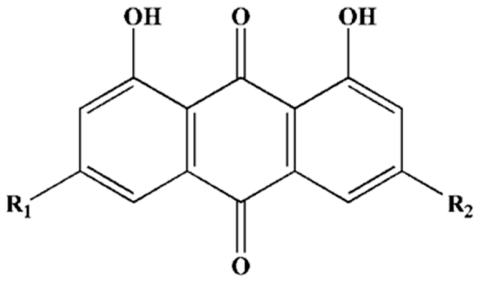
Supplementary Materials

Matrix effect and extraction recovery experiments:

According to the preparation method of mixed reference materials, the mixed reference materials with high, medium and low concentrations were prepared respectively. Then the peak areas (A1) of the five aglycones and internal standard were determined respectively according to "4.3.1 Apparatus and operation conditions". Blank plasma samples were spiked with mixed reference at the above high, medium and low concentrations and treated as the "4.3.2 Plasma sample preparation". Then the peak areas (A2) of the five aglycones and internal standard were determined respectively. The quality control samples with high, medium and low concentrations were directly determined according to the sample treatment method, Then the peak areas (A3) of the five aglycones and internal standard were determined respectively. Each concentration was measured in parallel for 6 times. The matrix effect is the ratio of the peak area (A2) to the peak area (A1) of the corresponding component. The extraction recovery ratio is the ratio of the peak area (A3) to the peak area (A2) of the corresponding components.



	R_1	R_2
Rhein	H	COOH
Emodin	OH	CH_3
Chrysophanol	H	CH_3
Aloe-emodin	H	CH_2OH
Physcion	OCH_3	CH_3
1,8-Dihydroxyanthraquinone	--	--

Figure S1. Chemical formula for five anthraquinones (Rhein, Emodin, Chrysophanol, Aloe-emodin, Physcion) and IS.

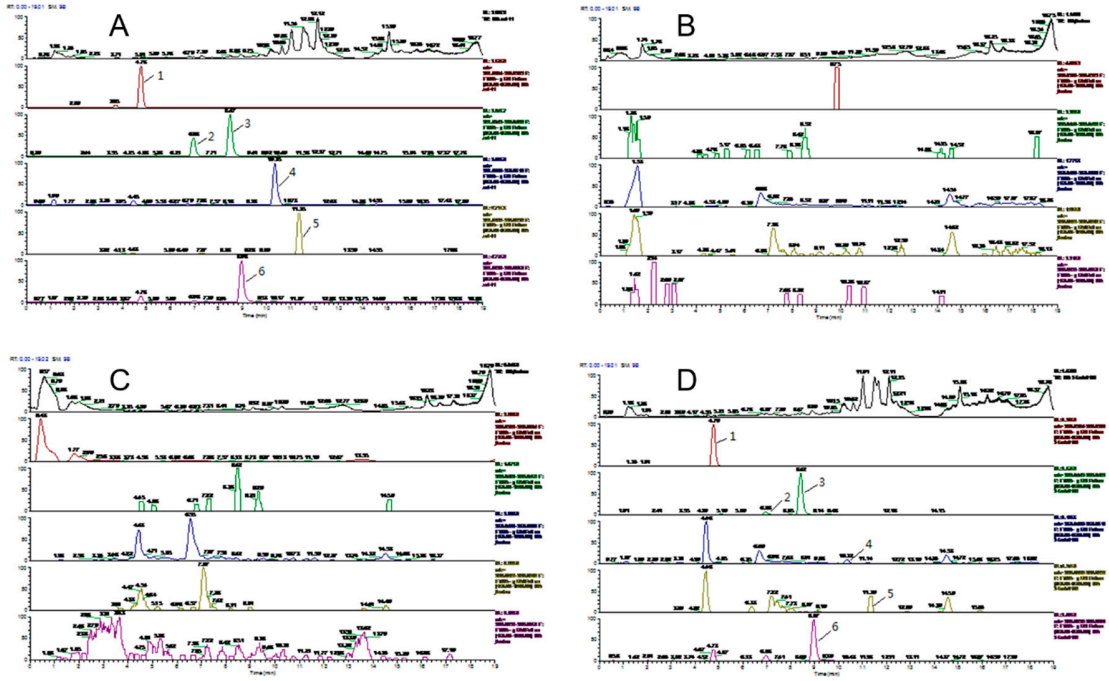


Figure S2. Six-channel chromatogram of five anthraquinones (Aloe-emodin, Aloe, Rhein, Chrysophanol, Pphyscion). A: Mixed reference. B: Blank methanol. C: Blank plasma sample. D: Drug-containing plasma sample.

Table S1. Calibration curves for the 5 anthraquinones in rat plasma

Analytes	Regression equation	r	Linear range (ng/mL)	LLOQ (ng/mL)	LOQ (ng/mL)
Aloe-emodin	$Y = 1.666\times10^{-2}x + 7.236\times10^{-3}$	0.9987	0.68-3424	0.68	0.026
Emodin	$Y = 2.318\times10^{-1}x - 1.214\times10^{-3}$	0.9971	0.74-3728	0.74	0.098
Rhein	$Y = 8.005\times10^{-4}x + 1.561\times10^{-3}$	0.9997	0.66-3312	0.66	0.098
Chrysophanol	$Y = 2.896\times10^{-2}x - 7.059\times10^{-3}$	0.9989	0.62-3104	0.62	0.538
Physcion	$Y=1.437\times10^{-1}x + 1.340\times10^{-1}$	0.9996	0.69-3456	0.69	0.317

Table S2. Intra-day and inter-day precision and accuracy for determination of the 5 anthraquinones in rat plasma (mean ± SD, n = 6, 5 replicates per day for 5 days)

Analytes	Spiked concentration (ng/mL)	Intra-day			Inter-day		
		Concentration (ng/mL)	Accuracy (%)	RSD (%)	Concentration (ng/mL)	Accuracy (%)	RSD (%)
Aloe-emodin	9.32	8.99 ± 0.55	-3.54	6.12	8.95 ± 0.59	-3.97	6.59
	93.2	90.19 ± 7.07	-3.23	7.84	87.03 ± 5.18	-6.62	5.95
	932	926.5 ± 10.53	-0.59	1.14	915.7 ± 8.6	-1.75	0.94
Emodin	7.76	6.91 ± 0.5	-10.95	7.24	6.79 ± 0.26	-12.50	3.83
	77.6	73.23 ± 1.48	-5.63	2.02	71.61 ± 3.55	-7.72	4.96
	776	784.2 ± 8.21	1.06	1.05	773.5 ± 13.34	-0.32	1.72
Rhein	8.28	7.77 ± 0.67	-6.16	8.62	6.75 ± 0.01	-18.48	0.15
	82.8	63.89 ± 4.24	-22.84	6.64	65.86 ± 3.19	-20.46	4.84
	828	792.2 ± 37.18	-4.32	4.69	796.2 ± 35.13	-3.84	4.41
Chrysophanol	8.56	7.65 ± 0.41	-10.63	5.36	7.72 ± 0.31	-9.81	4.02
	85.6	79.75 ± 4.39	-6.83	5.50	79.75 ± 4.39	-6.83	5.50
	856	856.9 ± 14.17	0.11	1.65	844.9 ± 11.91	-1.30	1.41
Physcion	8.64	7.09 ± 0.31	-17.94	4.37	7.08 ± 0.31	-18.06	4.38
	86.4	77.25 ± 3.76	-10.59	4.87	78.86 ± 6.17	-8.73	7.82
	864	881.4 ± 4.18	2.01	0.47	877.2 ± 9.06	1.53	1.03

Table S3. Matrix effects and extraction recovery of the 5 anthraquinones in rat plasma (mean ± SD, n = 6).

Analytes	Spiked concentration(ng/mL)	Matrix effect		Extraction recovery	
		Mean (%)	RSD (%)	Mean (%)	RSD (%)
Aloe-emodin	9.32	99.03	3.78	81.09	4.85
	93.2	99.85	5.32	88.76	3.82
	932	104.9	4.2	102.8	1.43
Emodin	7.76	102.8	3.98	82.17	3.2
	77.6	112.4	4.21	93.76	2.78
	776	103.9	4.78	99.81	3.65
Rhein	8.28	99.82	2.98	75.63	3.21
	82.8	100.3	3.45	83.54	5.43
	828	98.78	4.67	93.78	5.32
Chrysophanol	8.56	79.89	0.48	84.63	5.34
	85.6	88.92	6.32	87.56	6.56
	856	76.09	4.76	98.45	5.32
Physcion	8.64	115	6.34	79.04	4.58
	86.4	103.9	4.34	84.78	2.38
	864	110.4	4.89	101.7	3.4

Table S4. Stability of the 5 anthraquinones in rat plasma (mean ± SD, n = 6)

Analytes	Spiked concentration (ng/mL)	Short-term stability		Freeze/thaw stability		Long-term stability	
		Concentration (ng/mL)	Accuracy (%)	Concentration (ng/mL)	Accuracy (%)	Concentration (ng/mL)	Accuracy (%)
Aloe-emodin	9.32	8.88 ± 0.38	-4.72	8.73 ± 0.4	-6.33	8.65 ± 0.3	-7.19
	93.2	90.75 ± 4.54	-2.63	92.73 ± 2.0	-0.50	92.33 ± 1.35	-0.93
	932	925.8 ± 8.7	-0.67	925.6 ± 8.67	-0.69	931.2 ± 7.16	-0.09
Emodin	7.76	6.85 ± 0.33	-11.73	7.42 ± 0.46	-4.38	7.26 ± 0.36	-6.44
	77.6	74.67 ± 3.12	-3.78	76.0 ± 4.55	-2.06	72.39 ± 2.08	-6.71
	776	780.4 ± 10.12	0.57	767.1 ± 12.3	-1.15	766.1 ± 8.6	-1.28
Rhein	8.28	7.84 ± 0.45	-5.31	7.8 ± 0.35	-5.80	7.94 ± 0.28	-4.11
	82.8	77.33 ± 4.64	-6.61	80.73 ± 2.39	-2.50	80.24 ± 3.13	-3.09
	828	807.6 ± 29.1	-2.46	823.4 ± 9.51	-0.56	817.6 ± 15.28	-1.26
Chrysophanol	8.56	7.71 ± 0.35	-9.93	8.32 ± 0.2	-2.80	8.28 ± 0.17	-3.27
	85.6	80.45 ± 3.85	-6.02	84.54 ± 2.99	-1.24	83.24 ± 0.73	-2.76
	856	856.0 ± 11.72	0.00	856.5 ± 16.36	0.06	845.9 ± 5.72	-1.18
Physcion	8.64	7.16 ± 0.28	-17.13	7.80 ± 0.23	-9.72	7.42 ± 0.37	-14.12
	86.4	77.6 ± 3.15	-10.19	84.64 ± 3.86	-2.04	81.07 ± 4.91	-6.17
	864	877.4 ± 8.67	1.55	863.4 ± 8.44	-0.07	872.2 ±5.74	0.95

I Short-term stability was assessed by analyzing samples kept at 4 ℃ for 24 h.

II Freeze–thaw stability was evaluated at three consecutive freeze–thaw cycles (−20 ˚C to room temperature as one cycle).

III Long-term stability was studied by assaying samples following a period of 30 days of storage at -80 ˚C.