

Supplementary Information (SI)

Table S1. Sampling sites and times

Sample ID	Sampling site	Coordinates	Sampling time
S1	Obstetrics Department, Trung Khanh General Hospital Address: Group 9, Trung Khanh Town, Cao Bang	22°50'19"N 106°31'37"E	23/08/2024, 07:00– 09:00
S2	Pediatrics Department, Trung Khanh General Hospital Address: Group 9, Trung Khanh Town, Cao Bang	22°50'19"N 106°31'35"E	
S3	Tra Linh General Hospital Address: Cao Chuong, Trung Khanh, Cao Bang	22°48'26"N 106°19'51"E	
S4	Fish pond Address: Po Tau, Chi Vien, Trung Khanh, Cao Bang	22°49'04"N 106°37'07"E	
S5	Fish pond Address: Ban Da, Trung Khanh, Cao Bang	22°49'45"N 106°32'17"E	
S6	Quang Uyen General Hospital Address: Hoa Nam Street, Quang Uyen Town, Quang Hoa,	22°41'19"N 106°26'44"E	

	Cao Bang		
S7	Fish pond Address: Bang Hamlet, Duc Ly Commune, Ly Nhan District, Ha Nam	20°33'50"N 106°03'40"E	06/09/2024, 07:00– 09:00
S8	Fish pond Address: Duc Ly Commune, Ly Nhan District, Ha Nam	20°33'47"N 106°03'36"E	
S9	Poultry pond Address: Do Hamlet, Bac Ly, Ly Nhan, Ha Nam	20°33'42"N 106°04'35"E	
S10	Poultry pond Address: Yen Trach, Bac Ly, Ly Nhan, Ha Nam	20°33'34"N 106°04'24"E	
S11	Ly Nhan District General Hospital, Ha Nam Address: Vinh Tru Town, Ly Nhan, Ha Nam	20°34'06"N 106°01'48"E	
S12	Ha Nam Provincial General Hospital Address: Phu Ly City, Ha Nam	20°32'27"N 105°54'59"E	
S13	Pediatrics Department, Cao Bang Provincial General Hospital Address: Km2,	22°39'11"N 106°16'11"E	11/09/2024, 07:00– 09:00

	Dong Khe Road, Tan Giang Ward, Cao Bang City, Cao Bang		
S14	Endocrinology Department, Cao Bang Provincial General Hospital Address: Song Bang Ward, Cao Bang City	22°39'26"N 106°15'54"E	
S15	Cao Bang Traditional Medicine Hospital Address: Tan Giang Ward, Cao Bang City	22°39'23"N 106°15'46"E	

Table S2. Intra-day and Inter-day Repeatability (Single Concentration)

Analyte	Concentration (ppm)	Intra-day %RSD (n = 5)	Inter-day %RSD (n = 5)
Erythromycin	5	1.2	1.5
Clarithromycin	7	1.1	1.3
Azithromycin	10	1.3	1.4

Table S3. Effect of Deliberate Variations on Chromatographic Performance

Condition	Erythromycin [tR / N]	Clarithromycin [tR / N]	Azithromycin [tR / N]	Tf (E / C / A)	Rs (E–C / C–A)	%RSD Peak Area (E / C / A)
Nominal (Flow = 0.8 mL/min; pH = 6.0; Temp = 35°C)	[2.77 / 2120]	[3.34 / 2570]	[4.119 / 2840]	1.06 / 1.07 / 1.09	2.05 / 2.20	0.22 / 0.19 / 0.27
Flow = nominal + 0.1 mL/min	[2.76 / 2110]	[3.31 / 2550]	[4.118 / 2810]	1.05 / 1.06 / 1.08	2.00 / 2.15	0.22 / 0.19 / 0.27
Flow = nominal – 0.1 mL/min	[2.78 / 2130]	[3.36 / 2600]	[4.120 / 2890]	1.07 / 1.08 / 1.10	2.08 / 2.25	0.22 / 0.19 / 0.27
Column temp = nominal + 2 °C	[2.76 / 2100]	[3.33 / 2560]	[4.117 / 2760]	1.05 / 1.06 / 1.07	2.02 / 2.18	0.22 / 0.19 / 0.27
Column temp = nominal – 2 °C	[2.78 / 2140]	[3.35 / 2620]	[4.121 / 2840]	1.07 / 1.08 / 1.09	2.06 / 2.22	0.22 / 0.19 / 0.27
Mobile phase pH = nominal + 0.2	[2.79 / 2150]	[3.37 / 2650]	[4.119 / 2840]	1.08 / 1.09 / 1.10	2.10 / 2.28	0.23 / 0.19 / 0.27
Mobile phase pH = nominal – 0.2	[2.75 / 2080]	[3.31 / 2490]	[4.117 / 2760]	1.05 / 1.06 / 1.07	1.98 / 2.12	0.21 0.19 / 0.26

Note: E = erythromycin, A = azithromycin, C = clarithromycin (or your analyte names E, A, C as used in manuscript); %RSD (Area) calculated from 6 replicate injections under each condition; Rs = resolution; Tf = tailing factor; N = theoretical plate.

Table S4. Repeatability of Erythromycin, Clarithromycin, and Azithromycin Quantification in Real Samples (n = 5)

Sample	Number of injection	ERYTH		CLARITH		AZITH	
		Peak area (mAu)	Concentration (ppm)	Peak area (mAu)	Concentration (ppm)	Peak area (mAu)	Concentration (ppm)
S1	1	13421	11.273	802	1.014	787	1.007
	2	13421	11.273	804	1.016	787	1.007
	3	13421	11.273	805	1.017	789	1.008
	4	13421	11.273	809	1.020	892	1.093
	5	13421	11.273	801	1.013	781	1.002
	SD	0		3.114		47.5	
	RSD(%)	0		0.387 (%)		5.884 (%)	
S2	1	1097	1.277	879	1.077	791	1.010
	2	1097	1.277	871	1.070	793	1.012
	3	1092	1.273	875	1.073	791	1.010
	4	1093	1.274	873	1.072	792	1.011
	5	1095	1.276	873	1.072	794	1.012
	SD	2.28		3.033		1.304	
	RSD(%)	0.208 (%)		0.347 (%)		0.165 (%)	
S3	1	9019	7.703	1025	1.195	908	1.106
	2	9012	7.697	1023	1.193	904	1.103
	3	9013	7.698	1034	1.202	904	1.103
	4	9019	7.703	1029	1.198	903	1.102
	5	9019	7.703	1029	1.198	902	1.102
	SD	3.58		4.243		2.28	
	RSD(%)	0.04 (%)		0.413 (%)		0.252 (%)	
S4	1	2135	2.119	910	1.102	-	-
	2	2132	2.117	910	1.102	-	-
	3	2131	2.116	910	1.102	-	-
	4	2133	2.117	910	1.102	-	-
	5	2135	2.119	910	1.102	-	-
	SD	1.789		0		-	
	RSD(%)	0.084 (%)		0		-	
S5	1	-	-	-	-	4808	4.323
	2	-	-	-	-	4801	4.317
	3	-	-	-	-	4807	4.322
	4	-	-	-	-	4806	4.321

Sample	Number of injection	ERYTH		CLARITH		AZITH	
		Peak area (mAu)	Concentration (ppm)	Peak area (mAu)	Concentration (ppm)	Peak area (mAu)	Concentration (ppm)
	5	-	-	-	-	4808	4.323
	SD	-		-		2.915	
	RSD(%)	-		-		0.06 (%)	
S6	1	3215	2.995	1123	1.274	1507	1.600
	2	3214	2.994	1120	1.272	1501	1.595
	3	3213	2.993	1124	1.275	1503	1.597
	4	3214	2.994	1125	1.276	1505	1.599
	5	3213	2.993	1126	1.277	1505	1.599
	SD	0.837		2.302		2.28	
	RSD(%)	0.026 (%)		0.205 (%)		0.152 (%)	
S7	1	-	-	7091	6.111	-	-
	2	-	-	7099	6.118	-	-
	3	-	-	7096	6.115	-	-
	4	-	-	7095	6.115	-	-
	5	-	-	7093	6.113	-	-
	SD	-		3.033		-	
	RSD(%)	-		0.043 (%)		-	
S8	1	-	-	5897	5.144	-	-
	2	-	-	5893	5.140	-	-
	3	-	-	5894	5.141	-	-
	4	-	-	5895	5.142	-	-
	5	-	-	5897	5.144	-	-
	SD	-		1.789		-	
	RSD(%)	-		0.03 (%)		-	
S9	1	-	-	790	1.004	1246	1.385
	2	-	-	793	1.007	1241	1.381
	3	-	-	791	1.005	1241	1.381
	4	-	-	794	1.008	1243	1.383
	5	-	-	791	1.005	1245	1.384
	SD	-		1.643		2.28	
	RSD(%)	-		0.208 (%)		0.183 (%)	

Sample	Number of injection	ERYTH		CLARITH		AZITH	
		Peak area (mAu)	Concentration (ppm)	Peak area (mAu)	Concentration (ppm)	Peak area (mAu)	Concentration (ppm)
S10	1	-	-	902	1.095	1309	1.437
	2	-	-	903	1.096	1302	1.431
	3	-	-	904	1.097	1305	1.434
	4	-	-	905	1.098	1309	1.437
	5	-	-	902	1.095	1309	1.437
	SD	-		1.304		3.194	
	RSD(%)	-		0.144 (%)		0.244 (%)	
S11	1	3573	3.285	5027	4.439	8290	7.194
	2	3576	3.280	5023	4.435	8293	7.197
	3	3576	3.280	5025	4.437	8295	7.198
	4	3577	3.289	5027	4.439	8291	7.195
	5	3572	3.285	5029	4.440	8295	7.198
	SD	2.168		2.28		2.28	
	RSD(%)	0.06 (%)		0.045 (%)		0.183 (%)	
S12	1	5019	4.458	16034	13.360	9018	7.795
	2	5019	4.458	16038	13.363	9018	7.795
	3	5019	4.458	16034	13.360	9018	7.795
	4	5019	4.458	16035	13.360	9018	7.795
	5	5019	4.458	16032	13.358	9018	7.795
	SD	0		2.191		0	
	RSD(%)	0		0.014 (%)		0	
S13	1	1246	1.398	7890	6.759	4312	3.914
	2	1246	1.398	7892	6.761	4312	3.914
	3	1246	1.398	7896	6.764	4312	3.914
	4	1246	1.398	7895	6.763	4312	3.914
	5	1246	1.398	7896	6.764	4312	3.914
	SD	0		2.683		0	
	RSD(%)	0		0.034 (%)		0	
S14	1	3421	3.162	1432	1.525	8907	7.703
	2	3425	3.165	1436	1.528	8906	7.702
	3	3424	3.165	1435	1.527	8904	7.701
	4	3422	3.163	1437	1.529	8905	7.701
	5	3424	3.165	1435	1.527	8909	7.705
	SD	1.643		1.871		1.924	

Sample	Number of injection	ERYTH		CLARITH		AZITH	
		Peak area (mAu)	Concentration (ppm)	Peak area (mAu)	Concentration (ppm)	Peak area (mAu)	Concentration (ppm)
	RSD(%)	0.048 (%)		0.13 (%)		0.022 (%)	
S15	1	-	-	3210	2.966	2019	2.023
	2	-	-	3210	2.966	2016	2.020
	3	-	-	3210	2.966	2017	2.021
	4	-	-	3210	2.966	2012	2.017
	5	-	-	3210	2.966	2017	2.021
	SD	-		0		2.588	
	RSD(%)	-		0		0.128 (%)	

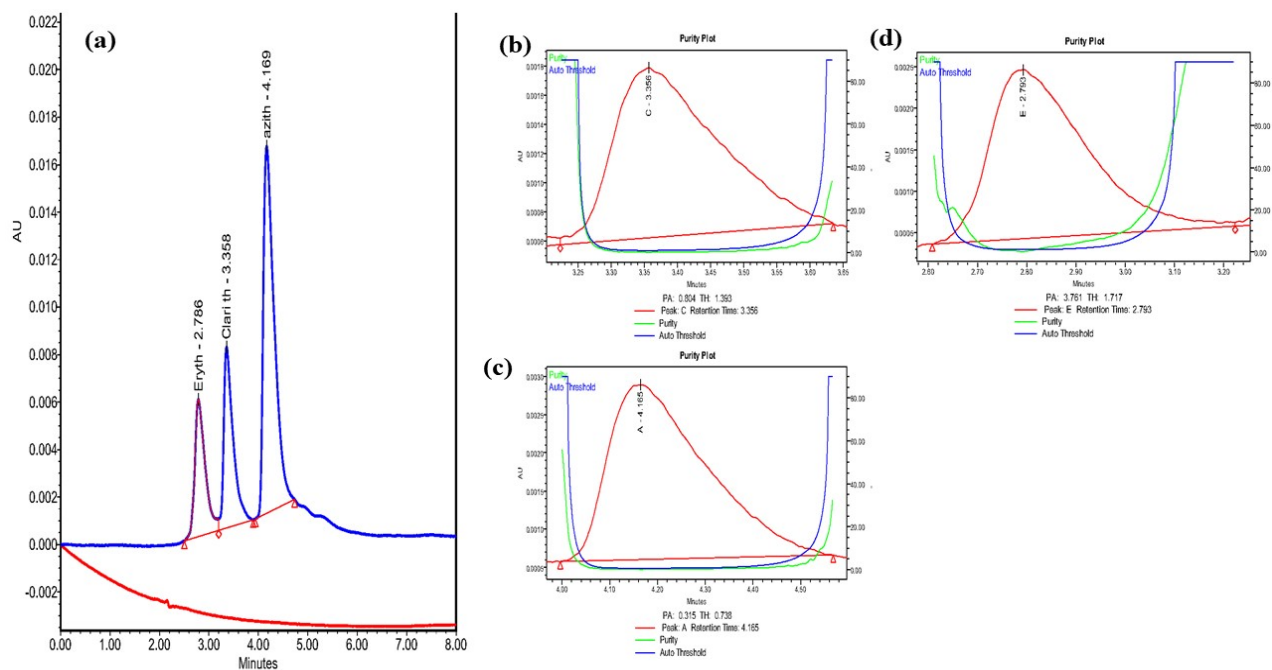


Figure S1. Selectivity (Specificity) assessment of the developed HPLC-PDA method. (a) Chromatogram of standard mixture of erythromycin (Eryth), clarithromycin (Clarith), and azithromycin (arith) at working concentrations, showing baseline separation with $R_s > 1.5$ between adjacent peaks (the blue line) and the chromatogram of blank (the red line). Purity plot of Peak purity of (b) clarithromycin (Clarith), (c) azithromycin (Arith) and (d) erythromycin (Eryth). Assessment using PDA detector confirmed spectral homogeneity of each analyte peak.