

An integrate pharmacokinetic study of Dengzhanxixin injection in rats by combination of multicomponent pharmacokinetics and anti-myocardial ischemic assay

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Supplementary Data

Table S1 The regression equation, linear range, r^2 , LOD and LOQ of thirteen compounds

Analyte	Regression equation (ng/mL)	Linear range (ng/mL)	r^2	LOD (ng/mL)	LOQ (ng/mL)
5-CQA	$Y=0.94x+0.022$	10-5000	0.991	4.5	10.0
3-CQA	$Y=1.10x+0.022$	10-5000	0.996	3.0	10.0
4-CQA	$Y=0.60x+0.006$	20-5000	0.991	9.0	20.0
4-CDOA	$Y=0.30x-0.007$	25-10000	0.992	4.5	25.0
Caffeic acid	$Y=0.73x+0.028$	10-5000	0.991	6.0	10.0
1,3-diCQA	$Y=0.79x+0.015$	10-5000	0.993	3.0	10.0
Scutellarin	$Y=0.75x+0.040$	25-5000	0.991	9.0	25.0
3,4-diCQA	$Y=0.25x+0.046$	40-10000	0.992	22.5	40.0
3,5-diCQA	$Y=0.34x+0.038$	40-10000	0.990	21.0	40.0
Erigoster B	$Y=3.78x+0.46$	20-5000	0.991	10.0	20.0
3,4-diCDOA (or 4,9-diCDOA)	$Y=4.17x+0.31$	30-5000	0.998	17.4	30.0
A-7-O-G	$Y=0.57x+0.026$	25-5000	0.990	14.4	25.0
4,5-diCQA	$Y=0.33x+0.093$	50-10000	0.993	14.4	50.0

Table S2 The precision and accuracy of thirteen compounds in rat plasma

Analyte	Concentration (ng/mL)	Intra-day (n=5)			Inter-day (n=5)		
		Detected (ng/mL)	RSD (%)	Accuracy (RE, %)	Detected (ng/mL)	RSD (%)	Accuracy (RE, %)
5-CQA	30	28.70±1.81	6.30	-4.33	31.14±2.11	6.78	3.79
	400	420.87±17.72	4.21	5.22	432.29±10.49	2.43	8.07
	4000	3601.01±187.07	5.19	-9.97	3712.10±103.10	2.78	-7.20
3-CQA	30	30.15±1.68	5.58	0.49	29.92±0.53	1.78	-0.26
	400	414.90±13.35	3.22	3.72	404.42±9.20	2.28	1.11
	4000	3793.00±250.50	6.60	-5.18	3705.02±86.70	2.34	-7.37
4-CQA	30	30.55±3.06	10.01	1.85	29.09±1.85	6.35	-3.05
	400	415.14±21.05	5.07	3.78	421.31±14.31	3.40	5.33
	4000	3776.69±224.45	5.94	-5.58	3899.84±107.84	2.77	-2.50
4-CDOA	60	59.45±1.78	3.00	-0.92	56.01±3.40	6.06	-6.65
	800	839.93±22.54	2.68	4.99	805.60±31.05	3.85	0.70
	8000	7502.89±194.99	2.60	-6.21	7193.98±286.14	3.98	-10.08
Caffeic acid	30	28.85±2.01	6.95	-3.82	28.29±2.02	7.12	-5.71
	400	456.20±17.10	3.75	14.05	441.12±20.55	4.66	10.28
	4000	3434.55±84.68	2.47	-14.14	3415.81±169.20	4.95	-14.60
1,3-diCQA	30	26.18±1.59	6.07	-12.72	26.74±0.98	3.66	-10.87
	400	432.77±17.49	4.04	8.19	427.57±9.23	2.16	6.89
	4000	3667.19±244.70	6.67	-8.32	3646.61±46.49	1.28	-8.83
Scutellarin	30	28.48±2.41	8.45	-5.08	26.16±2.83	10.82	-12.81
	400	449.39±22.80	5.07	12.35	418.88±30.43	7.26	4.72
	4000	3643.74±170.83	4.69	-8.91	3497.72±139.62	3.99	-12.56
3,4-diCQA	60	60.66±5.02	8.28	1.10	61.75±1.80	2.92	2.91
	800	761.89±15.04	1.97	-4.76	754.98±9.47	1.25	-5.63
	8000	7321.91±324.00	4.43	-8.48	7308.01±235.05	3.22	-8.65
3,5-diCQA	60	63.77±4.49	7.04	6.29	65.95±2.18	3.31	9.91
	800	681.46±18.91	2.77	-14.82	692.46±52.90	7.64	-13.44
	8000	6809.79±252.09	3.70	-14.88	6828.27±232.50	3.41	-14.65
Erigeron B	30	30.92±1.92	6.22	3.06	32.33±1.71	5.29	7.76
	400	345.75±14.73	4.26	-13.56	353.67±32.67	9.24	-11.58
	4000	3377.90±151.63	4.49	-15.55	3647.97±277.18	7.60	-8.80
3,4-diCDOA (or 4,9-diCDOA)	30	30.09±2.25	7.48	0.30	29.72±0.43	1.43	-0.93
	400	399.42±11.74	2.94	-0.14	410.42±15.66	3.82	2.60
	4000	4423.50±357.98	8.09	10.59	4448.60±103.76	2.33	11.22
A-7-O-G	30	27.84±2.08	7.49	-7.21	28.21±1.27	4.49	-5.98
	400	454.20±18.77	4.13	13.55	451.13±10.98	2.43	12.78
	4000	3402.58±88.68	2.61	-14.94	3379.51±21.52	0.64	-15.51

4,5-diCQA	60	65.99±2.68	4.06	9.98	65.64±1.04	1.58	9.40
	800	703.54±47.91	6.81	-12.06	698.01±49.98	7.16	-12.75
	8000	8553.69±331.69	3.88	6.92	8785.63±273.50	3.11	9.82

Table S3 The extraction recovery and matrix effect of thirteen compounds in rat plasma

Analyte	Concentration (ng/mL)	Extraction recovery(n=5)		Matrix effect(n=5)	
		Mean± SD (%)	RSD (%)	Mean±SD (%)	RSD (%)
5-CQA	30	112.66±7.38	6.55	131.42±4.94	3.76
	400	109.55±6.17	5.63	128.38±4.13	3.21
	4000	103.87±5.23	5.04	101.66±3.02	2.97
3-CQA	30	100.83±7.46	7.40	111.68±7.88	7.05
	400	107.86±4.32	4.01	101.7±2.57	2.53
	4000	99.98±4.41	4.41	98.2±3.85	3.93
4-CQA	30	102.50±8.29	8.09	97.76±7.09	7.25
	400	103.42±7.00	6.77	98.55±2.91	2.96
	4000	104.67±6.96	6.65	92.23±4.35	4.71
4-CDOA	60	109.44±7.30	6.67	96.06±6.70	6.98
	800	95.65±4.05	4.23	99.07±2.32	2.34
	8000	93.92±2.37	2.53	97.54±4.16	4.26
Caffeic acid	30	102.6±9.36	9.12	66.11±5.05	7.63
	400	94.07±4.13	4.39	82.7±1.31	1.59
	4000	89.30±4.60	5.15	80.64±4.86	6.03
1,3-diCQA	30	96.15±6.32	6.58	92.46±4.89	5.29
	400	96.47±4.96	5.14	97.64±4.86	4.98
	4000	95.11±4.53	4.76	97.25±5.15	5.29
Scutellarin	30	88.46±7.55	8.53	147.69±13.56	9.18
	400	86.33±7.35	8.52	107.33±10.47	9.76
	4000	79.60±6.59	8.27	86.59±5.96	6.89
3,4-diCQA	60	91.58±9.45	10.32	104.63±4.37	4.18
	800	99.39±1.83	1.85	101.17±2.09	2.06
	8000	103.1±6.27	6.08	100.86±3.16	3.13
3,5-diCQA	60	91.75±4.80	5.23	80.09±5.81	7.26
	800	98.95±3.43	3.46	96.37±5.57	5.78
	8000	97.98±3.77	3.85	88.44±3.03	3.43
Erigeron B	30	99.85±8.96	8.97	102.52±7.51	7.32
	400	95.40±5.73	6.01	95.51±5.26	5.51
	4000	82.73±7.56	9.14	90.54±8.24	9.10
3,4-diCDOA (or 4,9-diCDOA)	30	101.43±6.88	6.78	93.41±8.11	8.68
	400	101.24±5.59	5.52	102.67±3.31	3.23
	4000	98.96±5.99	6.05	109.68±5.38	4.91
A-7-O-G	30	107.25±3.02	2.82	92.39±10.54	11.40
	400	105.71±6.10	5.77	94.74±2.84	3.00
	4000	105.82±6.02	5.69	95.79±3.18	3.32
4,5-diCQA	60	109.31±3.69	3.37	86.65±6.12	7.06

800	104.39±4.63	4.43	97.03±5.04	5.19
8000	98.06±6.22	6.35	102.91±6.76	6.57

Table S4 The stability of thirteen compounds in rat plasma

Analyte	Spiked (ng/ml)	12 h in auto-sampler		Short-term stability (4°C, 12 h)		Freeze-thaw stability (three cycles)		Long-term stability (-80°C,30 days)	
		RE	RSD	RE	RSD	RE	RSD	RE	RSD
		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
5-CQA	30	8.23	3.42	-2.69	6.03	9.98	5.67	6.16	5.25
	400	1.10	2.28	-2.49	7.99	12.95	7.93	0.59	7.58
	4000	-10.00	4.11	-0.92	4.47	8.62	3.69	-1.38	1.92
3-CQA	30	-0.03	8.23	-1.64	7.65	-8.45	3.88	-5.73	3.36
	400	-1.62	2.88	9.94	4.05	-0.36	7.74	-9.98	3.60
	4000	-15.40	2.88	-10.45	4.77	1.19	4.34	-7.33	4.88
4-CQA	30	-5.73	5.74	0.52	5.38	-7.25	6.19	10.24	10.19
	400	-13.68	4.03	12.56	3.60	-1.14	5.14	-9.05	4.76
	4000	-14.96	3.28	0.37	4.28	2.02	3.72	-1.94	3.00
4-CDOA	60	-5.16	6.43	3.56	6.21	-12.60	7.47	3.05	8.33
	800	-4.05	2.34	11.57	7.40	3.53	2.37	14.17	3.37
	8000	-11.71	2.54	-2.68	2.07	1.83	3.15	12.26	10.88
Caffeic acid	30	-18.11	6.16	-0.20	6.58	-2.14	9.95	-9.71	10.57
	400	5.18	2.51	13.81	8.66	11.51	7.01	15.21	5.10
	4000	-13.69	5.88	9.04	5.26	5.57	5.03	0.56	4.38
1,3-diCQA	30	-5.24	7.61	0.49	6.74	-0.45	10.81	-10.61	8.04
	400	-1.49	4.13	4.57	5.85	2.78	4.45	3.20	4.45
	4000	-15.44	1.21	5.49	1.26	-5.43	4.81	-6.30	4.05
Scutellarin	30	-20.45	8.81	20.30	9.70	-7.17	15.82	-11.75	19.20
	400	0.37	2.42	9.11	7.56	2.09	4.37	-1.05	4.74
	4000	-15.53	6.55	5.81	2.31	-8.97	3.10	-8.45	3.27
3,4-diCQA	60	1.38	10.06	6.01	7.41	3.85	6.48	-8.41	4.83
	800	-3.71	2.67	14.95	3.58	-10.36	2.57	6.78	2.52
	8000	-11.03	4.62	5.43	3.16	5.78	2.03	7.83	3.38
3,5-diCQA	60	6.78	7.81	-10.10	7.17	-0.15	6.14	13.28	3.39
	800	-14.89	4.70	5.58	14.35	-15.29	3.02	-5.89	2.75
	8000	-13.39	6.67	11.47	2.21	4.18	3.17	12.76	3.77
Erigoster B	30	0.94	10.33	-5.94	13.29	1.04	14.51	-3.28	9.08
	400	10.32	1.69	-2.16	8.70	-12.19	7.95	-10.72	4.78
	4000	0.27	10.89	17.67	2.23	11.09	11.92	11.78	3.04
3,4-diCDOA (or 4,9-diCDOA)	30	14.41	9.49	-15.87	15.30	-7.38	12.31	3.89	6.92
	400	-7.72	5.80	14.40	9.36	-14.91	12.75	2.18	2.62
	4000	-9.28	5.20	12.75	2.85	-1.04	8.25	10.75	3.73
A-7-O-G	30	-8.10	5.17	12.23	9.05	9.66	5.67	-0.34	8.40
	400	13.11	3.34	14.84	5.25	15.27	10.10	14.12	5.67

4,5-diCQA	4000	-14.03	3.00	1.34	3.96	-0.44	3.02	-12.00	2.82
	60	-5.75	4.95	2.79	6.81	-3.70	9.55	5.86	8.28
	800	-7.96	3.50	13.96	4.16	-5.30	5.07	7.74	5.04
	8000	13.07	1.55	4.37	4.58	-7.70	1.64	2.12	3.27

Table S5 Fifteen compounds identified from EBI by LC-Q-TOF-MS

No.	t_R (min)	(-)ESI-MS m/z			Formula	Identification
		Observed	Calculated	Error (ppm)		
1	8.873	353.0848	353.0878	8.41	$C_{16}H_{17}O_9^-$	5-CQA ^a
2	11.816	381.0800	381.0827	7.04	$C_{17}H_{17}O_{10}^-$	3-CDOA
3	12.201	353.0854	353.0878	6.71	$C_{16}H_{17}O_9^-$	3-CQA ^a
4	13.038	353.0859	353.0878	5.44	$C_{16}H_{17}O_9^-$	4-CQA ^a
5	13.954	381.0807	381.0827	5.31	$C_{17}H_{17}O_{10}^-$	4-CDOA ^a
6	14.565	179.0338	179.0350	6.72	$C_9H_7O_4^-$	Caffeic acid ^a
7	15.321	381.0812	381.0827	4.02	$C_{17}H_{17}O_{10}^-$	9-CDOA
8	16.093	515.1165	515.1195	5.77	$C_{25}H_{23}O_{12}^-$	1,3-diCQA ^a
9	22.365	461.0709	461.0731	3.53	$C_{21}H_{17}O_{12}^-$	Scutellarin ^a
10	24.327	515.1154	515.1195	8.03	$C_{25}H_{23}O_{12}^-$	3,4-diCQA ^a
11	25.082	543.1107	543.1144	6.96	$C_{26}H_{23}O_{13}^-$	Erigoster B ^a
12	25.436	515.1153	515.1195	8.1	$C_{25}H_{23}O_{12}^-$	3,5-diCQA ^a
13	26.369	543.1106	543.1144	7.02	$C_{26}H_{23}O_{13}^-$	3,4-diCDOA (or 4,9-diCDOA) ^a
14	27.672	445.0750	445.0776	5.87	$C_{21}H_{17}O_{11}^-$	A-7-O-G ^a
15	28.395	515.1166	515.1195	5.68	$C_{25}H_{23}O_{12}^-$	4,5-diCQA ^a

^a compared with reference compounds.

Table S6 Fifteen compounds identified from EBI by LC-ESI-MS/MS

No.	t _R (min)	(-) ESI	LC/(-)ESI-MS ⁿ <i>m/z</i> (% base peak)	Identification
1	8.873	353[M-H] ⁻	MS ² [353]:191(100),179(35),173(12),161(10), 135(65),127(10),85(9)	5-CQA
2	11.816	381[M-H] ⁻	MS ² [381]:293(15),251(7),179(36),161(100), 135(40),133(60)	3-CDOA
3	12.201	353[M-H] ⁻	MS ² [353]:191(100),179(2),173(7),161(1), 155(1),135(20),93(4),85(8)	3-CQA
4	13.038	353[M-H] ⁻	MS ² [353]:191(100),179(80),173(70),161(30), 155(14),135(35),127(17),111(22),93(14),85(8)	4-CQA
5	13.954	381[M-H] ⁻	MS ² [381]:293(7),251(13),203(10),179(18), 161(100),135(35),133(55)	4-CDOA
6	14.565	179[M-H] ⁻	MS ² [179]:135(100)	Caffeic acid
7	15.321	381[M-H] ⁻	MS ² [381]:219(20),179(30),161(100), 135(20), 133(20)	9-CDOA
8	16.093	515[M-H] ⁻	MS ² [515]:353(43),335(20),191(100),179(90), 173(42),135(20)	1,3-diCQA
9	22.365	461[M-H] ⁻	MS ² [461]:285(100),267(2),257(3),241(2), 213(2),175(2)	Scutellarin
10	24.327	515[M-H] ⁻	MS ² [515]:353(37),335(40),255(5),191(29), 179(100),173(95),155(20),135(10)	3,4-diCQA
11	25.082	543[M-H] ⁻	MS ² [543]:381(100),363(20),293(20),251(18), 203(14),179(100),161(14),135(10),129(24)	Erigoster B
12	25.436	515[M-H] ⁻	MS ² [515]:353(100),335(20),191(97),173(50)	3,5-diCQA
13	26.369	543[M-H] ⁻	MS ² [543]:381(100),363(50),251(25),221(65), 203(50),179(50),161(51),135(26),129(50)	3,4-diCDOA (or 4,9-diCDOA)
14	27.672	445[M-H] ⁻	MS ² [445]:269(100),225(15)	A -7- <i>O</i> -G
15	28.395	515[M-H] ⁻	MS ² [515]:353(100),335(34),191(90),179(75), 173(17),135(26)	4,5-diCQA

Table S7 Assay results for EBI of different batches (Mean±SD, n=3)□

Analyte	Content (µg/mL)				
	20150246	20150940	20160143	20160347	20160435
5-CQA	83.75±0.64	80.25±0.80	58.29±0.93	60.78±0.67	76.86±1.09
3-CQA	56.51±0.23	54.82±0.56	40.24±0.70	42.62±0.46	52.31±0.49
4-CQA	63.93±1.08	61.53±0.48	46.11±0.31	49.42±1.62	59.40±0.99
4-CDOA	60.82±1.08	81.13±0.70	101.53±0.33	85.94±2.04	75.99±0.93
Caffeic acid	16.89±0.15	63.44±0.35	42.85±0.76	45.37±0.71	49.15±0.21
1,3-diCQA	8.31±0.07	8.26±0.17	4.31±0.06	5.32±0.13	7.13±0.25
Scutellarin	325.93±4.70	495.99±2.06	395.80±2.88	433.36±1.33	403.55±1.00
3,4-diCQA	43.17±0.69	113.67±1.06	64.73±0.66	97.36±1.44	82.84±1.30
3,5-diCQA	40.73±0.14	82.15±0.88	51.18±0.29	76.33±0.26	66.65±0.97
Erigeron B	38.96±0.41	158.17±1.56	149.22±1.31	193.27±0.81	105.72±0.63
3,4-diCDOA (or 4,9-diCDOA)	5.99±0.13	114.27±0.77	58.34±1.35	106.75±2.03	51.89±0.79
A-7-O-G	163.84±0.73	255.39±0.97	107.18±0.94	83.92±0.44	179.53±0.35
4,5-diCQA	136.11±0.21	326.95±0.64	189.05±1.80	250.78±0.51	225.60±0.48
Total	1044.94	1896.02	1308.83	1531.22	1436.62

the fifth day

the seventh day





Fig. S1 The electrocardiograms (ECGs) of each group on the fifth and seventh day of the trial. (a) control group, (b) model group, (c) EBI group, (d) Scutellarin group, (e) Caffeic acid group, (f) 3-CQA group, (g) A-7-O-G group, (h)5-CQA group, (i) 4-CQA group, (j) 4,5-diCQA group, (k) 3,5-diCQA group, (l) 3,4-diCQA group, and (m) 1,3-diCQA group.

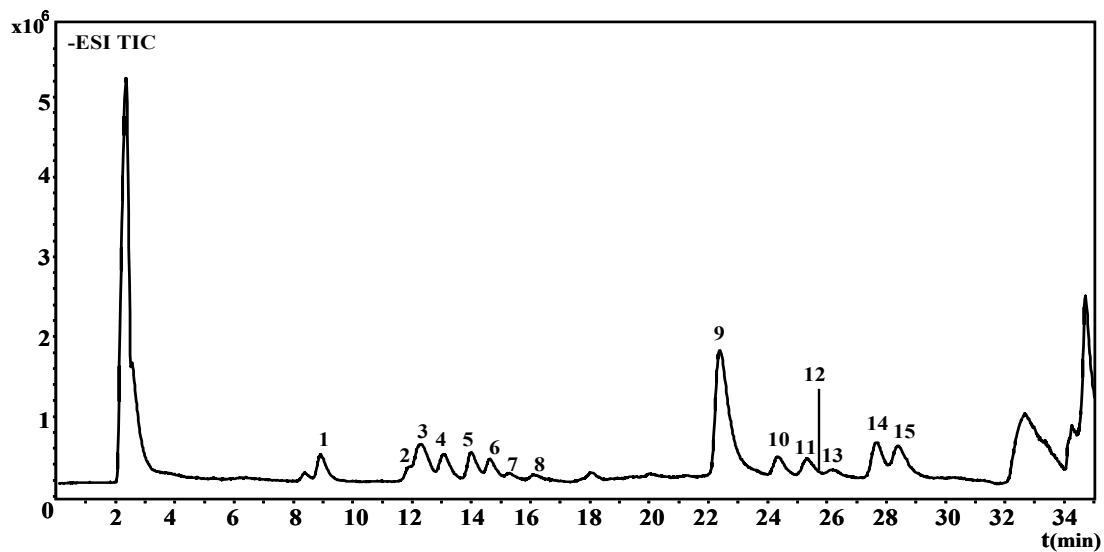


Fig. S2 LC-Q-TOF-MS total ion current (TIC) chromatogram in negative mode of EBI (peak 1, 5-CQA; peak 2, 3-CDOA; peak 3, 3-CQA; peak 4, 4-CQA; peak 5, 4-CDOA; peak 6, caffeic acid; peak 7, 9-CDOA; peak 8, 1,3-diCQA; peak 9, scutellarin; peak 10, 3,4-diCQA; peak 11, erigoster B; peak 12, 3,5-diCQA; peak 13, 3,4-diCDOA (or 4,9-diCDOA); peak 14, A-7-O-G; peak 15, 4,5-diCQA).