Supplementary Materials for

A highly sensitive LC-MS/MS method for quantification of AB-38b in rat plasma, liver and kidney tissue: Application to a pharmacokinetic study

Dandan Pan[a1], Libang Chen[a1], Haonan Luo[a], Haiyan Liu[d], Weihao Chen[a], Zhenzhou Jiang[c], Zhen Qiu[a], Nan Zhou[a], Yequan Zhou[a], Qiujin Xu[e], Tao Wang[a, b*], Qian Lu[a*], Tingting Yang[a*]

[a]Jiangsu Key Laboratory of New Drug Research and Clinical Pharmacy, Xuzhou Medical University, Xuzhou 221004, China

[b]Department of Pharmacy, The affiliated hospital of Xuzhou Medical University, Xuzhou 221006, China

[c]New Drug Screening Center, Jiangsu Center for Pharmacodynamics Research and Evaluation, China Pharmaceutical University, Nanjing 210009, China

[d]Hunan Creates Med Technology Co., Ltd, Changsha 410205, China

[e]Phase I Clinical Research Center, Xuzhou Central Hospital, Xuzhou 221009, China

*Corresponding author:

Tao Wang

Jiangsu Key Laboratory of New Drug Research and Clinical Pharmacy, Xuzhou Medical University, NO. 209. Tongshan Road, Xuzhou, Jiangsu 221004, China.

Department of Pharmacy, The affiliated hospital of Xuzhou Medical University, Xuzhou 221006, China

E-mail addresses: Misswt2011@126.com

Qian Lu

Department of Clinical Pharmacology, School of Pharmacy, Xuzhou Medical University, NO. 209. Tongshan Road, Xuzhou, Jiangsu 221004, China.

E-mail addresses: prairy@126.com

Tingting Yang

Department of Clinical Pharmacology, School of Pharmacy, Xuzhou Medical University, NO. 209.

Tongshan Road, Xuzhou, Jiangsu 221004, China.

E-mail addresses: tty@xzhmu.edu.cn; nanjing_cpu@126.com

[1]These authors equally contributed to this work.

*These authors contributed equally to this work.

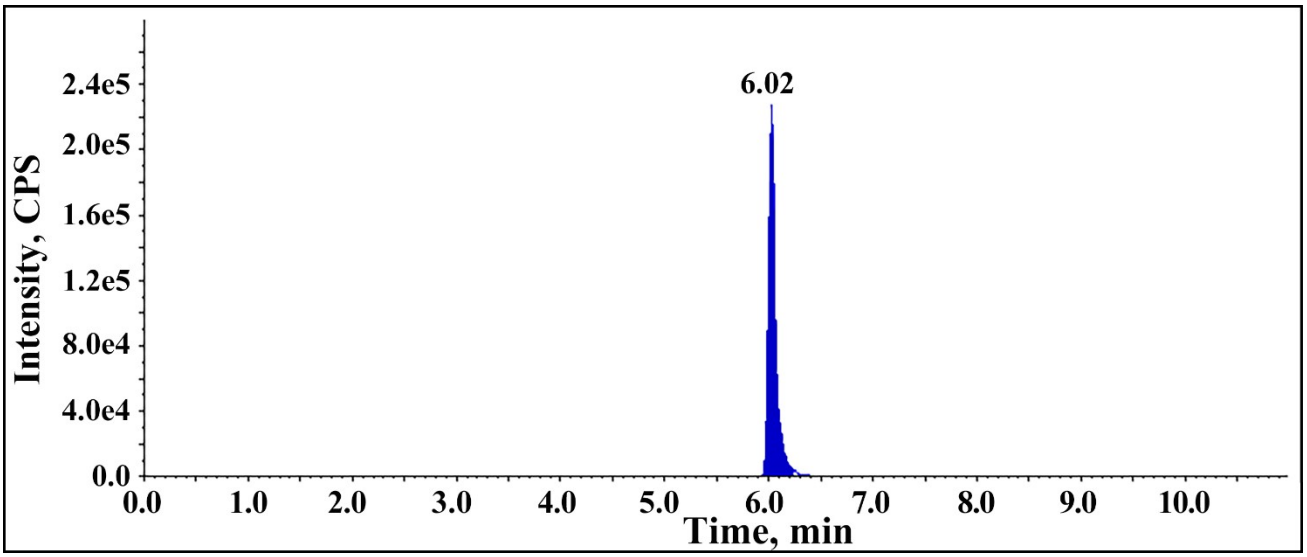

Figure. S1. The chromatogram of AB-38b for purity determination.

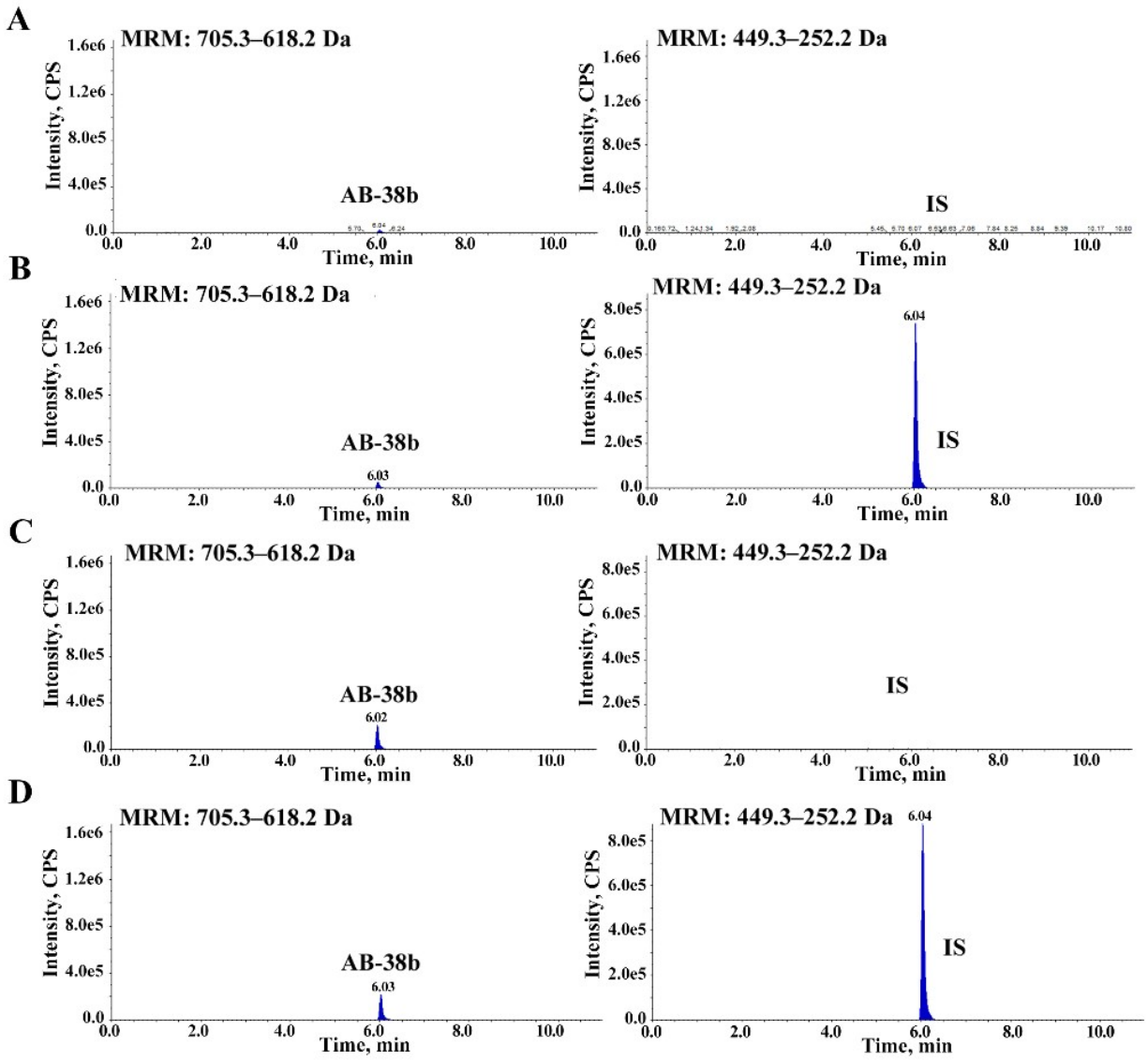

Figure. S2 Typical MRM chromatogram of AB-38b and IS (Tolvaptan) in the liver of rats. (A) A double blank sample; (B) A zero sample (blank sample spiked with IS); (C) Liver tissue homogenate sample spiked with AB-38b; (D) AB-38b at LLOQ with IS.

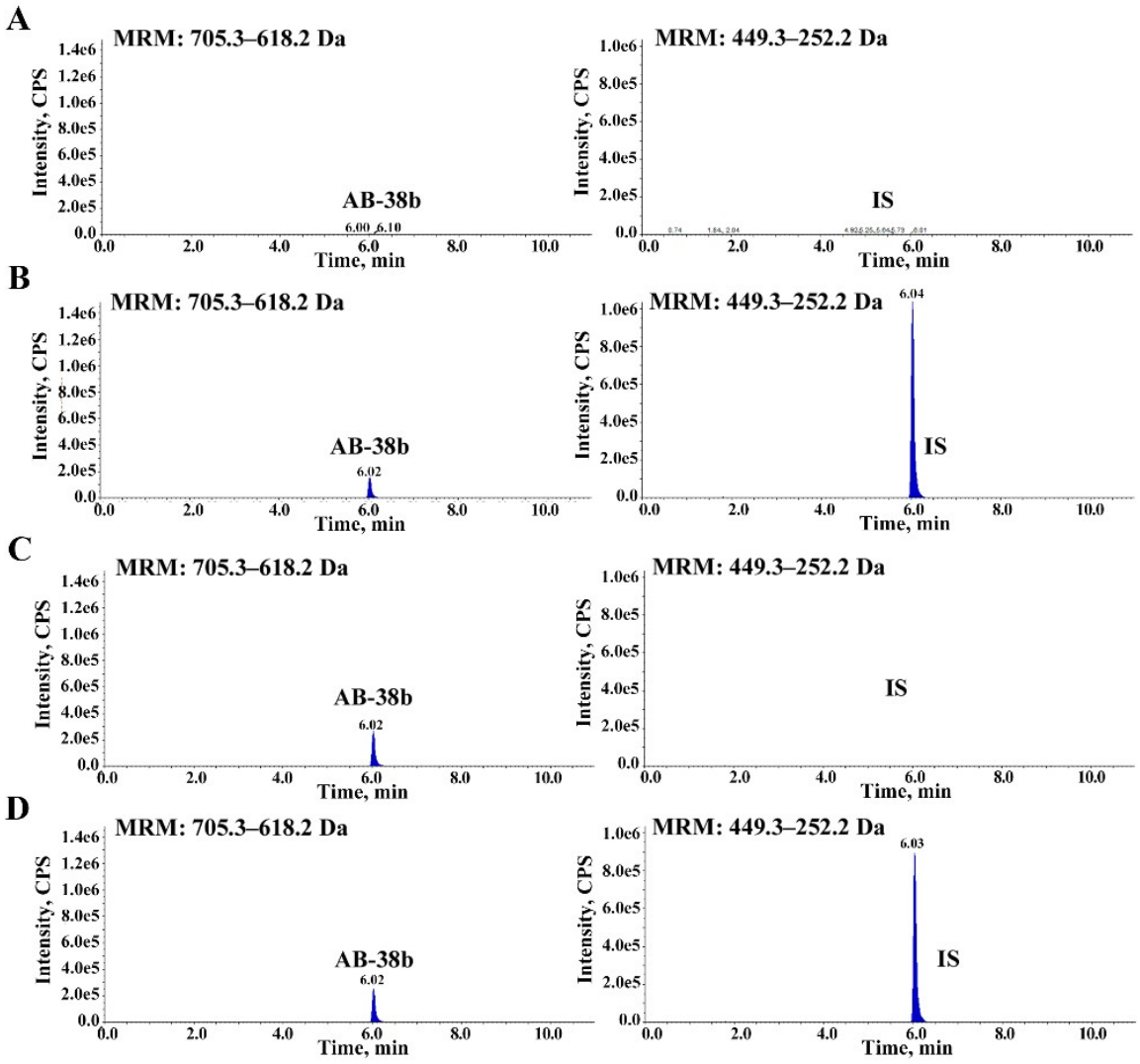

Figure.S3 Typical MRM chromatogram of AB-38b and IS (Tolvaptan) in the kidney of rats. (A) A double blank sample; (B) A zero sample (blank sample spiked with IS); (C) Kidney tissue homogenate sample spiked with AB-38b; (D) AB-38b at LLOQ with IS.

Table S1. Linear ranges, calibration curves, correlation coefficient and lower limits of quantification of AB-38b in different mixed matrices of rats (n = 5).

Sample	Tissue	Liner range (ng/mL)	Calibration curves	R^2	LLOQ
AB-38b	Liver	2.5-150	Y = 0.057X + 0.085	0.994	2.5
	Kidney	2.5-150	Y = 0.067X + 0.161	0.996	2.5

Table S2. Within-run and Between-run precision and accuracy for AB-38b in different mixed matrices of rats (n = 5).

Tissue	QC level	Concentration added (ng/mL)	Within-run (n = 5)			Between-run (n = 15)		
			Concentration Measured (ng/mL) (mean ± SD)	Accuracy (RE %)	Precision (CV %)	Concentration Measured (ng/mL) (mean ± SD)	Accuracy (RE %)	Precision (CV %)
Liver	LLOQ	2.5	2.65 ± 0.14	5.36	3.72	2.60 ± 0.08	4.16	3.11
	LQC	5	5.06 ± 0.14	1.80	2.82	5.14 ± 0.17	2.71	3.39
	MQC	25	24.40 ± 1.89	−2.40	7.75	25.59 ± 0.69	2.35	2.69
	HQC	100	97.26 ± 2.61	−2.74	2.68	101.99 ± 2.12	1.99	2.08
Kidney	LLOQ	2.5	2.53 ± 0.30	1.36	11.89	2.59 ± 0.13	0.48	4.84
	LQC	5	4.58 ± 0.22	−8.48	4.91	5.18 ± 0.09	0.72	1.68
	MQC	25	24.72 ± 2.80	−1.12	11.35	26.61 ± 1.05	8.80	3.96
	HQC	100	101.10 ± 5.90	1.10	5.84	104.13 ± 5.36	0.44	5.15

Table S3. Extraction recoveries of AB-38b in different mixed matrices of rats (n = 5).

Tissue	QC level	Concentration added (ng/mL)	Extraction recovery (n = 5)	
			Recovery (mean ± SD) (%)	Precision (CV %)
Liver	LQC	5	84.33 ± 2.90	3.44
	MQC	25	94.46 ± 8.61	9.12
	HQC	100	96.44 ± 7.97	8.27
	IS	200	99.64 ± 9.30	9.33
Kidney	LQC	5	83.52 ± 8.17	9.78
	MQC	25	97.80 ± 8.45	8.63
	HQC	100	97.47 ± 5.99	6.15
	IS	200	98.61 ± 11.24	11.40

Table S4. Matrix effect of AB-38b in different mixed matrices of rats (n = 5).

Tissue	QC level	Between 6 different batches of rats		
		Concentration added (ng/mL)	Concentration Measured (ng/mL) (mean ± SD)	Precision (CV %)
Liver	LQC	5	85.69 ± 5.43	6.34
	MQC	25	85.66 ± 2.27	2.65
	HQC	100	86.15 ± 1.28	1.49
Kidney	LQC	5	86.62 ± 2.64	3.04
	MQC	25	98.72 ± 7.20	7.29
	HQC	100	98.48 ± 9.78	9.94

Table S5. Dilution reliability of AB-38b in rat liver homogenate samples with high concentration (n = 6).

Tissue	Concentration added (ng/mL)	Dilution Factor	Concentration Measured (ng/mL) (mean ± SD)	CV (%)
Liver	500	5	102.03 ± 3.23	3.16

Table S6. The ratio of the upper limit of quantitation to the peak area of AB-38b or IS in blank rat plasma or tissue samples (n = 3).

Sample	Tissue	AB-38b Area			IS Area		
		ULOQ	Blank	%	ULOQ	Blank	%
AB-38b	Plasma	1.68E+07	1.19E+05	15.73	2.90E+06	3.70E+02	0.01
	Liver	3.03E+07	5.58E+04	9.48	4.02E+06	1.35E+03	0.03
	Kidney	2.79E+07	2.04E+05	16.01	3.15E+06	6.36E+02	0.02

Table S7. The stability of AB-38b in different mixed matrices of rats under various storage conditions (n = 5).

Tissue	Storage conditions	QC level	Concentration added (ng/mL)	Concentration Measured (ng/mL) (mean ± SD)	Accuracy (RE%)	Precision (CV%)
Liver	Injector stability (24 h at 4 ℃)	LQC	5	4.58 ± 0.40	−8.40	8.65
		MQC	25	24.68 ± 1.81	−1.28	7.32
		HQC	100	93.04 ± 6.05	−6.96	6.50
	short-term temperature stability (12 h, room temperature)	LQC	5	5.17 ± 0.27	3.44	5.24
		MQC	25	24.36 ± 0.95	−2.56	3.89
		HQC	100	93.88 ± 4.97	−6.12	5.30
	Freeze-thaw stability (3 cycles at −20 ℃)	LQC	5	4.74 ± 0.13	−5.16	2.78
		MQC	25	25.10 ± 0.44	0.40	1.76
		HQC	100	95.16 ± 3.01	−4.84	3.16
	Long-term stability (60 days at −80 ℃)	LQC	5	5.49 ± 0.19	9.80	3.49
		MQC	25	26.58 ± 1.93	6.32	7.27
		HQC	100	104.80 ± 5.81	4.80	5.54
Kidney	Injector stability (24 h at 4 ℃)	LQC	5	4.93 ± 0.37	−1.48	7.51
		MQC	25	27.04 ± 1.22	8.16	4.50
		HQC	100	102.32 ± 8.74	2.32	8.54
	short-term temperature stability (12 h, room temperature)	LQC	5	4.61 ± 0.37	−7.84	8.13
		MQC	25	25.46 ± 1.84	1.84	7.21
		HQC	100	101.88 ± 7.72	1.88	7.58
	Freeze-thaw stability (3 cycles at −20 ℃)	LQC	5	5.27 ± 0.23	5.40	4.45
		MQC	25	25.42 ± 1.66	1.68	6.55
		HQC	100	93.10 ± 4.87	−6.90	5.23
	Long-term stability (60 days at −80 ℃)	LQC	5	4.77 ± 0.35	−4.52	7.38
		MQC	25	24.52 ± 1.50	−1.92	6.10
		HQC	100	87.74 ± 2.32	−12.26	2.64

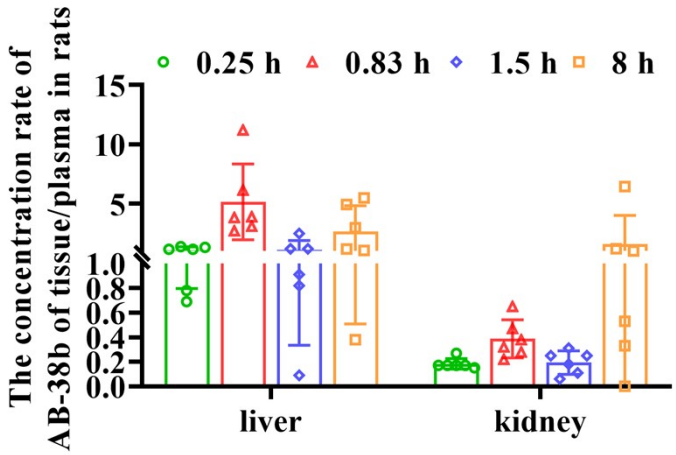

Figure.S4 The concentration rate of AB-38b of tissue/plasma in rats. The rats were given a single oral dose of 50 mg/kg (n = 6).