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Supplementary Information

Simultaneous Determination and Pharmacokinetics of Four Coumarins in Rat Plasma after Oral Administration of Traditional Chinese Medicine "YIGONG" Capsule by SPE-HPLC

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Table S1. Mean sum of squares and degrees of freedom used in F-test (ANOVA)

Source of variability	Sum of squares	Degrees of Freedom	Mean Sum of Squares
Total	$SQT = \Sigma\Sigma y_{ij}^2$	n	MQT = SQT/n
Correction ("b")	$FC = n\, y_{00}$	1	FC
Total correction	$SQC = \Sigma\Sigma(y_{ij}-y_{00})^2$	n–1	MQC = SQC/(n–1)
Due to regression ("a")	$SQR = \Sigma(y_i-y_{00})^2$	1	MQR = SQR
Residual	$SQE = \Sigma\Sigma(y_{ij}-y_i)^2$	n–2	MQE = SQE/(n–2)
Pure Error	$SQEP = \Sigma\Sigma(y_{ij}-y_{i0})^2$	$n- m_i$	$MQEP = SQEP/(n- m_i)$
Lack-of-fit	$SQL = \Sigma(y_i-y_{i0})^2$	m_i-2	$MQL = SQL/ (m_i-2)$

n = total number of measurements; m_i = i-concentration levels (7); y_{ij} = observed signal; y_{00} = mean of measured signals; y_i = predicted dependent variable; y_{i0} = mean of replicates of i-concentration level; "i" index refers to x-independent variable; "j" index refers to replicates in x-levels. First summation Σ ranges from i=1 to $i=m_i$. Second summation ΣΣ in SQC, SQE and SQEP ranges from i=1 to $j=n_i$.

Table S2. Linearity and regression efficiency tests

Test	$F_{critical}$	$F_{obtained}$	Condition
Adjustment of the linear model (ALM)	$F_{mi-2;\, n-mi;\, \alpha/2}$	MQL/MQEP	$F_{obtained} < F_{critical}$
Validity of the regression (VR)	$F_{1;\alpha-2;\alpha/2}$	MQR/MQE	$F_{obtained} >> F_{critical}$
	Efficiency of the regression (ER)		
Efficiency (r^2)		SQR/SQC	
Maximum efficiency (r^2_{max})		SQC/SQEP	

Table S3. Calibration curves for four coumarins of YGC content in rat plasma

Components	Calibration($y=ax+b$)	Linear range/ (μg mL^{-1})	r^2	r^2_{max}	ALM		VR		LOD/ (ng mL^{-1})	LOQ/ (ng mL^{-1})
					$F_{5,14,0.025}$	$F_{obtained}$	$F_{1,19,0.025}$	$F_{obtained}$		
psoralen	y = (112159±1265)x + (–84598 ± 124)	0.0097-3.10	0.9992	0.9999	3.66	2.77	5.92	23731	4.86	9.7
isopsoralen	y = (93295±188)x + (–81817 ± 235)	0.0071-2.80	0.9941	0.9998		2.73		3201	3.46	7.1
imperatorin	y = (79337±179)x + (–106203 ± 1134)	0.0067-0.81	0.9965	0.9998		2.68		5410	3.24	6.7
isoimperatorin	y = (68869±192)x + (–51119 ± 213)	0.0030-0.30	0.9987	0.9999		2.62		14596	1.30	3.0

LOD is defined as the concentration where the signal-to-noise ratio is 3, and LOQ is defined as the concentration where the signal-to-noise ratio is 10. The slope and intercept were given as the form of mean ± S.D.

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Table S4. Intra-day and inter-day precision for the assay of four coumarins in rats plasma (n = 6)

Nominal concentration / (μg mL^{-1})	Intra-day precision			Inter-day precision		
	Measured concentration Mean ± SD / (μg mL^{-1})	R.S.D / (%)	RE / (%)	Measured concentration Mean ± SD / (μg mL^{-1})	R.S.D / (%)	RE / (%)
psoralen						
3.100	3.140 ± 0.010	3.2	1.3	3.261 ± 0.143	4.4	5.2
0.310	0.337 ± 0.013	3.9	8.7	0.340 ± 0.028	8.2	9.7
0.031	0.032 ± 0.002	6.3	3.5	0.028 ± 0.002	7.1	– 9.7
isopsoralen						
2.800	2.696 ± 0.075	2.8	– 3.7	2.915± 0.134	4.6	4.1
0.280	0.278 ± 0.007	2.5	– 0.7	0.296 ± 0.014	4.7	5.7
0.028	0.025 ± 0.001	4.0	–10.7	0.024 ± 0.001	4.2	–14.3
imperatorin						
0.810	0.798 ± 0.045	5.6	– 1.5	0.755 ± 0.029	3.8	– 6.8
0.081	0.079 ± 0.002	2.5	– 2.5	0.075 ± 0.002	2.7	– 7.4
0.016	0.015 ± 0.001	6.7	– 6.3	0.014 ± 0.001	7.1	–12.5
isoimperatorin						
0.300	0.283 ± 0.016	5.7	– 5.7	0.279 ± 0.018	6.5	– 7.0
0.030	0.028 ± 0.001	3.6	– 6.7	0.031 ± 0.002	6.5	3.3
0.010	0.009 ± 0.0007	7.8	–10.0	0.0087 ± 0.0007	8.0	–13.0

Table S5. Absolute recovery of the developed method and repeatability of the extraction procedure (n = 6)

Components	Nominal concentration / (μg mL^{-1})	Measured concentation Mean ± SD / (μg mL^{-1})	Recovery / (%)	R.S.D / (%)
psoralen	3.100	2.855 ± 0.097	92.1	3.4
	0.310	0.296 ± 0.003	95.5	1.0
	0.031	0.027 ± 0.0016	87.1	6.0
isopsoralen	2.800	2.526 ± 0.078	90.2	3.1
	0.280	0.267 ± 0.005	95.4	1.9
	0.028	0.024 ± 0.0006	85.7	2.5
imperatorin	0.810	0.727 ± 0.0116	89.8	1.6
	0.081	0.069 ± 0.0009	85.2	1.3
	0.016	0.014 ± 0.0006	87.5	4.3
isoimperatorin	0.300	0.254 ± 0.012	84.7	4.7
	0.030	0.028 ± 0.001	93.3	3.6
	0.010	0.008 ± 0.0002	80.0	2.5

Table S6. Stability of four coumarins in rats plasma (n = 6)

Components	Nominal concentration / (μg mL^{-1})	Three freeze-thaw stability		Stored at –20 °C		Stored at ambient temperature	
		R.S.D / (%)	R.E. / (%)	R.S.D / (%)	R.E. / (%)	R.S.D / (%)	R.E. / (%)
psoralen	3.100	2.6	– 0.013	1.2	– 0.029	1.4	– 0.030
	0.310	1.9	0.113	4.8	0.018	2.7	0.103
	0.031	5.5	– 0.025	1.1	– 0.019	2.6	– 0.010
isopsoralen	2.800	2.7	– 0.014	3.1	– 0.009	4.8	– 0.007
	0.280	4.4	– 0.138	2.2	– 0.164	5.8	– 0.163
	0.028	11.4	0.044	1.1	0.044	6.7	0.007
imperatorin	0.810	6.2	0.057	1.4	0.004	1.9	0.008
	0.081	3.7	0.017	4.6	– 0.028	2.6	– 0.033
	0.016	10.6	– 0.006	12.8	– 0.021	2.2	0.037
isoimperatorin	0.300	8.5	– 0.050	4.6	– 0.030	2.7	– 0.040
	0.030	4.6	– 0.267	5.1	– 0.274	8.8	– 0.289
	0.010	8.7	– 0.157	8.6	– 0.157	7.2	– 0.175

Relative standard deviation (R.S.D) is expressed as: (standard deviation between observed concentrations/mean observed concentration) × 100; Relative error (R.E.) is expressed as: (mean observed concentration/nominal concentration) × 100.

Table S7. Pharmacokinetic parameters of four coumarins after oral administration of YGC content in rat, each value represents the mean ± SD (n=6)

Pharmacokinetic	psoralen	isopsoralen	imperatorin	isoimperatorin
K_{10} / (h^{-1})	0.230 ± 0.021	0.677 ± 0.086	0.249 ± 0.019	0.347 ± 0.046
K_{12} / (h^{-1})	0.866 ± 0.422	0.437 ± 0.023	1.536 ± 0.012	0.372 ± 0.034
K_{21} / (h^{-1})	0.584 ± 0.081	0.44 ± 0.053	1.179 ± 0.101	0.408 ± 0.097
$t_{1/2}$ / (h)	8.292 ± 0.211	3.831 ± 0.026	7.365 ± 0.254	4.841 ± 0.105
C_{max} / (μg mL^{-1})	2.452 ± 0.332	2.221 ± 0.242	0.684 ± 0.103	0.285 ± 0.035
T_{max} / (h)	0.75 ± 0.21	1.00 ± 0.32	2.00 ± 0.27	1.00 ± 0.36
AUC$_{0\to t}$ /(μg mL^{-1} h^{-1})	16.663 ± 2.675	8.885 ± 1.821	7.564 ± 1.324	1.743 ± 0.654
AUC$_{0\to\infty}$ /(μg mL^{-1} h^{-1})	19.127 ± 2.856	9.036 ± 1.956	8.322 ± 1.776	1.809 ± 0.994

K_{10}: central compartment elimination rate contrant; K_{12}: central to peripheral compartment rate contrant; K_{21} : peripheral to central compartment rate contrant; $t_{1/2}$: elimination terminal half life; C_{max}: maximum plasma concentration; T_{max}: time to reach the maximum plasma concentration; AUC$_{0\to t}$: area under the plasma concentration-time curve from zero to the time of the final measurable sample; AUC$_{0\to\infty}$: area under the plasma concentration-time curve from zero to infinity.