as Asian, Chinese or Korean ginseng) and *Panax quinquefolius* L. (also known as American or North American ginseng) are the two most recognized species around the world. Another is *Panax notoginseng* (Burk.) F. H. Chen. Other less known species in the ginseng genus include *Panax japonicus* C.A. Meyer (Japanese ginseng) and *Panax vietnamensis* Ha et Grushv (Vietnamese ginseng). Traditionally the ginseng root, available in white or red, is used. White ginseng is prepared by air-drying after harvest, and red ginseng is prepared by a steaming or heating process.^{3,4} The leaf, berry and other parts of ginseng are also medicinal sources.^{5–7}

Ginseng contains complex constituents. Thus, multidisciplinary methods are required to validate the analytical methods that support ginseng's use worldwide. In the past decade, advanced technologies for isolation and analysis of plants have revolutionized ginseng

research. The literature describes investigation of ginseng with pharmacognosy, phytochemistry, biosynthesis, and pharmacology.^{8–13} The aim of this review is to illustrate the recent advances in the isolation and analysis of ginseng, and to highlight their new applications, especially in species authentication, quality assessment, and interaction with biological systems. This review covers the literature between January 2000 and September 2010. The article is organized into three major sections: (1) preparation and isolation; (2) analytical advances; and (3) applications and challenges. Recent trends and emerging techniques are emphasized rather than routine methods. Information related to biosynthesis, pharmacology and clinical aspects is beyond the scope of this article.

2 Preparation and isolation

The first challenge in isolating and analyzing ginseng is the complexity of the sample matrices. The construction and maintenance of high quality chemical libraries in ginseng is needed, calling for state-of-the-art procedures for separation and isolation. Recent developments in this field include automated and solvent-free extraction techniques and more efficient isolation methods.

2.1 Sample extraction and preparation techniques

Efficient sample preparation can improve extraction efficiency and enrich the target analytes. Ginseng saponins have been extracted from ginseng with different solvents and methods. The conventional method uses heat-reflux,¹⁴ Soxhlet,¹⁵ shaking¹⁶ or ultrasound-assisted extraction (UAE).¹⁷ Soxhlet extraction at 80–90 °C for 20–24 h efficiently extracts saponins.¹⁵ Modern ultra-pressure or ultra-temperature extraction methods have been applied such as pressurized liquid extraction (PLE),¹⁸ microwave-assisted extraction (MAE),¹⁹ high-pressure MAE,²⁰ pressurized hot water extraction (PHWE)²¹ and supercritical fluid extraction (SFE).¹⁵ Compared with conventional methods, newer methods use less solvent, automate easily, take a short time and are more efficient.²² Table 1 lists the characteristics of nine different extraction techniques. Each has advantages and limitations depending on the projected use of results.

Plant material is considered first. Small sample particles have larger specific external areas, stronger solvent penetration, and thus higher extraction yield.²³ Extraction solvent and extraction volume are key in obtaining high recovery.²⁴ Among various solvent systems, 100% methanol has better extraction efficiency than water or 70% aqueous methanol extraction;²⁵ 50% ethanol has higher efficiency than an ethanol/glycerin/water mixture or 65% glycerin.²⁶ Adding non-ionic surfactants like Triton X-100 to solvent enhances the extraction yield.²⁷ Various extraction methods have been compared quantitatively.^{18,23,28} Extraction conditions affect results, and the best conditions for each technique should be optimized in comparison. For instance, efficiency differs between Soxhlet extraction for 2 h²³ and 20 h.¹⁵ Extraction efficiency also depends on the ginsenoside. Since degradation is possible during the extraction process, temperature is a factor to be considered.²⁹

2.2 Isolation and purification methods

A high quality chemical library of ginseng saponins is important for high throughput screening, research in the structure-activity relationship, and investigation of the mechanisms of this group of compounds. Because ginseng has many constituents, the isolation process can be time-consuming and tedious. New technologies are therefore welcome.

2.2.1 Liquid-solid isolation—Separation and isolation of ginseng saponins is commonly performed with liquid-solid column chromatography.^{30,31} Fig. 1 summarizes the procedures

for isolation of saponins from ginseng materials. Ginseng samples are extracted with methanol^{30,31} or ethanol^{32,33} once or several times, and the combined extracts are concentrated in vacuo to dryness. The residue is suspended in water and then partitioned with organic solvents to different solvent-soluble portions such as a *n*-hexane portion,³⁰ methylene chloride portion,^{30,34} ethyl-acetate portion,³⁵ *n*-butanol portion,³⁶ and an aqueous layer.^{31,34} The *n*-hexane and methylene chloride layers containing macromolecule and oil-soluble impurities are removed.^{30,34} The other portions are subjected to Diaion HP-20 column or silica gel column chromatography and eluted with a gradient solvent system to fractions and sub-fractions.^{31,34} The fractions are then repeatedly chromatographed with normal phase silica gel column chromatography,^{31,35} reversed-phase silica gel column chromatography,³¹ and MCI-gel CHP20P columns,³³ using a stepwise gradient elution of different solvent systems. The products are purified by preparative liquid chromatography,^{32,36} and their structures are identified by chemical and spectroscopic methods.

2.2.2 Liquid-liquid isolation—Liquid-liquid isolation techniques such as high-speed counter-current chromatography (HSCCC) and centrifugal partition chromatography (CPC) rely on the partition of a sample between two immiscible solvents. Without a solid support matrix, the irreversible adsorption of samples can be eliminated in the stationary phase of conventional column chromatography.³⁷ As methods of high efficiency and high recovery that are easy to scale-up, HSCCC and CPC have been used in preparative separation of ginseng saponins.^{38–43} Before separation with HSCCC, ginseng samples were extracted by organic solvents, and the saponin fraction was then enriched using column chromatography such as macroporous resin chromatography,³⁹ reversed-phase C₁₈ column,⁴¹ or medium-pressure liquid chromatography.⁴² A practical and effective strategy for a step by step selection of HSCCC conditions includes the selection of two-phase solvent systems; determination of partition coefficient (*K*) of analytes; preparation of the two-phase solvent system and sample solution; selection of elution mode, flow-rate and rotation speed; and on-line monitoring of the eluate.⁴⁴ The selection of a suitable solvent system is the most important step in HSCCC and may be estimated as 90% of the work.³⁷ Table 2 summarizes recent applications of HSCCC to isolation of saponins from ginseng products. Ginsenosides Rb1, Rg1, Re, Rf, Rd, Rg3, Rg5, Rk1, F4, Ro and notoginsenoside R1 have been isolated by HSCCC.

CPC is also a liquid-liquid partition chromatography without sorbent working in a constant-gravity field. Ginsenosides Rc, Rb1, and Re were isolated from *P. quinquefolius* by CPC using a two-phase solvent system of ethyl acetate–*n*-butanol–water (1 : 1 : 2).⁴³ Both CPC and HSCCC require the technician to be familiar with the experimental conditions and the practical separation procedure, which are unique.⁴⁴

2.2.3 Recently reported ginseng saponins—Most ginseng saponins belong to a family of steroids with a four trans-ring rigid steroid skeleton. They are also referred to as ginsenosides, triterpenoid saponins or dammarane derivatives. More than 200 saponins have been isolated from ginseng plants.¹¹ In addition to studies that focus on ginseng root, there have been studies of ginseng leaves and stems,^{5,45,46} flower buds,^{30,31,47} fruits,^{48,49} berries,⁵⁰ and seeds.⁵¹ Because steaming or heating changes the saponin profile of ginseng products,^{3,52} some researchers have isolated ginseng saponins from processed root,^{33,53} leaf,⁵⁴ flower-bud³⁴ and berry.⁵⁵

Ginseng saponins are divided into several groups. Two major groups are the protopanaxadiol (PPD)-type saponins with sugar moieties attached to the C-3 and/or C-20 and the protopanaxatriol (PPT) group with sugar moieties at C-6 and/or at C-20. Other groups include the ocotillol-type with a five-membered epoxy ring at C-20, the oleanane-type with a nonsteroidal structure, and the dammarane type with a modified C-20 side chain.

^{9,56} As techniques are developed for chemical purification and structural identification, novel ginseng saponins continue to be discovered. Figs. 2, Figs. 3, 4 and Table 3 show the structures of 123 new dammarane-type saponins isolated from various parts of *Panax* plants in the period from January 2000 to September 2010. They are placed in the order of the structure type. Compounds 4 and 91, both named as ginsenoside Rh5 shown in Table 3, have different structures. Apart from ginseng saponins, other novel compounds also have been characterized including polyacetylene,^{91–95} polysaccharide,⁹⁶ phenolic components,^{97,98} amino acids⁹⁹ and alkaloids.¹⁰⁰

2.3 Knockout technologies

The constituents and functions of ginseng are studied by isolation-to-bioassay or bioassay-guided isolation.¹ To prove whether the obtained constituents are active compounds of the extract, it is necessary to prepare an extract in which the assumed compound(s) is removed,¹⁰¹ called a “knockout” extract. The “knockout” concept has been widespread in genetic engineering for pharmacologic studies since 1989.¹⁰² In bioactivity comparisons, if a knockout extract has low biological activity compared with the activity of the original extract, the eliminated constituents can be considered bioactive compounds.¹⁰¹ Therefore, one goal of studies is to validate methods for preparing knockout extracts. Chemical chromatography and immunoaffinity chromatography have been introduced for this purpose.

2.3.1 Chemical chromatography—Some knockout extracts are prepared by column chromatography. For example, ginsenoside Re was believed to be the antihyperglycemic active compound in American ginseng berry extract.¹⁰³ To prepare the Rb1 knockout extract, ginseng berry extract was loaded onto an HP-20 chromatographic column. The column was eluted with water and then aqueous ethanol. The aqueous ethanol eluate portion was separated by reversed-phase preparative high-performance liquid chromatography (HPLC) to give three fractions: water fraction, Rb1 fraction, and the other saponin fraction. The Rb1 fraction was removed from the others. After mixing the water fraction and saponin portion, the Rb1 knockout extract was finally prepared.

To improve efficiency, Liu *et al.*¹⁰¹ developed an on-line chromatography technology to prepare knockout extract. As shown in Fig. 5, an HPLC technique was used to separate constituents in an herb extract, ensuring target compounds achieved baseline separation from adjacent peaks. Then a six-port valve-switching part cut and captured target compounds according to the chromatographic retention times. Ingredients were thus divided into two parts: the target compounds and others without the target. Based on valve-switching, main bioactive and characteristic components or a combination can be obtained. Fig. 5 also shows an application example with ginseng. With HPLC, ginseng extract underwent baseline separation of its major components.¹⁰⁴ Through the valve switching technique, original extract, Re and Rb1 knockout extract, and knockout compounds (Re + Rb1) can be obtained. A comparison of the bioactivities of the samples shows the overall efficacy of target peaks, the bioactive contribution of each component, and the interaction of multi-components.

2.3.2 Immunoaffinity chromatography—Immunoaffinity chromatography is a powerful technique to isolate and concentrate minor components of interest from complex mixtures.^{105,106} Its selectivity is derived from the use of an immobilized binding protein on a suitable solid phase support. In a continuous effort to produce monoclonal antibodies (MAbs) for naturally occurring bioactive compounds, Shoyama *et al.* prepared anti-ginsenoside Rb1,¹⁰⁷ Rg1,¹⁰⁸ Rd,¹⁰⁹ and Re MAbs.¹¹⁰ The antibody specificity was tested by determining the cross-reactivities of the MAbs with structurally similar compounds by competitive enzyme-linked immunosorbent assay (ELISA).^{111–113}

Fig. 6 shows a protocol for preparing ginsenoside Rb1 knockout ginseng extract.^{113–115} The first step is to obtain anti-ginsenoside Rb1 MAbs from mice through synthesis of antigen conjugates, immunization, hybridization and purification. Since anti-ginsenoside Rb1 MAbs have a sugar chain in the molecule, the second step is to produce aldehyde groups through oxidative cleavage by sodium periodide (NaIO_4).^{115,116} The oxidized product is treated with agarose gels conjugated with a hydrazine group to give hydrazone. The immunoaffinity column is produced with an affinity gel combined with MAbs.^{110,114} After method validation, ginseng extract is charged to the column, washed by phosphate buffer and then eluted by acetate buffer and 20% methanol to give pure ginsenoside Rb1. All washed buffer solutions are collected and deionized. The water solution is concentrated and applied to thin layer chromatography (TLC) or HPLC analysis. The immunoaffinity chromatographic method can establish one-step preparation of Rb1 knockout ginseng extract.

Compared with chemical chromatography for knockout, immunoaffinity chromatography enhanced analytical selectivity, decreased tedious steps in sample preparation, and improved sampling loading volume. Additionally, the run-time of chromatographic separations and optimal conditions can be saved. Yet, immunoaffinity chromatography has some limitations, namely complicated MAbs and column preparation, non-commercial availability of MAbs, and instability of immunoaffinity columns.

3 Analytical advances

In this section, we emphasize emerging techniques, general trends, and recent advances for analysis of ginseng. Some methods highlighted are high-performance thin-layer chromatography (HPTLC); hydrophilic interaction chromatography (HILIC); ultrahigh pressure liquid chromatography (UPLC); multidimensional chromatography; and on-line concentration electrokinetic chromatography for separation; and advanced mass spectrometry (MS) for detection, *i.e.* quadrupole (Q)-MS, ion trap (IT)-MS, time-of-flight (TOF)-MS instruments; and more recently, Q-IT, IT-TOF and Q-TOF.

3.1 Direct profiling technologies

With the development of chemometrics and informatics in multivariate statistical analysis, spectrometric and spectroscopic methods are frequently applied to profiling crude ginseng extracts without prior separation. Profiling without hyphenation technologies is useful for rapid evaluation of the composition, authentication of the ginseng plant species, quality control, and metabolomics.¹¹⁷ Major techniques are nuclear magnetic resonance (NMR), infrared (IR) and Raman spectroscopy.

3.1.1 Nuclear magnetic resonance (NMR) spectroscopy—The profiling of crude ginseng extracts can be performed by direct NMR analysis. This method, which requires limited sample preparation, is simple, comprehensive and reproducible. Direct NMR profiling is applied in metabolomic fingerprinting for quality control and authentication of different ginseng species, ages, and origins. With the aid of chemical shifts and coupling constants by ^1H NMR spectrum, constituents in ginseng can be identified. The concentration of each compound in the sample can be calculated from integration of the signals in the spectra without calibration curves.¹¹⁸ Multivariate data analysis sorts the discriminating signals from the complex spectra.¹¹⁹

There are several reports on the discrimination of ginsengs by NMR profiling. Sugar and methyl signals in ginseng saponins obtained from ^1H -NMR with principal component analysis (PCA) and cluster analysis can differentiate geographical origins and ages of *P. ginseng* roots.^{120,121} Ginseng saponins, saccharides, amino acids, fumaric acid and inositol were screened as important markers by two-dimensional (2-D) NMR with PCA to

discriminate between *P. ginseng* and *P. quinquefolius* preparations and white and red ginseng root.¹²²

Direct NMR profiling is powerful for recording changes in composition within a ginseng mixture. One of the main drawbacks of this method is its low sensitivity. It is not well suited for accessing minor changes in composition.

3.1.2 Infrared (IR) and Raman spectroscopy—IR is an important tool in organic chemistry to assess a compound's purity and its structure. Pattern recognition with IR has been applied to analyze ginseng composition and for species authentication and quality control. The main advantages of IR over the traditional chemical and chromatographic methods are its simplicity in routine operations. It is non-destructive and requires less sample preparation. One drawback of IR is the calibration step for quantitative analysis, which requires analysis of a set of samples covering the spectral variability in the sample.

Based on a unique IR spectral fingerprint in 2000-600 cm^{-1} , Yap *et al.* described a “2-6PC rule” for categorizing ginseng products to authenticate them from morphological adulterations¹²³ and to distinguish among *P. ginseng*, *P. quinquefolius* and *P. notoginseng*.¹²⁴ Near-infrared spectroscopy was used to determine the total sugar content in ginsengs.¹²⁵ Fourier transform infrared (FTIR) spectroscopy with support vector machine and wavelet transform technology identified the cultivation area of ginsengs¹²⁶ and authenticated American ginseng from Asian ginseng.¹²⁷ In a recent study, 2-D FTIR and 2-D IR correlation spectroscopy provided visual and colorful maps and dynamic structural information about chemical components to authenticate Asian, American ginseng and notoginseng,¹²⁸ to differentiate cultivated from wild ginseng,¹²⁹ to discriminate between the different geographical sources of American ginseng,¹³⁰ and to evaluate the different ages of ginseng samples.¹³¹

Raman spectroscopy with pattern recognition has been used to classify ginsengs according to species and processing method¹³² and to investigate polyacetylenes in American ginseng roots.¹³³

3.2 Separation-based methods

One emerging trend has been combining techniques. Combinations have been useful for profiling the composition of ginseng samples. Two major steps are involved, separation and detection. Separation of ginseng saponins is performed by TLC or HPTLC; HPLC, HILIC or UPLC; gas chromatography (GC); multidimensional chromatography; or electrophoresis (CE). Among these techniques, liquid chromatography is used most frequently. A comparison of different separation modes for ginseng analysis is founded in Table 4.

3.2.1 Thin layer chromatography (TLC) and high-performance TLC (HPTLC)—

For a considerable time, TLC or planar chromatography (PC) was called the “Cinderella” or the “princess” of chromatographic techniques. The main advantages of TLC are its versatility, speed, flexibility and low cost. It is used for tests of purity, quick qualification or species identification in pharmacopoeias and the pharmaceutical industry. To avoid harmful reagents recorded in 14th edition of the Japanese Pharmacopoeia, Ohno *et al.*¹³⁵ developed a reversed-phase TLC using a sodium sulfate/acetonitrile/methanol solution instead of a chloroform/methanol/water solution as the developing solvent with Rg1 as the marker. Detection of ginseng saponins by TLC is usually achieved by sulfuric acid alone or its mixture with aromatic aldehydes like vanillin or anisaldehyde.

Many of the TLC procedures have been carried out under high-performance conditions called HPTLC. In HPTLC, the plates are precoated for the stationary phase with a mean

particle size of 5 μm for better separation and reproducibility. HPTLC was developed for differentiation of the ginseng species. A fingerprint pattern with HPTLC discriminates white and red Asian ginseng, and American ginseng and notoginseng.^{154,155} With the introduction of densitometry, TLC also has become a useful tool for the quantitative analysis of ginseng saponins. A typical application was performed by Vanhaelen-Fastré *et al.*¹⁵⁶ who used HPTLC for simultaneous quantification of six major ginseng saponins in *P. ginseng*. Kevers *et al.*¹⁵⁷ developed an automatic HPTLC sampler and scanner that determines ginseng saponins and identifies the different species and sources. The major disadvantages of TLC and HPTLC are low accuracy and reproducibility.

3.2.2 Gas chromatography (GC)—GC is sensitive for detecting volatile chemical compounds or non-volatile compounds readily derivatized.¹ Most GC uses capillary columns (commonly fused-silica, 30 m $\times$ 0.25 mm $\times$ 0.25 μm). For detection, the flame ionization detector has remained popular. Structural information and selectivity available from mass spectrometry (MS) has made the combination of GC-MS more effective. The strategy for GC analysis of ginseng saponins is to hydrolyze the saponins before trimethylsilyl-derivatization of their genuine aglycones to simplify and improve sensitivity.¹¹

GC has been used for analysis of volatile organic compounds (*e.g.*, oils).^{136,158} Sample preparation is the crucial first step in GC analysis of volatile compounds in ginseng. Novel sample preparation techniques have been established to extract volatile compounds from the matrix, including headspace solid-phase microextraction¹³⁷ and solvent-free solid injection based on direct vaporization.¹⁵⁸ Another important use of GC has been to analyze multiresidue pesticides and their metabolites in ginseng samples. There are method reports that describe the screening of pesticides in dried ginseng powders, extracts and preparations.^{151,159,160} To make methods faster, automated, and cost-efficient, GC has been combined with high resolution time-of-flight (TOF) MS.¹⁶⁰

GC can also determine other active ingredients. There are methods for measuring semi-volatile components such as sesquiterpenes and polyacetylenes^{91,153} and for quantifying phenolic constituents after derivatization to their trimethylsilyl derivatives.¹⁶¹ GC is also a powerful tool to test endogenous metabolomics in biological samples for ginseng's therapeutic effects and toxicity.¹³⁸

As has been the general trend for a number of years, GC is decreasing in popularity as reflected by the number of new procedures being reported. Since the complex patterns of the different types of ginseng saponins cannot be evaluated with GC, it is rarely used in ginseng studies.

3.2.3 High-performance liquid chromatography (HPLC), hydrophilic interaction LC (HILIC) and ultra-performance LC (UPLC)—HPLC continues to be the most used technique in ginseng research because it is low cost, readily available, and easy to use. Typically, HPLC is performed under reversed-phase conditions with standard 150 or 250 mm $\times$ 4.6 mm columns packed with 5- μm porous silica-based C₁₈ sorbents. In the mobile phase, acetonitrile and water with buffer are used, and a gradient elution program is optimized. Although HPLC may be considered a mature technology, advances continue to improve the separation capability and productivity of the method and sample analysis.

HILIC has been gaining interest in the last few years as an alternative to HPLC.^{142–145} HILIC is especially useful for separation of polar and hydrophilic analytes as well as polar metabolites in biological samples. Separations are carried out in the hydrophilic stationary phase using isocratic hydro-organic eluents that contain high levels of the organic

component. Retention times increase with the hydrophilicity of the analytes. When combined with MS, high organic content in the mobile phase benefits the electrospray ionization process. The influence of column temperature and the choice of eluent components such as buffer are key to separation efficiency and selectivity.¹⁴⁵ In HILIC, a silica column (50 × 2.1 mm, 3 μm) was used to analyze dencichine (a neuro-excitatory non-protein amino acid) in different ginseng species with a run time of approximately 5 min. Dencichine was not retained by any of the reversed-phase packing.¹⁴² HILIC using Diol,¹⁴³ or polyvinyl alcohol-bonded,¹⁴⁵ 162 or polyamine-bonded¹⁴⁴ stationary phases was developed for the separation and quantitative determination of ginseng saponins in ginseng preparations. Shorter analysis time (15–30 min compared with more than 60 min in RP-HPLC) and better resolution were achieved. The elution window for the analysis of compounds difficult to separate can be broadened by the combination of HILIC with conventional HPLC.¹⁶³

UPLC that uses short columns packed with sub-2 μm particles has emerged as a powerful method in many laboratories. The high speed of chromatographic separation is 5–10 times faster than HPLC without reducing resolution.^{146,164,165} Existing HPLC conditions can be directly transferred to UPLC. Combining UPLC with an MS detector fulfills key requirements in terms of rapidity, sensitivity, selectivity, and peak-assignment certainty for the analysis of analytes at low concentrations in complex matrices.¹⁶⁶ UPLC has been popular for rapid separation of ginseng saponins in metabolomic fingerprinting,^{146–148} quality control,¹⁶⁷ and biological samples.^{168,169} A comparison was made between the performance of UPLC on a 100 × 2.1 mm, 1.7 μm column and of HPLC on a 250 × 4.6 mm, 5.0 μm column. Twenty-five ginseng saponins were identified within a 20-min run time with UPLC, while fewer markers were recognized by HPLC within 80 min.¹⁴⁸ At present, the major limitation of UPLC is the cost of dedicated equipment and consumables, keeping the instrument more of a research than a routine tool. To provide a more economical alternative, a simplified approach was recently introduced, in which a modified conventional HPLC system was connected directly with short columns to reduce time for analysis of complex herbal medicines.^{170,171} Direct connection offers opportunities for laboratories looking to improve separation without upgrading an existing HPLC system to an UPLC system.

3.2.4 Micellar electrokinetic chromatography (MEKC) and microemulsion EKC (MEEKC)—Ginseng saponins do not have charges, so capillary zone electrophoresis (CZE) is not applicable. Therefore, micellar electrokinetic chromatography (MEKC) and microemulsion electrokinetic chromatography (MEEKC) are used for the separation of neutral ginseng saponins. MEKC is efficient and fast, and running buffer composition can be tuned or changed to select separation.¹⁷² Compared to MEKC, the presence of a water-immiscible oil phase in the microemulsion droplets of MEEKC gives rise to some special properties, such as enhanced capacity for solubilization and an enlarged migration window.¹⁷³

In general, analysis of ginseng saponins with CE can be optimized by sampling and concentrating the ginseng samples, buffer additives, surface modifications and detections.¹⁷⁴ The concentration sensitivity of MEKC and MEEKC is poor because the best path is short and only a small volume of sample can be injected. To improve the sensitivity of MEKC, online concentration techniques with stacking procedures have been developed. Want *et al.*¹⁷⁵ developed a field-enhanced sample injection with reverse migrating micelles to separate and concentrate ten ginseng saponins in *P. notoginseng* on-line. Ginseng saponins achieved separation within 35 min, shorter than 65 min by HPLC. Sensitivity was one magnitude higher than with the method by Glöckl *et al.*¹⁴⁹ With high sensitivity two minor ginsenosides, Rh1 and Rg2, were determined.

Cao *et al.*¹⁵² used online dual sweeping based on borate complexation and enhancement of the organic solvent field enhancement for the preconcentration of ginsenosides Rf, Rg1, and Re in MEKC with nonionic Brij-35 micelles. Subsequently, they established a complex formation and acetonitrile sweeping technique in nonionic MEEKC for ginsenosides Rf, Rb2 and Re using a Brij-35 microemulsion.¹⁵¹ Pressure and electrokinetic injections of long sample plugs were also presented for simultaneous stacking of notoginsenoside R1, ginsenosides Rg1, Rf, Rh1, Rd, Rg3 in MEEKC.¹⁵⁰ Improvements in sensitivity were 50 to 200 folds.

In spite of some novel CE techniques, the application of CE for analysis of ginseng is limited. One example uses a CE microchip coupled with a polymerase chain reaction (PCR)—short tandem repeats for rapid authentication of ginseng species.¹⁷⁴ Most applications are for protein analysis to decipher ginseng's biological mechanisms.

3.2.5 Multidimensional chromatography—The separation power of traditional one-dimensional techniques is sometimes inadequate for complex samples. In such a case, multidimensional chromatography is used, and most multidimensional separations are 2-D. In 2-D separation, a sample is first separated via one method, and then the separated compounds are further subjected to an additional independent method. For complex samples like ginseng extracts, multidimensional chromatography offers more powerful separation ability, higher peak capacity and detection sensitivity than single-dimension chromatography. Multidimensional chromatography can be easily realized by the use of GC and TLC. The realization of multidimensionality in LC is difficult because of problems in switching the mobile phase from one system to another.¹⁷⁶

Shellie *et al.*¹⁵³ developed comprehensive 2-D GC (GC $\times$ GC) to identify qualitative differences between *Panax* species extracts. GC $\times$ GC revealed the presence of numerous common components and possible species-specific components. Qiu, *et al.*¹³⁶ described comprehensive GC $\times$ GC to analyze the chemical composition of volatile oil in *P. ginseng* at different ages. This method for PCA classifies and differentiates ginseng samples at different ages.

With 2-D chromatography, artifacts may form, polar and nonvolatile solvents may be unavailable in the mobile phase, and analysis time is extensive.

3.3 Hyphenated detection-based methods

After sample separation, the next step is to detect target compounds. Hyphenated detection-based methods include ultraviolet (UV) or diode-array detection (DAD), evaporative light scattering detection (ELSD), charged aerosol detection (CAD), MS and ELISA. Different detectors can be combined to obtain complementary information in one run, such as DAD-ELSD and DAD-MS. Table 5 compares the advantages and limitations of the various methods.

3.3.1 Ultraviolet (UV) or diode-array detection (DAD)—Analysis by UV continues to be the workhorse of the pharmaceutical laboratory. It is available in laboratories, inexpensive, and easy to use. Because of weak UV absorption by ginseng saponins, detection is usually set at 198–205 nm.^{3,50} Weak absorption affects the sensitivity because of the interference of other compounds that absorb short wavelengths. The choice of mobile phase and modifiers is also limited. UV detection system is collected with LC and CE for analysis of ginseng saponins. Instead of UV, DAD has become popular in many laboratories, since it monitors several wavelengths and provides “on-the-fly” spectra at once which maximizes sensitivity and assures peak purity.¹⁷⁷ UV and DAD are used to quantify ginseng saponins in extract and biofluids because of their accuracy and precision. In a recent

trend, UV/DAD is connected online with MS. UV/DAD-MS techniques yield information for rapid identification and quantification of constituents in ginseng.

3.3.2 Evaporative light scattering detection (ELSD)—The main problem encountered in performing UV analyses of ginseng is the relatively high level of baseline noise.^{13,55} ELSD is a universal, non-specific detector that provides a stable baseline even with gradient elution.¹⁴⁰ Furthermore, with volatile mobile-phase modifiers, such as HCOOH and CH₃COOH, better selectivity can be obtained.¹⁸ With ELSD non-chromophoric ginseng saponins such as 24(*R*)-pseudoginsenoside F11 can be analyzed.¹⁴⁰ Parameters to be settled for ELSD are the flow rate of the nebulizer gas and the drift tube temperature.^{178,179} The sensitivity of ELSD was found to be 5 times lower than that obtained with UV, with a minimum detectable concentration of 100–200 ng of ginseng saponins on the column.^{28,140}

HPLC-ELSD method has been developed for simultaneous monitoring of 12 ginseng saponins in different parts of *P. quinquefolius*,²⁸ 14 saponins in red *P. ginseng*,¹⁸⁰ and 19 saponins in black ginseng.¹⁷ Wan *et al.*¹⁸ described the chemical characteristics of three medicinal plants of the *Panax* genus by HPLC-ELSD. Quantitative evaluation of ginseng saponins using both UV and ELSD in American ginseng showed similar results, except that UV could not be applied to detect pseudoginsenoside F11.^{140,181} However, in light of its better sensitivity, easier handling and greater availability, UV is still recommended for the routine analysis of ginseng samples.

3.3.3 Charged aerosol detection (CAD)—CAD has been introduced as an alternative to ELSD for detection of non-UV and weakly UV-active compounds and for UV-absorbing compounds in the absence of standards. Both CAD and ELSD depend on mass, and the response generated does not depend on the spectral or physicochemical properties of the analyte. With a detection technique that generates universal response factors, there is potential for a single, universal standard for calibration against which all other compounds or impurities can be qualified.¹⁸² The main advantages of CAD are better sensitivity than an ELSD system, wider dynamic range, ease of use, and constancy of response factors.

Bai *et al.*¹⁸³ established an HPLC-CAD method for quantitative analysis of notoginsenoside R1, ginsenosides Rg1, Re, Rb1, Rg2, Rh1, and Rd in 30 batches of *P. notoginseng* samples. The sensitivity of CAD was higher than that of ELSD or UV. Wang *et al.*¹⁸⁴ compared LC-CAD, LC-ELSD and LC-UV methods to determine ginsenosides Rg1, Re, Rb1, Rc, Rb2, Rb3 and Rd in *P. ginseng*. CAD had higher sensitivity than UV and ELSD and better linearity and reproducibility than ELSD.

The limitations of CAD are the need for volatile buffers, non-volatility for analytes, and dependence on the changes in the eluent composition for response.

3.3.4 Mass spectrometry (MS) and multi-stage MS (MS/MS)—An emerging instrumental trend has been the application of MS and its hyphenation with chromatographic separation techniques. In ginseng and ginseng-treated samples, the most used of hyphenated techniques is HPLC-MS, and to a lesser extent, GC-MS. In a typical MS procedure, two key modules are the ion source, which can convert sample molecules in the gas phase into ions or move ions from the solution into the gas phase; and the mass analyzer, which sorts the ions by their masses. Several different ion sources are used depending on the information needed. Atmospheric pressure chemical ionization (APCI)^{185–187} and electrospray ionization (ESI),^{146,188–192} also known as “soft ionization,” are the most frequently used with LC in ginseng analysis. ESI is used more than APCI. Electron ionization or “hard ionization,” is the commonly used GC/MS technique.¹⁹³

Four types of mass analyzer are used for analysis of ginseng and ginseng-related samples, *i.e.*, quadrupole (Q),¹⁶⁰ ion trap (IT),¹⁹⁴ time-of-flight (TOF),^{147,148,164,195–197} and Fourier transform-ion cyclotron resonance (FT-ICR).^{198,199} Quadrupoles tend to be the simplest and least expensive mass analyzers. Quadrupole mass analyzers can operate in scanning mode or selected ion monitoring (SIM) mode. Because of scan mode's low sensitivity, SIM mode is used for quantifying and monitoring target compounds. SIM mode that provides information on a few ions is not suited for screening unknown compounds. Ion traps are also relatively inexpensive. Compared with losses with quadrupole, sensitivity losses during the full-scan mode can be avoided. An advantage of ion traps is that multiple stages of mass spectrometry can be performed without additional mass analyzers. Time-of-flight mass analyzers measure mass accurately and have high resolution and full-scan mass range. Accurate mass measurement, which gives the elemental composition of obtained ions, facilitates non-target component identification. Compared with quadrupole and ion trap, TOF is more expensive and has a narrower linear range for quantification. Like ion traps, FT-ICR mass analyzers can process mass reaction in multiple stages without additional analyzers. Like TOF, FT-ICR analyzers also have a wide mass range and a high mass resolution. They are, however, the most expensive among the mass analyzers.

Multi-stage MS (also called tandem MS or MS/MS or MSⁿ) combines the different designs of mass analyzers. MS/MS gives advanced structural information and has the sensitivity, specificity, and versatility. Triple quadrupole (QqQ),²⁰⁰ quadrupole ion trap (Q-IT),^{194,201} quadrupole time-of-flight (Q-TOF),^{164,196} or ion trap time-of-flight (IT-TOF)^{202,203} instruments are used for ginseng analysis. Hybrid systems such as Q-TOF-MS and IT-TOF-MS, which provide fragmentation information together with accurate mass measurements of product ions, are powerful tools for structural analysis. In MS/MS, collision-induced dissociation (CID) between analyte ions and neutral molecules forms fragment ions. Voltages are adjusted to increase collision energy to produce abundant fragmentation for predicting the structure of compounds. Another advantage of multi-stage MS is that nonanalyte ions are discarded in the first stage and thus sample cleanup is less. Multi-stage MS, however, is extremely expensive, which makes it impractical for most laboratories.

CID can also be achieved in single-stage mass spectrometers (commonly called in-source CID). The advantage of performing CID in single-stage instruments is simplicity and relatively low cost.²⁰⁴ One application is in-source CID in single-stage TOF-MS.²⁰⁵ The fragmentor in TOF-MS is crucial for efficient transmission of the ions to obtain the best balance between sensitivity and fragmentation.^{206,207} With dynamic adjustment of fragmentor from gentle ionization to intense fragmentation, molecular ions and abundant fragment ions are readily observed for unambiguous identification with similar or better performance than tandem MS.^{170,171} The disadvantage of CID in single-stage MS is that a specific precursor ion cannot be selected so the source of product ions cannot be determined.

One limitation of MS-based methods is the inherent lack of ionization of certain components. Another is that LC-MS ionization is instrument dependant, making it difficult to construct standard mass spectrum libraries for ginseng saponins. Equipment, especially for TOF-MS, must be calibrated accurately and regularly. As with most advanced technologies, generating useful and reliable data depend on the experience and the skill of the operators.

3.3.5 Enzyme-linked immunosorbent assay (ELISA)—ELISA is a biochemical test that measures the concentration of a compound using the reaction of an antibody or antibodies to its antigen. MAbs are often used. ELISA combines the advantages of the high specificity of antibodies and the great sensitivity of simple enzyme assays. Tanaka *et al.*^{114,208} developed a new immuno-staining ELISA method called Eastern blotting. In their

studies, ginseng extract was first subject to a silica gel TLC plate using developing solvents. After separation, the TLC plate was transferred to a polyvinylidene fluoride membrane or a polyethersulphone membrane. Then the membrane was treated by NaIO_4 and a buffer containing carrier protein like bovine serum albumin (BSA). The sugar moieties in ginseng saponins are oxidized by NaIO_4 to produce dialdehydes. Formed dialdehydes react with amino groups of lysine and/or arginine of the BSA that binds strongly to the adsorbent membrane. The membrane was further treated by a MAb and then a secondary antibody modified by enzyme. The aglycon part of the ginsenoside molecule was bounded by the corresponding MAb for visualization by the enzyme-labeled specific antibody. Finally, a substrate was added for staining, and NIH imaging software was used for quantification. The double staining of Eastern blotting for ginseng saponins can suggest the structure of unknown saponins, depending on the staining color related to the aglycone and the R_f value.

ELISA has been applied for single or simultaneous quantification of ginsenoside Re, Rb1, Rg1, Rd and Rc in various ginseng extracts.^{109,110,116,208,209} Eastern blotting has been used to identify chikusetsusaponins III and IV in *P. japonicas*.¹¹⁴ ELISA suffers from the complex preparation of MAbs, strong cross-reactivity caused by some structurally similar constituents, and narrow dynamic linear range for quantification.

4 Applications and challenges

As one of the most widely studied medicinal plants and herbs during the last decade, a great number of scientists have contributed to developing methods for analyzing ginseng. Advances in isolation and analysis technology have improved analysis time, efficiency, and method development. Application of these methods has been made to various aspects of ginseng and ginseng-related samples. We review species differentiation, quality assessment, and interaction with biological systems. Challenges in studying ginseng are also included.

4.1 Species authentication

Ginseng grows or is cultivated in different geographical locations. Although ginsengs are closely related in botanical taxonomy and contain similar chemical constituents, they differ in their effects on symptoms in people. In the herbal market, adulterants are sometimes part of samples.^{128,210} Because the roots of ginsengs are similar in appearance and many commercial ginseng products are sold as powder or shredded slices, identification of the origins of a product is not easy. Some new methods have been developed to differentiate ginseng species, mainly through chemical profiling or molecular markers.

4.1.1 Chemical profiling—Different ginseng species, ages, and geographical sources have respective pattern profiles. Metabolomic fingerprinting with multivariate data analysis is a reliable and comprehensive technology for authentication and identification. With application of HPTLC imaging,^{155,211} MS fingerprinting,^{147,148} or NMR profiling,^{120–122,212} ginseng saponins,^{147,148} amino acids,^{136,212} and polyacetylenes⁹¹ can account for variations.

The total ginsenoside content of *P. notoginseng* is higher than that of *P. quinquefolius* and much higher than that of *P. ginseng*. One difference among Asian ginseng, American ginseng and notoginseng is the unique presence of ginsenoside Rf in Asian ginseng.²¹³ Ginsenoside Rb2 is detected in abundance in Asian ginseng but in trace amounts in the other two ginsengs.¹⁸ Notoginsenoside R1 is a characteristic in notoginseng. Pseudoginsenoside F11 is a marker compound in American ginseng. F11 possesses the same molecular weight and has similar retention times as Rf.¹⁴¹ F11 without chromospheres cannot be detected by UV; HPLC-ELSD¹⁴⁰ or HPLC-MS¹⁶⁴ are capable of detecting both F11 and Rf. A UPLC/Q-TOF-MS was developed for 2-minute analyses of Rf and F11 in Asian ginseng-

adulterated American ginseng.¹⁶⁴ Establishing the ratios of Rg1/Re, Rg1/Rb1 and Rb2/Rb1 is also useful. Ratios less than 0.4 indicate American ginseng; higher ratios are characteristic of Asian ginseng.^{140,164} One exception is the high Rg1/Rb1 ratio found in wild American ginseng.²¹⁴ Other spectrometric methods have been reported for effective differentiation, such as 2-D correlation FTIR spectroscopy¹²⁸ and 2-D NMR spectroscopy.¹²²

4.1.2 Molecular marker—Inspecting genetic makeup is an alternative to defining botanical identity. The genetic makeup of an herbal species does not vary with its physical form or physiological and external conditions. With advances in molecular biotechnology, genetic tools are reliable for authentication of ginseng species at the DNA level. In 2003, Hon *et al.*²¹⁵ reviewed genetic methods used in the authentication of *Panax* species. These genetic tools included arbitrarily-primed PCR,²¹⁶ random amplified polymorphic DNA (RAPD),²¹⁷ sequence characterized amplified region (SCAR),²¹⁸ low-Cot DNA fingerprinting,²¹⁹ restriction fragment length polymorphism (RFLP),²¹⁷ amplified fragment length polymorphism (AFLP),²²⁰ mutant allele specific amplification,²²¹ direct amplification of length polymorphism (DALP) analysis,²²² and microsatellite marker technology.¹²

Zhu *et al.*²²³ revealed the phylogenetic relationship among thirteen *Panax* taxa by DNA sequence analysis on the chloroplast *trnK* gene and nuclear 18S rRNA gene. Subsequently, they developed a multiplex amplification refractory mutation system for the identification of five commonly used *Panax* species, *i.e.*, *P. ginseng*, *P. japonicus*, *P. quinquefolius*, *P. notoginseng* and *P. vietnamensis*.²²⁴ Qin *et al.*¹⁷⁴ developed a microchip electrophoresis method coupled with a PCR–short tandem repeats technique to discriminate between American and Asian ginseng and cultivated and wild American ginseng. Diao *et al.*²²⁵ developed a PCR-RFLP and amplification refractory mutation system for identifying *P. ginseng* from its adulterants. *P. ginseng* was successfully identified in single herbal medicines or preparations containing diverse components. Sasaki *et al.*²²⁶ developed a loop-mediated isothermal amplification PCR (LAMP) method that could be conducted under isothermal conditions. Lu *et al.*²²⁷ established nested PCR and DNA sequencing methods to identify *P. ginseng* in preparations. With this method, analytical time and costs were reduced. Table 6 summarizes the advantages and limitations of molecular biology methods.

4.2 Quality assessment

As global market demands for ginseng increase, quality control has become important. Unlike chemical drugs with a single entity, ginseng, like fruits and vegetables, has many constituents. Natural variations in soil and climate and preparation procedures affect the safety and batch-to-batch uniformity of ginseng products. Monitoring for quality should be done at selected steps for the dynamic coordination of all links. Good ginseng materials and manufacturing processes depend on good agricultural practices (GAPs) and good manufacturing practices (GMPs). Independent quality evaluation is an integral part of ginseng production. To standardize a ginseng extract, metabolomic fingerprinting is performed or marker compound(s) are determined.

4.2.1 Plant metabolomic fingerprinting—Metabolomic fingerprinting has been accepted internationally for assessment of herbal medicines. The basic concept of the fingerprint pattern is to consider the whole chromatographic profile or the whole spectrum as a feature.¹ The entire pattern of compounds can then be evaluated to determine not only the presence of desired markers but also the complete set of ratios of all detectable analytes.¹⁵⁵ Fingerprinting can match the inherent holistic properties of herbs and offers a logical tool for species authentication, quality evaluation, and lot-to-lot consistency.

Table 7 lists some examples of ginseng analysis using the metabolomic fingerprinting technique. The analytical tools include MS-based chromatography and NMR-based spectroscopy.¹¹⁸ Various constituents can be identified and quantified at low concentrations making MS a sensitive analytical tool for metabolic fingerprinting.^{136,146,148} Abundant fragment ions by multi-stage MS and high mass accuracy by TOF-MS offer structural information and the possibility of identifying unknown components. In NMR-based fingerprinting, the ¹H NMR spectrum provides a wealth of chemical information on ginseng.^{212, 238} NMR has the highest reliability in metabolomics.¹¹⁸ Apart from ginseng saponins, other important metabolites in ginseng like carbohydrates, amino acids and organic acids can be elucidated based on their chemical shifts in the ¹H NMR spectra. The concentration of each metabolite in the sample can be easily calculated from the integration of the signals in the spectra. There is no requirement for calibration curves to convert signal intensity into concentration. When the spectral complexity and signal overlap in the ¹H NMR spectra are high, the chemical structures of metabolites can be confirmed by diverse 2D-NMR spectroscopy.¹²²

When data obtained are analyzed by statistical methods, all relevant information must be extracted from a complex data set. Multivariate data analysis is used to recognize patterns and find discriminating signals. Among the multivariate data analysis, PCA^{146,238} and partial least squares-discriminant analysis (PLS-DA)^{148,212} have become the routine processing steps for raw analytical data. Marker signals for differentiation can then be identified, and the upper and lower limits of the signals are defined for quality control of ginseng.

4.2.2 Marker compound(s) quantification—Ginseng saponins are selected as the marker compound for the quality control and standardization of ginseng in official monographs. In 1993, the American Botanical Council started the Ginseng Evaluation Program.¹³ Ginsenosides Rb1, Rb2, Rc, Re, Rg1 and Rd were considered markers for *P. ginseng*. In 1999, the World Health Organization published monographs on selected medicinal plants (<http://apps.who.int/medicinedocs/en/d/Js2200e/19.html#Js2200e.19>). In that document, ginsenoside Rg1 of not less than 1.5% was selected as the marker compound. In 2002, the European Pharmacopoeia published a monograph on *P. ginseng* roots that chose ginsenosides Rb1 and Rg1 in amounts not less than 0.4% as markers. In 2004, the American Herbal Pharmacopoeia published monographs for *P. quinquefolius* and *P. ginseng* roots and extract. Ginsenosides Rb1, Rb2, Rc, Re, Rg1 and Rd were assigned as the marker compounds. Their total content, as evaluated by HPLC-UV, could not be less than 3% for *P. ginseng* extracts, 4% for *P. quinquefolius* roots, and 10% for the extracts. *P. ginseng* roots could not contain less than 0.2% Rg1 and 0.1% Rb1. In 2009, Health Canada published a monograph on *P. ginseng* (http://www.hc-sc.gc.ca/dhp-mps/prodnatur/applications/licen-prod/monograph/mono_panax_ginseng-eng.php). It stipulated that ginseng preparations must contain 0.024–0.294 g total ginseng saponins per daily dose.

For a comprehensive evaluation, laboratory methods use as many markers as possible. Kim *et al.*¹⁸⁰ evaluated white and red *P. ginseng* through simultaneous determination of 14 ginseng saponins by HPLC-ELSD. Sun *et al.*¹⁷ studied Korean white, red and black *P. ginseng* by measuring 19 ginseng saponins. Qian *et al.*²³⁹ used HPLC-DAD and MS for simultaneous determination of flavonoid, saponins and polyacetylenes in ginseng samples. Comparisons were made of ginseng for the effects of different origins, ages, cultivation methods, plant parts, and commercial manufacturer.^{3,104,139,214,240,241} Individual and total ginsenoside contents varied in different commercial ginseng products.¹³⁹ Ginsenoside profiles and content also varied depending on the plant parts tested. The contents, based on decreasing order, ranked as follows: leaf > root-hair > rhizome > root > stem.²⁸

To quantify marker compound(s), high quality ginsenoside libraries are required. Lack of reference standards, in spite of improved methods for determining compounds, makes it difficult to evaluate ginseng products. Some reference compounds are commercially available, but their cost and limited supply cannot meet the ginseng demand worldwide. Since most chemical compounds in an herb have a similar skeleton, researchers have introduced quantitative correction efficiencies that use a universal compound for calibration against other structurally similar components. In one study, a commercially available compound verticinone was used to quantify other twenty-six steroidal alkaloids.¹⁹² Compared with absolute quantitative determinations, an average accuracy of 98.5% was obtained. We attempted to use Rb1 and Rg1 to determine PPD-type and PPT-type ginseng saponins, respectively. Method validation and robustness tests are still under investigation.

Because the active compounds are unknown, the common practice is to select one or more major ginsenosides like Rb1 and Rg1 as active or markers. The drawback is less linkage between quality and activity. A combined method of quantifying marker compounds with profiling metabolomic fingerprinting might be a better way to link metabolic profiles with activity and to assure batch-to-batch consistency.

4.2.3 Online structural characterization—A challenge in plant metabolomic fingerprinting is the secondary metabolite identification. Traditionally, isolation to NMR-based identification has been the best strategy for absolute determination of structures. However, many constituents are not easily isolated and most reference compounds are unavailable in laboratories. The coupling of HPLC and MS has helped online structural characterization and rapid identification of compounds in complex herbal extracts.^{170,206,207,242} The application of tandem MS or, more recently, IT-TOF-MS and Q-TOF-MS, also has advanced the online identification of constituents in ginseng samples.^{186,187,190,243,244}

Fig. 7 shows one method for rapid identification of targeted and untargeted ginseng saponins. Available reference compound(s) are used to obtain characteristic fragmentation pathways and diagnostic ions of ginseng saponins. The diagnostic ions and pathways represent the mother nucleus and some substituent groups of saponins. For instance, our ongoing investigations revealed that all PPD-type ginseng saponins produce three common skeleton ions by TOF-MS, *i.e.*, m/z at 407.37, 425.37 and 443.38. Sugar types, numbers and linkage sequences also can be determined by simultaneous or successive losses of sugar moieties and sugar cross-ring cleavages.¹⁸⁹

Then, the complex ginseng extract is tested. Chromatographic separation by LC is important for subsequent identification. Diagnostic ions and fragmentation mechanisms from reference compounds are basic for screening known and unknown compounds in ginseng extract. Target compounds can be unequivocally identified by comparison of accurate retention times, molecular ions, and characteristic fragment ions with those of the reference compounds. Identification of untargeted ginseng saponins is complex. The first step is to determine the molecular ions of chromatographic peaks in ginseng extract. Then, different criteria are used to calculate the accurate molecular formula of each peak: acceptable accuracy threshold, the general rule of the number of nitrogen atoms, the dibasic ester (DBE) index, and “show isotopic” function. With the obtained molecular formula, screening for a hit is subsequently performed against various chemical databases. From the candidates collected, the characterized diagnostic ions can then be used for locating the candidates that contain such a substructure and/or substituent groups. Finally, a most possible structure can be determined from these candidates by fragmentation screening and matching.

Online structure characterization has been used to identify 152 saponins from *P. notoginseng* by LC/ESI-MSⁿ,¹⁸⁸ 35 ginseng saponins in *P. ginseng*¹⁸⁷ and 30 ginseng saponins in *P. quinquefolius*¹⁸⁶ were identified by LC-APCI-MS.

4.2.4 Dynamic chemical transformation—Dynamic structural and concentration changes of ginseng saponins take place during storage, processing, and extraction procedures. Transformation was observed in the class of malonyl ginseng saponins and neutral ginseng saponins (commonly referenced saponins).

Malonyl ginseng saponins, also called acidic ginseng saponins, are compounds with a malonyl group attached at the 6"-position of the glucosyl moiety. As early as 1983, four malonyl ginseng saponins, namely the malonyl-ginsenosides Rb1, Rc, Rb2 and Rd, were isolated from *P. ginseng*²⁴⁵ and later from *P. quinquefolius*.²⁴⁶ Malonyl-ginsenoside Ra3⁷² and malonyl-notoginsenoside R4⁷³ were recently isolated from the fresh roots of *P. ginseng*. Approximately fifteen malonyl ginseng saponins were found in ginseng extract by HPLC-MS/MS.^{186,187,194,244} Malonyl ginseng saponins, more polar and water soluble than neutral ginseng saponins, are extremely unstable and susceptible to thermal degradation during drying, storage, heating and extraction.^{29,247,248} In dried ginseng roots, malonyl ginseng saponins decreased as air temperature increased.²⁹ Malonyl ginseng saponins are absent or found in trace elements in red ginseng prepared by a steaming or heating process.²⁴⁸ Cold alcohol is recommended to extract malonyl ginseng saponins, and 40% ethanol has maximum extraction efficiency.²⁹ Malonyl ginseng saponins are thus not often found in commercial products prepared by hot extraction processes.¹⁹⁰

Malonyl-ginseng saponins make up much of the total saponins in ginseng. Their concentration accounts for approximately one-third of the total ginsenoside content in American ginseng roots, but relatively little in Asian and notoginseng.^{249,250} Malonyl-ginsenoside Rb1 is the most abundant.¹¹ The profiles of malonyl-ginseng saponins differ among different species.^{194,249} Because of their instability and the lack of suitable reference compounds, determining their profiles presents a challenge. Since they are usually invisible on HPLC-UV chromatograms, standard assay procedures may underestimate ginsenoside content by ignoring malonyl ginsenosides. Use of phosphate buffer is important for resolution of malonylated and other esterified ginseng saponins.^{247,251} If ELSD or MS is used for detection, ammonium acetate,²⁴⁴ ammonium formate,¹⁹⁰ and acetic acid¹⁹⁴ are recommended instead of phosphate. An alternative strategy is to determine malonyl ginseng saponins by an indirect two-step method that hydrolyzes these acidic saponins into their respective neutral saponins with KOH. However, the results may give only a rough estimate because other derivatives also might be transformed. Both APCI-MS^{186,187} and ESI-MS^{16,194,244} are useful for characterization of malonyl-ginsenosides in ginseng extracts. Fig. 8 shows the MS characterization of malonyl-ginsenoside Rb1 by tandem MS in negative ion mode. Fig. 8A lists the sequential mass spectra of deprotonated malonyl-ginsenoside Rb1, and Fig. 8B suggests the fragmentation pathways. The presence of a free carboxylic acid group means that malonyl ginseng saponins can be rapidly screened by displaying neutral losses of 44 Da (CO₂), 42 Da (CH₂=CO) and 60 Da (CH₃COOH).

Neutral ginseng saponins, commonly stable during drying and storage conditions,²⁵² degrade to new compounds under intensive heating or steaming. The conversion means that ginsenoside profiles of unprocessed and processed ginseng are different.^{52,55,253,254} As shown in Fig. 9, during steaming or heating, the polar ginseng saponins decreased, and less polar ginseng saponins increased. The structural changes in ginsenosides during the process are elimination of sugar chains and isomerization of the hydroxyl configuration at C-20 of the aglycones.⁵² The protopanaxadiol (PPD)-type ginsenosides selectively eliminate the C-20 sugar chain to produce Rg3.255.256 Rg3 is further transformed to Rk1 and Rg5 by

dehydration.³ A small amount of Rh2 observed in red ginseng implies that the elimination of C-3 sugar residue is relatively difficult in processing.⁵⁵ The protopanaxatriol (PPT)-type ginsenosides first lose the C-20 sugar residue and subsequently their terminal sugar unit at C-6 to form Rg2 and/or Rh1.²⁴⁸ Rh1 is further converted to Rk3 and Rh4 by dehydration at C-20. In summary, the elimination of sugar chains at C-20, then at C-6 or at C-3, and a subsequent dehydration reaction at C-20 are the effects of steaming.

4.2.5 Pesticide determination—Pesticide and heavy-metal contamination are safety concerns with ginseng products. Ginseng roots require 3–6 years of cultivation until harvest. During this time, disease control with pesticides against insects and environmental pollution lets the ginseng plants accumulate chemical contaminants. Multiresidue pesticides such as organochlorine,²⁵⁷ organophosphorus,¹⁹³ organohalogen,¹⁶⁰ and pyrethroid²⁵⁸ are found in excess of limits of detection or even over maximum residue limits in ginseng products. Few studies have been devoted to investigating contamination of ginseng with pesticides or toxic metals.

The steps in pesticide analysis are extraction, cleanup, separation and detection.²⁵⁹ Ginseng samples are blended with organic solvent alone, solvent mixtures, or water. Acetone,^{159,260} acetonitrile,^{159,261} methanol,²⁶² and ethyl acetate¹⁶⁰ are used. Pesticide extraction methods are Soxhlet extraction,^{257,263} solid-phase extraction (SPE),^{159,261} matrix solid-phase dispersion,^{61,196} and SFE.^{264,265} Traditional cleanup procedures fractionate extracts based on polarity, as in liquid-liquid partitioning^{257,258} and column chromatography, or adsorption chromatography using florisil^{196,260,266,267} and neutral alumina.^{260,268} SPE^{265, 268} and gel permeation chromatography¹⁶⁰ have become popular. GC is used for separation of large-scale multiresidue pesticides.^{159,160,266} Because some pesticides are highly polar, have low volatility and/or are thermo-labile, LC is used for analysis.⁶¹ Element-selective for electron capture,^{258,261,263,267} nitrogen-phosphorus,^{258,261} and flame photometrics are used.¹⁹³ The detectors are sometimes connected to combine results in a single run.^{261,263,267} MSⁿ and TOF-MS are the most used detection techniques.^{159,160,267,268}

In a survey of Asian and American ginseng extracts by Durnat *et al.*,²⁶⁹ 13 out of 30 samples contained pesticides greater than the maximum residue limits. When Hayward *et al.*^{159,160} analyzed 170 organohalogen and organophosphorous pesticides and their isomers and metabolites in dried ginseng roots, seven products contained combinations of pesticides.

4.3 Interaction with biological system

4.3.1 Pharmacokinetic parameters—Pharmacokinetic (PK) evaluation of ginseng is challenging because of diversity of ginseng saponins. Table 8 summarizes PK investigations on ginseng and ginseng saponins. Major parameters, $T_{\max}$, $T_{1/2}$, and F or bioavailability, are included.

Sample pretreatment and analysis is key in PK studies. A simple sample pretreatment uses protein precipitation and liquid-liquid extraction. Another sample pretreatment method is SPE that can remove interference and matrix effects. SPE suffers from lower extraction recoveries than liquid-liquid extraction. For analysis, LC-UV and LC-MS have been developed to assay ginseng saponins in biological samples. LC-UV is convenient but has low sensitivity with a lower limit of quantification (LLOQ) greater than 1000 ng/ml. LC-MS and MS-MS are more sensitive (LLOQ, 1–10 ng/ml) and specific. Segmental and SIM have better sensitivity and wider dynamic ranges than SIM alone.²⁹² PPD and PPT aglycone have weak ionization efficiency in MS compared with saponins that process sugar moieties.²⁹⁰ Digoxin is generally selected as the internal standard for MS quantification.

The oral bioavailability of PPD-type saponins (ginsenosides Ra3,²⁹³ Rb1,²⁸⁹ Rd,²⁷¹ Rg3,²⁷⁸ and Rh2²⁹⁴) and PPT-type saponins (ginsenosides Rg1,²⁷⁴ Re,²⁷⁰ Rh1,²⁷⁵ and notoginsenoside R1289) is less than 5%.²⁹⁵ Excretion of ginseng saponins at 0.2%–1.2% can be expected in human urine.²⁹⁶ Extensive metabolism in the gastrointestinal tract,^{279,297} poor membrane permeability,²⁹³ and lowered solubility of deglycosylated products²⁹⁸ limit intestinal absorption of ginseng saponins. Higher oral doses might saturate metabolism and increase the bioavailability.^{284,288} PPT-type saponins have better bioavailability than that of PPD group,^{288,289} perhaps because PPD-type saponins degrade faster than the PPT-type. IH-901 or compound K, an intestinal bacterial metabolite of PPD-type saponins, contributes to low bioavailability because of biliary excretion and hepatic metabolism via esterification with fatty acids.^{283, 284} Changing the pharmaceutical formulation may improve bioavailability. For instance, micronized Rh2 has a doubled bioavailability;²⁹⁸ the inclusion complex of IH901 and the water-soluble agent β -cyclodextrin offers 1.9-fold higher bioavailability than pure IH901 powder.²⁹⁹ The bioavailability of PPD aglycone and 25-OH-PPD is improved because of less metabolism and favorable membrane permeability.^{285–287}

The time to reach maximum concentration ($T_{\max}$) in plasma and tissues is generally less than 2 h, indicating that ginseng saponins are rapidly absorbed and readily distributed in the tissues.^{276,300} Levels of a ginseng saponin in the system depend on its elimination half-life ($T_{1/2}$). Most ginseng saponins and their deglycosylated products were excreted by the biliary system through active transport, which affected their $T_{1/2}$ values and systemic exposure.²⁹³ Time curves of ginseng saponins exhibited distinct multiple peaks after oral administration, indicating the involvement of enterohepatic recirculation.²⁸⁴

Tissue disposition demonstrated that liver and bile are responsible for systemic clearance of ginseng saponins from the circulation.^{283,284,300} Hepatic cytochrome P450 catalyzed ginsenoside metabolism has been described, and CYP3A4 catalyzed oxygenation metabolism played an important role in the hepatic disposition of ginsenosides. Table 8 shows that Ra3, Rb1, Rc and Rd have significantly longer $T_{1/2}$ values (18.57–39.4 h) than other ginseng saponins (0.2–4.77 h). Attachment of more sugar moieties in the PPD-type ginsenosides Ra3, Rb1, Rc and Rd blocks their access to biliary transporters and slows biliary excretion.²⁹³ Some PK parameters shown in Table 8 are far from mean values, such as $T_{\max}$ and $T_{1/2}$ of 25-OH-PPD and Rd. Whether this variation is caused by different factors or experimental conditions requires further evaluation.

4.3.2 Metabolic pathways—The metabolic pathways of PPD-type and PPT-type ginseng saponins on animals and humans are proposed in Fig. 10A and Fig. 10B, respectively. Identification of ginsenoside metabolites is generally based on MSⁿ experiments (LC-Q-IT-MS) and/or confirmation by accurate mass measurements (LC-Q-TOF-MS). Comparison of the MSⁿ fragment ions of the parent compounds with those obtained from the metabolites provides valuable clues to the parts of the molecule that undergo modification by degradation or combination or that remain unchanged. Sometimes, derivatization of functional groups or isolation of the metabolites from biofluids for further NMR assays can be performed.

The conversion of ginseng saponins in the gastrointestinal tract also has been studied using *in vitro* models such as acid conditions, enzymes, intestinal bacteria and microsomes.^{198,301} Because of high ginsenoside concentration used in the *in vitro* studies, the metabolic profiles may differ from *in vivo* results.³⁰² The main metabolic pathways *in vivo* include deglycosylation reactions in ginseng saponins by intestinal bacteria via stepwise cleavage of the sugar moieties.^{293,295,297} Oxygenation by intestinal and hepatic CYP450 enzymes is another metabolic pathway.^{275,281,303} Oxygenation generally occurred on the top-right aliphatic chain.²⁸² Parental metabolites that could be further esterified with fatty acids in

liver and tissues sustain fatty acid conjugates longer in the body.³⁰⁴ After oral administration of *P. notoginseng* extract to rats, the most abundant metabolites in rat feces were IH-901, Rg1 and F1.293 PPD and PPT aglycones were detected in low quantities in feces and not detected in plasma, bile and urine.²⁹³ After human volunteers ingested Ginsana G115 capsules (4% ginseng saponins), IH-901, Rh1 and F1 were the major metabolites found in the systemic circulation and excreted in urine.²⁹⁷ In some publications, 20(*S*)- and 20(*R*)-ginsenosides were not differentiated.^{278–281} Only a few studies have compared the metabolism and pharmacokinetics of 20(*S*)- and 20(*R*)-ginsenosides.²⁷⁶

Because of competitive absorption and metabolism among various components, differences can be observed between the administration of a single ginsenoside and of ginseng extract. The metabolic profile of a ginsenoside can also vary with different methods of administration. For instance, Rb1 is the dominant metabolite after intravenous injection of Rd, and Rg3 is the major metabolite after oral administration of Rd.²⁷³

4.3.3 Endogenous metabonomics—Metabonomics is the comprehensive systematic study of metabolite levels and changes in organisms over time as a consequence of stimuli such as disease or pharmaceutical interventions.³⁰⁵ Metabonomics has become a tool to evaluate the curative effects and the safety of herbal medicines including ginseng.^{138,306–308} Through metabonomic analysis, biomarkers are recognized and the basic dynamic variations of metabolic responses to herbal medicine intervention are revealed. With such information, the knowledge of a disease may improve, onset of disease may be predicted, and the clinical efficacy, safety, and mechanisms of action of complex herbal medicines can be evaluated.

The principal analytical techniques for metabonomic studies are based on NMR spectroscopy and mass spectrometry. All metabonomic studies result in complex, multivariate datasets that require visualization software and chemometric and bioinformatic methods for interpretation. Wang *et al.*¹³⁸ reported metabolic regulatory network alterations in response to acute cold stress in the rat and changes after ginsenoside intervention. Urinary metabolite profiling using GC-MS with multivariate statistical techniques revealed biochemical changes of these metabolites and demonstrated the protective effect of total ginseng saponins. Several metabolic pathways were identified in metabolic regulation and compensation to restore homeostasis. Wang *et al.*³⁰⁹ performed a metabonomics study on the effects of the ginsenoside Rg3 on liver-tumor-bearing rats. They combined column-switching with the orthogonal selectivity of hydrophilic interaction chromatography and reversed-phase chromatography. The administration of a single, high dose of Rg3 changed the metabolic pattern in cancer rats during the three days studied, and seventeen biomarker candidates were identified.

For such studies, complex data must be interpreted and combined with analytical methods. One problem is that the number of metabolites produced by a given system is difficult to predict. Given the potential interest in this field, metabonomics might be useful for a comprehensive evaluation of the systemic clinical efficacy, safety, and mechanism of action of ginseng.

4.3.4 Interaction with biomacromolecules—The interaction between small molecules (*e.g.*, ginsenosides) and biomacromolecules (*e.g.*, proteins and DNA) is one paradigm in pharmaceutical research.³¹⁰ To discover target candidates for new drugs, the mechanisms and binding parameters (*e.g.*, dissociation constants) between ginseng constituents and biomacromolecules should be characterized. Various strategies have emerged for the systematic and high-speed characterization of the interaction of small molecules with a biomacromolecule at the molecular level.³¹⁰ Most of the techniques study the simple binding system in which one constituent of ginseng binds to one biomacromolecular

receptor.^{311, 312} There is a trend towards library screening (*e.g.*, ginseng extract) or identifying interacting counterparts against known or unknown libraries.^{310,313}

Zou *et al.*^{314,315} proposed biological fingerprinting to investigate the binding characteristics of natural products with target biomacromolecules that are free or immobilized in affinity chromatography. Bioactive compounds that specifically bind target biomacromolecules can be screened from complex herbal medicines. Li *et al.*¹ developed holistic methods and integrated evaluation models to investigate biomacromolecule-binding compounds in ginseng preparations. Crude extract is divided into two major fractions, *i.e.*, fraction unrelated to bioactivities and fraction related to bioactivities. The latter fraction is then characterized by online HPLC-MS. Structural information and binding parameters of each captured compound can be determined. A good example is the recent development of 2-D turbulent flow chromatography (TFC) coupled on-line to LC-MS for solution-based ligand screening against multiple proteins, shown in Fig. 11.³¹³ This technique is an efficient tool for high-throughput screening of library ligands with low-to-high binding affinities and for evaluating the structure-activity relationship.

Wang *et al.*³¹⁶ screened anti-platelet aggregation agents from *P. notoginseng* using human platelet extraction and HPLC-DAD-ESI-MS/MS analysis. Five compounds that bind with human platelets were detected, and four were identified as adenosine, guanosine, ginsenoside Rh1 and F1. Liu *et al.*³¹⁷ studied the interaction of total saponins of *P. notoginseng* with human serum albumin (HSA) by FTIR spectroscopy. Ginseng saponins were shown to combine with HSA through C=O and C=N groups of polypeptide chain. The ginsenoside-protein combination caused a significant loss of α -helix structure and changes of the tyrosine residues in protein. Dasgupta *et al.*³¹⁸ compared the effects of five different ginseng species on serum digoxin measurement by immunoassays. Digibind, an antidigoxin antibody, can bind free digoxin-like immunoreactive components of ginseng. Thus, ginsenoside-biomacromolecules interaction reveals complex interconnected biological networks, which may help with discovery of new protein targets and drug candidates.

5 Concluding remarks and perspectives

Compounds isolated from ginseng are an abundant source of chemical diversity from which to discover active molecules. Synthesis or biosynthesis is the preferred way to produce novel compounds for pharmacological activities. Synthesis also helps us understand structure-activity relationships. Besides sample preparation and purification steps, current isolation protocols often comprise *in vitro* assays, which are frequently coupled on-line to HPLC or MS systems.

A major challenge of ginseng research is the complexity of chemical constituents, especially polar macromolecules. However, technical advances in analytical tools are promising. Chromatography is trending toward faster and more comprehensive separations, indicated by wide applications of UPLC, HILIC and multidimensional chromatography. Recent innovations have made mass spectrometers more sensitive and selective, and MS and tandem MS can now be applied in innumerable analytical fields of ginseng research.

One way to investigate ginseng is to view the extract as an indivisible whole, a self-organized and interactive multicomponent. Plant metabolomic fingerprinting and metabonomics of ginseng-treated biological samples are holistic approaches to quality control and activity evaluation, respectively. Other better integral analyses are needed to measure complex ginseng constituents, their metabolites and other related macromolecules.

Ginseng analysis requires expertise in chemistry, chemometrics, biology, and bioinformatics. With the exponentially increasing amount of data from ginseng plant and

ginseng-treated biological samples, collaboration among chemists, biologists, and computer scientists is important. It appears promising that new insights of ginseng will be gained in drug discovery and clinical utility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
Procedures for isolation of ginseng saponins from ginseng plant materials.

Fig. 2.
Chemical structures of recently isolated dammarane-type saponins from ginseng, compounds 1–71.

Fig. 3.
Chemical structures of recently isolated dammarane-type saponins from ginseng, compounds **72–95**.

Fig. 4.
Chemical structures of recently isolated dammarane-type saponins from ginseng, compounds **96–123**.

Fig. 5. Online chromatographic knockout technique, modified from Ref. ¹⁰¹. An example is shown for American ginseng, ginsenosides Re and Rb1 are knocked out simultaneously.

Fig. 6. Immunoaffinity chromatographic method for preparation of Rb1 knockout extract from *P. ginseng* extract, modified from Ref. ¹¹⁴. The red spot in the upper right shows Rb1. Lines 1, 2, and 3 in thin layer chromatography (TLC) plate located in the low right indicate crude extract, knockout extract, and purified Rb1, respectively.

Fig. 7.
General strategy for identification of ginseng saponins in ginseng samples by mass spectrometry (MSⁿ and accurate mass experiments).

Fig. 8. Mass spectrometry (MS) characterization of malonyl-ginsenoside Rb1 by tandem MS in negative ion mode. **(A)** Sequential mass spectrometric analysis of deprotonated malonyl-ginsenoside Rb1. **(B)** Interpretation of the fragmentation pattern. Experimental conditions were shown in Ref. ¹⁹⁴.

Fig. 9. HPLC-UV chromatograms of ginseng roots and chemical degradation in steaming process, modified from Ref. ²⁵⁶. **(A)** Ethanol extract of American ginseng, Asian ginseng and notoginseng root. **(B)** Ethanol extract of steamed ginseng roots at 120 °C for 4 h. Chromatographic separation was performed on a Prodigy ODS column. Detection wavelength was set at 202 nm. The mobile phase used acetonitrile and water for a gradient elution.

Fig. 10.

Proposed *in vivo* metabolic pathways of ginseng saponins. **(A)** protopanaxadiol-type saponins. **(B)** protopanaxatriol-type saponins. “→” denotes major pathways; “---→” denotes additional pathways. [P]: metabolites detected in plasma; [U]: metabolites in urine; [F]: metabolites in feces; [B]: metabolites in bile. ig: metabolites after oral administration; iv: metabolites after intravenous injection.

Fig. 11.

Schematic diagram of the two-dimensional turbulent flow column-switching LC/MS apparatus, modified from Ref. ³¹³. Ginseng extract or ginseng saponin is incubated with the target biomacromolecule. The first turbulent flow chromatography (TFC) that only retains low-molecular-weight analytes is used to separate free compounds and protein-ginsenoside complex. The captured complex is disrupted in reaction wire by dissociation solution. The compounds released are separated and enriched from biomacromolecules by the second TFC. Online-LC/MS is used to determine the structural information and affinity parameters of the binding compounds.

Table 1

Characteristics of nine extraction techniques for saponins from ginseng plant materials

Technique ^a	Core technology	Extraction efficiency	Operation	Speed	Automation	Cost	Ref.
HRE	Heating	Low	Simple	Low	Difficult	Very low	14
Soxhlet	Soxhlet extractor	High	Moderate	Very low	Possible	Low	15
SAE	Mechanical shaking	High	Simple	Moderate	Difficult	Low	16
UAE	Ultrasound	Low	Simple	Low	Difficult	Low	17
PLE	High pressure	Moderate	Complicated	Rapid	Easy	Moderate	18
MAE	Microwave	Moderate	Simple	Rapid	Difficult	Low	19
HP-MAE	High pressure and microwave	Moderate	Simple	Rapid	Difficult	Low	20
PHWE	High pressure and hot water	Moderate	Complicated	Moderate	Easy	Moderate	21
SFE	Supercritical fluid	Moderate	Complicated	Rapid	Easy	High	15

^a Abbreviations: HRE: heat reflux extraction; SAE: shaking-assisted extraction; UAE: ultrasound-assisted extraction; PLE: pressurized liquid extraction; MAE: microwave-assisted extraction; HP-MAE: high-pressure MAE; PHWE: pressurized hot water extraction; SFE: supercritical fluid extraction.

Table 2

Recent applications of HSCCC to saponin isolation from ginseng products

Material	Solvent system ^a (volume ratio)	Detection ^b	Obtained compound	Isolation efficiency ^c	Ref.
<i>P. notoginseng</i> , root	Hex- <i>n</i> -BuOH-H ₂ O (3 : 4 : 7)	TLC	Ginsenosides Rb1, Re, Rg1 and notoginsenoside R1	157, 13, 56, and 17 mg of Rb1, Re, Rg1 and R1 from 283 mg MeOH extract of five tablets	38
<i>P. ginseng</i> , root	CH ₂ Cl ₂ -MeOH-NH ₄ OAc-iPrOH (6 : 2 : 4 : 3)	ELSD	Ginsenosides Rf, Rd, Re, and Rb1	10.7, 11.0, 13.4 and 13.9 mg of Rf, Rd, Re and Rb1 from 480 mg enriched fraction by macroporous resin	39
<i>P. notoginseng</i> , root	CHCl ₃ -MeOH-2-BuOH-H ₂ O (5 : 6 : 1 : 4) EtOAc- <i>n</i> -BuOH-H ₂ O (1 : 1 : 2)	ELSD	Ginsenosides Rg1, Rd, Re, Rb1 and notoginsenoside R1	Not provided	40
Red <i>P. ginseng</i> , steamed root	CH ₂ Cl ₂ - MeOH-H ₂ O-iPrOH (6 : 6 : 4 : 1)	ELSD	Ginsenosides Rg3, Rk1, Rg5 and F4	32.2, 26.6, 28.6 and 8.1 mg of Rg3, Rk1, Rg5 and F4 from 350 mg enriched fraction by RP-C ₁₈ column	41
<i>P. ginseng</i> , root	EtOAc-iPrOH-0.1% formic acid H ₂ O (3 : 1 : 5)	UV	Ginsenoside Ro	61 mg Ro from 100 mg enriched sample by normal-phase MPLC	42

^a Abbreviations: Hex: *n*-hexane; BuOH: butanol; CH₂Cl₂: methylene chloride; MeOH: methanol; NH₄OAc: ammonium acetate; iPrOH: isopropanol; CHCl₃: chloroform; EtOAc: ethyl acetate.

^b Abbreviations: TLC: thin layer chromatography; ELSD: evaporative light scattering detection; UV: ultraviolet.

^c Abbreviations: RP: reversed-phase; MPLC: medium-pressure liquid chromatography.

Table 3

Isolated ginseng saponins reported from 2000–2010

No.	Name	Formula	Plant Material	Ref.
1	Floralginsenoside M	C ₅₃ H ₉₀ O ₂₂	Flower buds of <i>P. ginseng</i>	57
2	Floralginsenoside N	C ₅₃ H ₉₀ O ₂₂	Flower buds of <i>P. ginseng</i>	57
3	Floralquinqenoside E	C ₅₃ H ₉₀ O ₂₂	Flower buds of <i>P. quinquefolius</i>	35
4	Ginsenoside Rh5	C ₃₇ H ₆₄ O ₉	Roots and rhizomes of <i>P. vietnamensis</i>	58
5	Notoginsenoside FP1	C ₄₇ H ₈₀ O ₁₈	Fruit pedicels of <i>P. notoginseng</i>	59
6	Notoginsenoside M	C ₄₈ H ₈₂ O ₁₉	Roots of <i>P. notoginseng</i>	60
7	Notoginsenoside N	C ₄₈ H ₈₂ O ₁₉	Roots of <i>P. notoginseng</i>	60
8	Notoginsenoside Rw1	C ₄₆ H ₇₈ O ₁₇	Rhizomes of <i>P. notoginseng</i>	61
9	Notoginsenoside T3	C ₃₈ H ₆₆ O ₉	Acid hydrolysate roots of <i>P. notoginseng</i>	62
10	Notoginsenoside U	C ₄₂ H ₇₂ O ₁₄	Roots of <i>P. notoginseng</i>	63
11	Quinquenoside L17	C ₄₇ H ₈₀ O ₁₈	Leaves and stems of <i>P. quinquefolius</i>	5
12	Yesanchinoside D	C ₄₄ H ₇₄ O ₁₅	Underground part of <i>P. japonicus</i>	64
13	Yesanchinoside E	C ₅₄ H ₉₂ O ₂₃	Underground part of <i>P. japonicus</i>	64
14	Yesanchinoside F	C ₅₆ H ₉₄ O ₂₄	Underground part of <i>P. japonicus</i>	64
15	20(S)-acetylated Rg2	C ₄₄ H ₇₄ O ₁₄	Roots of <i>P. quinquefolius</i>	65
16	20(R)-acetylated Rg2	C ₄₄ H ₇₄ O ₁₄	Roots of <i>P. quinquefolius</i>	65
17	Malonylginsenoside Ra3	C ₆₂ H ₁₀₂ O ₃₀	Fresh roots of <i>P. ginseng</i>	66
18	Malonylnotoginsenoside R4	C ₆₂ H ₁₀₂ O ₃₀	Roots of <i>P. ginseng</i>	67
19	Notoginsenoside FP2	C ₅₈ H ₉₈ O ₂₆	Fruit pedicels of <i>P. notoginseng</i>	59
20	Notoginsenoside FT1	C ₄₇ H ₈₀ O ₁₇	Acid hydrolysate roots of <i>P. notoginseng</i>	68
21	Notoginsenoside L	C ₅₃ H ₉₀ O ₂₂	Roots of <i>P. notoginseng</i>	60
22	Notoginsenoside O	C ₅₂ H ₈₈ O ₂₁	Flower buds of <i>P. notoginseng</i>	69
23	Notoginsenoside P	C ₅₂ H ₈₈ O ₂₁	Flower buds of <i>P. notoginseng</i>	69
24	Notoginsenoside Q	C ₆₃ H ₁₀₆ O ₃₀	Flower buds of <i>P. notoginseng</i>	69
25	Notoginsenoside S	C ₆₃ H ₁₀₆ O ₃₀	Flower buds of <i>P. notoginseng</i>	69
26	Notoginsenoside T	C ₆₄ H ₁₀₈ O ₃₁	Flower buds of <i>P. notoginseng</i>	69
27	Quinquenoside L10	C ₄₇ H ₈₀ O ₁₇	Leaves and stems of <i>P. quinquefolius</i>	46
28	Quinquenoside L14	C ₄₇ H ₈₀ O ₁₇	Leaves and stems of <i>P. quinquefolius</i>	46
29	Yesanchinoside J	C ₆₁ H ₁₀₂ O ₂₈	Underground part of <i>P. japonicus</i>	70
30	Floralginsenoside A	C ₄₂ H ₇₂ O ₁₆	Flower buds of <i>P. ginseng</i>	71
31	Floralginsenoside C	C ₄₁ H ₇₀ O ₁₅	Flower buds of <i>P. ginseng</i>	71
32	Floralginsenoside H	C ₅₀ H ₈₄ O ₂₁	Flower buds of <i>P. ginseng</i>	47
33	Floralginsenoside J	C ₄₈ H ₈₂ O ₂₀	Flower buds of <i>P. ginseng</i>	47

No.	Name	Formula	Plant Material	Ref.
34	Floralginsenoside Ka	C ₃₆ H ₆₂ O ₁₁	Flower buds of <i>P. ginseng</i>	31
35	Floralginsenoside Tc	C ₅₃ H ₉₀ O ₂₄	Flower buds of <i>P. ginseng</i>	30
36	Floralquinquenoside B	C ₄₂ H ₇₂ O ₁₅	Flower buds of <i>P. quinquefolius</i>	35
37	Floralquinquenoside D	C ₄₂ H ₇₂ O ₁₅	Flower buds of <i>P. quinquefolius</i>	35
38	Floranotoginsenoside B	C ₅₃ H ₉₀ O ₂₄	Flowers of <i>P. notoginseng</i>	72
39	Floranotoginsenoside C	C ₅₃ H ₉₀ O ₂₄	Flowers of <i>P. notoginseng</i>	72
40	Ginsenoside I	C ₄₈ H ₈₂ O ₂₀	Flower buds of <i>P. ginseng</i>	73
41	Ginsenoside II	C ₄₈ H ₈₂ O ₂₀	Flower buds of <i>P. ginseng</i>	73
42	Ginsenoside SL1	C ₃₆ H ₆₂ O ₁₁	Steamed leaves of <i>P. ginseng</i>	74
43	Floralginsenoside B	C ₄₂ H ₇₂ O ₁₆	Flower buds of <i>P. ginseng</i>	71
44	Floralginsenoside D	C ₄₁ H ₇₀ O ₁₅	Flower buds of <i>P. ginseng</i>	71
45	Floralginsenoside E	C ₄₂ H ₇₂ O ₁₅	Flower buds of <i>P. ginseng</i>	71
46	Floralginsenoside F	C ₄₂ H ₇₂ O ₁₅	Flower buds of <i>P. ginseng</i>	71
47	Floralginsenoside G	C ₅₀ H ₈₄ O ₂₁	Flower buds of <i>P. ginseng</i>	47
48	Floralginsenoside I	C ₄₈ H ₈₂ O ₂₀	Flower buds of <i>P. ginseng</i>	47
49	Floralginsenoside K	C ₄₈ H ₈₂ O ₂₁	Flower buds of <i>P. ginseng</i>	47
50	Floralginsenoside O	C ₅₃ H ₉₀ O ₂₄	Flower buds of <i>P. ginseng</i>	57
51	Floralginsenoside P	C ₅₃ H ₉₀ O ₂₃	Flower buds of <i>P. ginseng</i>	57
52	Floralquinquenoside A	C ₃₆ H ₆₂ O ₁₁	Flower buds of <i>P. quinquefolius</i>	35
53	Floralquinquenoside C	C ₄₂ H ₇₂ O ₁₅	Flower buds of <i>P. quinquefolius</i>	35
54	Ginsenoside Rh6	C ₃₆ H ₆₂ O ₁₁	Leaves of <i>P. ginseng</i>	32
55	Floralginsenoside La	C ₄₈ H ₈₂ O ₁₉	Flower buds of <i>P. ginseng</i>	47
56	Floralginsenoside Lb	C ₄₈ H ₈₂ O ₁₉	Flower buds of <i>P. ginseng</i>	47
57	Floranotoginsenoside D	C ₅₃ H ₉₀ O ₂₃	Flowers of <i>P. notoginseng</i>	72
58	Ginsenoside Rg7	C ₃₆ H ₆₀ O ₉	Leaves of <i>P. ginseng</i>	32
59	Notopanaxoside A	C ₃₆ H ₆₂ O ₁₀	Roots of <i>P. notoginseng</i>	75
60	Notoginsenoside FT3	C ₄₇ H ₈₀ O ₁₈	Acid hydrolysate roots of <i>P. notoginseng</i>	68
61	Floranotoginsenoside A	C ₅₃ H ₉₀ O ₂₃	Flowers of <i>P. notoginseng</i>	72
62	Ginsenoside ST2	C ₃₆ H ₆₂ O ₁₀	Steamed leaves of <i>P. ginseng</i>	54
63	Notoginsenoside Rw2	C ₄₁ H ₇₀ O ₁₄	Rhizomes of <i>P. notoginseng</i>	61
64	Notoginsenoside ST5	C ₄₇ H ₈₀ O ₁₈	Steamed roots of <i>P. notoginseng</i>	33
65	Yesaninoside H	C ₅₃ H ₉₀ O ₂₃	Underground part of <i>P. japonicus</i>	70
66	Ginsenoside Ki	C ₃₆ H ₆₂ O ₁₀	Leaves of <i>P. ginseng</i>	76
67	Ginsenoside Km	C ₃₆ H ₆₂ O ₁₀	Leaves of <i>P. ginseng</i>	76
68	Quinquenoside L2	C ₄₈ H ₈₂ O ₁₉	Leaves and stems of <i>P. quinquefolius</i>	77

No.	Name	Formula	Plant Material	Ref.
69	Dammar-25(26)-ene-3,6,12,20,22,24-hexanol	C ₃₀ H ₅₂ O ₆	Leaves of <i>P. ginseng</i>	78
70	Floralginsenoside Kb	C ₄₅ H ₇₆ O ₁₉	Flower buds of <i>P. ginseng</i>	31
71	Floralginsenoside Kc	C ₄₅ H ₇₆ O ₂₀	Flower buds of <i>P. ginseng</i>	31
72	Floralginsenoside Ta	C ₃₆ H ₆₀ O ₁₀	Flower buds of <i>P. ginseng</i>	30
73	Vina-ginsenoside R25	C ₄₂ H ₇₀ O ₁₅	Roots and rhizomes of <i>P. vietnamensis</i>	58
74	Floralginsenoside Tb	C ₃₅ H ₆₂ O ₁₁	Flower buds of <i>P. ginseng</i>	30
75	Quinquenoside L9	C ₄₂ H ₇₄ O ₁₅	Leaves and stems of <i>P. quinquefolius</i>	79
76	Quinquenoside L16	C ₅₄ H ₉₄ O ₂₅	Leaves and stems of <i>P. quinquefolius</i>	46
77	25-OCH ₃ -PPD	C ₃₁ H ₅₆ O ₄	Leaves of <i>P. notoginseng</i>	80
78	25-OH-PPD	C ₃₀ H ₅₄ O ₄	Fruits of <i>P. ginseng</i>	48
79	25-OH-PPT	C ₃₀ H ₅₄ O ₅	Fruits of <i>P. ginseng</i>	48
80	Notoginsenoside FT2	C ₄₇ H ₈₂ O ₁₈	Acid hydrolysate roots of <i>P. notoginseng</i>	68
81	Notoginsenoside T4	C ₃₆ H ₆₂ O ₁₁	Acid hydrolysate roots of <i>P. notoginseng</i>	62
82	Quinquenoside L1	C ₄₈ H ₈₀ O ₁₈	Leaves and stems of <i>P. quinquefolius</i>	77
83	Quinquefoloside La	C ₅₄ H ₉₂ O ₂₃	Leaves of <i>P. quinquefolius</i>	45
84	Quinquefoloside Lc	C ₅₄ H ₉₂ O ₂₃	Leaves of <i>P. quinquefolius</i>	81
85	Dammar-(E)-20(22)-ene-3,12,25-triol	C ₃₀ H ₅₂ O ₃	Acid hydrolysate roots of <i>P. ginseng</i>	82
86	Notoginsenoside ST1	C ₃₆ H ₆₂ O ₁₀	Steamed roots of <i>P. notoginseng</i>	33
87	Ginsenoside Rg6	C ₄₂ H ₇₀ O ₁₂	Stem-leaves of <i>P. ginseng</i>	83
88	Ginsenoside Rs4	C ₄₂ H ₇₀ O ₁₂	Steamed roots of <i>P. notoginseng</i>	84
89	Ginsenoside Rs6	C ₄₂ H ₇₀ O ₁₂	Steamed roots of <i>P. notoginseng</i>	84
90	Isoginsenoside Rh3	C ₃₆ H ₆₀ O ₇	Fruits of <i>P. ginseng</i>	49
91	Ginsenoside Rh5	C ₃₆ H ₆₀ O ₉	Leaves of <i>P. ginseng</i>	32
92	Ginsenoside SL2	C ₄₂ H ₇₀ O ₁₄	Steamed leaves of <i>P. ginseng</i>	74
93	Ginsenoside ST1	C ₃₆ H ₆₀ O ₁₀	Steamed leaves of <i>P. ginseng</i>	54
94	Notoginsenoside ST2	C ₄₃ H ₇₄ O ₁₅	Steamed roots of <i>P. notoginseng</i>	33
95	Notoginsenoside ST3	C ₄₃ H ₇₄ O ₁₅	Steamed roots of <i>P. notoginseng</i>	33
96	Ginsenoside Rg8	C ₄₂ H ₇₀ O ₁₂	Roots of <i>P. quinquefolius</i>	85
97	Notoginsenoside T1	C ₃₆ H ₆₀ O ₁₀	Acid hydrolysate roots of <i>P. notoginseng</i>	62
98	Notoginsenoside T2	C ₃₆ H ₆₂ O ₁₀	Acid hydrolysate roots of <i>P. notoginseng</i>	62
99	Ginsenoside Rg1–12,23-epoxy	C ₄₂ H ₇₀ O ₁₄	Leaves of <i>P. ginseng</i>	86
100	Ginsenoside Rh9	C ₃₆ H ₆₀ O ₉	Leaves of <i>P. ginseng</i>	32
101	Quinquefoloside-Lb	C ₅₃ H ₈₈ O ₂₂	Leaves of <i>P. quinquefolius</i>	45
102	Ginsenoside Rk1	C ₄₂ H ₇₀ O ₁₂	Processed roots of <i>P. ginseng</i>	36
103	Ginsenoside Rk2	C ₃₆ H ₆₀ O ₇	Processed roots of <i>P. ginseng</i>	36

No.	Name	Formula	Plant Material	Ref.
104	Ginsenoside Rk3	C ₃₆ H ₆₀ O ₈	Processed roots of <i>P. ginseng</i>	36
105	Ginsenoside Rs5	C ₃₈ H ₆₂ O ₉	Steamed roots of <i>P. notoginseng</i>	84
106	Ginsenoside Rs7	C ₃₈ H ₆₂ O ₉	Steamed roots of <i>P. notoginseng</i>	84
107	Notoginsenoside T5	C ₄₁ H ₆₈ O ₁₂	Acid hydrolysate roots of <i>P. notoginseng</i>	62
108	Ginsenoside Rz1	C ₄₂ H ₇₀ O ₁₂	Steamed roots of <i>P. notoginseng</i>	53
109	Ginsenoside SL3	C ₄₂ H ₇₀ O ₁₄	Steamed leaves of <i>P. ginseng</i>	74
110	Ginsenoside Rh8	C ₃₆ H ₆₀ O ₉	Leaves of <i>P. ginseng</i>	32
111	Ginsenoside Rh7	C ₃₆ H ₆₀ O ₉	Leaves of <i>P. ginseng</i>	32
112	Yesaninoside G	C ₅₃ H ₈₈ O ₂₃	Underground part of <i>P. japonicus</i>	70
113	Yesaninoside I	C ₅₉ H ₁₀₀ O ₂₆	Underground part of <i>P. japonicus</i>	70
114	Hexanordammaran	C ₂₄ H ₄₀ O ₄	Leaves of <i>P. ginseng</i>	87
115	Notoginsenoside R10	C ₃₀ H ₅₀ O ₉	Steamed leaves of <i>P. ginseng</i>	88
116	Yesaninoside A	C ₄₄ H ₇₄ O ₁₆	Underground part of <i>P. japonicus</i>	64
117	Yesaninoside B	C ₄₈ H ₈₂ O ₂₀	Underground part of <i>P. japonicus</i>	64
118	Yesaninoside C	C ₄₇ H ₈₀ O ₁₉	Underground part of <i>P. japonicus</i>	64
119	Panaxadiene	C ₃₀ H ₄₈ O ₅	Seeds of <i>P. ginseng</i>	51
120	Polyacetyleneginsenoside Ro	C ₆₅ H ₁₀₀ O ₂₁	Roots of <i>P. ginseng</i>	89
121	Isodehydroprotopanaxatriol	C ₃₀ H ₅₀ O ₃	Acid hydrolysate roots of <i>P. ginseng</i>	90
122	20,25-epoxy-dammaran-2-en-6,12-diol	C ₃₀ H ₅₀ O ₃	Acid hydrolysate roots of <i>P. ginseng</i>	90
123	3-methyl-28-nordammaran-2-en-6,12-diol	C ₃₀ H ₅₀ O ₃	Acid hydrolysate roots of <i>P. ginseng</i>	90

Table 4

Separation modes employed in ginseng analysis

Method	Advantage	Limitation	Ref.
TLC/HPTLC	Easy to use	Low efficiency in separation	134,135
	Versatility	Low reproducibility	
	Low cost	Low accuracy in quantification	
GC	High sensitivity	Limited on volatile compounds	91,136–138
	High resolution	Time consumption in derivation	
	High speed	Thermal instability	
HPLC	Widely available	Extended analytical time	28,74,104,121, 139–141
	Relatively easy to use	Relatively high cost	
	High accuracy and precision		
HILIC	Suitable for hydrophilic molecules	Peak broadening	142–145
	Beneficial to ESI process	Short column lifetime	
	Weak interaction with stationary phase		
UPLC	High efficient separation	Back-pressure increase	146–148
	Reduced analytical time	Dedicated instrumentation	
	Reduced solvent consumption		
CE	Short analytical time	Low reproducibility	149–152
	Easy to automate	Insufficient detection sensitivity	
	Environmentally friendly	Complicated interfacing to MS.	
2-D chromatography	Powerful separation ability	Possibility of artifact formation	136,153
	High peak capacity	Extended analysis time	
	Excellent detection sensitivity	Limitations in mobile phase	

Abbreviations: HPTLC: high-performance thin layer chromatography; GC: gas chromatography; HPLC: high-performance liquid chromatography; HILIC: hydrophilic interaction liquid chromatography; UPLC: ultra-performance liquid chromatography; CE: electrophoresis; 2-D: two-dimensional; ESI: electrospray ionization; MS: mass spectrometry.

Table 5

Characterization of detection methods for ginseng analysis

Method ^a	Establishment	Sensitivity	Structure	Linear range	Operation	Precision	Accuracy	Cost
UV/DAD	Very easy	Moderate	Possible	Very wide	Simple	Fairly Good	Very good	Very low
ELSD	Easy	Low	No	Narrow	Fair	Good	Good	Low
CAD	Easy	Moderate	No	Wide	Fair	Good	Good	Low
MS	Not easy	High	Powerful	Narrow	Not simple	Low	Low	High
ELISA	Very complex	Very high	Possible	Very narrow	Simple	Low	Low	Low

^a Abbreviations: UV/DAD: ultraviolet or diode-array detection; ELSD: evaporative light scattering detection; CAD: charged aerosol detection; MS: mass spectrometry; ELISA: enzyme-linked immunosorbent assay.

Table 6

Advantages and limitations of DNA profiling methods for authentication of ginseng species

Method ^a	Principle	Advantage	Limitation	Ref.
RAPD	Use of small arbitrary primers to produce multiple DNA fragments	Technical simplicity, no sequence information required, polymorphism identification	Low reproducibility, extra amplified fragments	228–232
SCAR	Extension of RAPD primers based on polymorphic fragment sequences	Reduced competition between primer binding sites and high reproducibility	Low sensitivity	218
RFLP	Comparison of band profiles after restriction enzyme digestion of target DNA.	High reliability, no sequence information required	Large DNA amounts required, low polymorphism levels	70,217,225,233
AFLP	Polymorphism based on gain or loss of restriction site, or selective bases	High reproducibility, no prior sequence knowledge required	Difficulty in identifying homologous markers	220,234,235
DALP	Use of an arbitrarily primed-PCR to produce genomic fingerprints and to enable sequencing of DNA polymorphisms	High resolution fingerprint, new genetic markers characterization	Low reproducibility	222
DNA sequencing	Measurement of every base in the DNA sequence	Commonly used, application to primer designation	Time-consuming	227
Microsatellite	Determination of the short tandem repeats at a location	Highly polymorphic and codominant	Highly cost and time-consuming	220,236,237
LAMP	A single tube technique for the amplification of DNA	Reduced reaction time, high sensitivity	Low specificity	226

^a Abbreviations: RAPD: random amplified polymorphic DNA; SCAR: sequence characterized amplified region; RFLP: restriction fragment length polymorphism; AFLP: amplified fragment length polymorphism; DALP: direct amplification of length polymorphism; LAMP: loop-mediated isothermal amplification of polymerase chain reaction (PCR).

Table 7

Application of metabolomic fingerprinting to analysis of ginseng samples

Instrument ^a	Chemometrics ^b	Material	Result	Ref.
HPTLC fluorescent	CASE Software	<i>P. ginseng</i> , <i>P. quinquefolius</i> , <i>P. notoginseng</i>	Species authentication and stability of ginseng preparations achieved	155,211
¹ H-NMR	PCA	Fresh ginseng roots of different ages	Effective authentication and quality control performed	238
¹ H-NMR	PCA and cluster analysis	<i>P. ginseng</i> roots from different origins	Sugar signals and methyl signals separate showing origin-dependent and age-transitions	120,121
¹ H-NMR	PCA and PLS-DA	<i>P. ginseng</i> , <i>P. quinquefolius</i> from different origins, and Korean ginseng products	Glucose, fumarate, and various amino acids serving as biomarkers for quality assurance	212
2-D- <i>J</i> -resolved ¹ H-NMR	PCA	Different ages of <i>P. ginseng</i> and commercial ginseng capsules	Alanine, arginine, fumaric acid, inositol and ginseng saponins being important metabolites for differentiation	122
GC-GC-TOF-MS	PCA	Radix ginseng volatile oils of different ages	α -cadinol, α -bisabolol, thujopsene, and <i>n</i> -hexadecanoic acid increased with the age	136
UPLC-ESI-Q-TOF-MS	PCA	Different parts of <i>P. notoginseng</i>	Slight variations discriminated due to different geographical locations, cultivations and collection times	146
UPLC-ESI-Q-TOF-MS	PCA and PLS-DA	<i>P. ginseng</i> , <i>P. quinquefolius</i> , and <i>P. notoginseng</i>	Chikusetsusaponin IVa, Ro, Rc, Rb1, Rb2 and Rg2 accounted for variations among ginseng species	147
UPLC-ESI-Q-TOF-MS	PCA	<i>P. ginseng</i> from China and Korea, <i>P. quinquefolius</i> , <i>P. notoginseng</i> , and <i>P. japonicus</i>	Rf, F11, malonyl-Rb1, and Rb2 accounted for variations in species	148

^a Abbreviations: HPTLC: high-performance thin layer chromatography; 2-D NMR: two-dimensional nuclear magnetic resonance; GC-TOF-MS: gas chromatography time-of-flight mass spectrometry; UPLC-ESI-Q-TOF-MS: ultra-performance liquid chromatography electrospray ionization quadrupole-TOF-MS.

^b Abbreviations: CASE: computer-aided software engineering; PCA: principal component analysis; PLS-DA: partial least squares-discriminant analysis.

Table 8

Pharmacokinetic data on ginseng and ginseng saponins

Material ^a	Administration ^b (species)	Analytical method and LLOQ ^c (ng/ml)	T _{max} ^d (h)	T _{1/2} ^e (h)	F _f ^f (%)	Ref.
<i>P. ginseng</i> berry and Re	i.v. and i.g. (rat)	UPLC-ESI-MS, 2.5	0.4 ± 0.2	0.2–0.5	0.19–0.28	270
Rd	i.g. and i.v. (dog)	LC-ESI-MS, 5	3.0	24.2 ± 2.85 for i.g., 39.4 ± 12.0 for i.v.	0.26	271
Rd	i.v. (human)	LC-ESI-MS-MS, 3	0.5 ± 1.0	19.3 ± 3.4	–	272,273
Rgl	i.v. (rat)	LC-ESI-MS, 2–10	–	–	1.33	274
Rhl	i.g. and i.v. (rat)	LC-ESI-MS, 5	1.00 ± 0.00	0.43 ± 0.08 for i.g., 0.41 ± 0.05 for i.v.	1.01 ± 0.03	275
20(S)- and 20(R)-Rg2	i.v. (rat)	LC-UV, 3900–7800	–	0.64 ± 0.02 for 20S, 1.19 ± 0.06 for 20R	–	276
20(S)-Rg2	i.g. and i.v. (rat)	LC-ESI-MS, 5	–	0.53 ± 0.34	ND	277
20(S)-Rg3	i.g. and i.v. (rat)	LC-ESI-MS, 2–10	–	0.23	2.63	278,279
20(S)-Rg3	i.m. (human)	LC-ESI-MS/MS, 2.0–2.5	–	–	–	280
20(R)-Rg3	i.g. and i.v. (rat)	LC-ESI-MS, 30	–	0.31 for i.v.	ND	281
20(R)-Rh2	i.g. and i.v. (rat)	LC-ESI-Q-TOF-MS	–	0.27	ND	282
IH-901	i.g. and i.v. (rat)	LC-ESI-MS/MS, 5	0.63 ± 0.18	3.70 ± 1.38 for i.v.	4.54 ± 0.38	283,284
PPD	i.g. and i.v. (rat)	LC-APCI-MS, 1	2.50 ± 1.23	1.47 ± 0.30 for i.g., 2.73 ± 0.85 for i.v.	36.8 ± 12.4	285
PPD	oral (human)	LC-ESI-MS/MS, 01	1.28 ± 0.49	4.77 ± 2.05	–	286
25-OH-PPD	i.g. and i.v. (rat)	LC-ESI-MS/MS, 10	5.50 ± 4.70	3.9 ± 2.1 for i.g., 4.5 ± 2.6 for i.v.	64.8 ± 14.3	287
<i>P. notoginseng</i> extract	i.g. and i.v. (rat)	LC-UV, 1200–4000	–	18.57 for Rbl, 14.13 for Rgl	4.35 for Rbl, 18.4 for Rgl	288
<i>P. notoginseng</i> extract	i.g. and i.v. (rat)	LC-ESI-MS, 2.8–4.0	0.71 ± 0.19 for Rl, 0.75 ± 0.00 for Rgl, 0.88 ± 0.14 for Rd, 0.79 ± 0.10 for Re, 0.83 ± 0.13 for Rbl	1.11 ± 0.51 for Rl, 5.01 ± 2.09 for Rgl, 18.15 ± 9.14 for Rd, 1.01 ± 1.35 for Re, 20.15 ± 6.27 for Rbl	9.29 for Rl, 6.06 for Rgl, 2.36 for Rd, 7.06 for Re, 1.18 for Rbl	289
Ginseng saponins	i.g. (rat)	LC-ESI-MS, 8–50	–	–	–	290
“Shenmai”	i.v. (human)	LC-ESI-MS-MS, 1	–	2.09 ± 1.89 for Rgl	–	291

Material ^a	Administration ^b (species)	Analytical method and LLOQ ^c (ng/ml)	T _{max} ^d (h)	T _{1/2} ^e (h)	F _o ^f (%)	Ref.
"Shenmai"	i.v. (rat)	UPLC-SSIM-MS, 1–10	–	–	–	292

^aPPD: 20(S)-protopanaxadiol; IH-901 (or compound K): 20-O-(β-D-glucopyranosyl)-20(S)-protopanaxadiol; "Shenmai": a prescription prepared from Radix ginseng and Ophiopogonis.

^bi.v.: intravenous; i.g.: intragastrical; i.m.: intramuscular.

^cLLOQ: lower limit of quantification; UPLC: ultra-performance liquid chromatography; ESI-MS: electrospray ionization-mass spectrometry; APCI: atmospheric pressure chemical ionization; Q-TOF: quadrupole time-of-flight; SSIM: segmental and selected ion monitoring.

^dT_{max} time to reach the maximum plasma concentration after i.g. or oral administration.

^eT_{1/2} elimination half-life time.

^fF: oral bioavailability; ND: not detected in plasma after oral administration.