

## **Supporting Information of**

### **Study of bitespiramycin distribution in rats and cerebrospinal fluid of patients by a sensitive LC-MS/MS method with rapid sample preparation**

**Yujie Zhang <sup>1,†</sup>, Jingjie Cao <sup>1,†</sup>, Jiahan Su <sup>1</sup>, Tingting He <sup>1</sup>, Qianru Wang <sup>1</sup>, Feng Wei <sup>2</sup>, Xin Guo <sup>3</sup>, Qibing Mei <sup>1,3,\*</sup> and Jing Zeng <sup>1,\*</sup>**

<sup>1</sup> School of Pharmacy, Southwest Medical University, Luzhou, China.

<sup>2</sup> Shanghai Tonglian Pharmaceutical Co. Ltd., Shanghai, China.

<sup>3</sup> Luzhou New Drug Safety Evaluation Research Center, Luzhou, China.

\* Correspondence: authors: qbmei@swmu.edu.cn, zengjing@swmu.edu.cn

† These authors contributed equally to this work.

**Table S1 The concentrations of SPMs I, II, III and ISV-SPMs I, II, III in rats' plasma after intragastric administration of 100 mg/kg of bitespiramycin (Mean  $\pm$  SD, n = 6)**

Components	Concentration (ng/mL)					
	1h	3h	6h	12h	24h	48h
ISV-SPM I	9.40 $\pm$ 9.05	12.02 $\pm$ 2.75	5.08 $\pm$ 1.38	N.D.	N.D.	N.D.
ISV-SPM II	59.13 $\pm$ 23.37	73.83 $\pm$ 10.58	25.05 $\pm$ 15.21	4.37 $\pm$ 1.68	2.42 $\pm$ 1.04	1.34 $\pm$ 0.71
ISV-SPM III	29.45 $\pm$ 10.34	37.42 $\pm$ 4.12	13.15 $\pm$ 8.48	2.24 $\pm$ 1.23	1.34 $\pm$ 0.72	0.71 $\pm$ 0.53
SPM I	105.55 $\pm$ 101.49	103.73 $\pm$ 13.66	33.65 $\pm$ 16.43	5.13 $\pm$ 0.48	2.68 $\pm$ 0.42	0.87 $\pm$ 0.30
SPM II	728.33 $\pm$ 540.15	658.00 $\pm$ 128.69	338.67 $\pm$ 53.09	101.42 $\pm$ 13.34	48.38 $\pm$ 10.33	14.78 $\pm$ 5.06
SPM III	1728.33 $\pm$ 1315.68	1766.67 $\pm$ 635.25	970.00 $\pm$ 264.78	363.50 $\pm$ 78.90	126.80 $\pm$ 28.97	47.12 $\pm$ 17.00

**Table S2 The concentrations of ISV-SPM I in rats' tissues after intragastric administration of 100 mg/kg of bitespiramycin (Mean  $\pm$  SD, n = 6)**

Tissues	Concentration (ng/g)					
	1h	3h	6h	12h	24h	48h
Brain	N. D.	0.75 $\pm$ 0.34	N. D.	N. D.	N. D.	N. D.
Lung	64.50 $\pm$ 40.24	217.1 $\pm$ 45.20	129.20 $\pm$ 48.23	37.74 $\pm$ 7.07	28.60 $\pm$ 11.65	7.36 $\pm$ 1.31
Prostate	3.86 $\pm$ 2.60	19.74 $\pm$ 14.47	68.03 $\pm$ 29.33	51.00 $\pm$ 5.05	37.40 $\pm$ 9.48	13.93 $\pm$ 2.69
Testis	N. D.	2.00 $\pm$ 0.65	1.68 $\pm$ 0.56	0.82 $\pm$ 0.33	0.90 $\pm$ 0.36	N. D.
Womb	19.31 $\pm$ 10.58	42.14 $\pm$ 12.06	50.25 $\pm$ 14.57	16.99 $\pm$ 8.05	19.83 $\pm$ 12.41	8.78 $\pm$ 7.15
Ovary	23.92 $\pm$ 16.64	71.55 $\pm$ 25.72	127.05 $\pm$ 48.24	29.27 $\pm$ 6.65	21.01 $\pm$ 7.53	9.16 $\pm$ 3.70
Bladder	2.85 $\pm$ 2.85	9.24 $\pm$ 5.12	13.97 $\pm$ 8.29	3.98 $\pm$ 3.09	4.31 $\pm$ 2.89	1.44 $\pm$ 0.65

**Table S3 The concentrations of ISV-SPM II in rats' tissues after intragastric administration of 100 mg/kg of bitespiramycin (Mean  $\pm$  SD, n = 6)**

Tissues	Concentration (ng/g)					
	1h	3h	6h	12h	24h	48h
Brain	2.10 $\pm$ 0.89	4.69 $\pm$ 2.04	3.00 $\pm$ 1.12	0.35 $\pm$ 0.21	0.75 $\pm$ 0.85	N. D.
Lung	1407.00 $\pm$ 278.56	2968.00 $\pm$ 879.65	1727.00 $\pm$ 332.09	218.60 $\pm$ 69.47	201.10 $\pm$ 77.54	48.59 $\pm$ 5.26
Prostate	55.92 $\pm$ 37.97	231.033 $\pm$ 90.26	432.67 $\pm$ 178.16	145.37 $\pm$ 23.87	190.73 $\pm$ 103.69	63.03 $\pm$ 21.64
Testis	2.43 $\pm$ 2.35	13.37 $\pm$ 2.70	12.58 $\pm$ 3.31	4.21 $\pm$ 0.92	6.53 $\pm$ 1.58	4.94 $\pm$ 2.47
Womb	144.85 $\pm$ 66.88	190.15 $\pm$ 49.01	194.00 $\pm$ 71.19	59.60 $\pm$ 22.74	74.20 $\pm$ 24.74	34.44 $\pm$ 25.84
Ovary	188.45 $\pm$ 99.16	265.10 $\pm$ 76.33	382.75 $\pm$ 87.20	56.95 $\pm$ 12.89	63.60 $\pm$ 30.06	24.79 $\pm$ 10.73
Bladder	43.84 $\pm$ 9.15	117.73 $\pm$ 51.80	197.07 $\pm$ 122.68	27.66 $\pm$ 6.82	29.89 $\pm$ 12.48	12.12 $\pm$ 4.57

**Table S4 The concentrations of ISV-SPM III in rats' tissues after intragastric administration of 100 mg/kg of bitespiramycin (Mean  $\pm$  SD, n = 6)**

Tissues	Concentration (ng/g)					
	1h	3h	6h	12h	24h	48h
Brain	2.56 $\pm$ 1.08	5.24 $\pm$ 2.14	4.40 $\pm$ 2.31	1.05 $\pm$ 0.58	1.79 $\pm$ 1.89	0.54 $\pm$ 0.16
Lung	1741.00 $\pm$ 328.27	3573.00 $\pm$ 1081.71	2444.00 $\pm$ 660.74	318.70 $\pm$ 109.78	296.20 $\pm$ 134.05	73.77 $\pm$ 8.99
Prostate	85.31 $\pm$ 62.68	293.33 $\pm$ 80.25	527.33 $\pm$ 190.34	160.20 $\pm$ 26.55	244.47 $\pm$ 130.41	85.87 $\pm$ 26.13
Testis	3.16 $\pm$ 2.85	15.84 $\pm$ 3.02	12.34 $\pm$ 2.74	4.46 $\pm$ 1.03	7.80 $\pm$ 2.16	5.42 $\pm$ 2.85
Womb	182.05 $\pm$ 82.36	254.50 $\pm$ 63.83	250.05 $\pm$ 71.54	62.35 $\pm$ 23.15	81.55 $\pm$ 24.80	39.10 $\pm$ 26.51
Ovary	299.00 $\pm$ 170.41	358.40 $\pm$ 92.98	472.50 $\pm$ 108.94	60.95 $\pm$ 12.37	76.75 $\pm$ 37.06	31.60 $\pm$ 13.07
Bladder	56.93 $\pm$ 13.99	156.93 $\pm$ 68.33	244.13 $\pm$ 122.23	38.53 $\pm$ 8.15	49.81 $\pm$ 18.95	20.67 $\pm$ 6.51

**Table S5 The concentrations of SPM I in rats' tissues after intragastric administration of 100 mg/kg of bitespiramycin (Mean  $\pm$  SD, n = 6)**

Tissues	Concentration (ng/g)					
	1h	3h	6h	12h	24h	48h
Brain	N. D.	1.60 $\pm$ 0.93	0.68 $\pm$ 0.30	0.85 $\pm$ 0.33	0.60 $\pm$ 0.37	0.76 $\pm$ 0.19
Lung	114.02 $\pm$ 67.75	264.80 $\pm$ 62.49	230.20 $\pm$ 62.38	326.40 $\pm$ 101.61	239.30 $\pm$ 81.40	130.05 $\pm$ 56.79
Prostate	47.34 $\pm$ 46.94	147.53 $\pm$ 59.90	251.97 $\pm$ 136.15	318.67 $\pm$ 70.04	143.37 $\pm$ 29.00	73.07 $\pm$ 8.61
Testis	9.73 $\pm$ 9.91	43.13 $\pm$ 22.42	17.15 $\pm$ 4.15	21.15 $\pm$ 5.68	15.02 $\pm$ 3.17	11.55 $\pm$ 2.16
Womb	288.25 $\pm$ 162.62	624.00 $\pm$ 133.77	795.50 $\pm$ 195.03	183.95 $\pm$ 56.90	166.65 $\pm$ 79.97	88.40 $\pm$ 52.58
Ovary	159.30 $\pm$ 105.38	459.05 $\pm$ 170.08	963.50 $\pm$ 413.22	163.10 $\pm$ 78.20	95.80 $\pm$ 28.62	48.10 $\pm$ 13.55
Bladder	81.13 $\pm$ 48.35	139.40 $\pm$ 70.63	175.00 $\pm$ 108.28	105.18 $\pm$ 80.16	83.80 $\pm$ 38.39	53.11 $\pm$ 15.34

**Table S6 The concentrations of SPM II in rats' tissues after intragastric administration of 100 mg/kg of bitespiramycin (Mean  $\pm$  SD, n = 6)**

Tissues	Concentration (ng/g)					
	1h	3h	6h	12h	24h	48h
Brain	13.89 $\pm$ 6.39	49.10 $\pm$ 18.26	22.28 $\pm$ 12.83	14.80 $\pm$ 6.05	19.33 $\pm$ 7.45	11.97 $\pm$ 5.18
Lung	2598.00 $\pm$ 1048.62	6728.00 $\pm$ 2060.06	6662.00 $\pm$ 1037.53	4260.00 $\pm$ 1121.50	2992.00 $\pm$ 695.75	1112.50 $\pm$ 421.18
Prostate	882.63 $\pm$ 712.74	3086.00 $\pm$ 1207.64	5633.33 $\pm$ 1966.06	4810.00 $\pm$ 1264.83	2956.67 $\pm$ 859.99	1450.33 $\pm$ 175.52
Testis	100.70 $\pm$ 88.77	581.67 $\pm$ 207.52	729.00 $\pm$ 186.97	482.67 $\pm$ 68.44	403.33 $\pm$ 63.39	241.13 $\pm$ 41.26
Womb	2565.50 $\pm$ 1064.35	3715.00 $\pm$ 643.27	3929.00 $\pm$ 909.20	2251.00 $\pm$ 686.47	2393.50 $\pm$ 972.49	1158.50 $\pm$ 726.15
Ovary	1157.00 $\pm$ 446.48	1786.00 $\pm$ 491.13	2880.00 $\pm$ 671.90	928.00 $\pm$ 181.88	825.50 $\pm$ 412.62	237.65 $\pm$ 81.08
Bladder	945.73 $\pm$ 481.23	1764.00 $\pm$ 528.94	3448.67 $\pm$ 1900.76	1302.00 $\pm$ 514.03	963.33 $\pm$ 489.50	569.67 $\pm$ 315.48

**Table S7 The concentrations of SPM III in rats' tissues after intragastric administration of 100 mg/kg of bitespiramycin (Mean  $\pm$  SD, n = 6)**

Tissues	Concentration (ng/g)					
	1h	3h	6h	12h	24h	48h
Brain	19.95 $\pm$ 9.69	56.67 $\pm$ 23.90	38.35 $\pm$ 25.03	48.22 $\pm$ 24.92	33.21 $\pm$ 13.39	23.96 $\pm$ 9.17
Lung	7846.00 $\pm$ 2711.43	20250.00 $\pm$ 2540.98	20150.00 $\pm$ 2341.51	13300.00 $\pm$ 3269.57	8750.00 $\pm$ 1560.65	3700.33 $\pm$ 1449.72
Prostate	996.07 $\pm$ 766.55	3716.67 $\pm$ 1554.32	6973.33 $\pm$ 2019.69	6360.00 $\pm$ 1464.19	4370.00 $\pm$ 1004.69	2523.33 $\pm$ 228.53
Testis	75.47 $\pm$ 67.63	388.67 $\pm$ 44.10	409.00 $\pm$ 94.15	296.33 $\pm$ 38.06	247.57 $\pm$ 48.39	159.20 $\pm$ 24.42
Womb	2645.00 $\pm$ 1055.27	4940.00 $\pm$ 1147.82	5410.00 $\pm$ 1668.03	3346.50 $\pm$ 956.57	3751.50 $\pm$ 1422.66	1905.50 $\pm$ 1243.33
Ovary	1736.50 $\pm$ 624.10	3245.50 $\pm$ 1007.69	5410.00 $\pm$ 1113.43	2003.50 $\pm$ 404.00	1799.50 $\pm$ 912.43	580.95 $\pm$ 194.30
Bladder	843.33 $\pm$ 391.50	1818.67 $\pm$ 502.55	4078.67 $\pm$ 2147.62	1662.00 $\pm$ 463.38	1252.00 $\pm$ 415.61	656.00 $\pm$ 146.73

**Table S8 The concentration and penetrability of SPMs I, II, III and ISV-SPMs I, II, III in vitro (Mean  $\pm$  SD, n = 6)**

Time	Concentration (ng/ml)					
	ISV-SPM I	ISV-SPM II	ISV-SPM III	SPM I	SPM II	SPM III
2h	53.77 $\pm$ 15.05	24.98 $\pm$ 4.85	25.13 $\pm$ 5.33	90.37 $\pm$ 20.79	85.42 $\pm$ 19.14	78.65 $\pm$ 16.67
4h	90.37 $\pm$ 15.81	40.38 $\pm$ 5.50	40.43 $\pm$ 6.46	128.33 $\pm$ 23.64	124.15 $\pm$ 21.30	113.38 $\pm$ 18.93
8h	138.83 $\pm$ 15.33	61.95 $\pm$ 5.95	63.27 $\pm$ 7.41	191.00 $\pm$ 15.44	185.33 $\pm$ 14.65	173.50 $\pm$ 13.05
Time	Penetrability (%)					
	ISV-SPM I	ISV-SPM II	ISV-SPM III	SPM I	SPM II	SPM III
2h	8.07 $\pm$ 2.26	7.49 $\pm$ 1.45	7.54 $\pm$ 1.60	13.56 $\pm$ 3.12	12.81 $\pm$ 2.87	11.80 $\pm$ 2.50
4h	13.56 $\pm$ 2.37	12.11 $\pm$ 1.65	12.13 $\pm$ 1.94	19.25 $\pm$ 3.55	18.62 $\pm$ 3.20	17.01 $\pm$ 2.84
8h	20.82 $\pm$ 2.30	18.59 $\pm$ 1.79	18.98 $\pm$ 2.22	28.65 $\pm$ 2.32	27.80 $\pm$ 2.20	26.03 $\pm$ 1.96

**Table S9. The tissue-to-plasma concentration ratio (Ct/Cp) determined 1 hours following oral administration of bitespiramycin at a dosage of 100 mg/kg.**

	Brain	Lung	Prostate	Testis	Womb	Ovary	Bladder
ISV-SPM I	/	6.86	0.41	/	2.05	2.54	0.30
ISV-SPM II	0.04	23.80	0.95	0.04	2.45	3.19	0.74
ISV-SPM III	0.09	59.12	2.90	0.11	6.18	10.15	1.93
SPM I	/	1.08	0.45	0.09	2.73	1.51	0.77
SPM II	0.02	3.57	1.21	0.14	3.52	1.59	1.30
SPM III	0.01	4.54	0.58	0.04	1.53	1.00	0.49

**Table S10. The tissue-to-plasma concentration ratio (Ct/Cp) determined 3 hours following oral administration of bitespiramycin at a dosage of 100 mg/kg.**

	Brain	Lung	Prostate	Testis	Womb	Ovary	Bladder
ISV-SPM I	0.06	18.06	1.64	0.17	3.51	5.95	0.77
ISV-SPM II	0.06	40.20	3.13	0.18	2.58	3.59	1.59
ISV-SPM III	0.14	95.48	7.84	0.42	6.80	9.58	4.19
SPM I	0.02	2.55	1.42	0.42	6.02	4.43	1.34
SPM II	0.07	10.22	4.69	0.88	5.65	2.71	2.68
SPM III	0.03	11.46	2.10	0.22	2.80	1.84	1.03

**Table S11. The tissue-to-plasma concentration ratio (Ct/Cp) determined 6 hours following oral administration of bitespiramycin at a dosage of 100 mg/kg.**

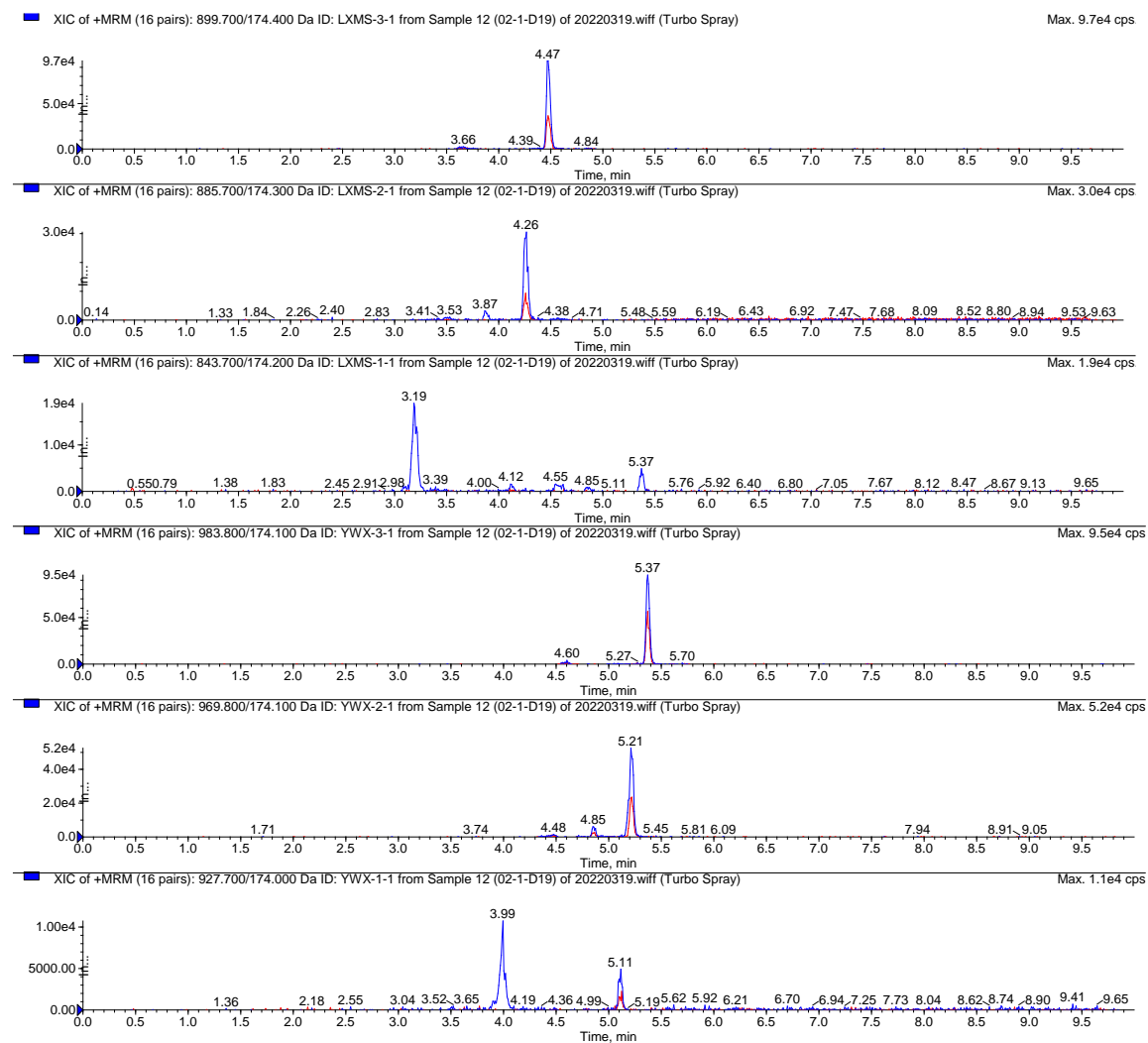
	Brain	Lung	Prostate	Testis	Womb	Ovary	Bladder
ISV-SPM I	/	25.43	13.39	0.33	9.89	25.01	2.75
ISV-SPM II	0.12	68.94	17.27	0.50	7.74	15.28	7.87
ISV-SPM III	0.33	185.86	40.10	0.94	19.02	35.93	18.57
SPM I	0.02	6.84	7.49	0.51	23.64	28.63	5.20
SPM II	0.07	19.67	16.63	2.15	11.60	8.50	10.18
SPM III	0.04	20.77	7.19	0.42	5.58	5.58	4.20

**Table S12. The tissue-to-plasma concentration ratio (Ct/Cp) determined 12 hours following oral administration of bitespiramycin at a dosage of 100 mg/kg.**

	Brain	Lung	Prostate	Testis	Womb	Ovary	Bladder
ISV-SPM I	/	/	/	/	/	/	/
ISV-SPM II	0.08	50.02	33.27	0.96	13.64	13.03	6.33
ISV-SPM III	0.47	142.28	71.52	1.99	27.83	27.21	17.20
SPM I	0.17	63.63	62.12	4.12	35.86	31.79	20.50
SPM II	0.15	42.00	47.43	4.76	22.19	9.15	12.84
SPM III	0.13	36.59	17.50	0.82	9.21	5.51	4.57

**Table S13. The tissue-to-plasma concentration ratio (Ct/Cp) determined 48 hours following oral administration of bitespiramycin at a dosage of 100 mg/kg.**

	Brain	Lung	Prostate	Testis	Womb	Ovary	Bladder
ISV-SPM I	/	/	/	/	/	/	/
ISV-SPM II	/	36.26	47.04	3.69	25.70	18.50	9.04
ISV-SPM III	0.76	103.90	120.94	7.63	55.07	44.51	29.11
SPM I	0.87	149.48	83.99	13.28	101.61	55.29	61.05
SPM II	0.81	75.27	98.13	16.31	78.38	16.08	38.54
SPM III	0.51	78.53	53.55	3.38	40.44	12.33	13.92



**Figure S1 The MRM chromatograms six components of the patient cerebrospinal fluid**