# Herbal Metabolic Profiling of Raw and Steamed *Panax notoginseng*

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## Introduction

Herbal medicine is a commonly practiced form of traditional medicine. *Panax notoginseng* (Burk.) F.H. Chen is a highly valued and important Chinese medicinal herb (Fig. 1). While the raw form is traditionally used for its haemostatic and cardiovascular properties [1], the steamed form is used to increase production of various blood cells in anemic conditions [2]. Due to their contrasting pharmacological actions, consuming the wrong form of herb may lead to undesirable clinical outcomes. Quality control of this herb is hence paramount. We conceived and tested the feasibility of herbal metabolic profiling, using UPLC/TOFMS, to measure qualitatively multiparametric metabolite responses of raw *P. notoginseng* to thermal stimulus.

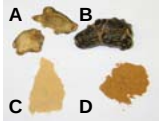



Fig. 1
(A) Slices of raw *P. notoginseng* root, (B) raw *P. notoginseng* root, (C) raw and (D) steamed *P. notoginseng* powders.

## Experimental

**LC/MS Conditions:** ACQUITY UPLC™ system (Waters Corp., MA, USA) and 100 x 2.1 mm C18 1.7 µm column were used. Mobile phase constitutes (A) $H_2O$ (0.1% formic acid) and (B) ACN (0.1% formic acid). The isocratic and gradient conditions were optimized in 12 min at flow rate of 0.5 mL/min and column temperature at 45ºC. TOFMS (Waters, Manchester, UK) was optimized for both ES- and ES+ modes using leucine enkephalin and calibrated using sodium formate (400-1500 Da). Accurate mass measurement was achieved via LockSpray™ and the use of leucine enkephalin as reference compound for both ES- and ES+ modes. Dynamic Range Enhancement (DRE™) was applied throughout the experiment for extended mass accuracy.

**Sample Preparation:** Mixture containing ginsenosides Rb1, Rc, Rd, Re, Rg1 and notoginsenoside R1 was prepared in 50% (v/v) methanol for method development/validation. Raw and steamed (9 h) powdered samples were extracted via ultrasonication using 70% (v/v) methanol. The extract was dried, reconstituted and injected as previously described [3]. 6 individual extractions were performed to generate 6 replicates each of raw and steamed samples for the metabolic profiling experiments.

**Data Analysis**: UPLC/TOFMS ES- raw data were analyzed to identify potential discriminant variables. Resulting 3D data comprising peak number (RT-*m/z* pair), sample name, and ion intensity were analyzed by PCA using MarkerLynx software (Waters, Manchester, UK). Marker ions, determined from the ion intensity plots, were tentatively assigned to the ginsenosides based on the possible calculated mass, empirical formula, mass accuracy (mDa and ppm), double bond equivalent (DBE) and i-FIT value of the potential candidate ions and data-mining using an in-house ginsenoside database.

## Results and Discussion

The retention time reproducibility of the UPLC method was determined to be consistently high for injections of both standard mixture solution and complex herbal extracts (CV < 0.15% for all tested ginsenosides, n = 18, ES- mode). This was important in ensuring that the right datasets were built for analysis by PCA without RT correction. As compared to HPLC, faster chromatography via UPLC resulted in more detected peaks (Fig. 2).

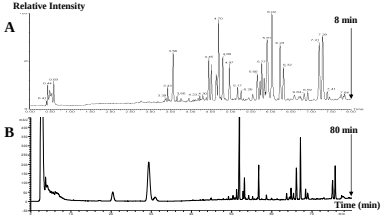
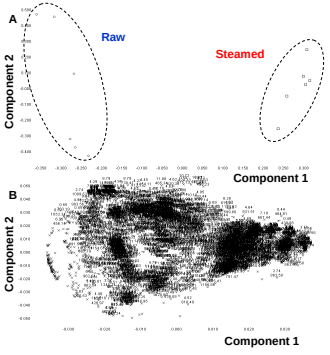


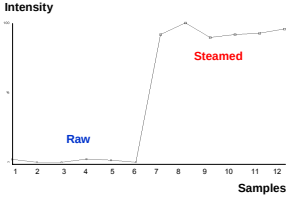

Fig. 2
(A) UPLC/TOFMS ES- total ion current chromatogram (TIC) and (B) HPLC/UV chromatogram (203 nm) of steamed *P. notoginseng*.

Mass accuracy was consistently high for selected ginsenosides in both raw and steamed herbal extracts (< 5 RMS ppm for all tested ginsenosides, n = 12). The PCA scores plot in Fig. 3A could be readily divided into two clusters: raw and steamed (9h) *P.notoginseng*, confirming that the steaming process led to changes in the levels and occurrence of ginsenosides. It was predicted that the total number of analytes, detected using UPLC/TOFMS profiling of both raw and steamed *P. notoginseng*, was close to 400 or more. It was found that the ion intensity plot (Fig. 4) as compared to loadings plot



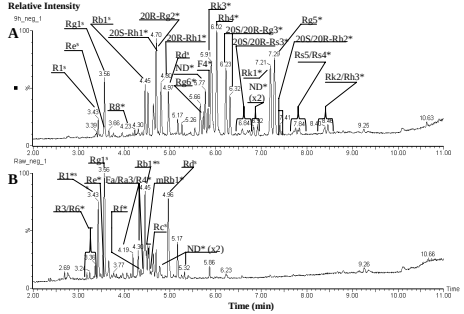

◄ Fig. 3. (A) Scores plot of raw (○) and steamed groups (□) and (B) loadings plot obtained via PCA of *P. notoginseng* extracts.

Fig. 4. Representative ion intensity plot for a marker ion with RT 6.02 min. ▼




(Fig. 3B), is a relatively useful instrument for biomarker screening. 8 major markers (20S-Rg3, 20R-Rg3, 20S-Rh1, 20R-Rh1, Rk3, Rh4, Rk1, Rg5) identified in the steamed *P. notoginseng* were consistent with our previous findings [3]. However, as shown in Figure 5, significantly more markers (marked with asterisk) were detected and assigned in this study. In the steamed and raw *P. notoginseng* extracts, Figure 5A and B, respectively, 20 and 10 markers, respectively, were tentatively assigned (*), while the identities of 3 and 2 confirmed markers, respectively, were not determined (ND*). Importantly, UPLC was successful in resolving (20S) and (20R) epimers of ginsenosides Rg2, Rh1, Rg3 and Rs3 within a significantly shorter analysis time (5 times reduction in RT). Geometric isomers at the C-20 position i.e. ginsenosides Rg6 and F4, Rk3 and Rh4, Rk1 and Rg5, and Rs5 and Rs4 were also clearly resolved through this system.

Fig. 5. Total ion current chromatograms (TIC) of UPLC/TOFMS analysis of (A) steamed (9 h) and (B) raw *P. notoginseng*. Marked peaks denote different ginsenosides confirmed using standards (ˢ), ginsenoside markers tentatively assigned using the experimental data (*) and markers with no suggested identity (ND*).




## Conclusion

UPLC/TOFMS had been demonstrated to be a powerful tool for herbal metabolic profiling to discriminate differentially processed herbs such as raw and steamed *P. notoginseng*. Three important aspects of the UPLC/TOFMS approach for the metabolite profiling of complex herbal extracts are high retention time reproducibility, high chromatographic resolution and accurate mass measurement. The herbal metabolic profiling approach is promising for the quality control of herbs subjected to internal (genetic, diurnal, seasonal) and external (climate, processing, cultivation practice) variations and the holistic standardization of herbal extract for clinical studies.

## References:

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