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A comparative evaluation of the plasma and DBS-based LC-MS/MS methods for the simultaneous analysis of nine antibiotics for application to pharmacokinetic evaluations and precision dosing in neonates

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SUPPLEMENTARY MATERIAL

1. LC-MS/MS condition

Tune File Values

Source Type: HESI

Capillary Temp (C): 350.00

Source Heater Temp (C): 420.00

Sheath Gas Flow (arb): 50.00

Aux Gas Flow (arb): 16.00

Sweep Gas Flow (arb): 0.00

POSITIVE POLARITY

Source Voltage (kV): 4.00

Source Current (uA): 100.00

Capillary Voltage (V): 47.00

Tube Lens (V): 100.00

Skimmer Offset (V): 0.00

Multipole RF Amplifier (Vp-p): 400.00

NEGATIVE POLARITY

Source Voltage (kV): 4.00

Source Current (uA): 100.00

Capillary Voltage (V): -2.00

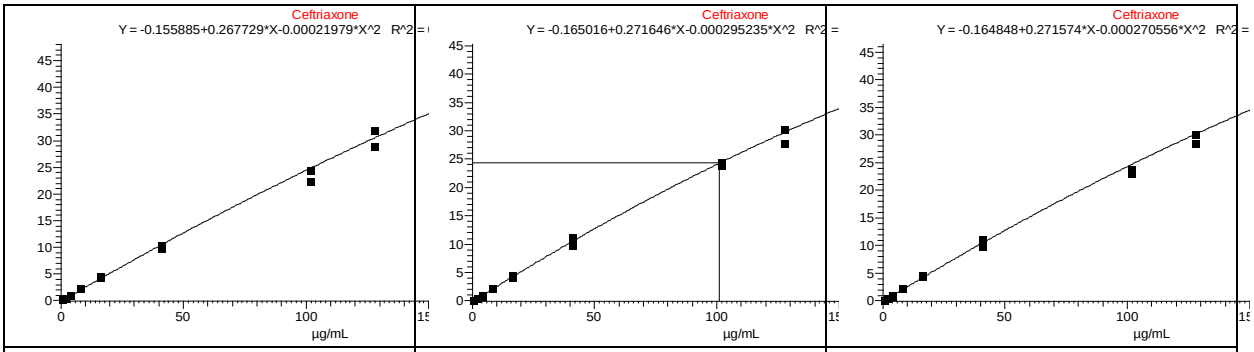
Tube Lens (V): -43.39

Skimmer Offset (V): 0.00

Multipole RF Amplifier (Vp-p): 400.00

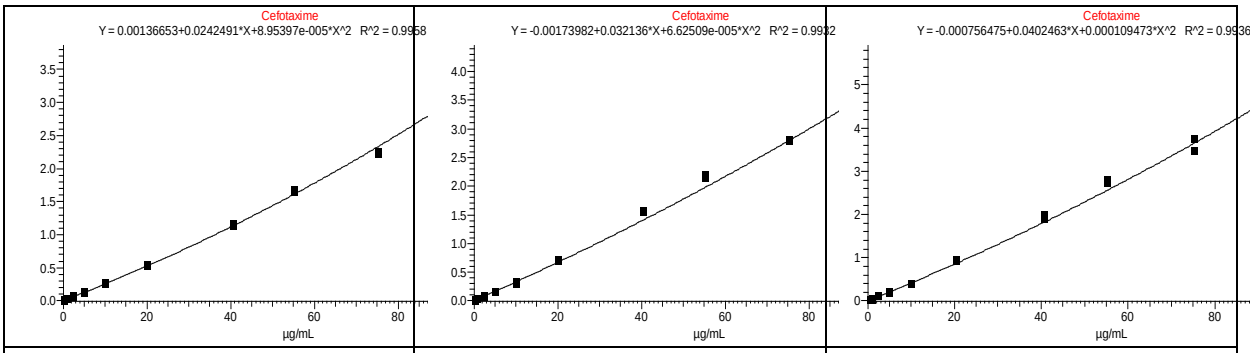
Multipole 00 Offset (V): -8.00	Multipole 00 Offset (V): 7.50
Lens 0 Voltage (V): -9.00	Lens 0 Voltage (V): 7.50
Multipole 0 Offset (V): -9.25	Multipole 0 Offset (V): 8.50
Lens 1 Voltage (V): -13.00	Lens 1 Voltage (V): 18.00
Gate Lens Offset (V): -38.00	Gate Lens Offset (V): 74.00
Multipole 1 Offset (V): -12.50	Multipole 1 Offset (V): 15.50
Front Lens (V): -14.00	Front Lens (V): 8.75

The curve of each analyte of interest was performed on 3 different days using fresh independent standard stock in the calibration range as shown in Table 7. The back-calculation was performed as a quantitative area ratio method, Quadratic regression with 1/X^2 weighing factor. All the analytes LLOQ levels were within 20% of respective nominal concentration, and other calibrators were within 15% of respective nominal concentration. The correlation coefficient of all the analytes was NLT 0.99, indicating the method linear at a defined concentration range.



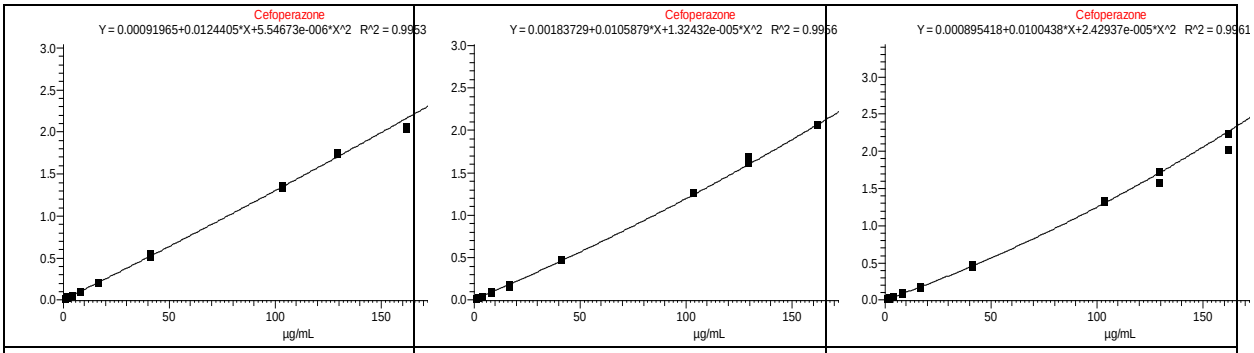
Calibration Curve-I	Calibration Curve-II	Calibration Curve-III

Figure S1: Calibration Curves from Three CC runs for Ceftriaxone.



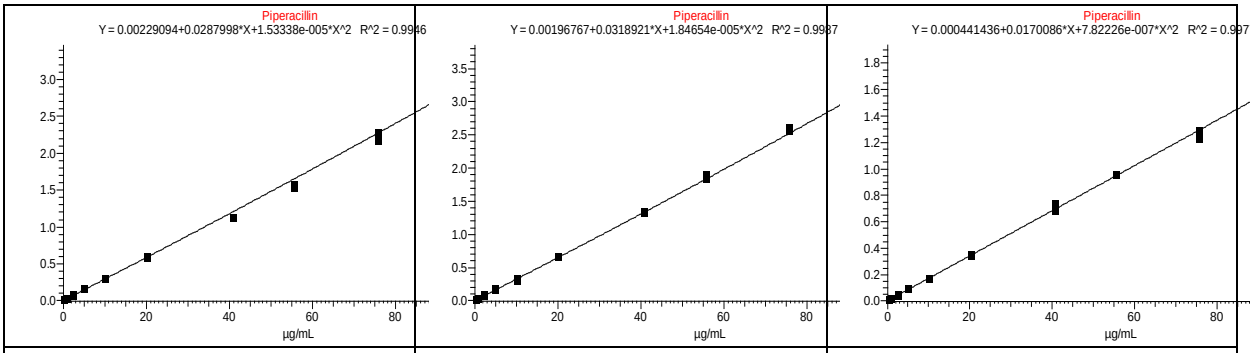
Calibration Curve-I	Calibration Curve-II	Calibration Curve-III

Figure S2: Calibration Curves from Three CC runs for Cefotaxime.



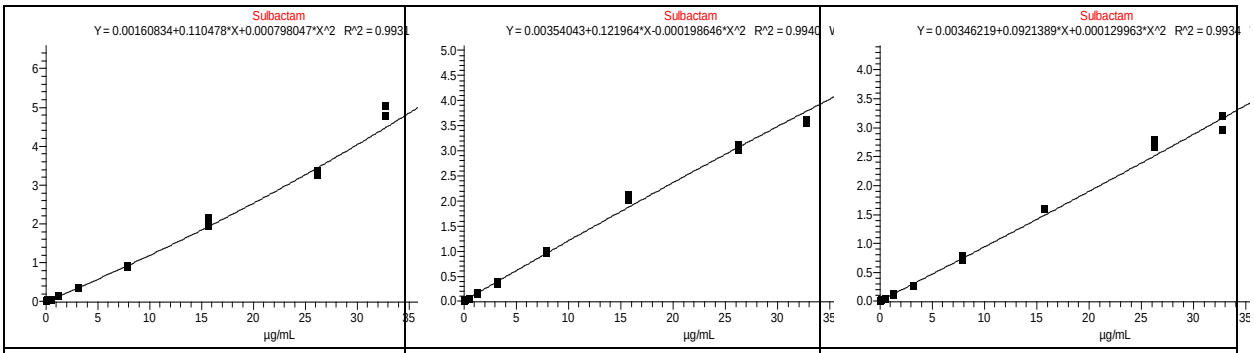
Calibration Curve-I	Calibration Curve-II	Calibration Curve-III

Figure S3: Calibration Curves from Three CC runs for Cefoperazone.



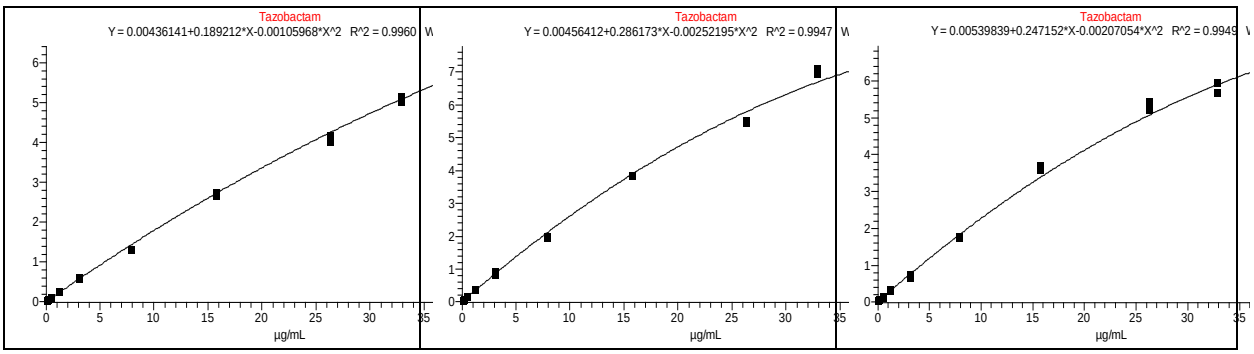
Calibration Curve-I	Calibration Curve-II	Calibration Curve-III

Figure S4: Calibration Curves from Three CC runs for Piperacillin.



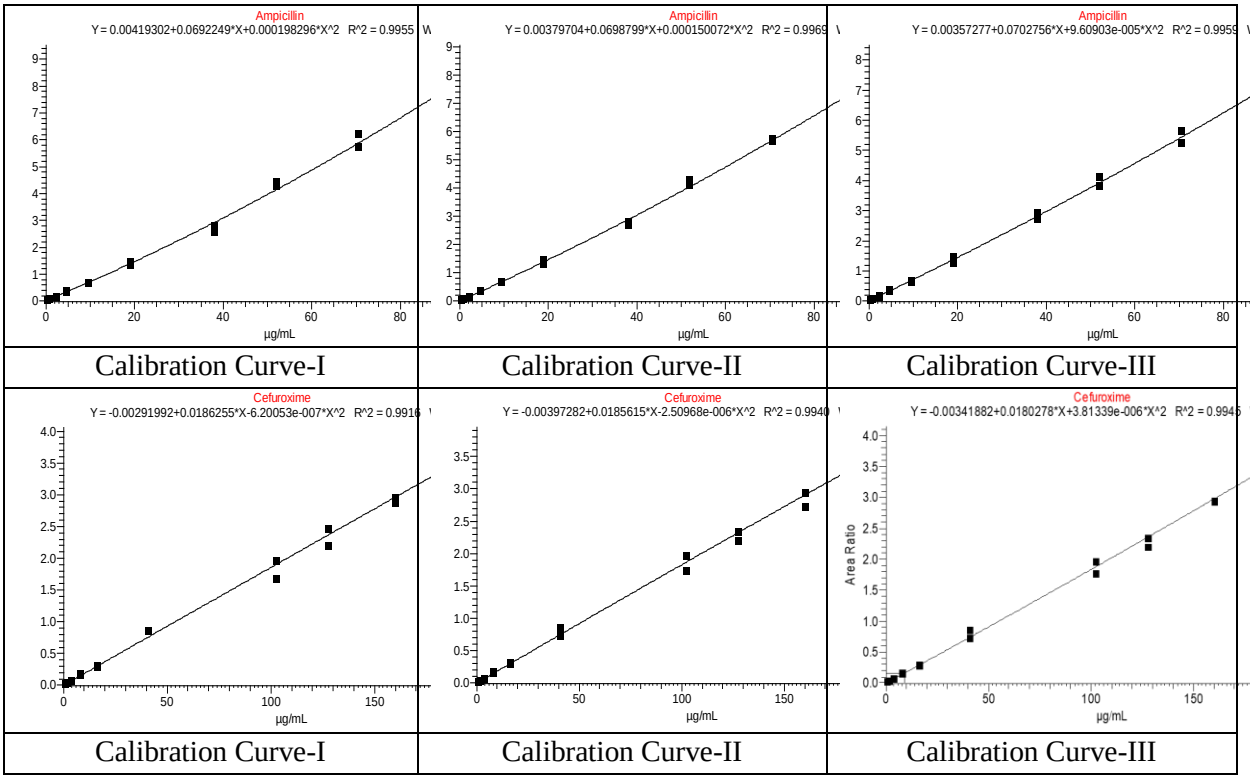
Calibration Curve-I	Calibration Curve-II	Calibration Curve-III

Figure S5: Calibration Curves from Three CC runs for Sulbactam.



Calibration Curve-I	Calibration Curve-II	Calibration Curve-III

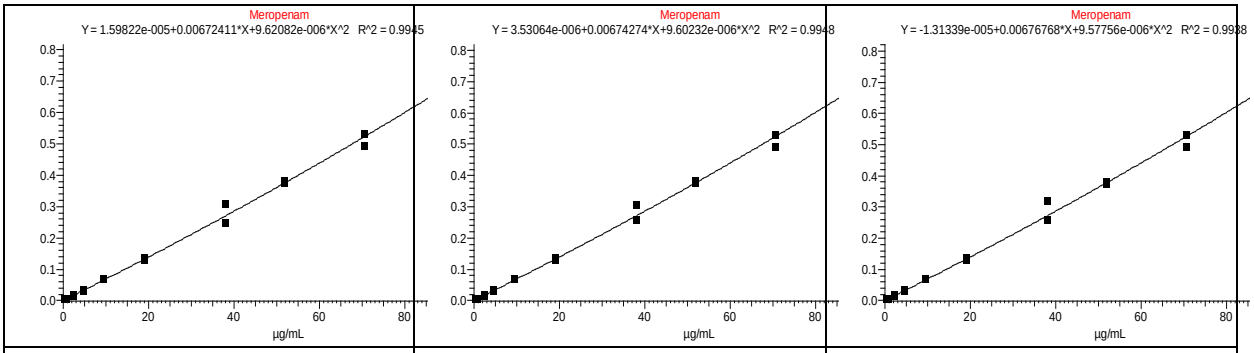
Figure S6: Calibration Curves from Three CC runs for Tazobactam.



Calibration Curve-I	Calibration Curve-II	Calibration Curve-III
Calibration Curve-I	Calibration Curve-II	Calibration Curve-III

Figure S7: Calibration Curves from Three CC runs for Ampicillin.

Figure S8: Calibration Curves from Three CC runs for Cefuroxime.



Calibration Curve-I	Calibration Curve-II	Calibration Curve-III

Figure S9: Calibration Curves from Three CC runs for Meropenem.

Table S1: Intercept, Slope, X-value, and R^2 values of three calibration curves in plasma matrix.

Drug	Run	Intercept, Slope, X-value, R^2
Ceftriaxone	Calibration Curve-I	Y = -0.159128+0.268283*X-0.000223365*X^2 R^2 = 0.9951
	Calibration Curve-II	Y = -0.165016+0.271646*X-0.000295235*X^2 R^2 = 0.9955
	Calibration Curve-III	Y = -0.164848+0.271574*X-0.000270556*X^2 R^2 = 0.9956
Cefotaxime	Calibration Curve-I	Y = 0.00136653+0.0242491*X+8.95397e-005*X^2 R^2 = 0.9958
	Calibration Curve-II	Y = -0.00173982+0.032136*X+6.62509e-005*X^2 R^2 = 0.9932
	Calibration Curve-III	Y = -0.000756475+0.0402463*X+0.000109473*X^2 R^2 = 0.9936
Cefoperazone	Calibration Curve-I	Y = 0.00091965+0.0124405*X+5.54673e-006*X^2 R^2 = 0.9953
	Calibration Curve-II	Y = 0.00183729+0.0105879*X+1.32432e-005*X^2 R^2 = 0.9956
	Calibration Curve-III	Y = 0.000895418+0.0100438*X+2.42937e-005*X^2 R^2 = 0.9961
Piperacillin	Calibration Curve-I	Y = 0.00229094+0.0287998*X+1.53338e-005*X^2 R^2 = 0.9946
	Calibration Curve-II	Y = 0.00196767+0.0318921*X+1.84654e-005*X^2 R^2 = 0.9987
	Calibration Curve-III	Y = 0.000441436+0.0170086*X+7.82226e-007*X^2 R^2 = 0.9977
Sulbactam	Calibration Curve-I	Y = 0.00160834+0.110478*X+0.000798047*X^2 R^2 = 0.9931
	Calibration Curve-II	Y = 0.00354043+0.121964*X-0.000198646*X^2 R^2 = 0.9940
	Calibration Curve-III	Y = 0.00346219+0.0921389*X+0.000129963*X^2 R^2 = 0.9934
Tazobactam	Calibration Curve-I	Y = 0.00436141+0.189212*X-0.00105968*X^2 R^2 = 0.9960
	Calibration Curve-II	Y = 0.00456412+0.286173*X-0.00252195*X^2 R^2 = 0.9947
	Calibration Curve-III	Y = 0.00539839+0.247152*X-0.00207054*X^2 R^2 = 0.9949
Cefuroxime	Calibration Curve-I	Y = -0.00291992+0.0186255*X-6.20053e-007*X^2 R^2 = 0.9916
	Calibration Curve-II	Y = -0.00397282+0.0185615*X-2.50968e-006*X^2 R^2 = 0.9940
	Calibration Curve-III	Y = -0.00341882+0.0180278*X+3.81339e-006*X^2 R^2 = 0.9945
Ampicillin	Calibration Curve-I	Y = 0.00419302+0.0692249*X+0.000198296*X^2 R^2 = 0.9955
	Calibration Curve-II	Y = 0.00379704+0.0698799*X+0.000150072*X^2 R^2 = 0.9969
	Calibration Curve-III	Y = 0.00357277+0.0702756*X+9.60903e-005*X^2 R^2 = 0.9959
Meropenem	Calibration Curve-I	Y = 1.59822e-005+0.00672411*X+9.62082e-006*X^2 R^2 = 0.9945
	Calibration Curve-II	Y = 3.53064e-006+0.00674274*X+9.60232e-006*X^2 R^2 = 0.9948
	Calibration Curve-III	Y = -1.31339e-005+0.00676768*X+9.57756e-006*X^2 R^2 = 0.9938

Table S2: Calculated % recovery after back calculation from linearity plot for each calibrator in plasma matrix.

Analyte	Calibrator	CC1	CC1	CC2	CC2	CC3	CC3	CC4	CC4	CC5	CC5	CC6	CC6	CC7	CC7	CC8	CC8	CC9	CC9	CC10	CC10
Ceftriaxone	Linearity-1	101.74	93.56	110.46	96.00	108.36	110.27	85.17	89.69	108.27	102.70	92.75	91.21	99.25	104.83	107.46	95.84	101.36	106.77	96.15	97.57
	Linearity-2	102.07	93.44	107.41	97.92	113.83	107.14	87.70	87.60	102.15	104.94	92.84	90.79	105.27	106.00	99.16	100.75	104.64	103.66	96.67	96.00
	Linearity-3	90.70	106.97	106.94	92.58	107.13	109.66	92.29	105.36	89.18	103.62	89.21	100.11	106.60	98.17	102.16	98.95	97.50	103.77	99.44	99.61
Cefotaxime	Linearity-1	85.78	111.87	112.84	95.25	90.75	100.62	103.08	96.66	102.40	97.15	98.89	102.41	100.25	101.87	103.31	102.06	97.04	96.01	97.88	103.56
	Linearity-2	96.34	109.71	100.59	94.93	88.22	94.54	96.03	101.17	88.19	100.01	102.42	104.54	108.80	109.90	109.27	107.49	100.67	100.19	93.13	92.33
	Linearity-3	98.16	109.00	102.03	89.15	92.06	96.73	90.94	105.52	90.40	93.97	109.20	107.42	103.55	108.00	107.80	106.03	95.57	102.35	95.11	95.27
Cefoperazone	Linearity-1	96.48	101.52	117.97	87.44	94.36	105.73	99.87	95.06	98.48	98.51	99.15	106.36	99.32	100.89	101.55	102.97	94.47	95.67	101.21	102.92
	Linearity-2	112.73	87.70	109.33	91.19	100.34	96.38	108.50	96.99	89.96	98.79	101.52	101.33	101.10	102.10	101.43	105.31	99.87	99.93	97.70	97.34
	Linearity-3	98.29	99.23	99.34	109.57	91.19	96.74	107.64	101.44	92.64	106.13	99.03	102.56	101.72	102.17	93.79	100.46	91.38	99.09	102.82	105.06
Piperacillin	Linearity-1	109.62	91.25	102.59	89.68	100.12	109.23	108.33	104.44	101.23	101.15	97.70	101.36	93.17	92.80	94.69	93.23	95.47	100.12	104.66	109.13
	Linearity-2	97.80	103.98	94.42	100.03	99.90	104.44	104.11	101.71	92.36	97.73	100.96	99.18	99.63	100.50	100.06	103.02	102.74	101.54	97.29	98.56
	Linearity-3	99.80	104.97	97.92	91.01	97.03	101.06	106.37	108.21	96.35	97.48	97.61	101.46	105.57	97.12	100.18	100.67	94.46	99.92	102.73	100.08
Sulbactam	Linearity-1	94.56	104.44	113.89	92.40	89.55	100.15	105.10	94.60	100.22	95.92	98.22	100.86	109.80	101.02	95.69	97.89	110.10	105.68	88.57	100.03
	Linearity-2	107.42	97.91	87.00	106.44	91.88	96.02	108.46	97.69	90.93	99.03	102.09	105.03	107.84	114.08	101.76	98.14	95.43	93.61	102.68	96.62
	Linearity-3	100.79	102.44	97.51	97.35	89.07	108.03	93.01	111.37	91.55	89.56	105.91	98.19	107.00	107.31	106.12	110.78	93.68	101.34	95.98	92.80
Tazobactam	Linearity-1	100.04	95.34	96.06	107.57	101.62	110.42	107.92	101.16	100.27	97.11	90.35	91.20	96.88	100.60	97.44	93.75	101.67	98.49	104.39	110.54
	Linearity-2	102.41	89.59	100.43	109.33	106.15	111.82	100.56	97.88	93.14	102.38	92.92	91.89	98.15	98.16	93.29	91.80	106.24	110.77	109.57	105.07
	Linearity-3	94.38	105.73	101.49	96.21	94.58	110.25	101.38	105.74	87.84	96.45	97.03	94.85	111.28	106.46	103.79	110.24	94.21	101.11	96.00	87.37
Ampicillin	Linearity-1	95.95	96.19	112.23	107.24	90.40	97.03	106.64	100.96	100.17	97.57	95.31	104.08	95.64	89.51	103.09	106.66	98.00	104.92	96.22	102.03
	Linearity-2	91.73	103.08	98.84	93.56	91.93	107.60	98.73	103.09	85.19	93.80	94.38	92.20	108.63	103.81	101.14	107.59	91.56	98.46	93.35	84.72
	Linearity-3	95.42	92.66	110.65	105.72	88.71	95.28	105.02	99.35	99.16	96.53	90.59	104.49	102.09	94.93	96.85	103.83	95.70	102.53	93.63	103.74
Meropenem	Linearity-1	100.32	108.41	87.20	99.32	86.12	98.27	98.50	108.16	104.45	103.79	99.32	104.40	112.56	92.09	99.84	101.90	94.55	101.34	97.68	101.48
	Linearity-2	100.43	108.50	87.16	99.24	85.96	98.07	98.27	107.91	104.19	103.53	99.07	104.14	112.30	95.41	99.62	101.67	94.35	101.12	101.29	97.49
	Linearity-3	100.57	108.61	87.10	99.13	85.75	97.82	97.97	107.57	103.84	103.19	98.74	103.80	116.57	95.11	99.32	101.37	94.08	100.84	97.24	101.02
Cefuroxime	Linearity-1	105.21	107.41	88.28	89.06	90.87	93.73	112.79	104.02	99.60	95.74	112.14	112.08	103.15	88.54	92.21	103.93	99.52	97.04	102.08	102.61
	Linearity-2	102.49	106.16	91.37	92.15	92.60	95.48	113.98	105.16	100.46	96.57	96.84	113.14	104.72	92.74	94.08	100.18	101.60	93.79	101.23	105.29
	Linearity-3	100.47	105.21	102.43	106.20	88.94	93.32	94.47	97.42	110.57	107.57	94.85	98.37	98.26	114.55	103.89	94.28	92.93	98.70	98.98	98.54

2. Accuracy and Precision

Within-run accuracy and precision were evaluated by analysing six replicates of QCs (LLOQ, LQC, MQC and HQC) concentration levels in each analytical run. Between-run accuracy and precision were evaluated by analysing each QC concentration level in three analytical runs over three days and calculated by combining the data from all runs.

Table S3: Accuracy and Precision of Ceftriaxone.

Nominal Conc. (µg/mL)		Plasma Conc. (µg/mL)				DBS Conc. (µg/mL)			
		LLOQ	LQC	MQC	HQC	LLOQ	LQC	MQC	HQC
Within run accuracy and precision									
Batch 1 n=6	Accuracy (%)	99.22	106.75	105.91	103.81	NA			
	Precision (%CV)	6.38	6.05	6.99	7.43				
Batch 2 n=6	Accuracy (%)	87.43	99.76	106.85	105.36				
	Precision (%CV)	4.68	5.79	3.98	6.51				
Batch 3 n=6	Accuracy (%)	111.18	106.13	105.25	103.71				
	Precision (%CV)	2.47	4.35	4.05	3.15				
Between run accuracy and precision									
n=18	Accuracy (%)	99.28	104.21	106.00	104.29	NA			
	Precision (%CV)	11.19	5.99	4.92	5.68				

Table S4: Accuracy and Precision of Cefotaxime.

Nominal conc. (µg/mL)		Plasma conc. (µg/mL)				DBS conc. (µg/mL)			
		LLOQ	LQC	MQC	HQC	LLOQ	LQC	MQC	HQC
Within run accuracy and precision									
Batch 1 n=6	Accuracy (%)	102.75	107.29	103.84	104.22	NA			
	Precision (%CV)	8.15	6.02	4.10	1.72				
Batch 2 n=6	Accuracy (%)	116.50	103.14	110.35	106.60				
	Precision (%CV)	7.96	5.88	2.18	2.18				
Batch 3 n=6	Accuracy (%)	106.96	104.23	106.61	96.63				
	Precision (%CV)	3.70	3.25	2.59	5.74				
Between run accuracy and precision									
n=18	Accuracy (%)	109.09	104.89	106.93	102.48	NA			
	Precision (%CV)	8.41	5.20	3.83	5.41				

Table S5: Accuracy and Precision of Cefoperazone.

Nominal conc. (µg/mL)		Plasma conc. (µg/mL)				DBS conc. (µg/mL)			
		LLOQ	LQC	MQC	HQC	LLOQ	LQC	MQC	HQC
Within run accuracy and precision									
Batch 1 n=6	Accuracy (%)	98.02	104.55	99.41	102.97	NA			
	Precision (%CV)	11.17	6.06	5.64	2.19				
Batch 2 n=6	Accuracy (%)	94.99	94.79	102.32	100.03				
	Precision (%CV)	8.16	7.71	2.32	1.49				
Batch 3 n=6	Accuracy (%)	98.11	99.23	94.08	91.76				
	Precision (%CV)	5.60	6.84	2.61	5.20				
Between run accuracy and precision									
n=18	Accuracy (%)	96.98	99.52	98.60	98.26	NA			
	Precision (%CV)	8.02	7.64	5.07	5.82				

Table S6: Accuracy and Precision of Piperacillin.

Nominal conc. (µg/mL)		Plasma conc. (µg/mL)				DBS conc. (µg/mL)			
		LLOQ	LQC	MQC	HQC	LLOQ	LQC	MQC	HQC
Within run accuracy and precision									
Batch 1 n=6	Accuracy (%)	101.71	106.35	96.34	102.10	NA			
	Precision (%CV)	3.92	4.56	5.99	3.42				
Batch 2 n=6	Accuracy (%)	102.60	99.52	102.24	100.00				
	Precision (%CV)	2.46	5.07	4.06	3.08				
Batch 3 n=6	Accuracy (%)	94.40	93.66	88.13	93.23				
	Precision (%CV)	7.35	6.04	2.67	2.74				
Between run accuracy and precision									
n=18	Accuracy (%)	99.44	99.85	95.57	98.44	NA			
	Precision (%CV)	6.03	7.24	7.54	4.92				

Table S7: Accuracy and Precision of Sulbactam.

Nominal conc. (µg/mL)		Plasma conc. (µg/mL)				DBS conc. (µg/mL)			
		LLOQ	LQC	MQC	HQC	LLOQ	LQC	MQC	HQC
Within run accuracy and precision									
Batch 1 n=6	Accuracy (%)	92.60	106.37	108.28	108.36	NA			
	Precision (%CV)	7.60	5.43	3.25	3.89				
Batch 2 n=6	Accuracy (%)	96.92	98.34	109.57	101.37				
	Precision (%CV)	8.10	8.24	3.19	6.78				
Batch 3 n=6	Accuracy (%)	103.45	94.44	90.60	92.79				
	Precision (%CV)	5.37	8.59	2.92	4.32				
Between run accuracy and precision									
n=18	Accuracy (%)	97.95	99.72	102.82	100.84	NA			
	Precision (%CV)	8.04	8.66	9.15	8.10				

Table S8: Accuracy and Precision of Tazobactam.

Nominal conc. (µg/mL)		Plasma conc. (µg/mL)				DBS conc. (µg/mL)			
		LLOQ	LQC	MQC	HQC	LLOQ	LQC	MQC	HQC
Within run accuracy and precision									
Batch 1 n=6	Accuracy (%)	92.09	106.66	104.28	104.63	NA			
	Precision (%CV)	6.52	3.91	4.06	6.23				
Batch 2 n=6	Accuracy (%)	93.63	99.47	97.70	99.74				
	Precision (%CV)	7.15	4.84	7.19	8.72				
Batch 3 n=6	Accuracy (%)	103.82	104.74	98.59	96.04				
	Precision (%CV)	9.32	5.73	5.77	7.93				
Between run accuracy and precision									
n=18	Accuracy (%)	96.78	103.62	100.19	100.13	NA			
	Precision (%CV)	9.33	5.49	6.18	8.05				

Table S9: Accuracy and Precision of Ampicillin.

Nominal conc. (µg/mL)		Plasma conc. (µg/mL)				DBS conc. (µg/mL)			
		LLOQ	LQC	MQC	HQC	LLOQ	LQC	MQC	HQC
Within run accuracy and precision									
Batch 1 n=6	Accuracy (%)	97.66	99.37	100.38	99.17	NA			
	Precision (%CV)	5.38	2.70	4.42	2.79				
Batch 2 n=6	Accuracy (%)	96.94	94.92	98.42	104.45				
	Precision (%CV)	5.22	3.52	2.43	5.72				
Batch 3 n=6	Accuracy (%)	97.68	96.95	103.7	100.40				
	Precision (%CV)	6.95	3013	3.98	4.02				
Between run accuracy and precision									
n=18	Accuracy (%)	97.43	97.08	100.83	98.23	NA			
	Precision (%CV)	5.52	3.48	4.11	4.82				

Table S10: Accuracy and Precision of Meropenem.

Nominal conc. (µg/mL)		Plasma conc. (µg/mL)				DBS conc. (µg/mL)			
		LLOQ	LQC	MQC	HQC	LLOQ	LQC	MQC	HQC
Within run accuracy and precision									
Batch 1 n=6	Accuracy (%)	94.92	98.92	100.38	98.92	NA			
	Precision (%CV)	3.52	3.41	4.42	2.99				
Batch 2 n=6	Accuracy (%)	96.95	97.66	95.13	104.45				
	Precision (%CV)	3.13	5.51	6.28	2.29				
Batch 3 n=6	Accuracy (%)	97.68	96.94	103.70	100.31				
	Precision (%CV)	6.95	5.22	3.98	4.15				
Between run accuracy and precision									
n=18	Accuracy (%)	96.52	97.84	99.74	99.22	NA			
	Precision (%CV)	6.69	4.52	5.85	3.15				

Table S11: Accuracy and Precision of Cefuroxime.

Nominal conc. (µg/mL)		Plasma conc. (µg/mL)				DBS conc. (µg/mL)			
		LLOQ	LQC	MQC	HQC	LLOQ	LQC	MQC	HQC
Within run accuracy and precision									
Batch 1 n=6	Accuracy (%)	97.66	99.37	100.38	99.17	NA			
	Precision (%CV)	5.51	2.70	4.42	2.79				
Batch 2 n=6	Accuracy (%)	96.94	94.92	98.42	104.45				
	Precision (%CV)	5.22	3.52	2.43	5.72				
Batch 3 n=6	Accuracy (%)	97.68	96.95	102.18	100.82				
	Precision (%CV)	6.95	3.13	1.68	3.59				
Between run accuracy and precision									
n=18	Accuracy (%)	97.43	97.08	100.33	98.37	NA			
	Precision (%CV)	5.52	3.48	3.25	4.79				

3. Recovery of analytes from Plasma Matrix

Table S12: Recovery from plasma and DBS matrix.

Parameter	Plasma samples					DBS samples				
	LLOQ	LQC	MQC	HQC	Mean	LLOQ	LQC	MQC	HQC	Mean
Ceftriaxone (n=6)										
Recovery (%)	86.65	94.87	92.91	100.47	93.725	37.96952	47.95796	51.45948	60.97864	49.5914
%CV	3.9	4.58	2.44	2.01	6.08	20.12	11.45	18.45	8.56	19.16
Cefotaxime (n=6)										
Recovery (%)	90.31	89.19	79.75	89.68	87.2325	66.15372	68.43072	78.44952	77.16	72.54849
%CV	8.54	4.24	2.63	1.12	5.74	12.65	10.47	4.65	7.48	8.49
Cefoperazone (n=6)										
Recovery (%)	96.72	94.89	96.16	91.98	94.9375	62.475	49.10152	58.39775	65.71344	58.92193
%CV	8.95	3.51	2.53	2.31	2.23	14.65	7.98	11.1	10	12.22
Piperacillin (n=6)										
Recovery (%)	84.06	94.44	81.09	92.8	88.0975	76.42552	86.74792	81.68792	88.61072	83.36802
%CV	3.85	4.3	2.67	3.23	7.41	12.22	4.75	15.42	6.54	6.57
Sulbactam (n=6)										
Recovery (%)	90.42	93.05	92.44	94.55	92.615	56.18552	68.9266	68.30928	74.3212	66.93565
%CV	5.37	4.13	1.82	5.71	1.85	15.46	8.566	5.46	10.12	11.44
Tazobactam (n=6)										
Recovery (%)	82.87	99.6	95.29	94.5	93.065	58.62444	75.5552	71.19348	74.268	69.91028
%CV	3.73	1.91	2.63	5.01	7.69	10.89	12.13	6.312	5.73	11.08

Ampicillin										
Recovery (%)	93.14	97.01	96.566	94.15	95.2165	69.01768	72.93412	76.48479	73.8956	73.08305
%CV	2.34	6.55	4.42	3.464	1.96	9.637	7.164	6.79	4.89	4.24
Meropenem										
Recovery (%)	87.45	93.455	94.15	90.68	91.43375	63.2594	69.33646	70.0398	70.20352	68.2098
%CV	5.82	4.86	2.35	3.55	3.34	10	4.661	6.42	2.97	4.87
Cefuroxime										
Recovery (%)	94.56	97.45	92.45	93.166	94.4065	70.45472	73.3794	68.3194	72.84862	71.25054
%CV	6.45	2.18	3.45	5.42	2.34	4.656	5.75	2.45	4.98	3.27
Ceftiofur (n=24)										
Recovery (%)	93.88					72.611				
%CV	3.22					15.145				

4. Dilution integrity

The Dilution Integrity (DI) WS was prepared in such a way that 5% spiking into blank plasma resulted in two times of ULOQ of the respective analyte, as shown in Table 20. Two dilution factors, 1:3 and 1:4, in 5 replicate samples were performed, and the accuracy and precision of analytes from nominal concentration was evaluated. All of the dilution integrity samples of all analytes resulted in accuracy within ±15% of nominal concentration and precision below 15% CV.

Table S13: Accuracy and Precision of Dilution Integrity Standards.

Analyte	Concentration (µg/mL)	Dilution 1:3		Dilution 1:4	
		% Accuracy	%CV	% Accuracy	%CV
Ceftriaxone	400.00	103.17	2.05	108.47	4.33
Cefuroxime	400.00	92.54	5.04	104.8	5.98
Cefoperazone	400.00	106.36	3.55	110.81	2.37
Piperacillin	200.00	97.19	4.18	104.10	4.25
Cefotaxime	200.00	108.23	4.11	111.08	2.91
Sulbactam	80.00	101.87	3.06	107.66	4.01
Tazobactam	80.00	102.83	2.57	100.22	6.89
Ampicillin	200.00	93.26	4.65	90.78	5.35
Meropenem	200.00	89.54	3.49	96.80	3.97

5. Short-term Stability study of analytes in plasma matrix

Table S14: Short-term Stability study of analytes in plasma matrix

Analyte/ Condition	Level	Ceftriaxone		Cefotaxime		Cefoperazone		Piperacillin		Sulbactam		Tazobactam		Ampicillin		Meropenem		Cefuroxime		Ceftiofur (IS)	
		%Assay	SD	%Assay	SD	%Assay	SD	%Assay	SD	%Assay	SD	%Assay	SD	%Assay	SD	%Assay	SD	%Assay	SD	%Assay	SD
Standard Stock Solution		99.38	2.23	100.05	3.66	99.54	3.48	99.50	3.32	98.79	1.79	100.03	4.06	99.44	3.16	99.30	2.27	98.89	1.68	100.16	4.21
Working Standard Solution (2-8°C, 30 Day)	LQC	99.68	2.72	99.45	3.47	97.82	2.73	98.85	2.41	97.37	2.08	98.61	3.46	97.99	2.04	98.57	1.92	97.45	2.37	99.61	1.86
	HQC	99.56	2.04	98.15	2.88	98.01	3.13	99.21	1.71	97.11	2.22	98.76	3.27	98.48	2.35	98.77	4.26	97.89	2.22		
Bentch-top (room temperature, 24h)	LQC	99.31	3.82	98.92	2.08	98.14	2.22	99.47	3.61	98.00	1.70	98.50	2.60	96.57	1.84	94.68	2.13	98.67	2.32	NA	
	HQC	98.19	2.43	98.06	3.55	99.03	3.03	97.75	2.28	96.86	2.60	97.07	3.02	98.49	3.88	96.58	2.98	98.55	3.06		
Autosampler (10°C, 36h)	LQC	97.12	2.33	98.42	1.89	97.14	2.63	98.62	1.74	98.18	2.15	96.55	3.54	95.58	3.37	95.43	4.83	97.60	3.25	99.55	4.58
	HQC	98.79	1.84	98.90	3.27	98.37	3.94	99.37	3.70	97.74	1.61	97.17	4.26	97.65	3.59	94.95	4.29	98.36	1.78		
Processed-sample (2-8°C, 72 h)	LQC	97.68	3.98	97.47	3.96	96.87	3.54	99.96	3.60	96.42	2.87	95.97	3.12	96.45	2.96	94.65	3.22	97.33	3.03	98.87	3.72
	HQC	97.32	1.94	96.43	3.37	98.68	3.23	95.13	3.79	99.02	2.69	99.73	2.08	94.60	1.63	96.80	5.03	98.78	1.55		
Freez-thaw (-70°C, 3 cycles)	LQC	96.56	3.30	94.55	2.87	96.91	3.33	97.72	2.84	95.64	2.22	97.54	3.95	93.78	3.57	94.45	5.40	96.40	4.05	NA	
	HQC	96.77	2.52	96.46	2.97	98.04	2.12	96.26	3.34	94.96	2.06	99.53	1.66	95.45	4.18	93.85	2.14	95.54	2.47		

NA: Not applicable

6. Long-term Stability study of analytes in plasma matrix at -70°C

Table S15: Long-term Stability study of analytes in plasma matrix at -70°C

Analyte	Piperacillin		Tazobactam		Cefoperazone		Sulbactam		Cefotaxime		Ceftriaxone		Ampicillin		Meropenem		Cefuroxime	
Time (Days)	%Change	SD	%Change	SD	%Change	SD	%Change	SD	%Change	SD	%Change	SD	%Change	SD	%Change	SD	%Change	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	-0.41	0.062	-0.27	0.012	-0.16	0.064	-0.67	0.12	-0.23	0.04755	-0.46	0.098	-0.35	0.0945	-0.27	0.1032	-0.46	0.101
7	-0.46	0.0904	-0.4	0.098	-0.24	0.0984	-1.2	0.204	-0.3	0.0945	-0.51	0.114	-0.75	0.221	-0.83	0.069	-0.93	0.165
15	-0.96	0.1405	-0.78	0.1054	-1.05	0.45	-1.67	0.3478	-0.56	0.1078	-0.72	0.1246	-1.81	0.245	-1.98	0.132	-1.37	0.245
30	-1.36	0.26	-1.9	0.1645	-1.65	0.224	-3.01	0.1425	-1.68	0.1704	-2.64	0.245	-2.86	0.142	-2.556	0.32	-2.78	0.148
60	-3.86	0.14	-4.06	0.1274	-3.4	0.2054	-5.98	0.3664	-3.34	0.324	-3.93	0.2954	-4.06	0.414	-5.91	0.2794	-4.5	0.09