Supporting Information

An integrated platform for high-throughput pharmacokinetic study of glycosides using boronic acid-functionalized 96-well glass plate

Ningning Zhao, [a,b] Qianqian Gu, [a,b] Zhiqiang Liu, [a,b,c] Fengrui Song, [a,b] Zifeng Pi [a] and Shu Liu [a,*]

[a] National Center of Mass Spectrometry in Changchun and Jilin Province Key Laboratory of Chinese Medicine Chemistry and Mass Spectrometry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, 130022, People's Republic of China;

[b] University of Science and Technology of China, Hefei 230029, China.

[c] State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, 130022, China.

Materials and methods

Tenuifoliside A, Sibiricose A6, 3, 6'-disinapoyl sucrose, Tenuifolin, Polygalaxanthone III, Rb2, Rb3, Rc, Rd, F1, Rh1(s), Rh1(r), Rb1, Rg1, Rg2(s), Rg2(r), Rg3, Re, Rf, Rh2(s), Rh2(r) and Compound K (CK) were from the College of Pharmacy, Jilin University. White ginseng and radix polygala were obtained from Tongrentang in Changchun. Rutin, Dioscin (IS), adenosine, caffeic acid, cynarine, amygdalin, aesculin, and polydatin were obtained from Push Bio-technology Corporation (Chengdu, China). Purities of all standards were above 98.0%. HPLC-grade acetonitrile, methanol and formic acid were provided from Fisher Scientific (Loughborough, UK). A Milli-Q water (18.2 MΩ) purification system (Milford, MA, USA) was used to generate ultrapure water. Aminopropyl triethoxy silane (APTES), 4-Formylphenylboronic acid (FPBA), 3-aminophenylboronic acid monohydrate (APBA), sodium cyanoborohydride, 1, 6-Hexamethylenediamine, anhydrous methanol, glutaraldehyde and heparin were obtained from Aladdin Industrial Corporation (Shanghai, China). Disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4) and acetic acid were purchased by Sinopharm Chemical Reagent Co.

Synthesis of APBA, TBA and FPBA functional glass plates

Synthesis of APBA and TBA functional glass plate

Vial@APBA and Vial@TBA plates were synthesized through the modified method.[1] Briefly, the plate was treated by CO_2-plasma (power, 80 W; time, 5 min) to give rise to carboxyl-activated holes by a Harrick PlasmaFlo PDC-FMG-2. Later, 30 uL APTES (in 300 uL water/ethanol v/v 1:1) was dropped into each hole. After stirring at 60℃ for 24 h, the solvent was discarded by a pipette and rinsed three times in turn by water, ethanol. Subsequently, glutaraldehyde anhydrous methanol solution (1%) was added holes stirring for 6 h at room temperature. After water and ethanol washing three times, the active holes were gained. The APBA (0.6 mmol) and 1, 6-hexanediamine (0.6 mmol) were dissolved in 10 mL anhydrous methanol at 40℃ for 12 h to form a molecular team (TBA). Meanwhile, the 300 μL of APBA and TBA solvent was added to the former plate reacting for 12 h, respectively. The solvent was discarded and all the holes were washed in turn by water and ethanol, and then dried under vacuum.

Synthesis of FPBA functional glass plate

The procedure for the synthesis of Vial@APBA plate was as follows: The plate modified APTES NPs were same. Then all the holes were added into 100 μL anhydrous methanol containing 1 mg FPBA and 1 mg sodium cyanoborohydride, the mixture was mechanically stirred (100 rpm) for 24 h at room temperature. The final plate was washed water and ethanol, and dried under vacuum. For method validation and PK experiments, the mixture of FPBA and sodium cyanoborohydride were 300 μL reacted in all the holes.

Materials characterization

Fourier transform infrared spectroscopy (FT-IR) was operated with a Bruker IFS 66 V/S spectrometer. X-ray photoelectron spectroscopy (XPS) was carried out on VG ESCALAB 250. The Energy Dispersive X-Ray Spectroscopy (EDS) and Boron analysis for Vial@FPBA were operated by a JSM-6460LV scanning electron microscopy (SEM).

Samples processing

Parallel and regeneration experiments were tested as follows, 100 μL diluted rutin, Rg3 and Tenuifoliose A and 200 μL water were added into the BA-functionalized 96-well glass plate, followed by stirring for 1 h at room temperature. Later, the solvent was discarded and plates were washed three times by ethanol. Finally, the 100 μL of methanol-acetic acid solvent (9:1, v/v) with 25 ng mL^{-1} IS was used to elution targets under strong stirring for 60 min and transferred the solvent to a clean tube for analysis by UHPLC-MS/MS.

Initial experiments were carried out as follows, 100 μL drug-free plasma spiked with 50 μL diluted rutin, Rg3 and tenuifoliose A and 150 μL water, were added into the FPBA-functionalized 96-well glass plate. The subsequent procedures were same in above section. PK experiments were implemented with 100 μL real plasma added to 200 μL water.

UHPLC-MS/MS analysis

All of the experiments were performed by a UHPLC-MS system consisted of a Ultra High Performance Liquid Chromatography LC-30A coupled with a triple quadrupole mass spectrometer LC-MS-8050 with ESI source (Shimadzu Corp., Kyoto, Japan). The ACQUITY UPLC C18 column (50 mm × 2.1 mm, 1.7 μm) was used at a flow rate of 300 μL min^{-1} at 25℃. The gradient of initial experiments for rutin, Tenuifoliside A and Rg3 was as follows: 0.0–3.0 min, 10–40% A; 3.0–5.0 min, 40–50% A; 5.0–8.0 min, 50–60% A; 8.0–10.0 min, 60–100% A. For rutin experiment, the gradient was as follows: 0–1 min, 10–40% A; 1–3 min, 40–100% A; 3–5 min, 100–100% A. For radix polygala experiment, the gradient was as follows: 0–2 min, 10–20% A; 2–3 min, 20–30% A; 3–6 min, 30–40% A; 6–8 min, 40–60% A; 8–9 min, 60–100% A; 9–10 min, 100–100% A. For white ginseng experiment, the gradient was as follows: 0–1.5 min, 10–20% A; 1.5–4 min, 20–28% A; 4–6 min, 28–30% A; 6–10 min, 30–30% A; 10–13 min, 30–32% A; 13–14 min, 32–40% A; 14–18 min, 40–60% A; 18–21 min, 60–100% A; 21–23 min, 100–100% A. For extensive application of the Vial@FPBA, the gradient was as follows: 0–1 min, 5–5% A; 1.5–4 min, 16–16% A; 4.5–5.5 min, 80–100% A; 6–7 min, 100–100% A. Acetonitrile (A) and 0.1% aqueous formic acid solutions (V/V) (B) were used for the mobile phase. 5 μL of sample was injected to UHPLC-MS/MS. The MS conditions of quantification analysis were optimized in negative ion mode with MRM mode. The precursor ion, product ion, Q1 (voltage promotes the ionization of precursor ion), collision energy (CE) and Q3 (voltage promotes the ionization of product ion) of 23 targeted compounds and IS were optimized in Table S1. The operation parameters were as follows: interface temperature 300 ℃, DL temperature 250℃, heat block temperature 400 ℃, nebulizing gas 3 L min^{-1}, drying gas 10 L min^{-1} and heating gas 10 L min^{-1}.

Method validation

A full validation (specificity, linearity, calibration range, precision, accuracy, LODs, LOQs, accuracy, precision, matrix effects, extraction recovery and stability) following the general principles of the FDA guidelines was performed for the assay in rat plasma.

Preparation of calibration standards and quality control (QC)

A series of working solutions of rutin, Tenuifoliside A, Sibiricose A6, 3, 6'-disinapoyl sucrose, Tenuifolin, Polygalaxanthone III, Rb2, Rb3, Rc, Rd, F1, Rh1(s), Rh1(r) ,Rb1, Rg1, Rg2(s), Rg2(r), Rg3, Re, Rf, Rh2(s), Rh2(r) and CK were obtained as follows: 1-3000 ng mL^{-1}. For different experiments, we prepared 4 type QC samples. For initial experiment, low, medium, and high concentrations for rutin, Tenuifoliside A and Rg3 were prepared spiked into plasma. For rutin experiment, low, medium, and high concentrations for rutin were prepared spiked with plasma. For radix polygala experiment, low, medium, and high concentrations for Tenuifoliside A, Sibiricose A6, 3, 6'- disinapoyl sucrose, Tenuifolin, and Polygalaxanthone III were prepared spiked with plasma. For white ginseng experiment, low, medium, and high concentrations for Rb2, Rb3, Rc, Rd, F1, Rh1(s), Rh1(r), Rb1, Rg1, Rg2(s), Rg2(r), Rg3, Re, Rf, Rh2(s), Rh2(r) and CK were prepared, and added to plasmas. The IS was prepared to 25 ng mL^{-1} in every samples.

Specificity, linearity, LODs and LOQs

The initial linearity, calibration curves, correlation coefficients, LODs and LOQs were summarized in Table S2.

The blank plasma, free-drug plasma spiked with QC samples, and real plasma from rats gavaged rutin, radix polygala extracts and white ginseng extracts, were used to assess specificity of method. For methanol extraction and Vial@FPBA method, calibration curves of various compounds at a series of concentrations were processed based on the form of $y = ax + b$. The LODs and LOQs of the analytes were obtained in Table S4, which are defined at a signal-to-noise ratio (S/N) of 3 and 10 times.

Accuracy and precision

Spiked samples with three concentration levels of QC samples were assayed for intra-day and inter-day precision and accuracy of each compound. Six replicates of 23 compounds were analyzed on the same day, and duplicate samples were determined on three consecutive days for inter-day precision. Here, RSD and RE were noted precision and accuracy, respectively.

Matrix effects and extraction recovery

Matrix effects and extraction recoveries at three QC levels (n=6) were analyzed by drug-free plasma samples spiked with three analytes, respectively.

Stability

The stabilities of proposed method were investigated for blank plasma spiked with 23 compounds at 25℃ for 24 h (short−term stability), at −20℃ for 30 days (long−term stability), and at three times of freeze−thaw cycles with three QC levels (n=6), respectively.

Application of the method in a pharmacokinetic study

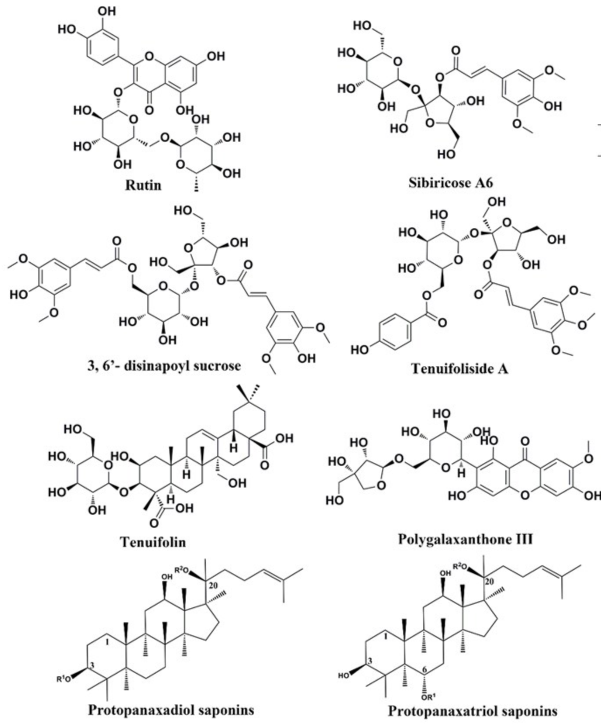
In this research, 3 groups of rats (rutin group, radix polygala group, and white ginseng group, each group including 6, 250 ± 20 g) were provided by Jilin Institute of Pharmacy (Jilin, China), namely rutin group, radix polygala group and white ginseng group. All the rats were maintained at a temperature of 23 ± 2℃ and a relative humidity of 55 ± 5%. After adapting one week, the rutin group, radix polygala group and white ginseng group were gavaged rutin (15 mg kg^{-1}), ethanol (75%) extracts of white ginseng (4 g kg^{-1}) and ethanol (75%) extracts of radix polygala (4 g kg^{-1}) at body weight for 5 days, individually. Later, all animals were fasted overnight but accessed water freely. Heparinized blood was collected from ophthalmic veins for 500 μL at 0, 0.083, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24 and 48 h. After centrifuging (4000 rpm, 10 min, 4℃), the collected supernatants were stored at −80℃ until analysis. All the experimental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Ethics Committee for the Use of Experimental Animals of Jilin University.

The obtained a series of time points each group samples (rutin group, radix polygala group and white ginseng group) were uniformly divided into 2 groups. In control group, 100 μL of thawed plasma samples were extracted by 400 μL of methanol. After vertexing a few second, the mixture was centrifuged and the obtained supernatants were dried by N_2 stream. The 100 μL of samples were re-dissolved with methanol (containing 25 ng mL^{-1} IS) until to analysis. And in our FPBA group, same samples were placed into FPBA-functionalized 96-well glass plate with 200 μL of water. The subsequent procedures were same in above Sample Processing section.

The UHPLC-MS/MS method was used to determine the concentrations of detected compounds in rat plasma after gavage of rutin, white ginseng extracts, and radix polygala extracts. Plasma concentration−time (C−T) profile was plotted by using a PK Solver 2.0 software, and PK parameters were calculated by following pharmacokinetic parameters: C_{max} (the highest peak plasma concentration), T_{max} (time at C_{max}), AUC_{0-t} h (AUC from time 0 h to last time), $AUC_{0-\infty}$ (AUC from time 0 h to infinity), mean residence time (MRT), and $t_{1/2}$ (elimination half-life).

Extension applications of Vial@FPBA for cis-diol compounds

The aglycones (flavonoid, triterpenoid, sapogenin, sucrose ester, polygalaxanthone), and we have added the analysis of other cis-diol groups, including adenosine, caffeic acid, cynarine, amygdalin, aesculin and polydatin. The low, medium, and high concentrations for the six standards were prepared spiked into plasma. We analyzed the plasma spiked with the supplement compounds of low, medium and high concentrations by our proposed method, in comparison with single sample analysis of methanol method in Table S11, 12.



Analyte	R^1	R^2	Type	sugars
Rb2	Glc-(1→2)-Glc-	Ara(*f*)-(1→6)-Glc-	PPD	4
Rb3	Glc-(1→2)-Glc-	Xyl-(1→6)-Glc-	PPD	4
Rc	Glc-(1→2)-Glc-	Ara(*f*)-(1→6)-Glc-	PPD	4
Rd	Glc-(1→2)-Glc-	Glc	PPD	3
F1	H	Glc	PPT	1
Rh1(s)	Rha-(1→2)-Glc-(s)	H	PPT	2
Rh1(r)	Rha-(1→2)-Glc-(r)	H	PPT	2
Rb1	Glc-(1→2)-Glc-	Glc-(1→6)-Glc-	PPD	4
Rg1	Glc	Glc	PPT	2
Rg2(s)	Rha-(1→2)-Glc-	H-(s)	PPT	2
Rg2(r)	Rha-(1→2)-Glc-	H-(r)	PPT	2
Rg3	Glc-(1→2)-Glc-	H	PPD	2
Re	Rha-(1→2)-Glc-	Glc	PPT	3
Rf	Glc-(1→2)-Glc-	H	PPT	2
Rh2(s)	Glc	H-(s)	PPD	1
Rh2(r)	Glc	H-(r)	PPD	1
CK	H	Glc	PPD	1

Fig. S1. Chemical structures of rutin, Sibiricose A6, 3, 6'-disinapoyl sucrose, Tenuifoliside A, Tenuifolin, Polygalaxanthone III, Protopanaxadiol (PPD) saponins and Protopanaxatriol (PPT) saponins (left), and substitution pattern, saponins types and numbers of sugar in ginsenosides (right). Here, PPD-type: containg PPD saponins; PPT-type: containg PPT saponins in ginsenosides structure.

Note: Glc, β-D-glucopyranose; Ara(p), α-L-arabinofpyranosyl; Rha, α-L-rhamnopyranosyl; Xyl, β-D-xylopyranosyl; Ara(f), α-L-arabinofuranosyl.

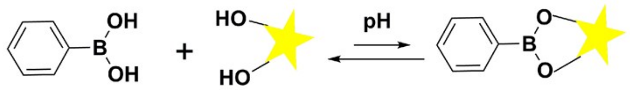


Fig. S2. Boronate-affinity mechanism (reversible covalent interaction) depending on pH, leaving intact enriched molecules after release.

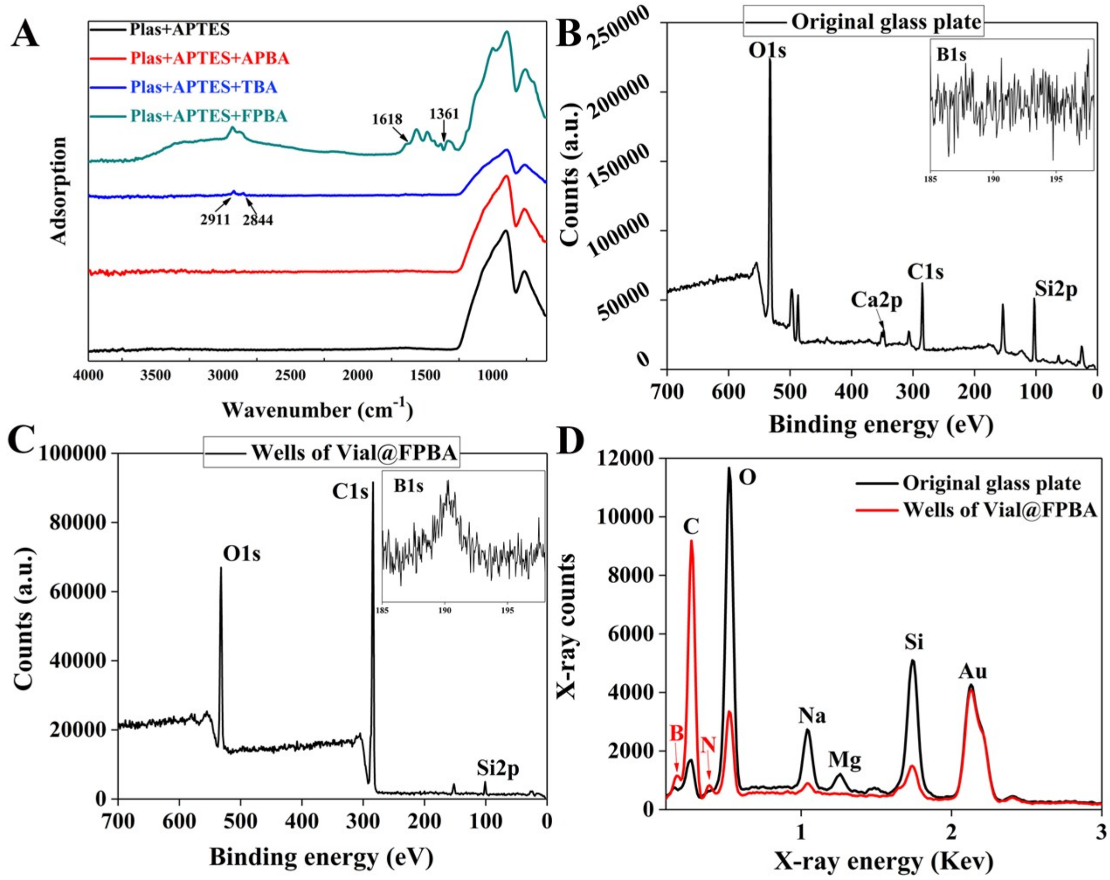


Fig. S3. FTIR spectra of APTES, APBA, TBA and FPBA functional glass plates (A), the XPS spectra for original glass plate (B) and the well of Vial@FPBA (C), EDS image for the well of original glass plate and Vial@FPBA (D).

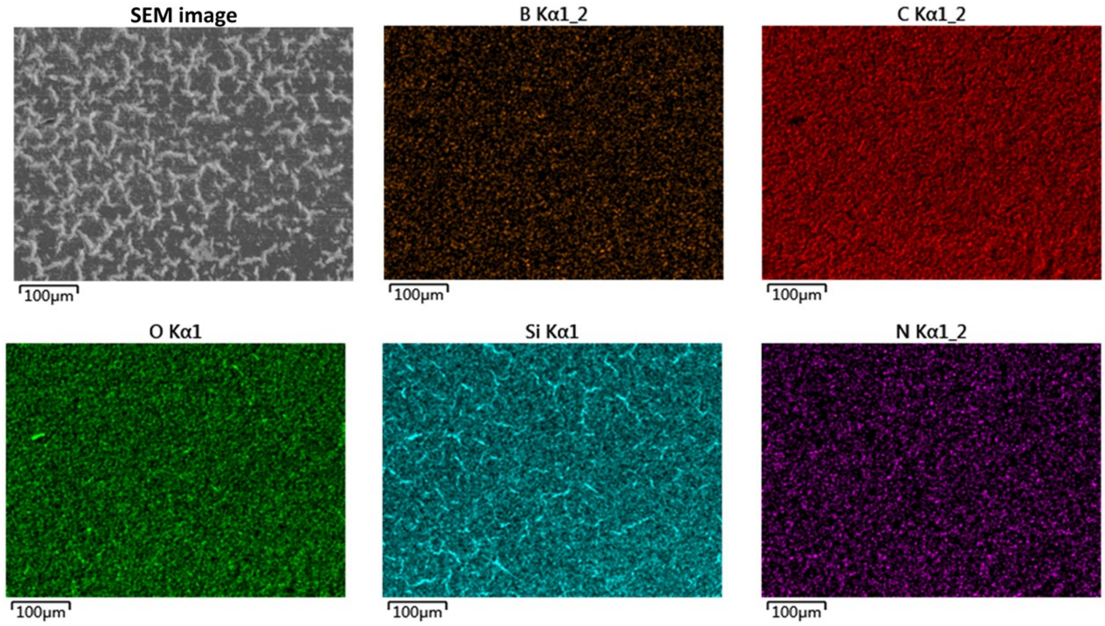


Fig. S4. SEM mapping images for Vial@FPBA.

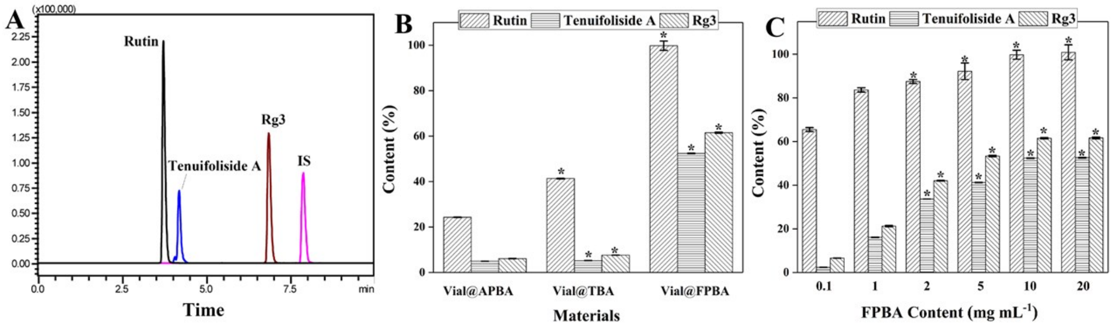


Fig. S5. MRM chromatograms of the 3 analytes and IS in initial experiment (A), enrichment content of three types of BA-functional 96-well glass plate for rutin, Tenuifoliside A and Rg3 (B), and effects of synthesis condition: FPBA amount (C).

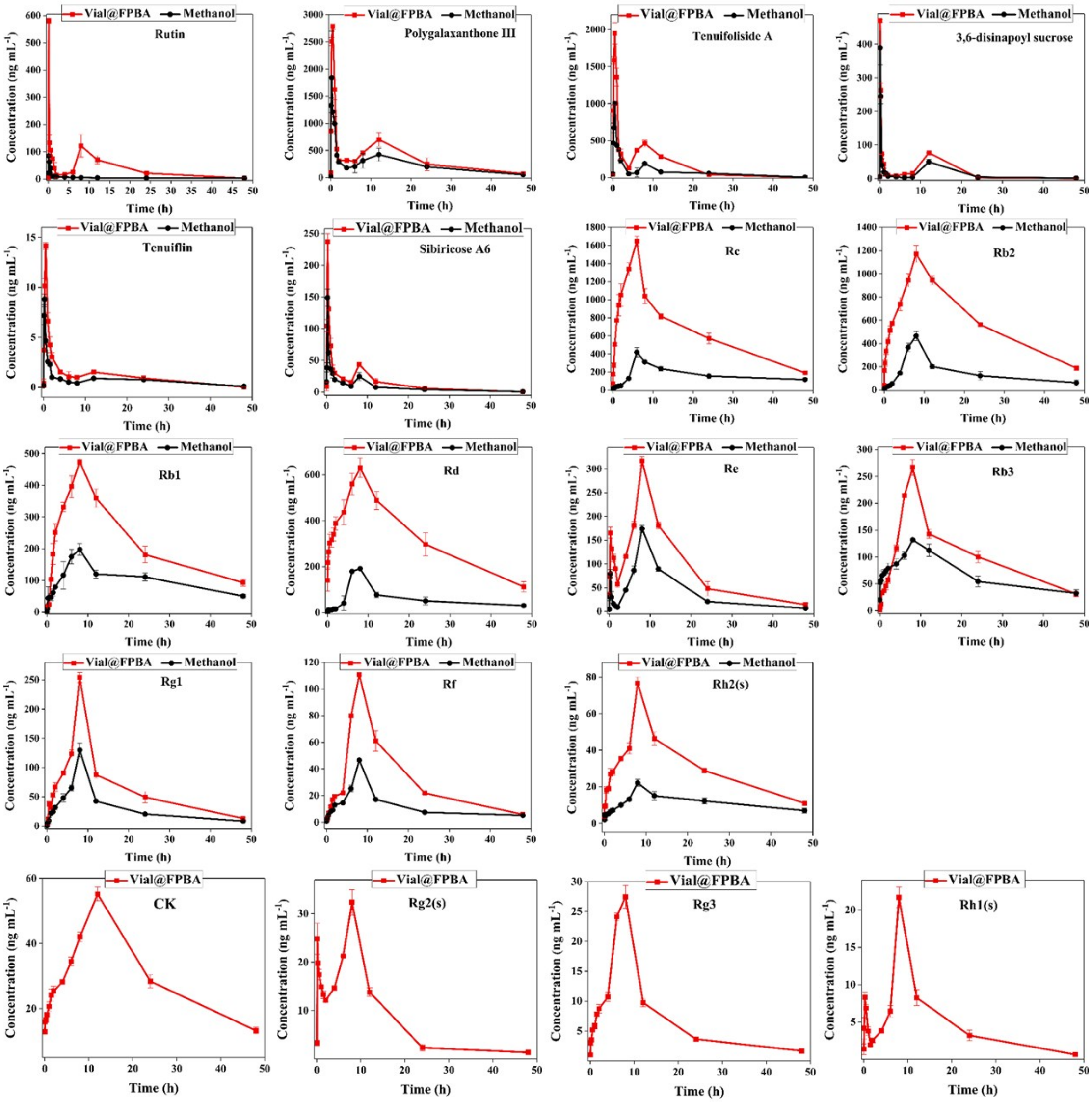


Fig. S6. The mean plasma concentration-time profiles of the 19 compounds in rats following i.g. administration of rutin, white ginseng extracts and polygala extracts by our proposed method *vs.* methanol method.

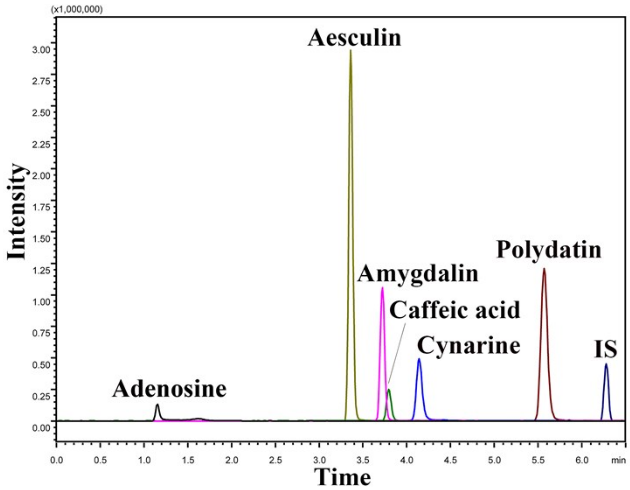


Fig. S7. MRM chromatograms of the 6 extensional analytes and IS.

Table S1 Element composition for the well of Vial@FPBA (n=3).

Element	Wt_{well} % Mean±SD	At_{well} % Mean±SD	Wt_{batch} % Mean±SD	At_{batch} % Mean±SD	RSD_{well} %	RSD_{batch} %
B	11.36±0.42	13.93±0.46	11.17±0.19	13.77±0.54	3.7	1.73
C	56.06±0.22	61.90±0.13	56.08±0.30	61.60±0.44	/	/
N	4.82±0.21	4.56±0.19	4.75±0.08	4.56±0.26	/	/
O	18.23±0.27	15.11±0.27	17.96±0.24	14.94±0.28	/	/
Si	9.54±0.56	4.51±0.28	9.15±0.41	4.45±0.28	/	/

RSD_{well} : RSD for wells of one single Vial@FPBA.

RSD_{batch} : RSD for batches of different Vial@FPBA plates.

Table S2 Optimized precursor and product ion pairs, Q1, CE and Q3, and RT of 23 analytes with dioscin (IS) in the negative ion mode for all experiments.

Analyte	Precursor ion (m/z)	product ion (m/z)	Q1 (V)	CE (V)	Q3 (V)	tR (min)
Rutin	609	300.1	24	37	20	2.82/3.67
Tenuifoliside A	681.1	443.15	20	25	23	4.13/4.66
Sibiricose A6	547	205.2	28	25	13	1.99
3, 6'- disinapoyl sucrose	753.2	205.2	30	34	24	4.36
Tenuifolin	679.3	455.3	26	27	24	6.35
Polygalaxanthone III	567	297.05	22	30	14	3.86
Rb2	1123.5	1077.4	34	28	34	12.19
Rb3	1123.5	1077.4	34	25	34	12.78
Rc	1123.5	1077.4	34	36	32	10.61
Rd	991.5	945.45	24	30	28	14.99
F1	683.4	637.55	26	20	22	12.05
Rh1(s)	683.4	637.45	26	21	36	8.94
Rh1(r)	683.4	637.45	26	20	36	9.58
Rb1	1153.5	1107.5	34	22	34	9.46
Rg1	845.4	799.5	26	25	24	5.11
Rg2(s)	829.4	783.4	24	21	22	8.95
Rg2(r)	829.4	783.4	24	20	22	9.46
Rg3	829.4	783.45	32	22	24	6.80/17.57
Re	991.4	945.5	30	24	28	5.18
Rf	845.4	799.45	26	21	24	7.61

Rh2(s)	667.4	621.45	26	15	36	19.18
Rh2(r)	667.4	621.5	26	20	36	19.64
CK	667.3	621.4	26	20	34	19.23
Dioscin	913.4	867.35	22	24	27	9.582/3.757/18.575/7.75
Adenosine	312.1	134.15	12	16	12	1.36
Caffeic acid	179.25	135.2	15	16	15	4.2
Cynarine	515	190.95	20	40	27	4.38
Amygdalin	502.25	456.1	20	12	24	4.15
Aesculin	339.05	177.1	20	25	20	3.87

Note: Different retention time (tR) respectively represented initial optimized condition, and rutin, white ginseng extracts, radix polygala extracts conditions, and extention application of cis-diol compounds conditions.

Table S3 The linear ranges, regression data, LODs and LOQs of the 3 compounds for initial optimization of Vial@FPBA platform (n=6).

Analyte	**Range (ng mL^{-1})**	**Calibration curves**	**Correlation coefficient (r^2)**	**LODs (ng mL^{-1})**	**LOQs (ng mL^{-1})**
Rutin	2.5-5000	Y = 0.9220X - 0.3092	0.9980	0.005	0.01
Tenuifoliside A	1-5000	Y = 0.3772X - 0.0683	0.9995	0.125	0.25
Rg3	10-2500	Y = 2.14295X + 0.4239	0.9986	0.05	0.1

Table S4 Parallelism experiments of Vial@FPBA for the enrichment of rutin, Tenuifoliside A and Rg3 (n=6).

Analyte	Precision (RSD, %)	Accuracy (RE, %)
Rutin	3.48	-0.23
Tenuifoliside A	3.32	-4.02
Rg3	2.82	-2.87

Table S5 Linear ranges, calibration curves, correlation coefficient, LODs and LOQs for the 23 analytes with methodology of Vial@FPBA in rat plasma.

Analyte	**Range (ng mL^{-1})**	**Calibration curves**	**Correlation coefficient (r^2)**	**LODs (ng mL^{-1})**	**LOQs (ng mL^{-1})**
Rutin	0.1-1000	Y = 1.2389X + 0.0962	0.9985	0.0001	0.0005
Sibiricose A6	10-2500	Y = 0.1147X + 0.0014	0.9990	0.05	0.1
3, 6'- disinapoyl sucrose	0.5-1000	Y = 0.6371X + 0.0031	0.9997	0.01	0.025
Tenuifoliside A	10-2500	Y = 0.0874X - 0.0371	0.9921	0.01	0.025
Tenuifolin	0.5-500	Y = 0.6911X + 0.0420	0.9995	0.01	0.025
Polygalaxanthone III	0.5-3000	Y = 0.2296X - 0.0006	0.9995	0.025	0.05
Rb2	2.5-2500	Y = 0.2220X - 0.0110	0.9987	0.05	0.1
Rb3	5-1000	Y = 0.2202X - 0.0091	0.9997	0.05	0.5
Rc	0.5-2500	Y = 0.1516X - 0.0098	0.9993	0.1	0.25
Rd	1-1000	Y = 0.6409X - 0.0111	0.9999	0.01	0.05
F1	2.5-1000	Y = 0.3711X - 0.0116	0.9997	0.1	0.5
Rh1(s)	1.25-1000	Y = 0.0850X - 0.0032	0.9999	0.5	1
Rh1(r)	5-1000	Y = 0.1129X - 0.0058	0.9995	1	5
Rb1	2.5-1000	Y = 0.3597X - 0.0122	0.9997	0.005	0.01
Rg1	2.5-1000	Y = 0.3755X - 0.0143	0.9997	0.1	0.5
Rg2(s)	1-1000	Y = 0.9560X - 0.0374	0.9990	0.05	0.75

Rg2(r)	1-1000	Y = 0.9617X - 0.0087	0.9990	0.08	0.75
Rg3	1-1000	Y = 0.9014X - 0.0261	0.9998	0.01	0.05
Re	1-1000	Y = 0.5442X - 0.0066	0.9995	0.01	0.05
Rf	2.5-1000	Y = 1.0993X + 0.0518	0.9999	0.01	0.025
Rh2(s)	1-500	Y = 0.2653X - 0.0050	0.9995	0.5	2.5
Rh2(r)	1-500	Y = 0.0508X + 0.0015	0.9995	2.5	5
CK	1-500	Y = 0.3029X - 0.0077	0.9994	0.1	1

Table S6 Intra-day and inter-day precision and accuracy for the determination of 23 analytes in rat plasma (n=6).

Analyte	Nominal conc. (ng mL^{-1})	Intra-day		Inter-day	
		Precision (RSD, %)	Accuracy (RE, %)	Precision (RSD, %)	Accuracy (RE, %)
Rutin	1000	6.84	0.06	5.06	0.42
	250	5.17	8.83	5.01	7.42
	5	8.74	-6.08	4.60	-9.38
Sibiricose A6	2500	7.56	-1.53	5.71	-2.44
	250	6.90	0.88	7.52	-3.63
	10	8.21	0.08	1.82	-7.56
3, 6’-disinapoyl sucrose	500	1.35	-1.55	1.13	-2.52
	100	1.64	-1.49	1.11	-0.97
	5	1.58	-1.75	0.56	-1.36
Tenuifoliside A	1000	0.90	9.00	5.85	-0.90
	250	0.30	2.97	2.05	0.06
	20	0.82	8.19	4.86	-0.13
Tenuifolin	500	4.42	-2.51	3.45	0.13
	100	5.41	-2.57	7.66	-2.70
	5	2.84	-0.69	4.22	-5.14
Polygalaxanthone III	3000	0.95	-7.69	0.58	-7.02
	500	1.13	-7.79	0.49	-7.72
	5	0.76	-7.36	0.98	-8.04
Rb2	2500	2.53	1.33	3.14	0.61
	250	0.05	2.81	0.88	0.74
	10	3.10	0.58	0.75	1.15
Rb3	1000	3.21	1.68	2.37	0.06
	250	2.18	3.33	2.80	0.51
	10	4.54	1.65	3.29	1.39
Rc	2500	5.40	1.80	7.58	1.29

	250	2.63	4.77	4.56	-1.57
	5	4.95	-0.66	1.21	-4.12
	1000	1.77	3.09	0.88	2.10
Rd	250	2.46	3.27	1.00	0.06
	10	1.58	3.90	1.41	0.04
	1000	1.61	-0.06	2.16	-0.37
F1	250	0.42	0.86	5.57	3.33
	10	1.74	-0.66	4.32	6.08
	1000	3.70	0.12	2.05	2.08
Rh1(s)	250	5.21	-0.12	3.20	2.11
	10	3.16	-1.61	3.09	3.47
	1000	8.62	2.51	10.27	-0.42
Rh1(r)	250	1.02	7.59	2.21	3.57
	10	11.29	0.36	0.74	4.85
	1000	3.64	2.03	3.71	3.49
Rb1	250	4.89	2.65	2.33	-2.07
	10	1.18	-0.06	0.80	-0.79
	1000	2.88	2.74	0.56	1.05
Rg1	250	3.74	3.39	9.64	-0.51
	10	3.18	3.79	7.94	3.89
	1000	8.69	-4.35	0.76	0.44
Rg2(s)	250	11.27	-6.48	5.64	3.42
	10	10.52	-7.02	0.39	0.05
	1000	4.23	-4.73	4.33	-7.52
Rg2(r)	250	1.86	-2.85	5.73	-6.57
	10	4.19	-5.94	6.96	-5.72
	1000	8.73	-4.87	8.70	-5.15
Rg3	250	2.87	2.32	3.16	1.91
	10	7.21	-2.08	5.07	-4.62
Re	1000	8.22	-3.11	3.85	-3.98

	250	4.50	-5.74	0.16	-1.48
	10	3.05	-3.18	3.69	-4.09
	1000	2.17	-4.34	1.02	-4.29
Rf	250	1.58	-3.51	1.21	-2.77
	10	2.59	-4.59	5.28	-4.27
	500	3.62	-4.39	5.20	-2.81
Rh2(s)	100	1.40	-5.91	6.46	-4.46
	10	2.42	-5.22	0.98	-8.01
	500	8.22	-3.01	2.76	-5.53
Rh2(r)	100	3.74	-6.16	2.95	-5.80
	10	4.49	-0.53	3.60	-5.94
	500	2.07	-6.94	2.22	-3.87
CK	100	2.83	-7.23	2.96	-4.36
	10	0.74	-5.87	5.05	-5.73

Table S7 Recoveries and matrix effect of the 23 analytes in rat plasma (n=6).

Analyte	Nominal conc. (ng mL^{-1})	Recoveries (%, n=6)		Matrix effect (%, n=6)	
		Mean±SD	RSD	Mean±SD	RSD
Rutin	1000	101.17±5.89	5.82	97.85±9.59	9.80
	250	108.84±3.61	3.32	109.49±4.40	4.02
	5	94.46±7.74	8.19	93.49±5.68	6.08
Sibiricose A6	2500	98.48±0.74	0.75	98.89±2.65	2.68
	250	99.71±2.48	2.49	98.62±3.17	3.21
	10	100.50±1.09	1.09	100.15±0.98	0.98
3, 6’-disinapoyl sucrose	500	95.47±3.90	4.08	93.21±2.18	2.33
	100	100.84±2.01	1.99	100.08±2.15	2.15
	5	101.98±0.54	0.53	92.98±1.84	1.98
Tenuifoliside A	1000	104.00±1.87	1.80	93.33±3.02	3.24
	250	104.87±3.66	3.49	106.09±2.83	2.67
	20	95.38±8.66	9.08	91.33±7.15	7.83
Tenuifolin	500	95.99±2.26	2.35	97.27±1.25	1.29
	100	97.05±0.97	0.99	94.60±2.52	2.67
	5	96.49±0.15	0.16	95.48±3.77	3.95
Polygalaxanthone III	3000	92.75±1.99	2.15	91.63±0.67	0.73
	500	93.54±2.04	2.18	95.08±1.01	1.06
	5	93.07±2.71	2.91	94.73±1.01	1.07
Rb2	2500	99.23±1.17	1.18	101.48±4.35	4.29
	250	101.22±4.73	4.67	98.14±0.37	0.38
	10	98.97±1.55	1.56	99.87±2.85	2.86
Rb3	1000	102.48±6.55	6.39	100.81±1.05	1.04
	250	104.50±3.6	3.53	101.12±1.0	1.02

		9		3	
	10	103.02±1.60	1.55	99.91±0.04	0.04
Rc	2500	101.72±0.09	0.09	97.42±3.27	3.35
	250	100.86±1.30	1.29	97.29±3.08	3.17
	5	99.60±0.19	0.19	98.19±4.36	4.44
Rd	1000	100.46±2.69	2.67	91.48±1.11	1.21
	250	92.56±2.64	2.85	91.91±1.73	1.89
	10	92.60±2.69	2.91	92.24±1.31	1.42
F1	1000	91.00±1.03	1.13	92.00±2.44	2.66
	250	98.87±0.81	0.82	92.32±2.42	2.62
	10	91.73±2.59	2.82	93.51±0.54	0.57
Rh1(s)	1000	93.55±0.75	0.80	95.86±2.75	2.86
	250	91.41±3.77	4.13	97.05±0.97	0.99
	10	90.88±3.02	3.32	95.43±0.89	0.93
Rh1(r)	1000	95.42±1.26	1.32	94.29±4.84	5.13
	250	94.99±0.65	0.68	92.31±3.38	3.66
	10	96.06±4.05	4.22	96.21±5.72	5.94
Rb1	1000	98.00±3.18	3.25	94.17±0.39	0.42
	250	91.94±3.54	3.85	92.78±1.57	1.69
	10	93.06±1.96	2.11	91.67±3.14	3.43
Rg1	1000	95.08±8.71	9.16	97.45±13.65	14.00
	250	97.51±8.86	9.09	91.80±5.66	6.16
	10	96.90±9.70	10.01	88.72±1.29	1.46
Rg2(s)	1000	92.88±2.53	2.72	91.79±5.95	6.48
	250	92.72±4.36	4.71	92.79±7.37	7.94
	10	98.37±12.35	12.56	97.76±1.89	1.93
Rg2(r)	1000	100.00± 6.40	6.40	96.13±7.78	8.09
	250	96.50±2.88	2.99	93.00±3.36	3.61

	10	95.88±5.52	5.75	98.50±4.42	4.49
	1000	97.05±5.48	5.65	98.23±7.11	7.24
Rg3	250	103.36±2.63	2.54	102.79±2.65	2.58
	10	99.06±5.94	6.00	97.06±4.50	4.64
	1000	102.99±3.06	2.97	101.48±2.24	2.20
Re	250	104.54±2.09	2.00	97.02±5.64	5.81
	10	102.96±4.33	4.20	99.89±3.72	3.73
	1000	97.58±0.71	0.73	87.38±1.24	1.42
Rf	250	94.42±6.35	6.72	97.56±1.01	1.03
	10	94.88±9.37	9.88	90.88±2.30	2.53
	500	97.28±3.18	3.26	96.15±3.54	3.68
Rh2(s)	100	96.60±4.17	4.31	93.86±5.53	5.89
	10	99.10±0.63	0.64	97.00±1.41	1.46
	500	96.53±2.36	2.44	96.79±3.27	3.38
Rh2(r)	100	97.55±2.20	2.25	100.73±8.87	8.80
	10	102.77±5.98	5.82	95.24±1.08	1.13
	500	101.00±0.81	0.80	97.36±1.41	1.45
CK	100	97.07±4.75	4.89	98.67±2.47	2.50
	10	99.33±1.68	1.69	97.07±4.75	4.89

Table S8 Stabilities for 23 analytes throughout the entire experimental process (n=6).

Analyte	Nominal conc. (ng mL^{-1})	Short term stability		Long term stability		Freeze–thaw stability	
		Precision (RSD, %)	Accuracy (RE, %)	Precision (RSD, %)	Accuracy (RE, %)	Precision (RSD, %)	Accuracy (RE, %)
Rutin	1000	3.03	0.22	0.94	0.36	0.86	0.60
	250	3.38	0.83	0.92	0.07	0.13	1.12
	5	3.15	1.01	0.65	0.74	1.42	0.21
Sibiricose A6	2500	7.12	-0.63	5.93	2.22	9.76	0.56
	250	6.60	-3.02	2.47	4.68	1.81	-0.52
	10	4.20	2.43	5.85	-5.95	1.74	0.23
3, 6'-disinapoyl sucrose	500	0.55	-0.58	3.97	-1.94	1.84	0.65
	100	1.68	-2.14	4.77	-2.37	4.89	-3.97
	5	0.80	-1.21	1.92	-0.16	6.72	-2.66
Tenuifoliside A	1000	3.33	-2.50	2.34	-2.92	5.18	-2.08
	250	3.97	-2.89	2.00	0.09	0.84	-5.09
	20	3.07	-1.45	3.18	-3.49	1.58	-8.25
Tenuifolin	500	5.65	-2.00	3.29	-0.23	1.19	-2.65
	100	6.11	-3.36	5.34	-6.09	0.95	-2.49
	5	6.90	-1.82	0.89	-2.81	0.24	-3.31
Polygalaxanthone III	3000	1.56	-7.66	2.19	-2.52	2.40	-4.55
	500	2.61	-3.43	1.09	-3.67241	4.36	-3.19
	5	3.18	-3.60	1.96	-1.56897	1.31	-5.29
Rb2	2500	1.24	0.81	0.38	0.38	0.37	0.21
	250	0.49	0.27	0.53	0.33	0.17	0.59
	10	1.29	0.11	0.56	-0.40	3.80	2.75
Rb3	1000	0.20	-1.11	4.75	-4.80	2.51	4.38
	250	3.49	1.25	13.11	1.40	4.59	3.04
	10	9.52	0.40	8.38	4.60	4.41	1.00
Rc	2500	4.82	0.12	6.32	-2.84	1.01	0.49
	250	6.03	-0.71	4.47	4.81	1.25	0.77

	5	7.62	0.81	10.78	0.47	1.33	0.83
	1000	0.67	0.57	1.41	0.04	1.36	-6.27
Rd	250	0.74	-0.43	0.67	0.57	1.70	-6.61
	10	1.00	0.06	0.74	-0.43	1.64	-6.65
	1000	3.52	0.34	0.84	0.36	0.88	-0.75
F1	250	7.84	3.58	3.77	-1.67	1.23	-0.84
	10	2.78	-1.19	2.93	-2.26	0.41	-0.27
	1000	1.28	0.30	3.79	1.82	1.47	-3.91
Rh1(s)	250	4.37	2.56	5.04	-1.09	6.70	-0.18
	10	3.12	0.91	5.23	0.82	0.50	-6.25
	1000	2.27	2.65	1.53	3.90	1.21	-4.74
Rh1(r)	250	3.01	3.20	10.25	-4.17	1.70	-4.84
	10	8.84	-1.10	11.77	-3.04	0.38	-5.72
	1000	2.39	-2.99	6.08	1.09	8.41	-5.38
Rb1	250	3.19	-2.42	1.85	-1.98	5.17	-7.62
	10	4.44	0.49	4.24	2.37	2.47	-9.42
	1000	13.43	0.21	5.38	-3.28	2.47	-7.64
Rg1	250	5.52	-5.62	2.29	-5.42	2.42	-8.33
	10	3.83	-3.48	3.09	-1.74	2.73	-8.15
	1000	6.98	4.96	2.32	-4.88	3.48	-7.61
Rg2(s)	250	6.59	5.23	1.85	-2.03	2.60	-8.04
	10	2.95	-3.50	4.17	-3.60	0.76	-9.40
	1000	1.23	-1.93	2.83	-7.87	4.83	-7.72
Rg2(r)	250	2.63	-2.88	2.20	-6.87	2.60	-9.21
	10	0.74	-8.02	3.42	-7.66	1.86	-9.69
	1000	2.97	-6.53	4.80	-2.21	3.89	0.41
Rg3	250	3.92	-2.25	0.78	-4.91	2.26	-3.89
	10	4.43	-1.54	5.38	-0.61	1.49	-3.37
Re	1000	2.22	-3.93	1.67	-5.47	1.37	-2.40
	250	1.51	-3.05	3.78	-4.02	0.74	-3.85

	10	2.68	-4.13	2.41	-4.97	2.01	-5.13
	1000	2.44	-5.04	0.36	-5.63	3.27	-4.62
Rf	250	0.78	-6.03	2.19	-3.90	2.86	-6.38
	10	1.09	-6.11	2.55	-4.14	3.03	-5.71
	500	2.92	-5.64	0.16	-8.47	6.06	-4.11
Rh2(s)	100	2.01	-7.04	4.29	-5.71	1.97	-6.92
	10	4.12	-5.61	2.18	-7.14	7.07	-3.30
	500	6.85	-0.50	1.83	-7.72	4.60	-0.03
Rh2(r)	100	9.57	-2.30	0.90	-5.92	4.91	-0.01
	10	7.75	-1.10	1.36	-4.77	0.97	-0.05
	500	0.80	-5.15	5.63	0.14	4.99	-0.86
CK	100	6.99	-0.78	1.34	0.03	5.59	-1.92
	10	6.20	-0.24	4.31	-0.72	4.13	0.93

Table S9 Comparison of this work with methanol method.

Analyte	Methanol method			This work		
	Range (ng mL^{-1})	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	Range (ng mL^{-1})	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
Rutin	2.5-1000	0.005	0.01	0.1-1000	0.0001	0.0005
SibiricoseA6	5-1000	0.25	0.5	10-2500	0.05	0.1
3, 6'- disinapoyl sucrose	0.5-1000	0.125	0.25	0.5-1000	0.01	0.025
Tenuifoliside A	1-1000	0.125	0.25	10-2500	0.01	0.025
Tenuifolin	5-500	0.125	0.25	0.5-500	0.01	0.025
Polygalaxanthone III	5-2500	0.8	1	0.5-3000	0.025	0.05
Rb2	2.5-2500	0.05	0.25	2.5-2500	0.05	0.1
Rb3	5-1000	0.05	1	5-1000	0.05	0.5
Rc	5-1000	4	8	0.5-2500	0.1	0.25
Rd	1-1000	0.25	0.5	1-1000	0.01	0.05
F1	2.5-500	0.5	0.75	2.5-1000	0.1	0.5
Rh1(s)	5-1000	5	10	1.25-1000	0.5	1
Rh1(r)	2.5-1000	5	10	5-1000	1	5
Rb1	2.5-1000	0.025	0.05	2.5-1000	0.005	0.01
Rg1	5-1000	1	10	2.5-1000	0.1	0.5
Rg2(s)	2.5-1000	0.1	0.25	1-1000	0.05	0.75
Rg2(r)	2.5-1000	1	2.5	1-1000	0.08	0.75
Rg3	2.5-500	0.05	0.1	1-1000	0.01	0.05
Re	2.5-1000	0.1	0.75	1-1000	0.01	0.05
Rf	5-1000	0.025	0.05	2.5-1000	0.01	0.025
Rh2(s)	2.5-500	5	10	1-500	0.5	2.5
Rh2(r)	2.5-500	5	10	1-500	2.5	5
CK	2.5-500	1	5	1-500	0.1	1

Table S10 The pharmacokinetic parameters (X±SD, n=6) of 19 analytes in rat plasma for rutin, white ginseng extracts and radix polygala extracts by two enrichment methods.

Method	Analyte	Tmax (h)	Cmax (ng mL^{-1})	$t_{1/2}$ (h)	$AUC_{0\rightarrow t}$ (ng h mL^{-1})	$AUC_{0\rightarrow \infty}$ (ng h mL^{-1})	$MRT_{0\rightarrow t}$ (h)
Vial@FPBA	Rutin	0.08	582.38±39.97**	7.78±0.95**	1659.02±238.83**	1696.39±254.02**	13.99±1.03
Methanol		0.08	83.57±2.65	24.79±3.55	220.35±4.37	282.60±24.11	35.50±9.18
Vial@FPBA	Polygalaxanthone III	0.46±0.04	2973.50±386.83	6.39±0.55**	16015.33±658.76*	16155.46±709.70	13.07±0.54**
Methanol		0.23±0.03	1683.91±225.81	12.45±0.50	12171.17±826.28	13190.60±952.05	18.52±0.06
Vial@FPBA	Tenuifoliside A	0.50	1948.94±144.18**	5.06±0.19**	7827.19±328.44**	7842.08±333.00**	8.31±0.17**
Methanol		0.50	1136.25±41.96	7.88±0.44	3805.52±61.15	3852.89±65.38	11.71±0.57
Vial@FPBA	3,6-disinapoyl sucrose	0.08	466.71±3.92	4.33±0.50	997.87±62.43	1002.80±56.56	10.86±0.04*
Methanol		0.08	389.46±51.21	8.31±0.70	749.51±59.45	777.62±54.88	14.23±0.38
Vial@FPBA	Tenuifolin	0.44±0.06	17.82±1.47	6.80±0.90*	42.25±3.67	47.47±3.32	13.73±0.30*
Methanol		0.50	8.00±1.00	10.17±0.34	33.95±2.45	35.22±2.47	16.29±0.23
Vial@FPBA	SibiricoseA6	0.25	237.06±12.78	4.90±0.05**	644.46±26.98**	646.62±25.28**	9.17±0.40*
Methanol		0.25	149.03±13.41	6.76±0.27	414.81±4.12	418.04±4.66	10.80±0.46
Vial@FPBA	Rc	6.00	1647.58±211.29*	16.84±0.83*	30674.37±5682.39	35331.74±5654.69	23.67±1.39*
Methanol		6.00	781.71±3.78	23.14±1.75	13921.02±1515.08	18930.70±2606.74	35.70±2.21
Vial@FPBA	Rb2	8.00	1172.89±102.64**	15.48±0.49**	28211.56±3727.10**	32425.05±4410.53**	24.21±0.60*
Methanol		8.00	363.63±28.28	21.11±0.62	5590.68±108.03	9308.77±18.94	28.62±1.92
Vial@FPBA	Rb1	8.00	474.36±8.74**	17.44±0.95**	10608.62±887.63**	12960.69±1230.38**	27.58±1.58**
Methanol		8.00	188.50±20.45	26.17±1.16	4957.67±162.81	6913.38±270.03	38.50±0.83
Vial@FPBA	Rd	8.00	630.59±42.35	16.63±1.85	15505.05±1907.50	18242.94±1701.22	25.55±3.14
Methanol		8.00	190.63±3.19	22.70±4.29	2774.99±165.33	3746.71±232.32	36.62±4.25
Vial@FPBA	Re	8.00	316.43±9.31**	9.19±0.79**	4295.00±396.56**	4487.42±464.32**	15.34±1.48

Methanol		8.00	174.00±7.35	14.04±0.73	1609.45±9.46	1742.21±3.83	17.93±0.21
Vial@FPBA	Rb3	8.00	266.87±24.66**	16.10±1.91	4919.38±827.90	5641.51±848.87	24.21±0.25
Methanol		10.00±0.83	133.27±3.35	20.82±0.93	3065.35±348.99	3929.85±689.10	32.57±1.06
Vial@FPBA	Rg1	8.00	254.04±0.62 **	12.97±0.48 **	3080.79±237.03 **	3325.34±284.86**	18.63±1.23*
Methanol		8.00	129.97±2.11	15.96±1.16	1495.16±50.37	1691.52±80.88	21.38±0.89
Vial@FPBA	Rf	8.00	110.88±0.83**	10.42±0.56**	1532.61±56.99**	1620.93±63.20**	17.37±0.06**
Methanol		8.00	46.64±1.34	22.68±1.59	583.11±19.63	753.54±4.68	30.87±1.88
Vial@FPBA	Rh2(s)	8.00	76.72±6.34**	17.28±0.61**	1661.89±144.28**	1979.59±145.38*	26.14±1.23**
Methanol		8.00	22.07±1.92	25.82±2.69	607.42±5.33	866.82±39.71	37.88±1.64
Vial@FPBA	CK	12.00	55.18±5.11	18.06±0.62	1430.67±85.67	1776.94±113.73	29.60±0.47
Methanol		/	/	/	/	/	/
Vial@FPBA	Rg2(s)	8.00	32.37±2.62	9.26±0.04	380.63±26.87	398.64±28.09	13.56±0.43
Methanol		/	/	/	/	/	/
Vial@FPBA	Rg3	8.00	28.14±2.37	15.05±0.63	386.81±3.36	409.10±42.37	19.30±1.69
Methanol		/	/	/	/	/	/
Vial@FPBA	Rh1(s)	8.00	21.58±4.79	11.43±1.56	218.60±34.45	232.19±30.91	17.29±1.37
Methanol		/	/	/	/	/	

Note: a list of the metabolites via Mean±SD Student's t-test: * $p < 0.05$, ** $p < 0.01$, for Vial@FPBA method, vs. Methanol group.

"/" represented no discovered and calculated data.

Table S11 Linear ranges, calibration curves, correlation coefficient for adenosine, caffeic acid, cynarine, amygdalin, aesculin, and polydatin.

Analyte	**Range (ng mL^{-1})**	**Calibration curves**	**Correlation coefficient (r^2)**
Adenosine	10-1000	Y = 1.3551X + 0.1084	0.9995
Caffeic acid	10-1000	Y = 2.0552X + 0.4055	0.9990
Cynarine	5-1000	Y = 5.1118X - 0.6247	0.9957
Amygdalin	10-1000	Y = 8.6409X - 0.1153	0.9998
Aesculin	5-1000	Y = 18.8570X + 1.5493	0.9989
Polydatin	10-1000	Y = 14.9635X + 1.5845	0.9996

Table S12 Recoveries of adenosine, caffeic acid, cynarine, amygdalin, aesculin, and polydatin in rat plasma by two methods (n=6).

Analyte	Nominal conc. (ng mL^{-1})	Recoveries (Methanol method) (%, n=6)		Recoverie (Vial@FPBA) (%, n=6)	
		Mean±SD	RSD	Mean±SD	RSD
Adenosine	1000	91.07±1.60	1.76	98.97±7.24	7.32
	100	83.83±2.11	2.51	95.80±10.90	11.38
	10	/	/	92.79±7.12	7.67
Caffeic acid	1000	94.67±6.48	6.85	93.60±11.57	12.37
	100	85.77±4.57	5.33	95.99±13.79	14.36
	10	/	/	89.01±9.53	10.71
Cynarine	1000	93.31±7.23	7.75	91.27±5.90	6.47
	100	87.45±2.20	2.51	98.05±12.42	12.67
	10	81.53±1.29	1.59	89.26±1.04	1.17
Amygdalin	1000	91.50±7.92	8.65	97.49±6.07	6.23
	100	88.90±12.65	14.23	95.51±4.86	5.09
	10	81.45±8.24	10.11	94.14±5.37	5.71
Aesculin	1000	96.29±5.68	5.90	108.20±9.69	8.95
	100	95.33±12.86	13.49	100.61±5.58	5.54
	10	78.42±8.58	10.94	100.86±5.91	5.86
Polydatin	1000	92.47±4.25	4.59	93.01±4.18	4.50
	100	84.61±11.80	13.95	103.97±7.40	7.12
	10	82.04±6.18	7.53	90.56±4.68	5.17

Note: /: The concentrations of detected analytes low LODs of instrument by methanol method, we didn't detect the concentration of the sample.

Reference

1 H. Zhu, H. Yao, K. Xia, J. Liu, X. Yin, W. Zhang and J. Pan, *Chem. Eng. J.*, 2018, **346**, 317.