

Supporting Information

J. Chem. Metrol. 15:2 (2021) 163-171

Development of a quick reverse phase liquid chromatographic method with photodiode-array detection for quantitative determination of chlorthalidone, metoprolol succinate and telmisartan in tablet formulation

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Method Development Trials

Trial 1

Mobile Phase: Acetonitrile: Phosphate buffer (pH = 5.5)

Ratio: (50: 50 v/v)

Flow rate: 1 mL/min

Observation: Poor resolution, poor reproducibility, merging of peak

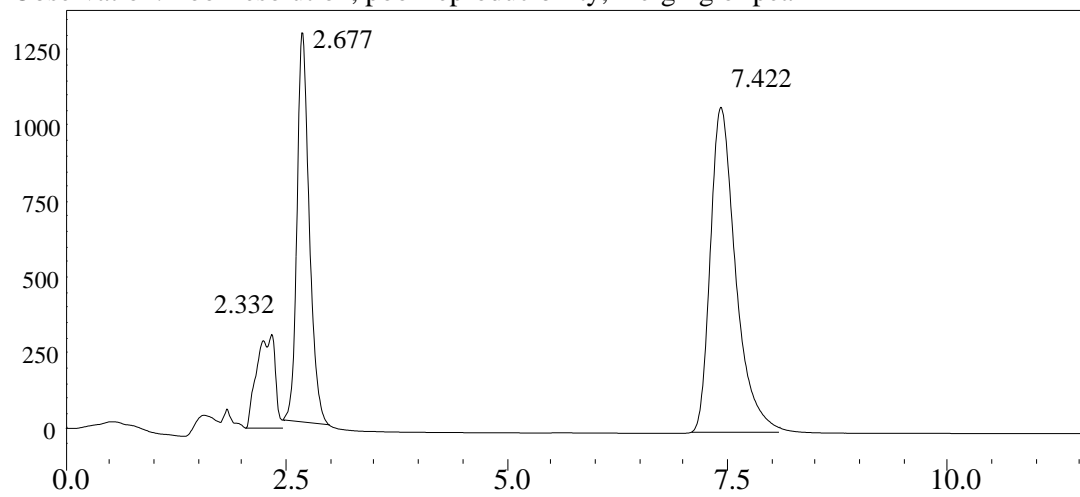


Figure S1: Trial 1 HPLC chromatogram

Trial 2

Mobile Phase: Acetonitrile: Phosphate buffer (pH = 4.0)

Ratio: (40: 60 v/v)

Flow rate: 1 mL/min

Observation: Broad peak, poor reproducibility.

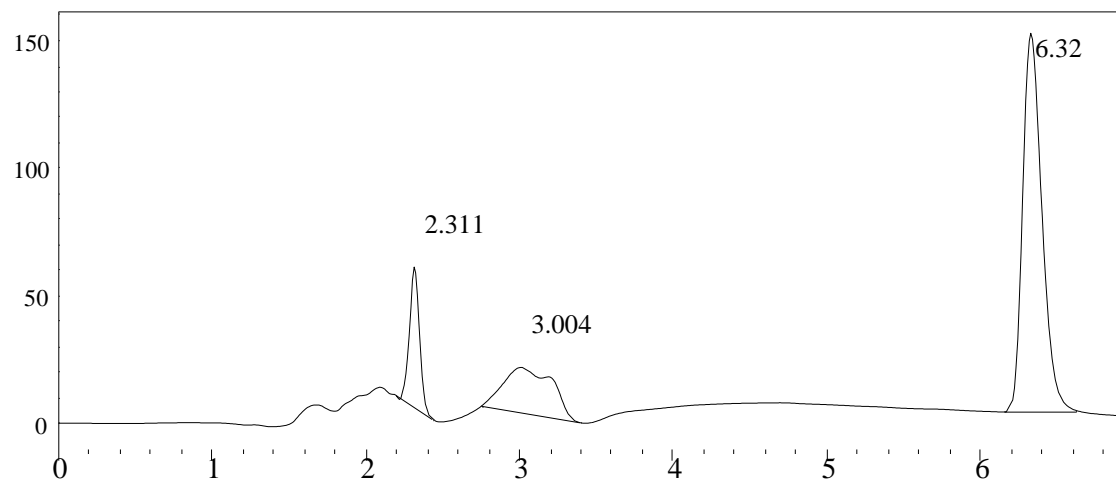


Figure S2: Trial 2 HPLC chromatogram

Trial 3

Mobile Phase: Acetonitrile: Phosphate buffer (pH = 3.0): Methanol

Ratio: (30: 50: 20 v/v)

Flow rate: 1 mL/min

Observation: Merging of peak

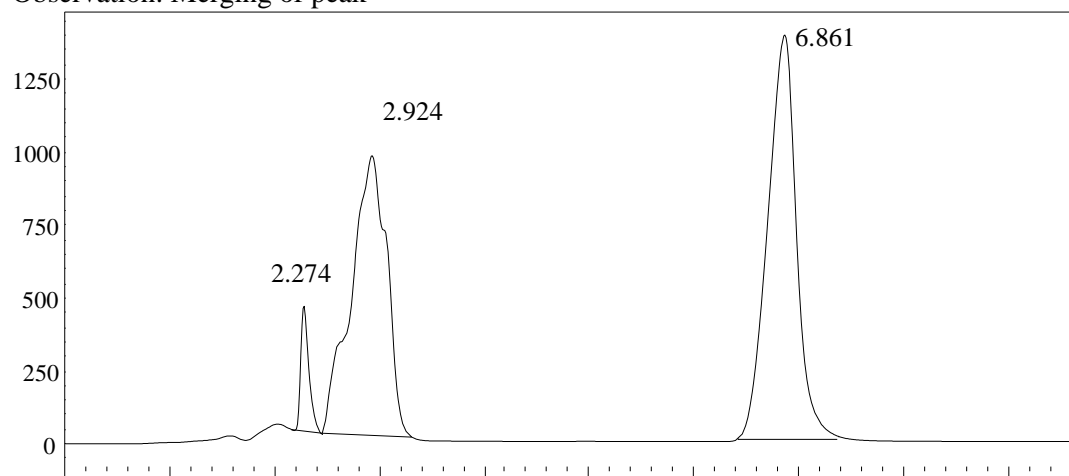


Figure S3: Trial 3 HPLC chromatogram

Trial 4

Mobile Phase: Acetonitrile:
O-phosphoric acid buffer (pH = 2.3)
Ratio: (50: 50 v/v)
Flow rate: 1 mL/min Observation:
No peak separation

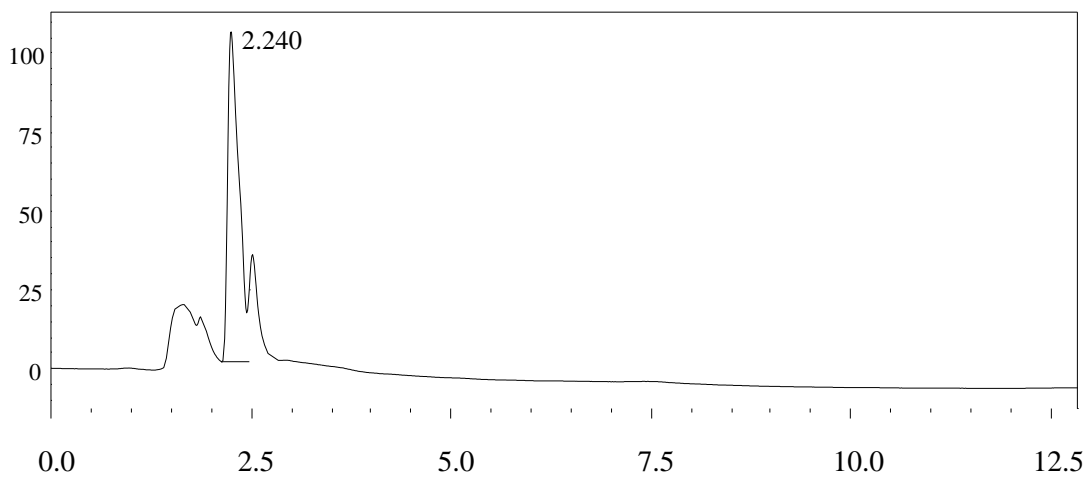


Figure S4: Trial 4 HPLC chromatogram

Trial 5

Mobile Phase: Acetonitrile: Phosphate buffer (1.0 mM) (pH = 2.5)

Ratio: (40: 60 v/v)

Flow rate: 1 mL/min

Observation: Less theoretical plates in CHL & MET, capacity of buffer affected the peak properties

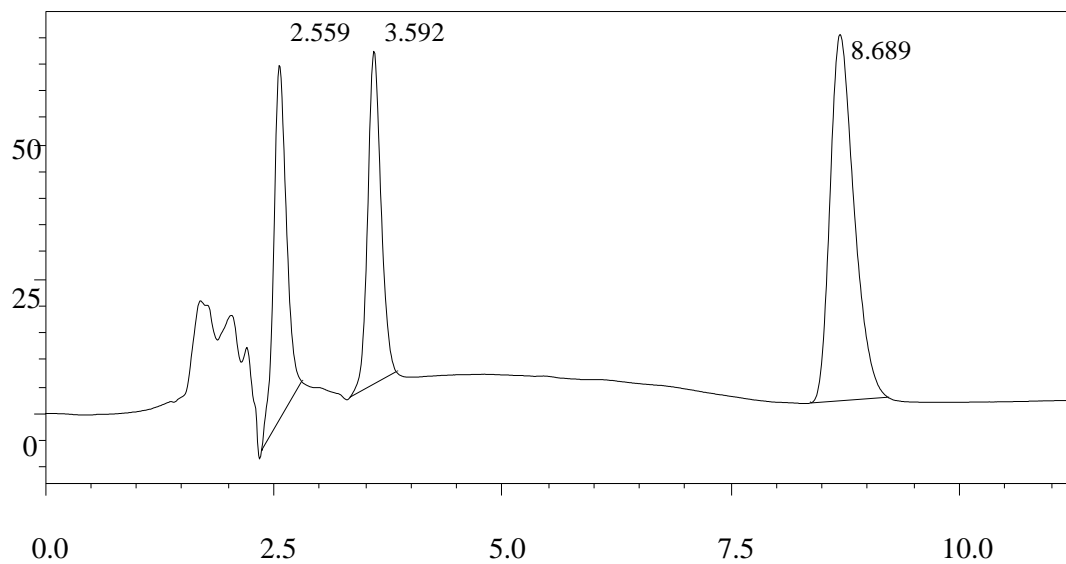


Figure S5: Trial 5 HPLC chromatogram

Mobile Phase: Acetonitrile: Phosphate buffer (0.005 mM) (pH = 2.5)

Ratio: (50: 50 v/v)

Flow rate: 0.7 mL/min

Observation: Satisfactory peak separated which fulfils the system suitability parameters.

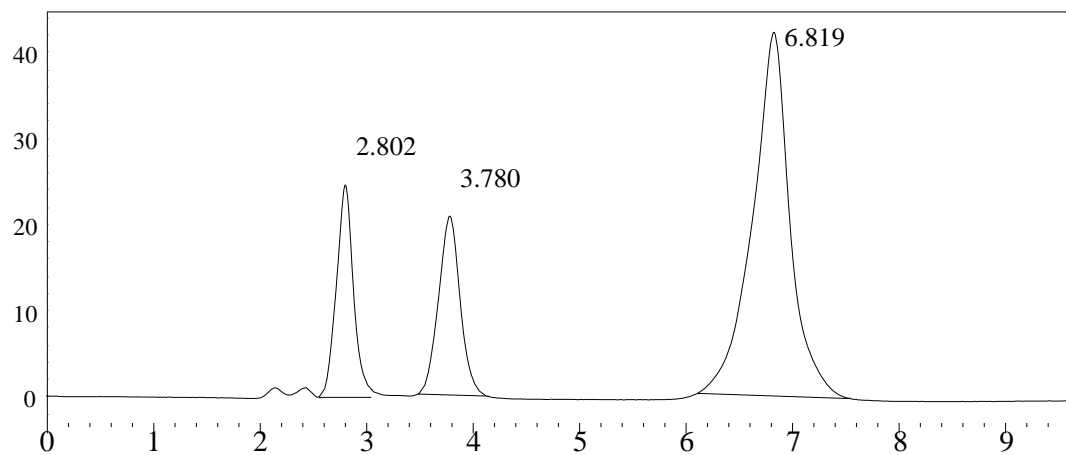


Figure S6: Optimized chromatographic condition

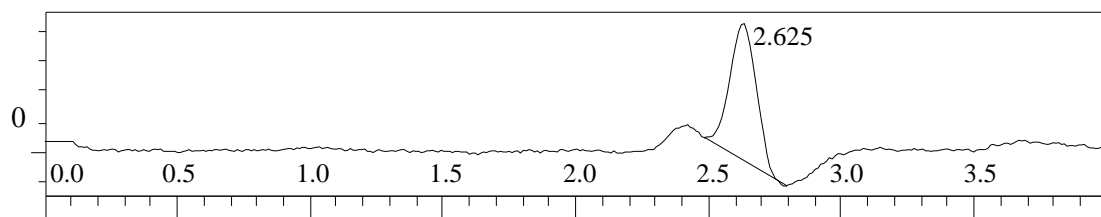


Figure S7: Interference of blank

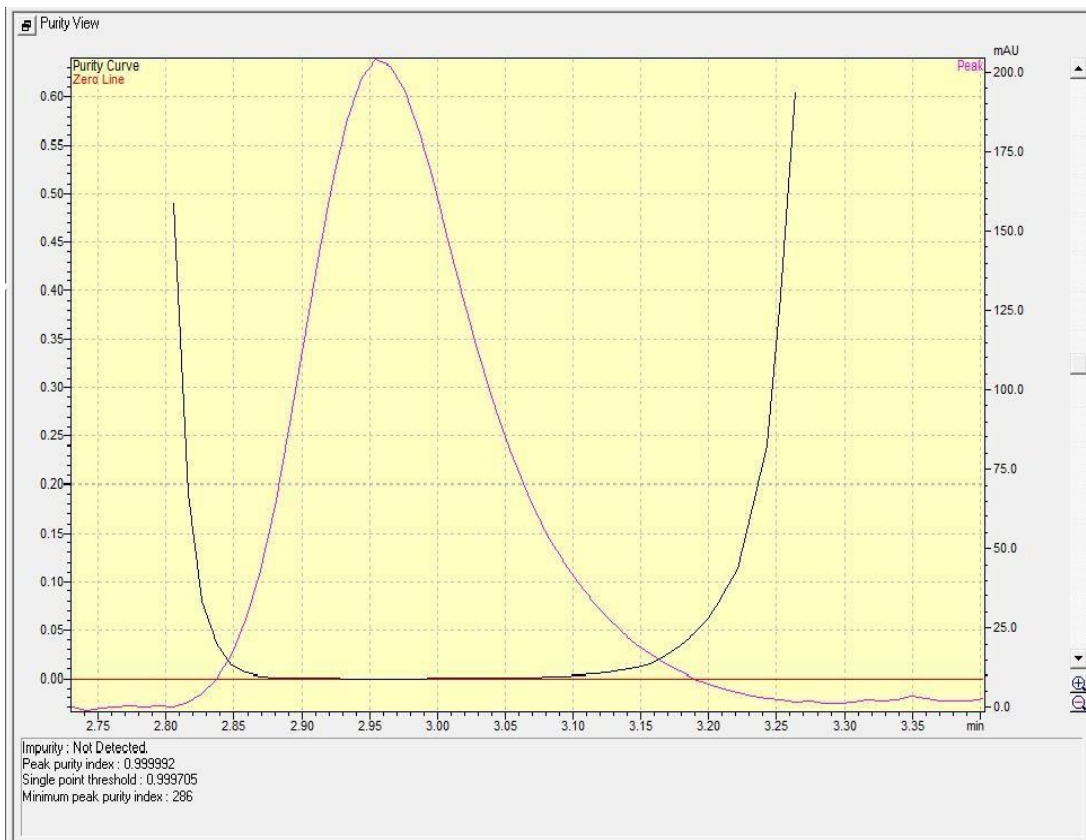


Figure S8: Peak purity index of CHL

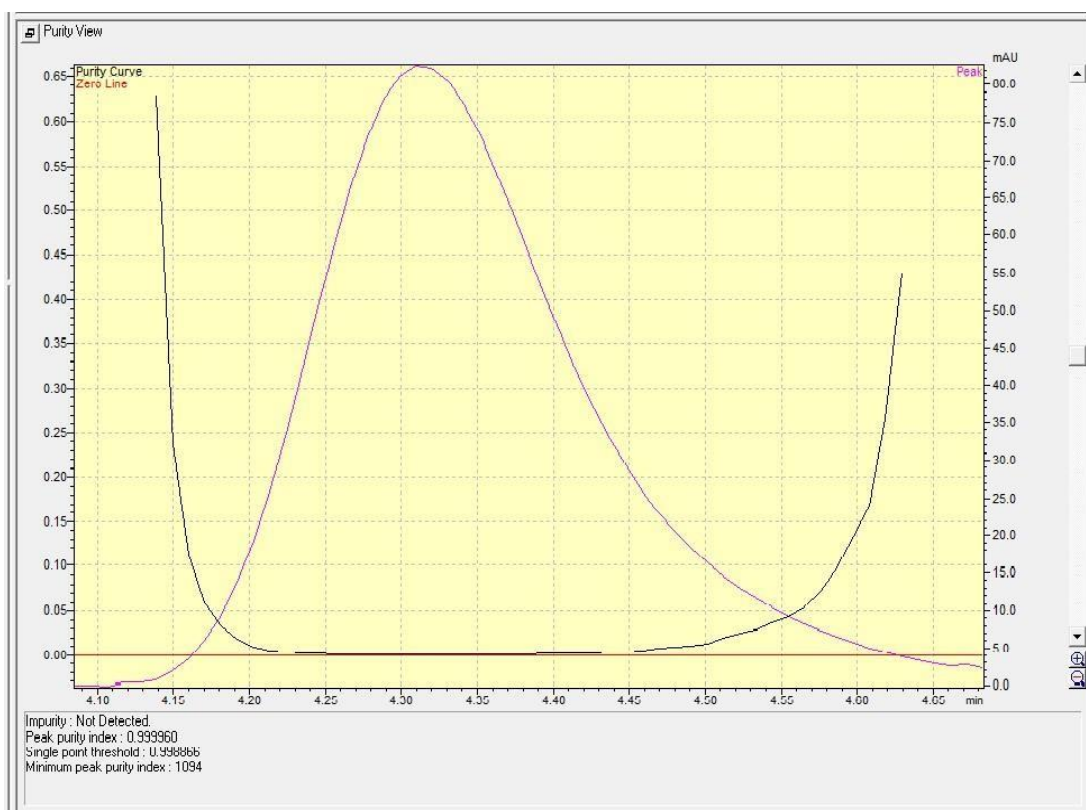


Figure S9: Peak purity index of MET

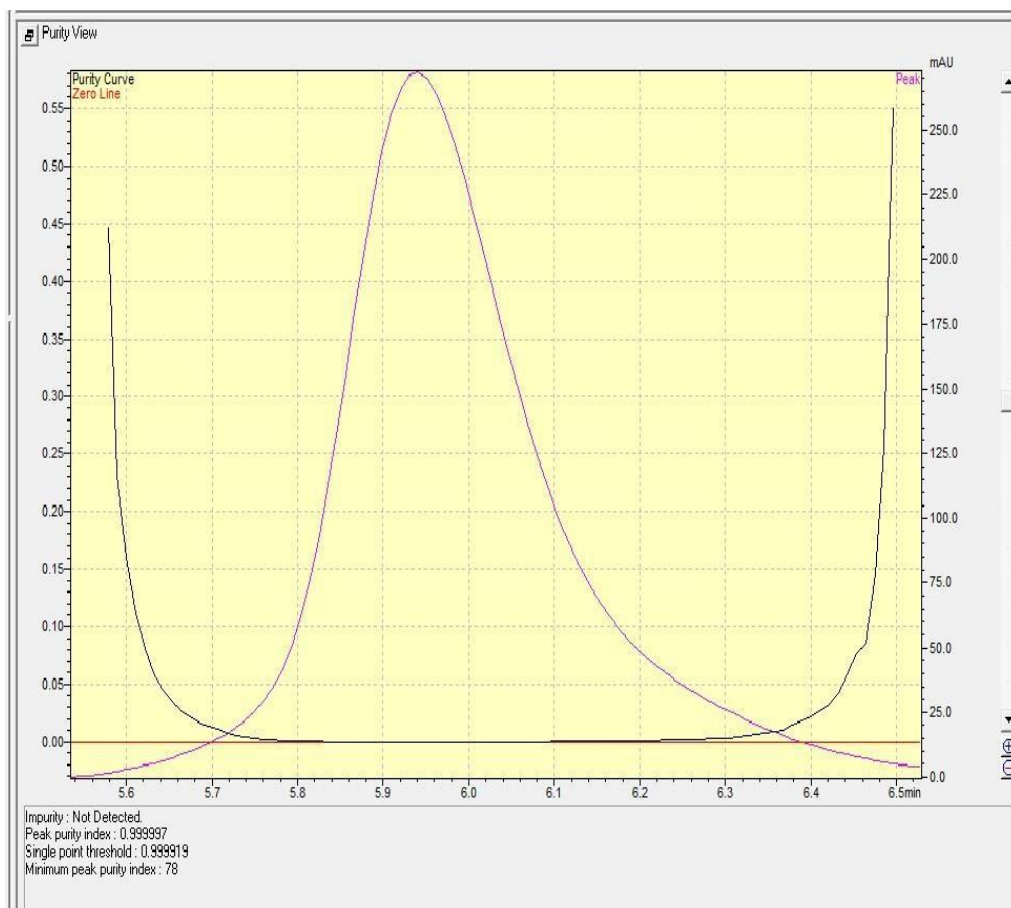


Figure S10: Peak purity index of TEL

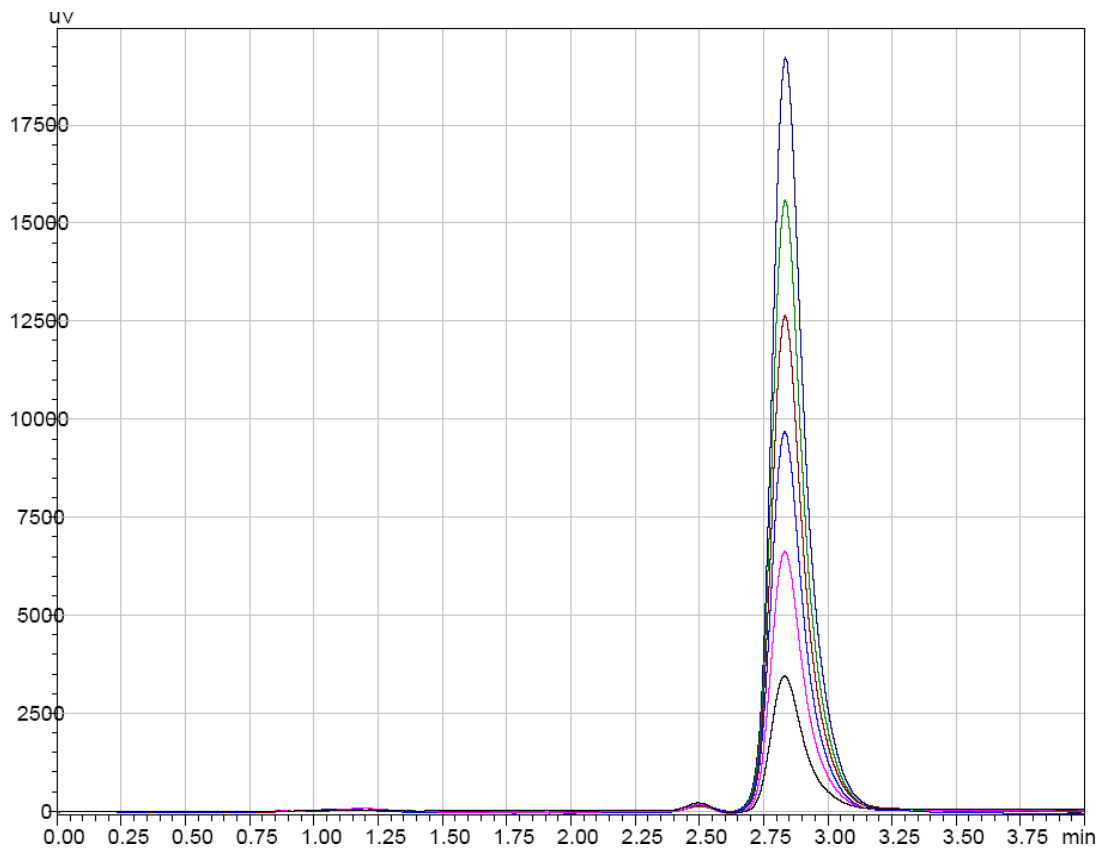


Figure S11: Overlay chromatograms of CHL (3.21-18.72) $\mu\text{g/mL}$

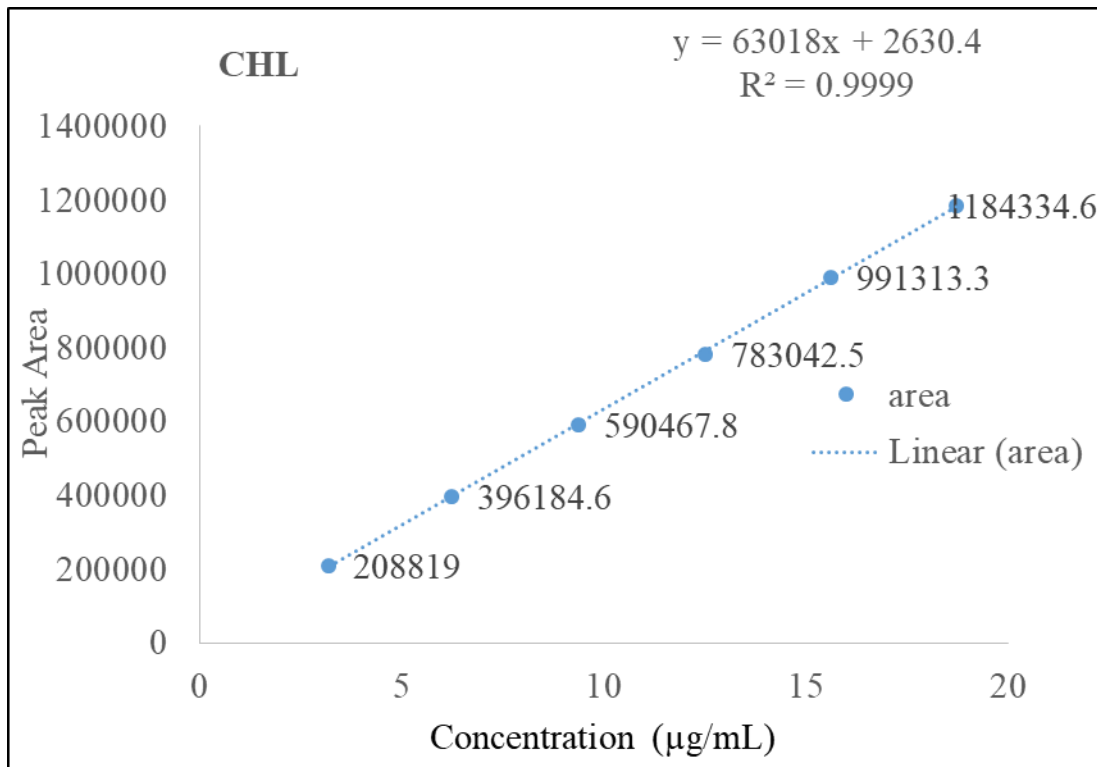


Figure S12: Calibration curve of CHL (3.21-18.72) µg/mL

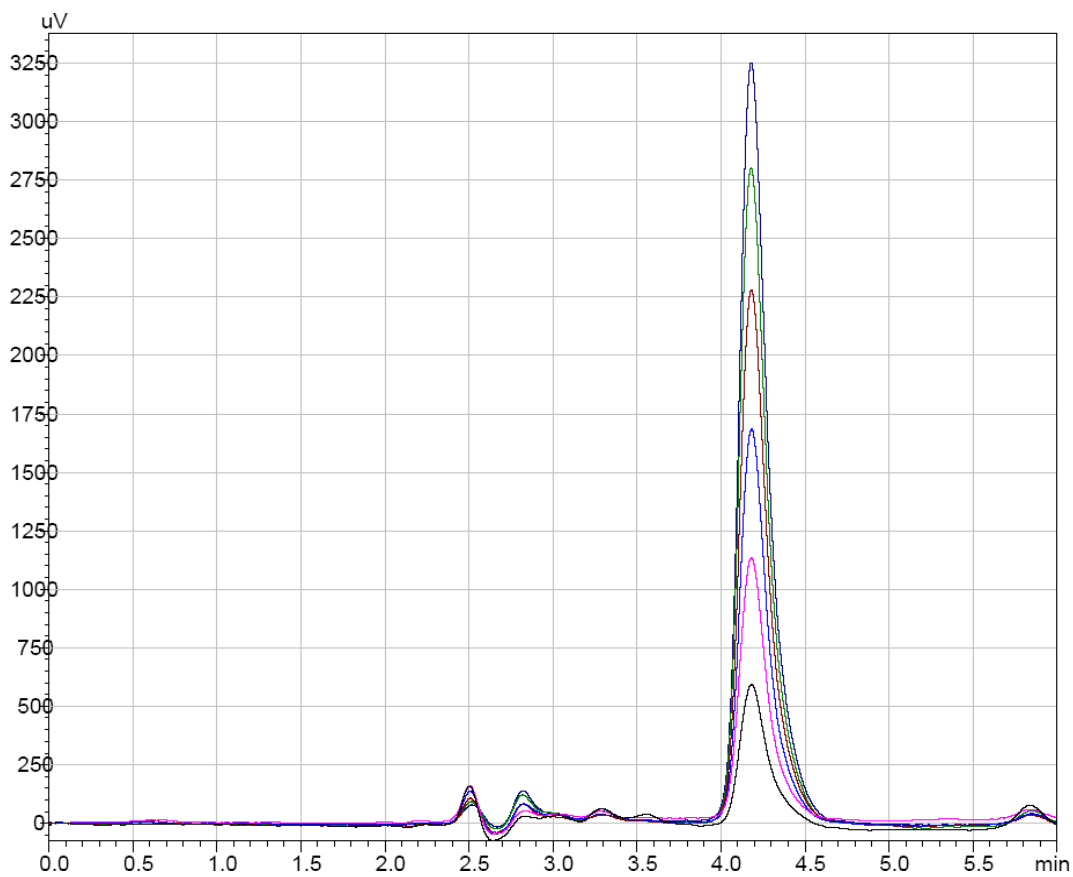


Figure S13: Overlay chromatograms of MET (6.25-37.50) µg/mL

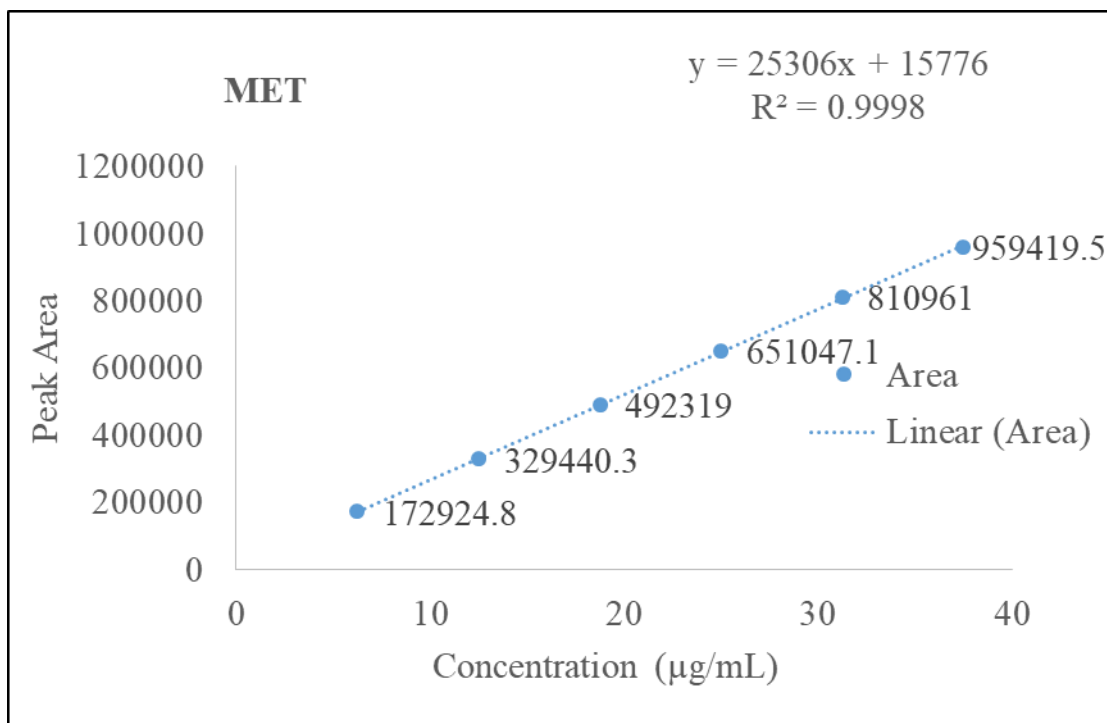


Figure S14: Calibration curve of MET (6.25-37.50) µg/mL

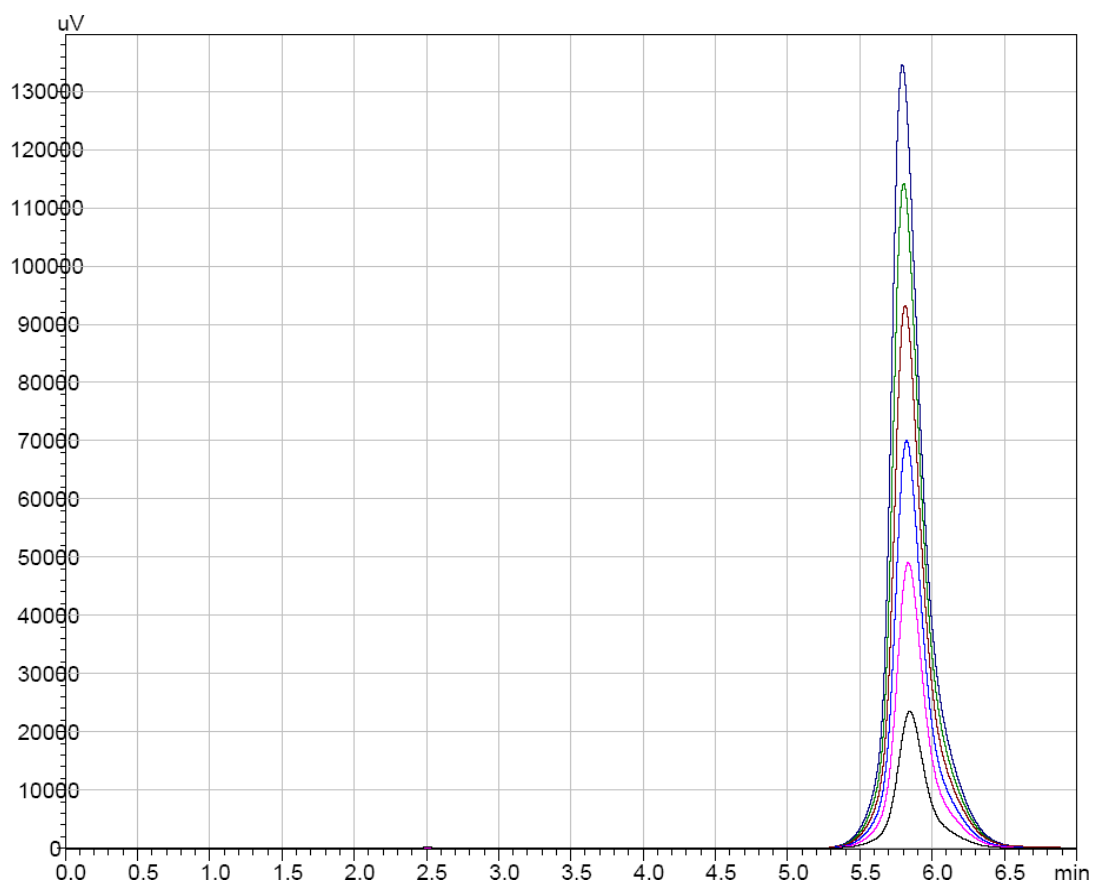


Figure S15: Overlay chromatograms of TEL (10-60) $\mu\text{g/mL}$

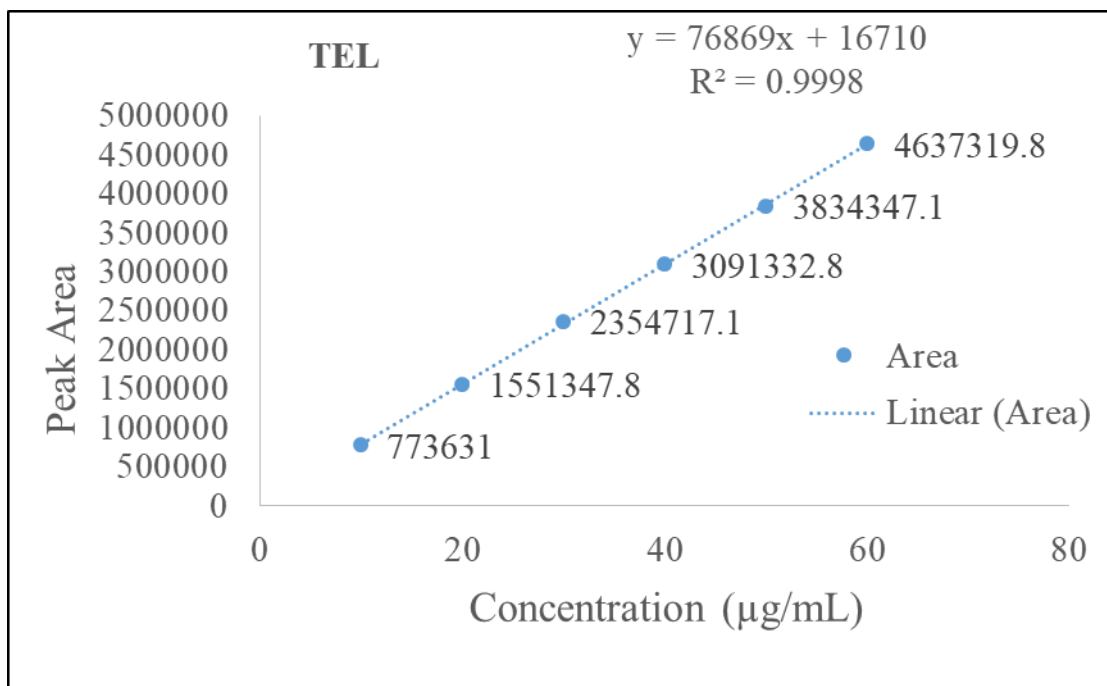


Figure S16: Calibration curve of TEL (10-60) µg/mL

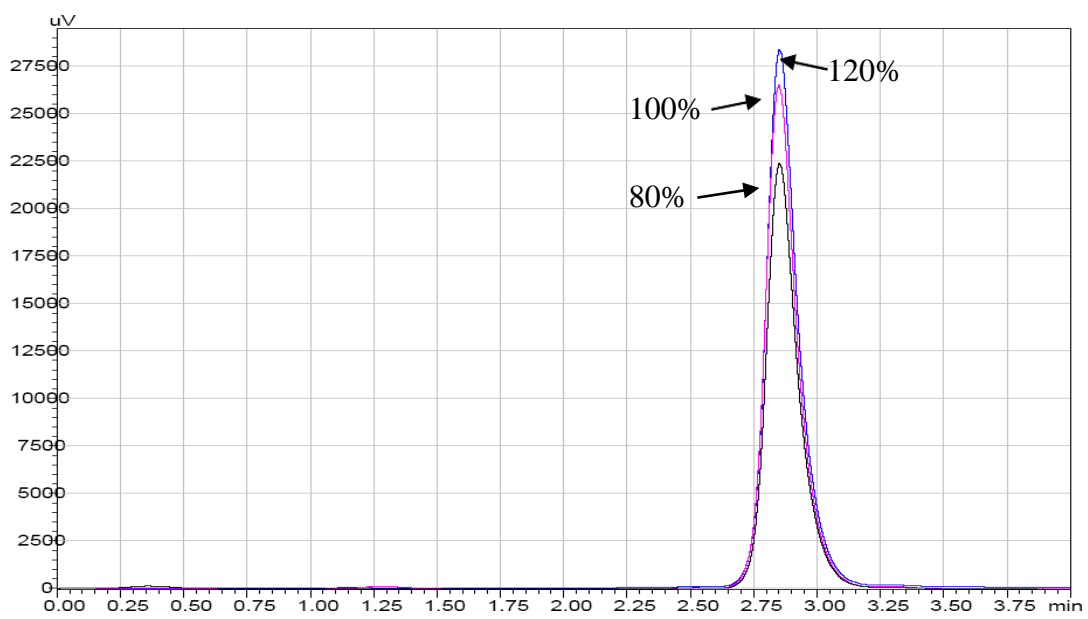


Figure S17: Overlay chromatogram of Accuracy (CHL)

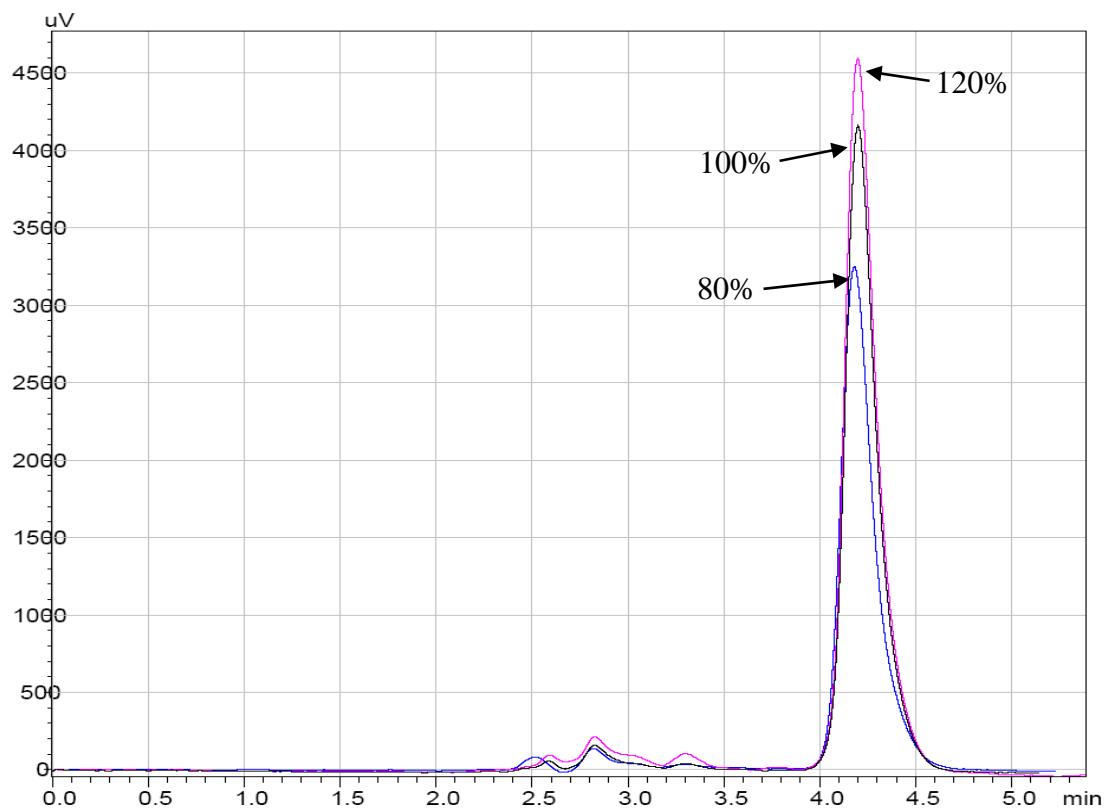


Figure S18: Overlay chromatogram of Accuracy (MET)

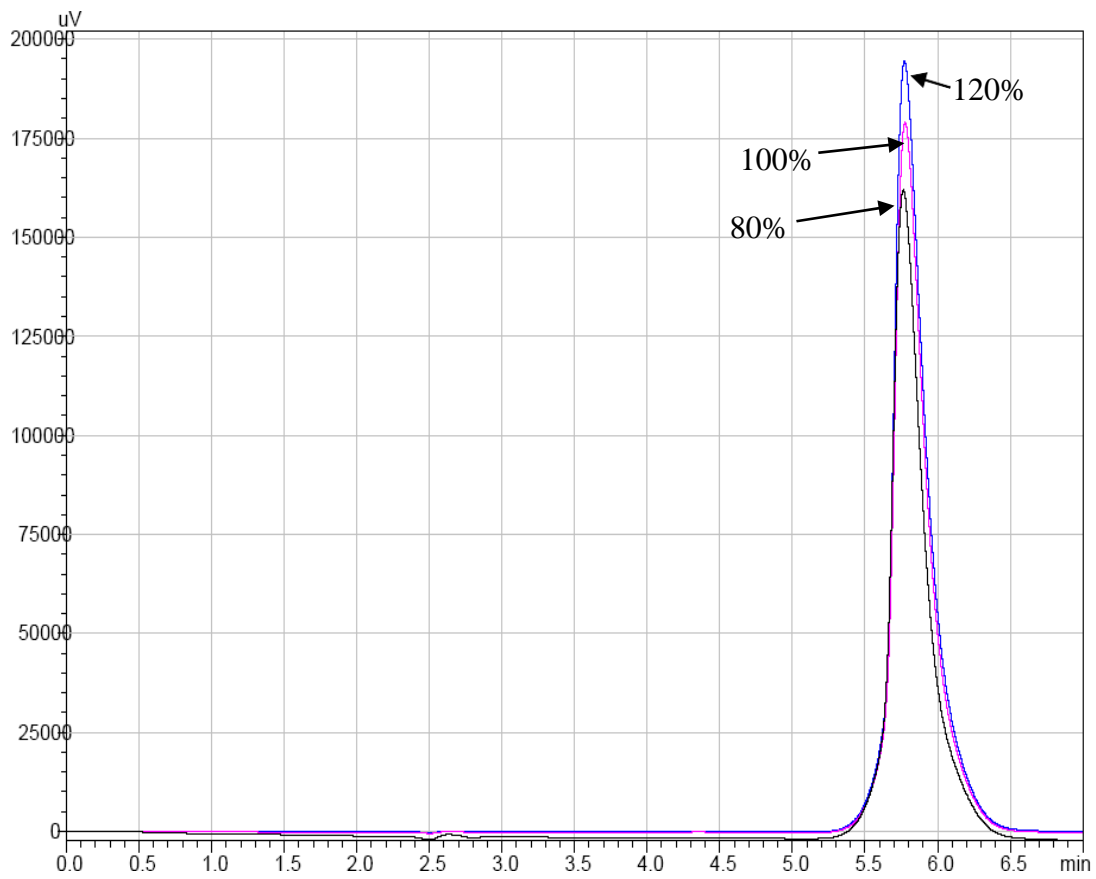


Figure S19: Overlay chromatogram of accuracy (TEL)

Table S1: System suitability data for CHL, MET and TEL

Parameter	Drug	Mean ± * SD	%RSD
Retention Time (min)	CHL	2.96 ± 0.008	0.280
	MET	4.31 ± 0.008	0.188
	TEL	5.94 ± 0.007	0.127
Theoretical Plate	CHL	2262.41 ± 15.36	0.679
	MET	3118.32 ± 25.57	0.820
	TEL	3608.58 ± 24.56	0.680
Tailing Factor	CHL	1.29 ± 0.01	1.259
	MET	1.38 ± 0.02	1.676
	TEL	1.29 ± 0.01	0.798
Resolution	CHL	10.37 ± 0.02	0.283
	MET	4.78 ± 0.01	0.405
	TEL	4.645 ± 0.02	0.486
Capacity factor	CHL	2.37 ± 0.01	0.596
	MET	3.47 ± 0.01	0.482
	TEL	4.755 ± 0.02	0.575

(*Mean n=6)

Table S2: % Assay and peak purity data for CHL, MET and TEL

Drugs	Label Claim (% w/w)	Mean Area	%Assay ± * SD	%RSD	3 Point Purity
CHL	12.5	785061.5	99.32 ± 0.697	0.70	0.999992
MET	25.0	647908.2	99.91 ± 1.189	1.19	0.999960
TEL	40.0	3122073	100.99 ± 0.974	0.96	0.999997

(*Mean assay of n=6)

Table S3: Linearity / Range data for CHL, MET and TEL

Sr. No.	CHL			MET			TEL			
	Conc. (µg/mL)	Mean Peak area ± * SD	%RSD	Conc. (µg/mL)	Mean Peak area ± * SD	%RSD	Conc. (µg/mL)	Mean Peak area ± * SD	%RSD	
1	3.21	208819 ± 2133.82	1.02	6.25	172924.83 ± 1476.37	0.85	10	773631 ± 5462.26	0.70	
2	6.25	396184.6 ± 3328.23	0.84	12.50	329440.3 ± 2409.58	0.73	20	1551347.83 ± 10917.83	0.70	
3	9.37	590467.8 ± 4778.21	0.80	18.75	492319 ± 3392.48	0.68	30	2350437.16 ± 12189.28	0.51	
4	12.50	783042.5 ± 3417.18	0.43	25.00	651047.16 ± 4309.21	0.66	40	3091332.83 ± 13482.83	0.43	
5	15.62	991313.3 ± 3479.11	0.35	31.25	810961 ± 5322.74	0.65	50	3834347.16 ± 11209.05	0.29	
6	18.72	1184334.6 ± 3897.73	0.32	37.50	954419.5 ± 2213.99	0.23	60	4637319.83 ± 9667.75	0.20	
Linearity Equation				Linearity Equation				Linearity Equation		
Y = 63018X + 2630.4				Y = 25306X + 15776				Y = 76869X + 16710		
R ² = 0.9999				R ² = 0.9998				R ² = 0.9998		

(*Mean area of n=6)

Table S4: Accuracy data for CHL

Level (%)	Sample conc. (µg/mL)	Std. Conc. (µg/mL)	Total Conc.	Mean Peak Area ± *SD	%RSD	Amount recovered	% Recovery
80	6.25	5.0	11.25	711112.33 ± 1841.87	0.25	11.24	99.93
100	6.25	6.25	12.5	783686.33 ± 1671.61	0.21	12.39	99.15
120	6.25	7.5	13.75	867322 ± 1469.89	0.16	13.72	99.79

(*Mean area of n=3)

Table S5: Accuracy data for MET

Level (%)	Sample Conc. (µg/mL)	Std. Conc. (µg/mL)	Total Conc.	Mean Peak Area ± *SD	%RSD	Amount recovered	% Recovery
80	12.5	10	22.5	580327.33 ± 865.07	0.14	22.30	99.15
100	12.5	12.5	25.0	643428.33 ± 1345.76	0.20	24.80	99.21
120	12.5	15	27.5	707723.66 ± 968.86	0.13	27.34	99.42

(*Mean area of n=3)

Table S6: Accuracy data for TEL

Level (%)	Sample conc. (µg/mL)	Std. Conc. (µg/mL)	Total Conc.	Mean Peak Area ± *SD	%RSD	Amount recovered	% Recovery
80	20	16	36	2786457.33 ± 9662.31	0.34	36.03	100.08
100	20	20	40	3079164.33 ± 6400.45	0.20	39.83	99.59
120	20	24	44	3400294.33 ± 4853.98	0.14	44.01	100.03

(*Mean area of n=3)

Table S7: Repeatability data for CHL, MET and TEL

Concentration ($\mu\text{g/mL}$)			Mean Peak area \pm * SD			%RSD		
CHL	MET	TEL	CHL	MET	TEL	CHL	MET	TEL
3.21	6.25	10	206351.4 \pm 2699.49	175649.1 \pm 1547.52	776922 \pm 4574.07	1.30	0.88	0.58
9.37	18.25	30	587468.4 \pm 2584.80	496780.1 \pm 3811.59	2387815 \pm 10368.06	0.43	0.76	0.43
15.62	31.25	50	994466.8 \pm 4109.09	813447.2 \pm 2717.01	3886535 \pm 11811.97	0.41	0.33	0.30

(*Mean area of n=3)

Table S8: Intermediate precision data for CHL, MET and TEL

Concentration ($\mu\text{g/mL}$)			Mean Peak area \pm * SD			%RSD		
CHL	MET	TEL	CHL	MET	TEL	CHL	MET	TEL
3.21	6.25	10	205134.9 \pm 3303.666	177045.4 \pm 1941.515	777841.8 \pm 7297.636	1.61	1.09	0.93
9.37	18.25	30	583704 \pm 5675.538	495706.6 \pm 4503.209	2378543 \pm 21212.15	0.97	0.90	0.89
15.62	31.25	50	992483.4 \pm 6486.412	811136.8 \pm 5085.795	3850643 \pm 30347.97	0.65	0.62	0.78

(*Mean area of n=3)

Table S9: LoD/LoQ data for CHL, MET and TEL

Parameters	CHL	MET	TEL
LoD($\mu\text{g/mL}$)	0.59	1.21	2.12
LoQ($\mu\text{g/mL}$)	1.79	3.66	6.44

(n=3)

Table S10: Robustness data for CHL, MET and TEL

Parameter	Factor	Drug	Mean area ± *SD	% Recovery	% RSD
Wavelength (225nm)	223 nm	CHL	800552.33± 9085.67	101.29	1.134
	(-2 nm)	MET	648245.66 ± 3855.57	99.97	0.594
		TEL	3117934.6± 35107.42	100.86	1.125
		CHL	780885.66± 1581.00	98.79	0.202
	(+2 nm)	MET	648579± 3528.47	100.02	0.544
		TEL	3081268± 27750.63	99.66	0.900
CHL		788219± 2712.97	99.72	0.344	
Flow Rate (0.7 mL/min)	0.66	CHL	788219± 2712.97	99.72	0.344
	(-5%)	MET	647245.66± 3158.71	99.81	0.488
		TEL	3141268± 14450.86	101.61	0.460
		CHL	777552.33± 4410.62	98.37	0.567
	(+5%)	MET	645912.33± 2979.84	99.60	0.461
		TEL	3077934.66± 37209.55	99.55	1.208
CHL		784885.66± 5613.16	99.30	0.715	
Mobile Phase Composition (Buffer: ACN) (40:60)	39.2	CHL	784885.66± 5613.16	99.30	0.715
	(-2%)	MET	649245.66± 8249.81	100.12	1.270
		TEL	3091268± 52399.21	99.99	1.695
		CHL	784552.33± 4658.06	99.26	0.593
	(+2%)	MET	649245.66± 3101.52	100.12	0.477
		TEL	3107934.66± 40472.47	100.53	1.302

(*Mean area of n=3)