Electronic Supplementary information

Enhanced one-step sample pretreatment method for extraction of ginsenosides from rat plasma using tailor-made deep eutectic mixture solvents

Ningning Zhao[a,b], Shu Liu*[a], Junpeng Xing[a], Zhong Zheng[a], Zifeng Pi[a], Fengrui Song[a], Zhiqiang Liu*[a,b]

Table S1 Optimized precursor/product ion pairs and MRM parameters of the 15 analytes with dioscin (IS) in the negative ion mode.

Analyte	MRM transition (m/z) Precursor ion→product ion	Dwell time(s)	Cone energy (V)	Collision energy (V)	tR (min)
Rg1	799.54→637.45	0.05	34	30	5.9550
Re	945.54→100.95	0.05	60	46	6.0600
Rf	799.54→100.95	0.05	52	36	9.9550
Rb1	1107.58→112.99	0.05	74	50	13.835
Ro	955.47→613.10	0.05	70	48	19.920
Rc	1077.50→191.00	0.05	74	38	16.495
Rb2	1077.51→100.95	0.05	74	42	20.145
Rb3	1077.51→100.95	0.05	72	58	21.005
Rd	991.60→100.98	0.05	20	51	27.785
CK	621.2→160.95	0.05	40	16	30.480
Rh1	683.46→637.41	0.05	29	24	13.240
Rh2	667.4→621.43	0.05	35	21	30.395
Rg2	829.54→783.48	0.05	25	18	12.910
Rg3	783.5→100.95	0.05	54	32	29.730
PPD	459.33→375.26	0.05	52	25	30.420
Dioscin	867.38→721.05	0.05	56	26	30.010

Table S2 The linear range, regression data, and LOQs of the fifteen ginsenosides. (n = 6)

Analyte	Range (ng mL^{-1})	Calibration curves	Correlation coefficient (r^2)	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
Rg1	5-1250	y=0.1248x+0.0014	0.9964	0.1	3
Re	5-2500	y=0.2960x-0.0015	0.9943	0.1	5
Rf	1-1250	y=0.3687x-0.0003	0.9910	0.5	1
Ro	20-2500	y=0.1442x-0.0031	0.9923	0.5	20
Rb1	40-2500	y=0.0379x-0.0016	0.9916	0.1	8
Rc	20-2500	y=0.2811x-0.0085	0.9910	0.1	15
Rb2	40-1250	y=0.0631x-0.0023	0.9944	0.25	25
Rb3	20-1250	y=0.0834x-0.0012	0.9940	0.25	10
Rd	10-1250	y=0.1856x-0.0009	0.9967	0.1	10
CK	5-1250	y=0.1189x-0.0001	0.9916	0.1	5
Rh1	20-2500	y=0.0359x-0.0001	0.9991	0.5	15
Rh2	20-1250	y=0.0981x-0.0012	0.9965	0.25	15
Rg2	1-1250	y=0.4964x-0.0012	0.9914	0.1	0.8
Rg3	1-1250	y=0.6037x+0.0011	0.9932	0.25	0.4
PPD	40-5000	y=0.0238x-0.0006	0.9994	0.1	40

LOD, limit of detection; LOQ, limit of quantification.

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17 **Table S3** Intra-day and inter-day precisions and accuracies for the determination of fifteen

18 ginsenosides from the samples (mean±SD, n = 6)

Analytes	Nominal conc. (ng mL^{-1})	Intra-day		Inter-day	
		Precision (RSD, %)	Accuracy (RE, %)	Precision (RSD, %)	Accuracy (RE, %)
	1250	7.29	-2.00	5.49	-5.54
Rg1	50	6.59	-0.77	10.31	-0.94
	5	11.19	1.38	7.77	3.29
	2500	2.89	-2.67	2.95	-3.81
Re	100	8.56	4.14	10.30	5.05
	5	7.06	-2.05	11.85	-2.78
	1250	7.28	-6.37	4.69	-2.90
Rf	50	7.25	-3.89	8.51	0.34
	1	6.39	-3.32	4.15	-8.24
	2500	6.18	5.30	8.72	5.43
Ro	200	6.53	-5.70	5.08	-7.70
	20	10.22	-4.52	5.02	-7.08
	2500	8.42	-2.55	11.90	-3.08
Rb1	400	6.56	-7.31	3.45	-3.53
	40	8.20	-11.25	9.82	-8.82
	2500	8.20	-5.14	6.91	-1.61
Rc	200	7.75	-2.92	2.38	-9.30
	20	8.83	-7.08	11.66	-3.15
	1250	8.51	-0.63	11.78	-1.89
Rb2	200	5.19	-11.62	4.57	-9.64
	40	8.18	-2.15	1.69	-4.98
	1250	7.17	5.72	3.49	9.81
Rb3	100	10.62	-2.17	1.71	-4.11
	20	6.36	-6.39	10.18	-7.72
	1250	1.91	-8.42	0.32	-9.42
Rd	100	6.43	2.39	8.50	5.85
	10	7.79	-2.46	5.34	-8.55
	1250	8.29	3.29	6.99	-0.76
CK	50	6.73	-5.80	11.09	-7.70
	5	5.39	-3.99	3.25	-7.36
	2500	7.63	2.77	10.79	2.85
Rh1	200	7.32	0.55	2.49	-5.72
	20	7.18	-0.38	2.57	-5.08
	1250	6.95	-1.93	6.75	0.85
Rh2	100	6.05	2.55	3.23	5.80
	20	9.14	6.73	11.32	9.23
	1250	5.84	-0.79	5.53	1.64
Rg2	50	8.17	2.61	10.24	7.30
	1	7.85	-3.53	1.97	-8.09
	1250	4.15	-4.73	1.73	-6.91
Rg3	50	6.33	-8.15	5.05	-12.32
	1	8.08	-7.22	8.52	-12.00
	5000	3.37	-6.36	4.75	-6.51
PPD	500	8.43	1.98	9.74	2.45
	50	4.25	0.73	3.28	4.01

Table S4 The stability of fifteen ginsenosides in rat plasma under different storage conditions.

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, %)
	1250	10.32	-2.06	4.84	1.61	4.98	1.51
Rg1	50	8.00	-2.65	2.32	4.57	7.24	1.11
	5	9.60	4.73	1.84	10.41	7.77	3.29
	2500	1.01	-1.09	3.96	-3.10	0.50	-0.74
Re	100	1.36	-3.53	11.65	4.12	1.14	11.80
	5	3.16	3.07	8.71	-5.08	11.85	-2.78
	1250	5.63	-9.71	10.30	-6.50	2.87	-11.51
Rf	50	9.86	-0.55	1.35	-6.59	2.34	-7.24
	1	10.20	-4.01	6.06	-1.32	4.15	-8.24
	2500	4.49	8.49	4.24	1.98	2.28	6.76
Ro	200	4.17	-7.12	0.92	-10.44	9.96	-4.28
	20	7.01	-8.33	1.99	-11.62	5.02	-7.08
	2500	7.35	-6.37	4.58	1.79	3.58	-3.94
Rb1	400	7.80	-10.80	11.23	-8.45	3.89	-3.83
	40	4.43	-5.46	5.40	-11.79	9.82	-8.82
	2500	4.52	-9.31	11.41	-4.51	2.30	-10.76
Rc	200	11.24	-3.13	8.86	-1.60	7.36	-2.71
	20	9.64	-1.85	2.04	-9.84	11.66	-3.15
	1250	2.99	4.09	8.81	-4.08	1.51	2.99
Rb2	200	2.27	-11.13	2.30	-8.22	8.67	-12.11
	40	1.58	-7.15	3.27	-6.02	1.69	-4.98
	1250	10.08	5.03	6.61	2.32	5.23	1.29
Rb3	100	5.69	-8.94	7.40	-7.78	10.21	4.60
	20	1.18	-1.90	9.01	-8.54	10.18	-7.72
	1250	2.15	-7.82	2.47	-8.02	1.07	-7.11
Rd	100	1.00	-1.21	9.49	5.15	8.31	5.99
	10	6.76	-0.33	12.08	-3.78	5.34	-8.55
	1250	11.73	2.87	4.76	7.77	5.63	7.13
CK	50	9.21	-9.02	1.89	-1.78	3.07	-2.58
	5	0.74	-4.73	3.99	-6.86	3.25	-7.36
	2500	5.34	6.66	5.46	-1.18	2.72	4.65
Rh1	200	10.18	-0.19	7.71	1.47	7.46	1.28
	20	11.55	1.48	8.99	3.21	2.57	-5.08
	1250	9.41	-0.92	2.66	-5.74	4.86	-4.22
Rh2	100	10.13	0.98	6.91	-1.44	1.00	4.12
	20	2.56	-1.31	13.86	7.44	11.32	9.23
	1250	2.46	-4.00	7.98	-0.03	1.24	-4.83
Rg2	50	1.92	-1.81	12.16	5.96	10.62	7.04
	1	10.13	0.37	12.09	-0.91	1.97	-8.09
	1250	5.68	-4.21	3.95	-3.07	2.78	-2.28
Rg3	50	10.77	-8.48	5.74	-5.35	2.09	-7.83
	1	9.30	-11.48	0.78	-6.18	8.52	-12.00
	5000	1.99	-4.71	2.76	-7.85	1.00	-5.38
PPD	500	0.14	9.40	9.60	2.34	1.25	-5.44
	50	6.45	1.78	3.18	-0.63	3.28	4.01

Table S5 The pharmacokinetic parameters (X±SD, n = 6) of 10 analytes in rat plasma.

Method	Analyte	T_{max} (h)	C_{max} (ng mL^{-1})	$t_{1/2}$ (h)	AUC_{0-t} (ng h mL^{-1})	$AUC_{0-\infty}$ (ng h mL^{-1})	MRT_{0-t} (h)
DES	Rg3	4	35.16±0.3	34.85±4.07	455.07±21.56	539.70±46.39	34.63±5.00
Methanol		4	17.43±0.3	29.13±5.47	311.19±20.35	353.11±41.75	29.59±6.15
DES	Rg2	7.90±0.13	12.69±0.1	23.35±1.69	246.45±7.19	274.18±12.53	29.32±1.91
Methanol		8	9.25±0.12	21.20±0.86	246.80±8.22	279.60±11.56	32.45±1.34
DES	Rf	2	11.42±0.28	23.61±2.28	303.31±21.34	350.97±36.32	33.56±3.65
Methanol		2	/	/	/	/	/
DES	Rg1	8	45.82±0.35	18.16±0.61	1184.18±22.59	1265.73±32.14	27.01±0.81
Methanol		8	40.67±0.26	20.49±0.64	957.74±18.48	1066.31±33.80	30.38±1.03
DES	Re	8	10.65±0.53	91.09±3.6	1677.51±181.43	1695.02±184.40	16.61±1.53
Methanol		8	/	/	/	/	/
DES	Rd	8	967.60±6.3	29.22±0.44	19931.20±215.63	24316.85±407.75	43.08±0.62
Methanol		8	171.15±4.8	42.53±1.52	7713.91±215.34	10727.41±507.01	61.04±2.08
DES	Rc	8	753.99±10.5	33.56±0.94	14919.27±256.54	18226.68±453.06	41.15±1.85
Methanol		8	257.16±13.6	33.80±2.21	9581.93±273.48	12332.97±534.68	48.91±2.35
DES	Rb3	11.86±0.31	196.85±5.41	27.37±1.08	7575.08±207.52	9062.30±385.71	44.08±2.59
Methanol		12	150.63±4.89	28.02±1.39	6482.72±198.46	7713.89±384.70	43.04±3.68
DES	Rb2	8	1164.89±10.59	18.86±0.22	26062.09±75.74	28921.64±330.47	29.71±0.35
Methanol		8.12±0.47	532.85±15.21	29.80±0.74	13274.34±300.78	15972.61±412.35	41.01±1.03
DES	Rb1	8	964.91±10.57	23.03±0.23	29315.69±203.59	33882.43±360.32	34.66±0.35
Methanol		8	608.26±5.78	25.05±0.29	24964.42±369.25	29885.36±380.34	38.29±0.44