An enzymatic protocol for absolute quantification of analogues: application to specific protopanoxadiol-type ginsenosides

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Electronic Supplementary Information

Relative response factors (*F*) calculation

The enzymatic hydrolysis coupled with the CCMM could be applied for calculating the *F* for the analytes of interest. The detailed procedures were according to the following equations. In LC-ESI/MS analysis, the calibration curves could be described as follows:

$$y = ax + b \quad (1)$$

Where *y* is the response area ratio for the interested analyte to IS, and *x* is the mass concentration. The *x* could be calculated as follows:

$$x = n \times M \quad (2)$$

Where *n* and *M* is the actual molar concentration and molar mass, respectively. *n* could be directly determined by the enzymatic hydrolysis. *F* was typically defined as the following equation[20]:

$$F = a_1/a_2 \quad (3)$$

Where a_1 and a_2 are the slopes of the CCAC for the analytes of interest. After concentration calibration, the CCAC was replaced by the CCMM to calculate the value of *F*, which was expressed as the Eq. 4:

$$F = \left[a_1^{'}/(x_1/R_1)\right]/\left[a_2^{'}/(x_2/R_2)\right] \quad (4)$$

Where $a_1^{'}$ and $a_2^{'}$ are the slopes of the CCMM for the interested analytes. x_1/R_1 and x_2/R_2 are the actual mass concentration for analytes in matrix materials. Thus, the represented form of Eq. 4 is the same to the Eq. 3. x_1 and x_2 are their mass concentrations. R_1 and R_2 are the collections recoveries for analytes. The collection recovery (*R*) could be calculated by the following equation based on the modified

NSRE[5]:

$$R = x_d/x_t = [(y_d^{'} - b^{'})/a^{'}]/(m \times C_0 v_0/v_R) = (y_d^{'} - b^{'})/(m \times a^{'}) \quad (5)$$

Where x_t and x_d is the theoretical crude extract concentration and the detected crude extract concentration for collected interested analytes,[5,22] respectively. The $y_d^{'}$, $b^{'}$ and $a^{'}$ is the detected response ratios as well as the slopes and intercepts of their corresponding CCMM, respectively. C_0 is the original Sanqi extract concentration (100 mg mL^{-1}), v_0 is the injection volume for collection (20 μL) and v_R is reconstituted volume (2000 μL). The m is the dilution folds. When the determined R and x were inserted in Eq. 4, F could be successfully calculated.

Supplementary Figures

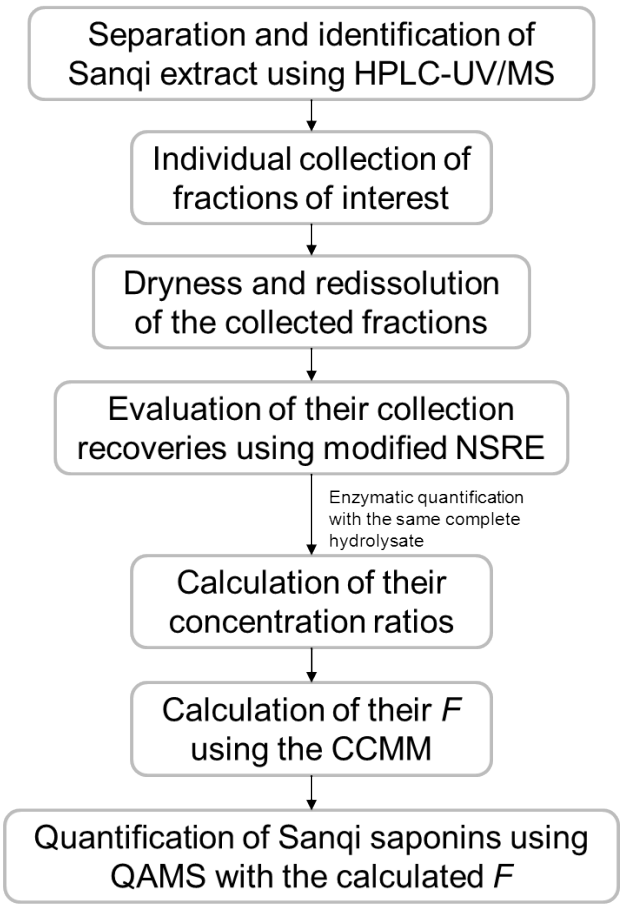

Fig. S1 The protocol for simultaneous quantification of analogues in complex extract. The significant advantage of this protocol is only using minimal collected extract fractions and the complete hydrolyte. The relative response factors (*F*) calculations depended on the enzymatic hydrolysis, the modified non-standard recovery evaluation (NSRE) strategy and the calibration curves matrix materials (CCMM). The economical method of the quantitative analysis of multi-components with a single marker (QAMS) coupled with the calculated *F* was employed for absolute quantification of specific protopanaxatriol type ginsenosides in Sanqi (*Panax notoginseng*) extract.

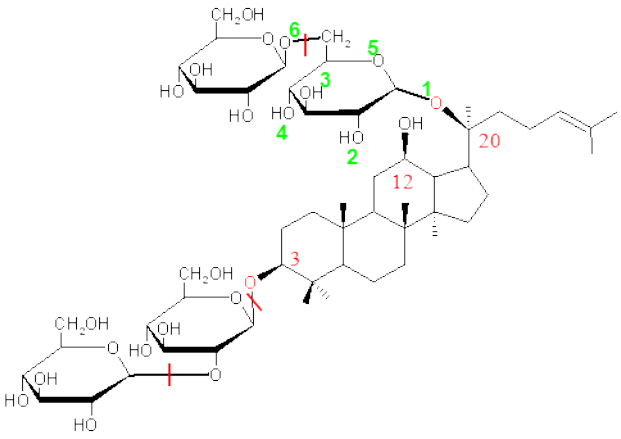

Fig. S2 The structure of GRb_1 and the marked hydroxyl group in the skeleton of protopanoxadiol (PPD) and the side sugar moieties.

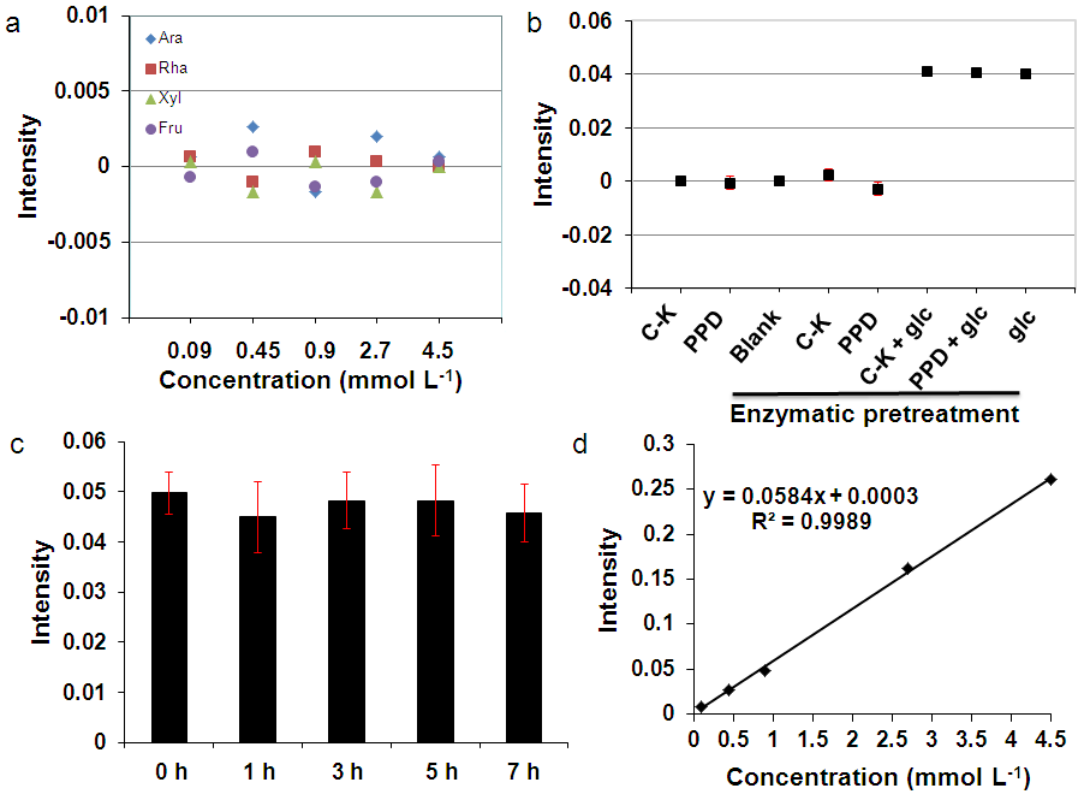

Fig. S3 Glucose assay kits analysis. (a) The selectivity of this method for glucose, rather than arabinose (Ara), rhamnose (Rha), xylose (Xyl) and fructose (Fru); (b) the assay was not disturbed by the other hydrolysates, including compound K (C-K) and protopanoxadiol (PPD); (c) the stability of glucose; (d) the calibration curve of glucose.

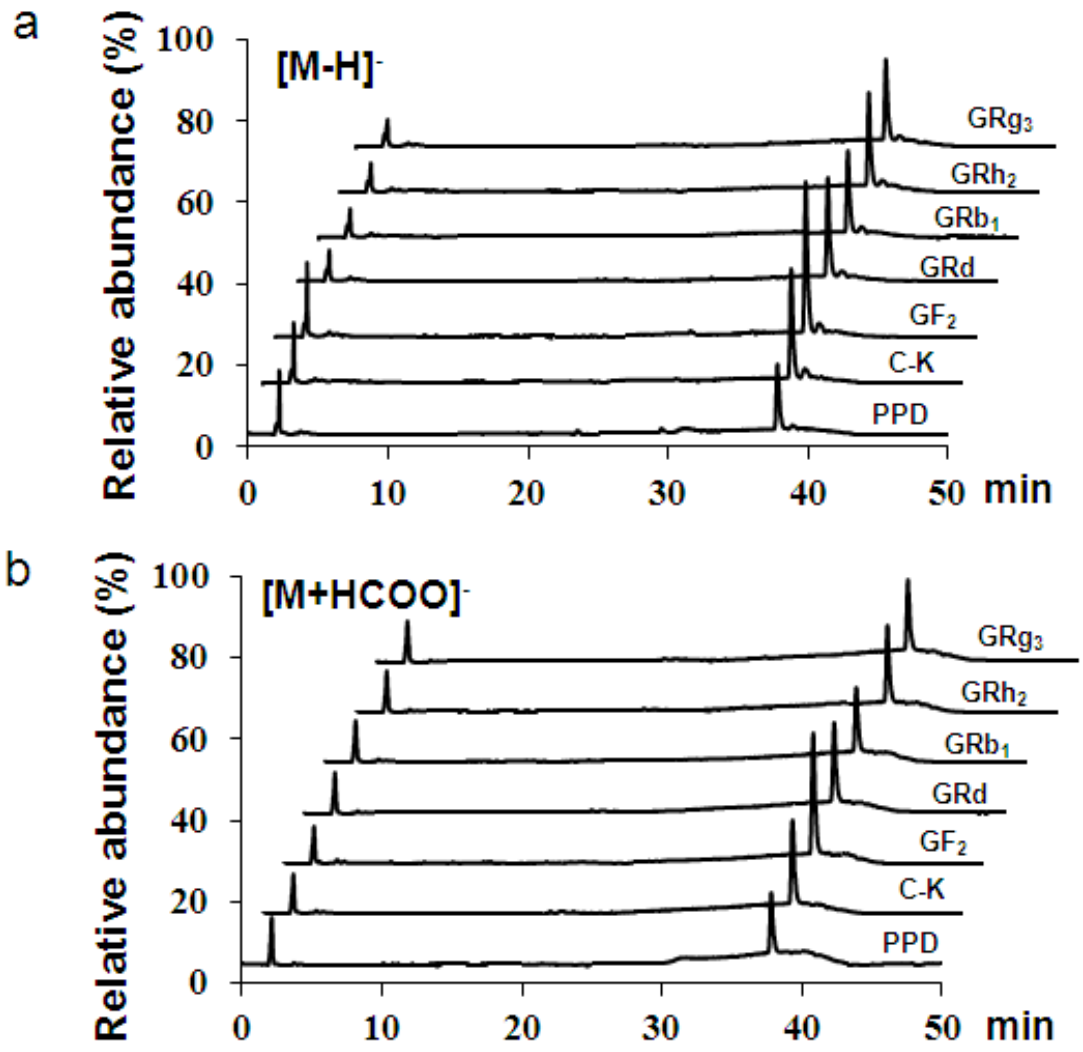

Fig. S4 The $[M-H]^-$ (a) and $[M+HCOO]^-$ (b) ions of the C-25, 26 hydroxylated side products were not detected.

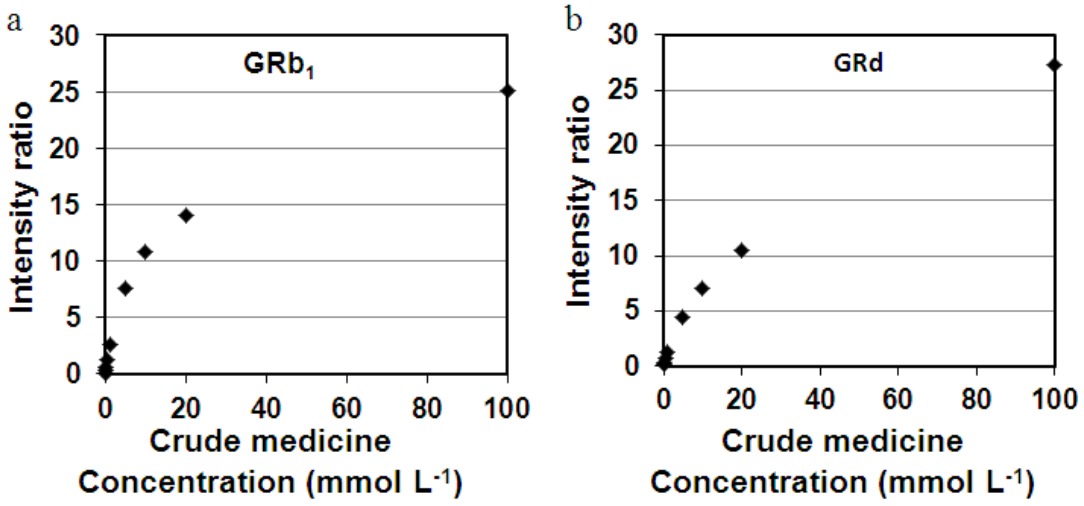

Fig. S5 The mass spectrometry response saturation for GRb_1 (a) and GRd (b).

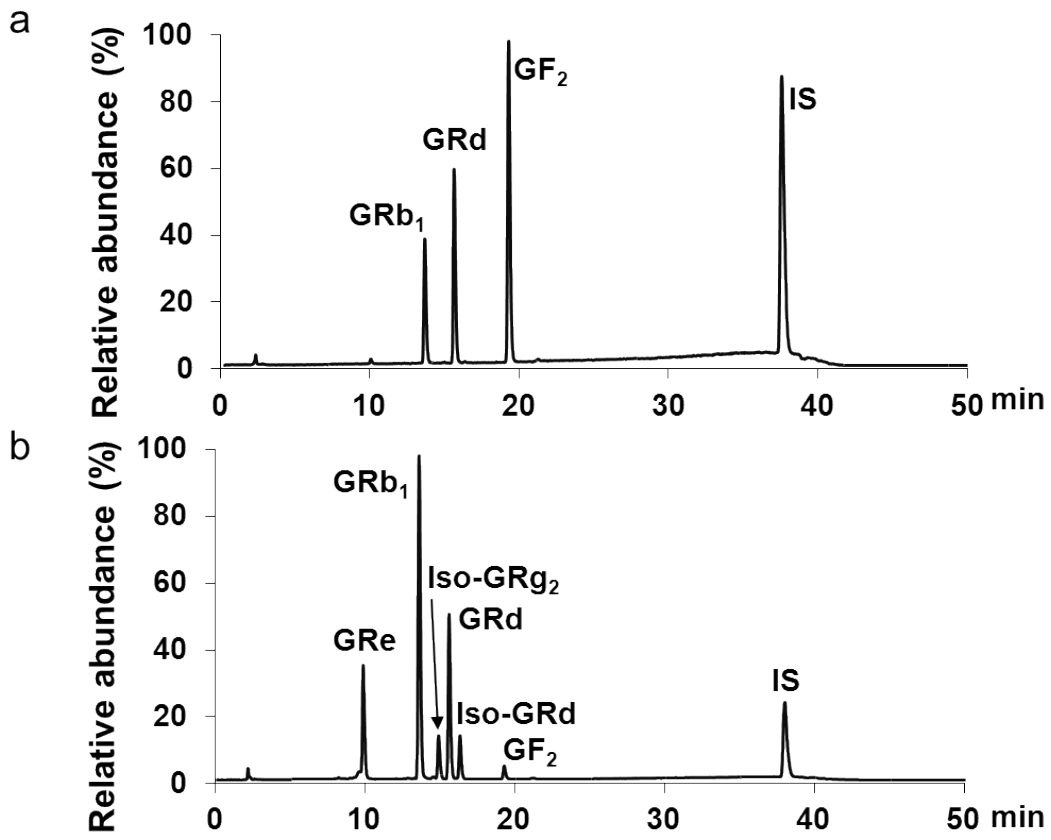

Fig. S6 The chromatographic spectra of the chemical standards and *Panax notoginseng* determined by the LC-MS. The compounds eluted at 9.71 min (*m/z* 991.5, GRe) and 14.62 min (*m/z* 829.5, Isomer of ginsenoside Rg_2, Iso-GRg_2) were typical PPT-type ginsenosides.