

## Supporting Information

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### 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

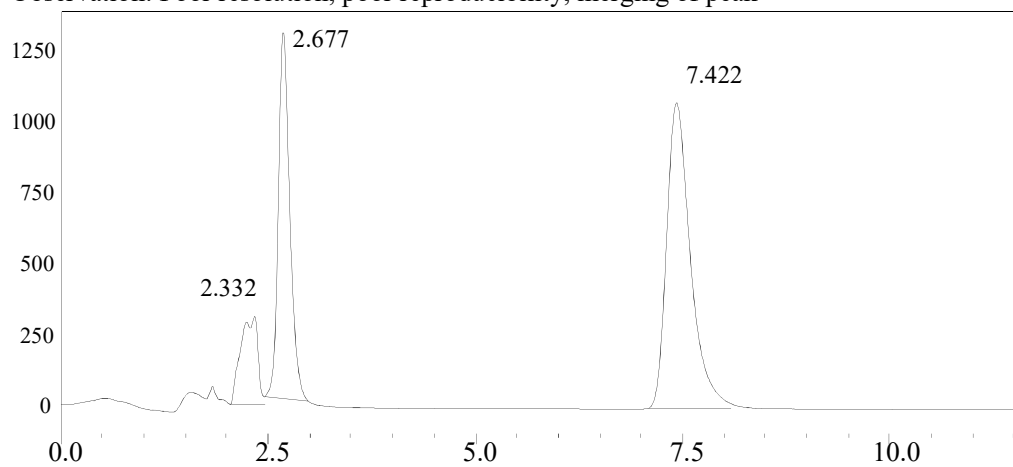


Figure S1: Trial 1 HPLC chromatogram

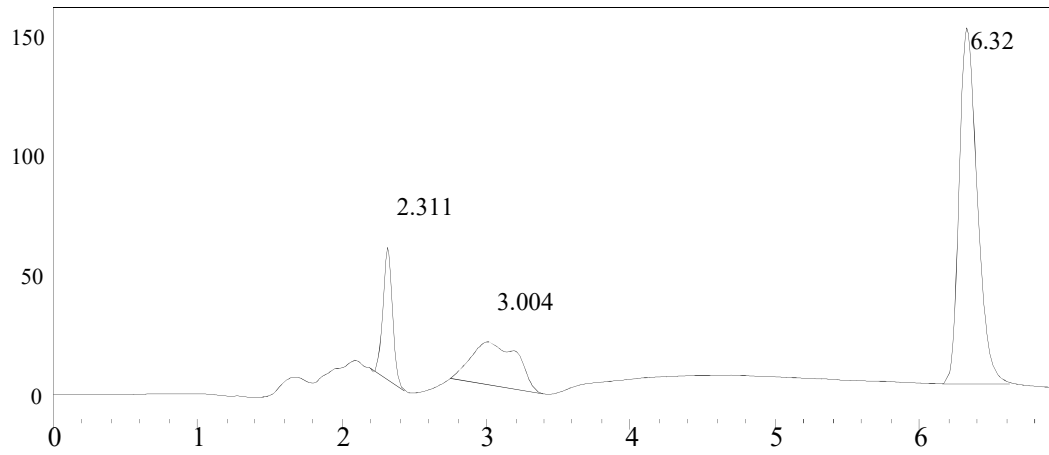
**Trial 2**

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

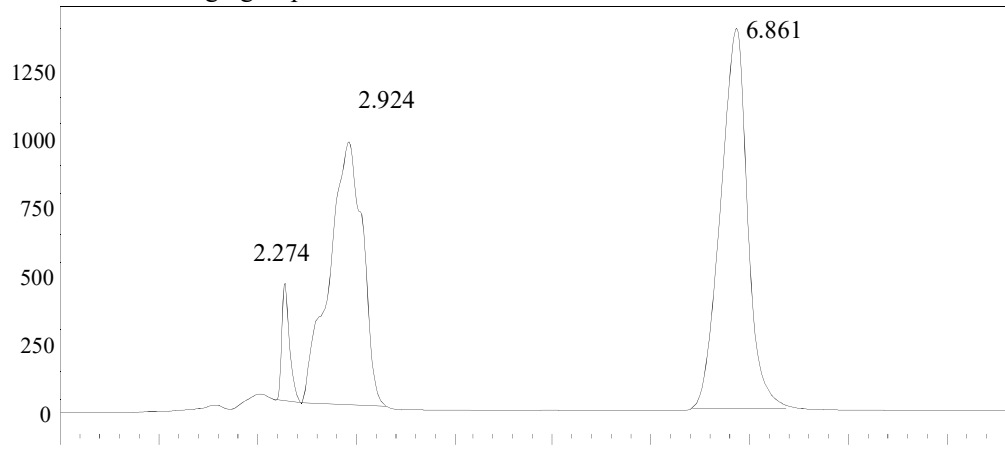
**Trial 3**

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

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

Flow rate: 1 mL/min

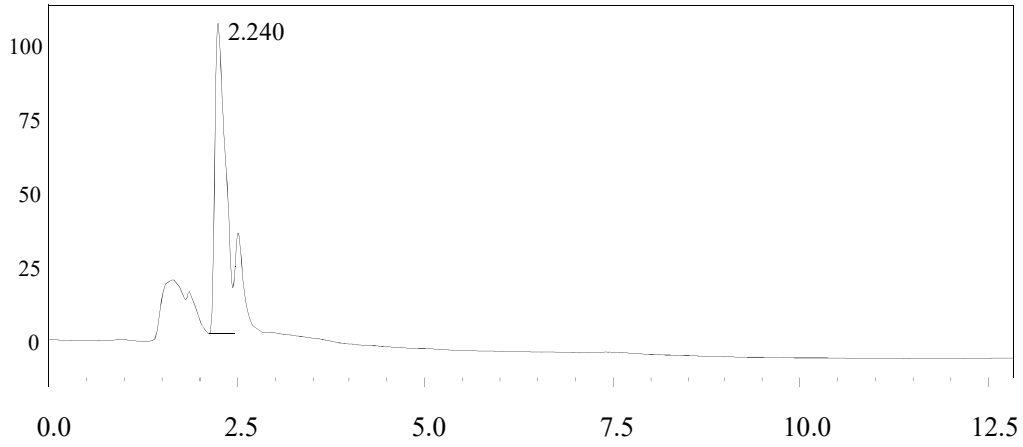
Observation: Merging of peak



**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

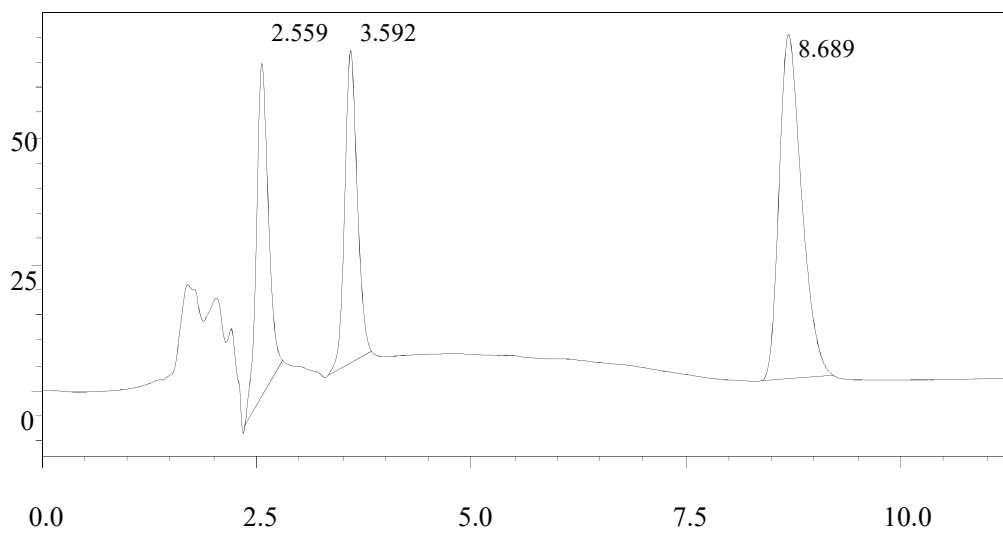
### Trial 5

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



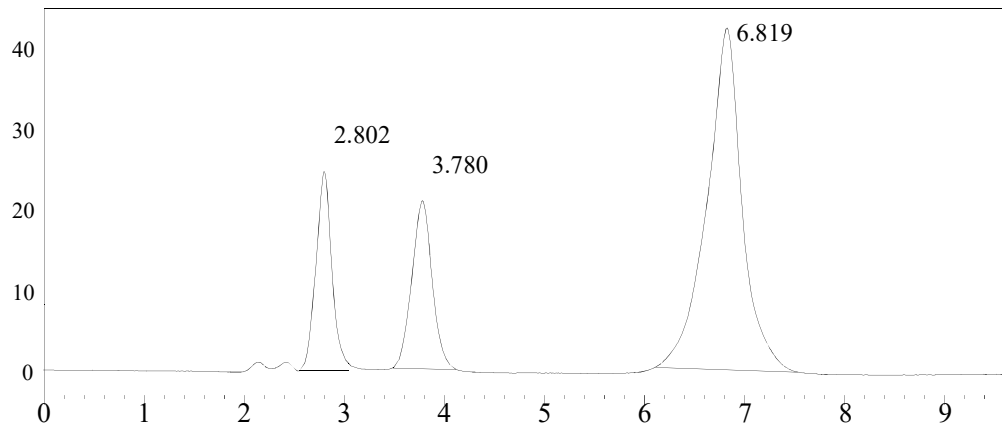
**Figure S5:** Trial 5 HPLC chromatogram

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

Ratio: (50: 50 v/v)

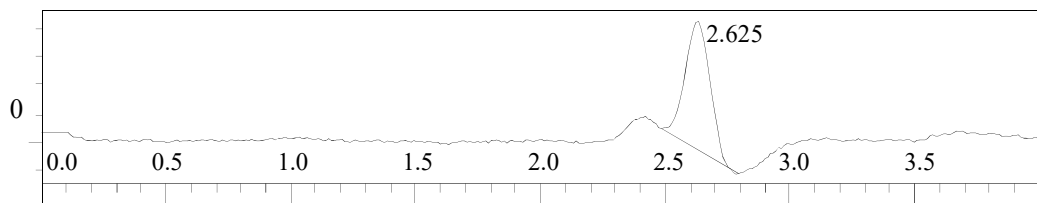
Flow rate: 0.7 mL/min

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

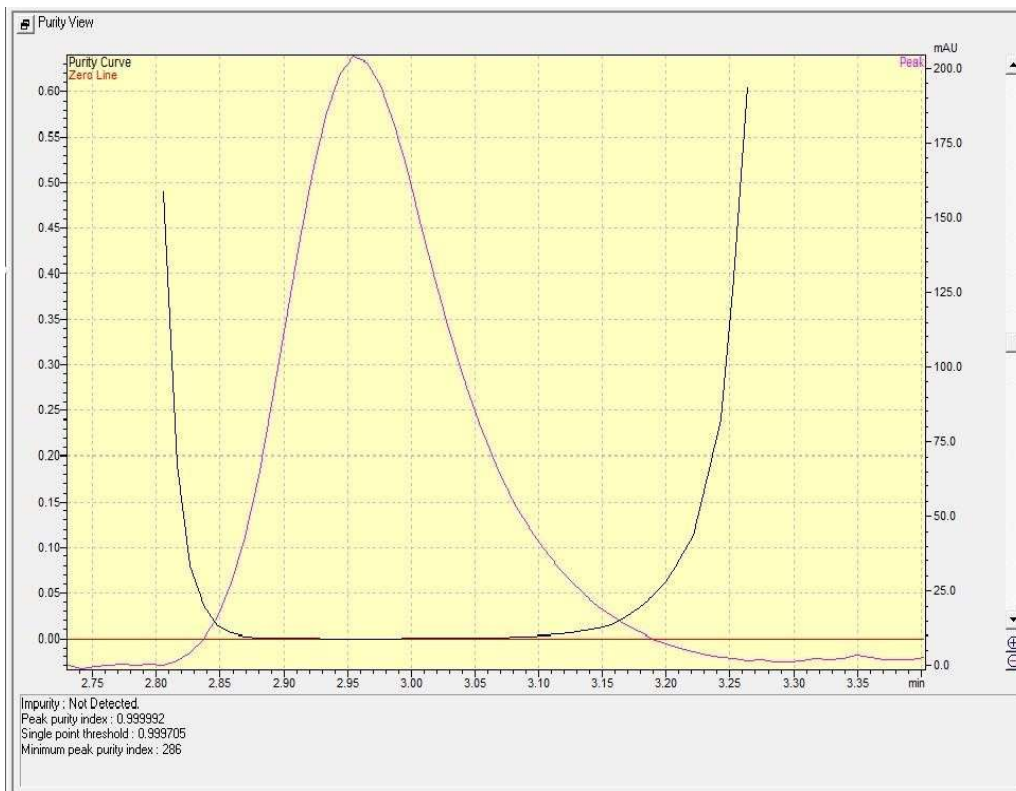


**Figure S6:** Optimized chromatographic condition

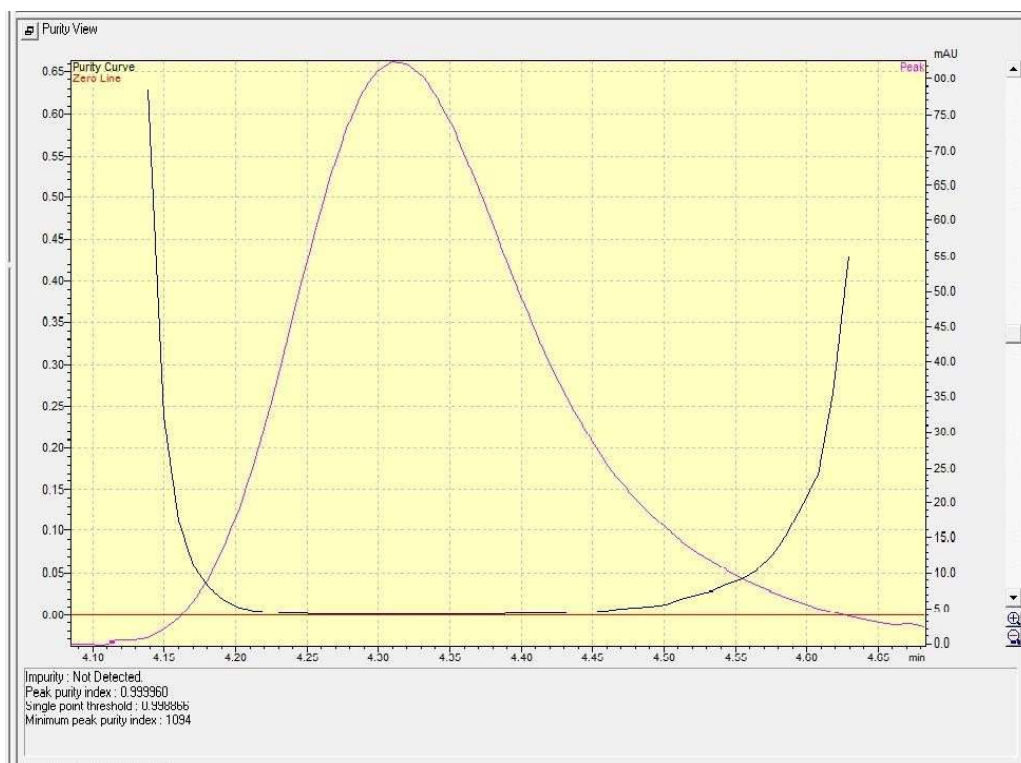




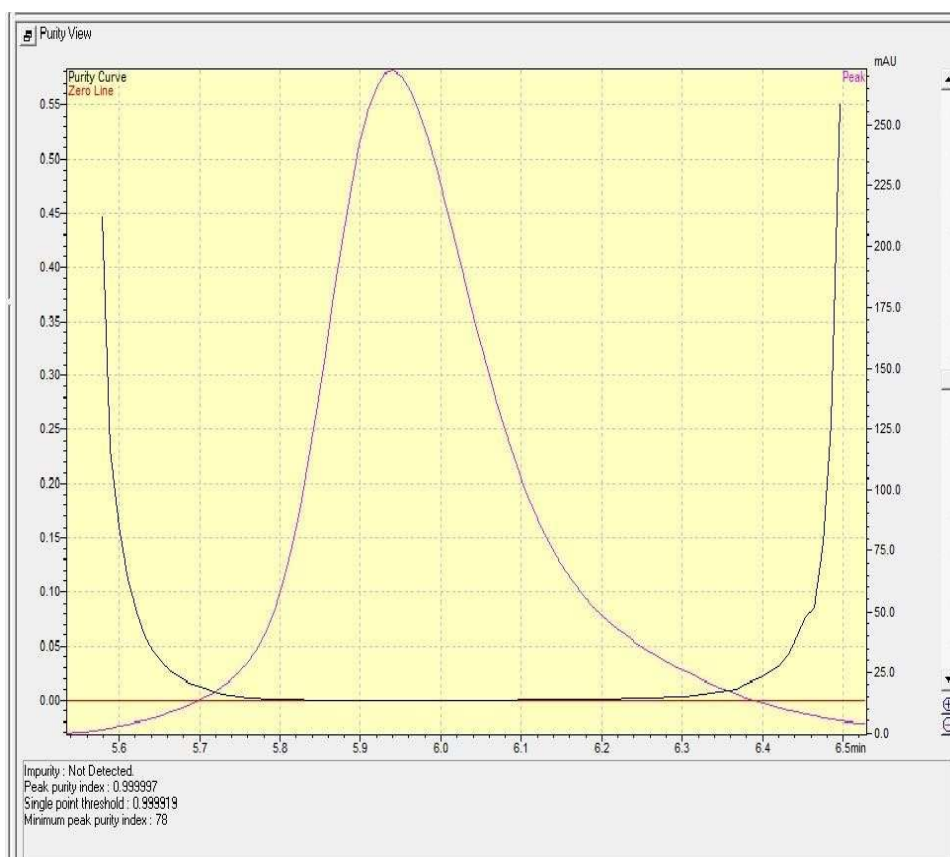
**Figure S7:** Interference of blank



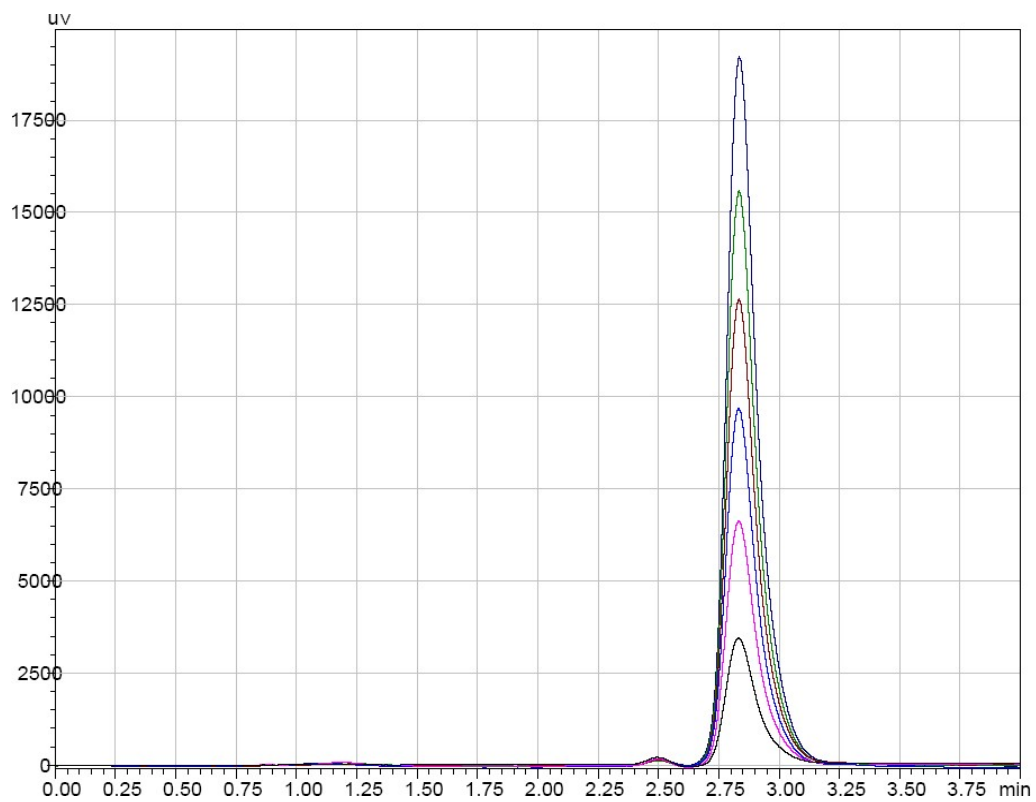
**Figure S8:** Peak purity index of CHL



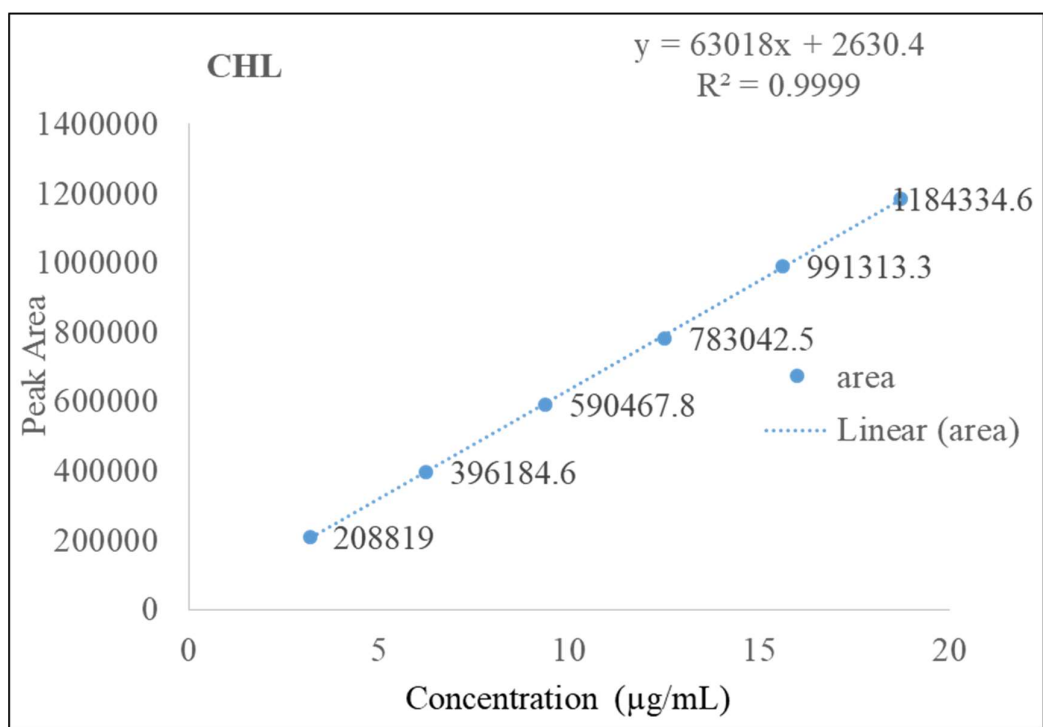
**Figure S9:** Peak purity index of MET



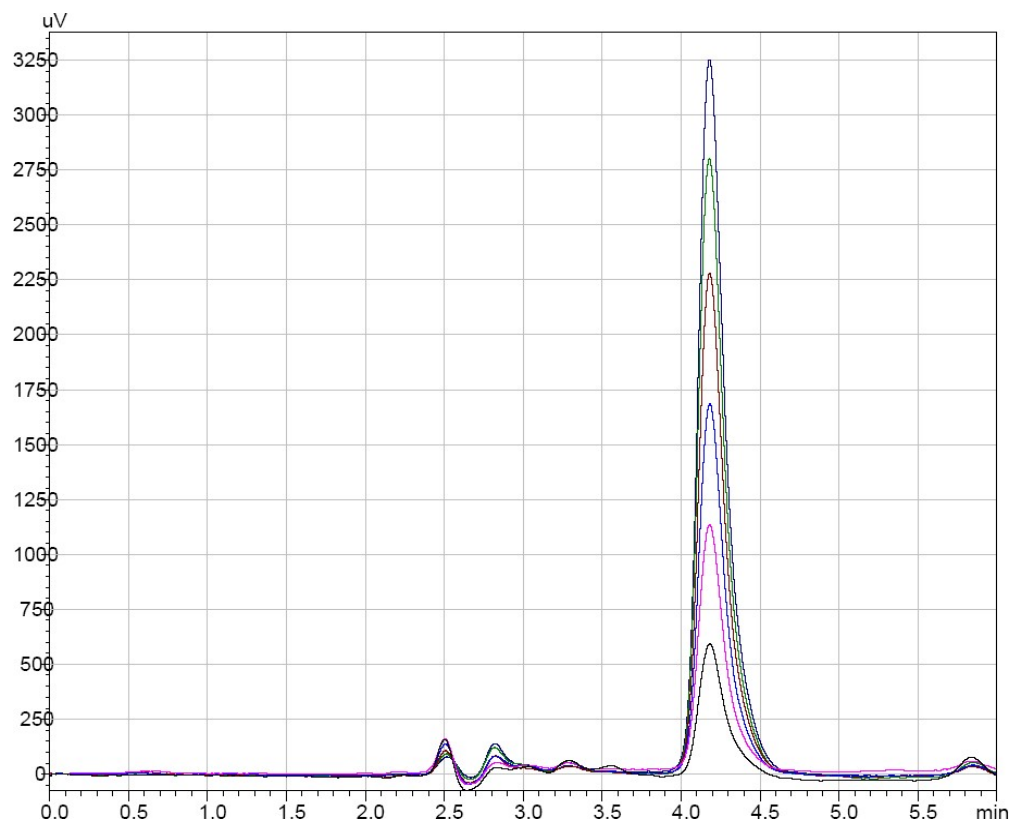
**Figure S10:** Peak purity index of TEL



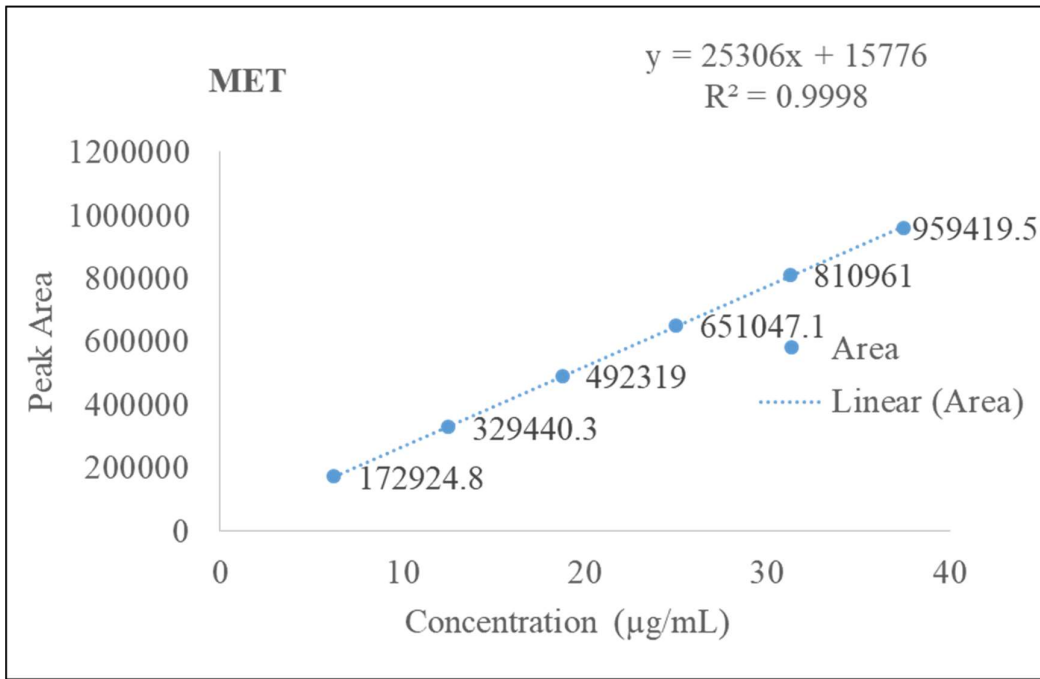
**Figure S11:** Overlay chromatograms of CHL (3.21-18.72)  $\mu\text{g/mL}$



**Figure S12:** Calibration curve of CHL (3.21-18.72)  $\mu\text{g/mL}$

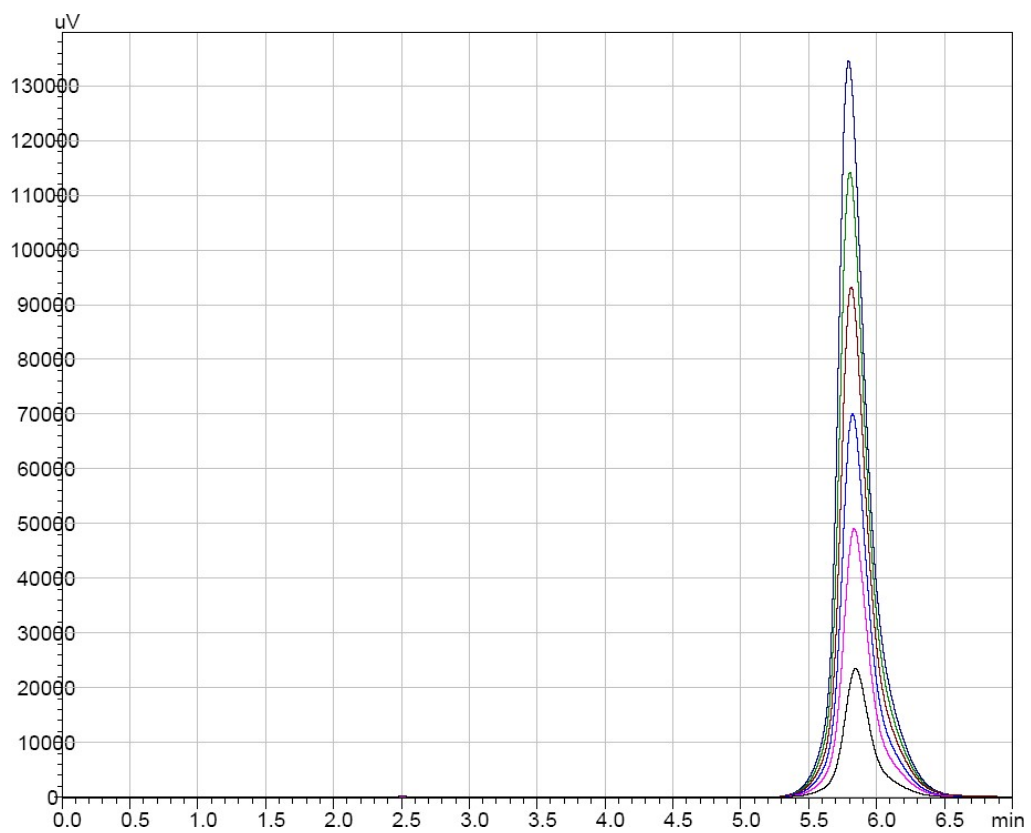


**Figure S13:** Overlay chromatograms of MET (6.25-37.50) µg/mL

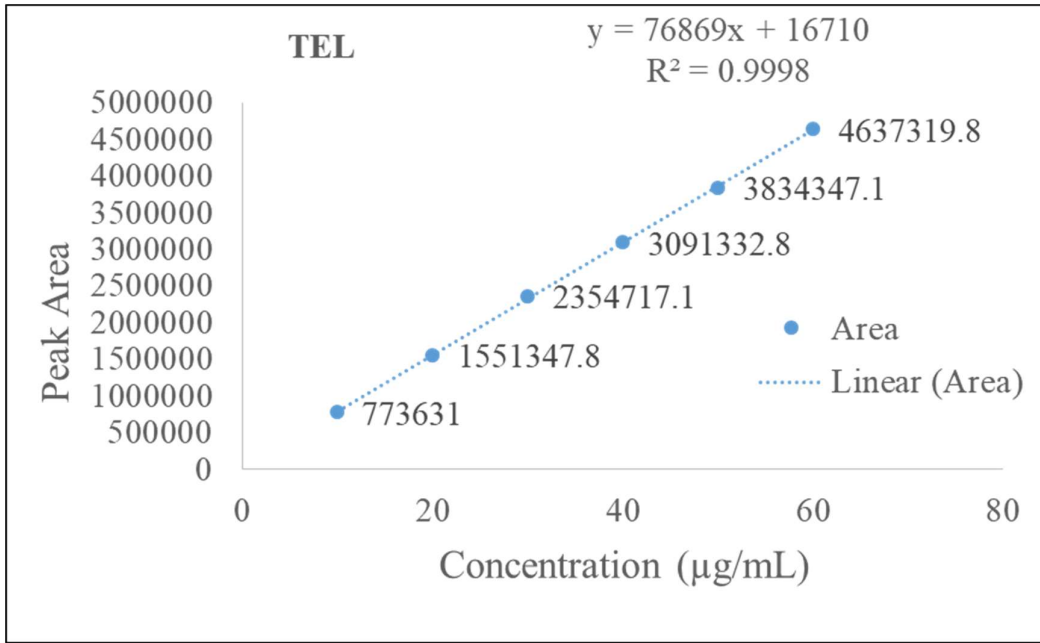


**Figure S14:** Calibration curve of MET (6.25-37.50)  $\mu\text{g/mL}$

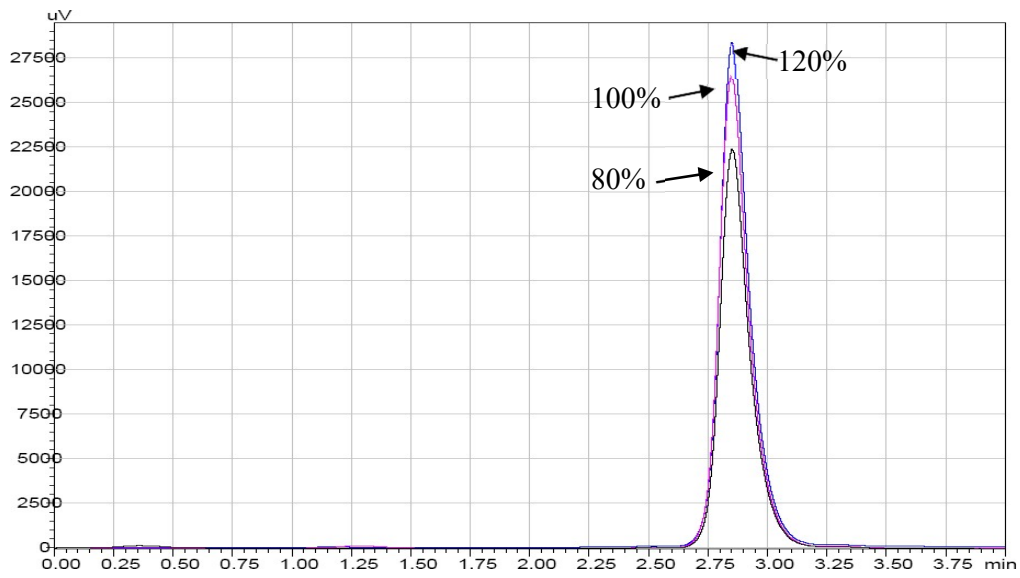




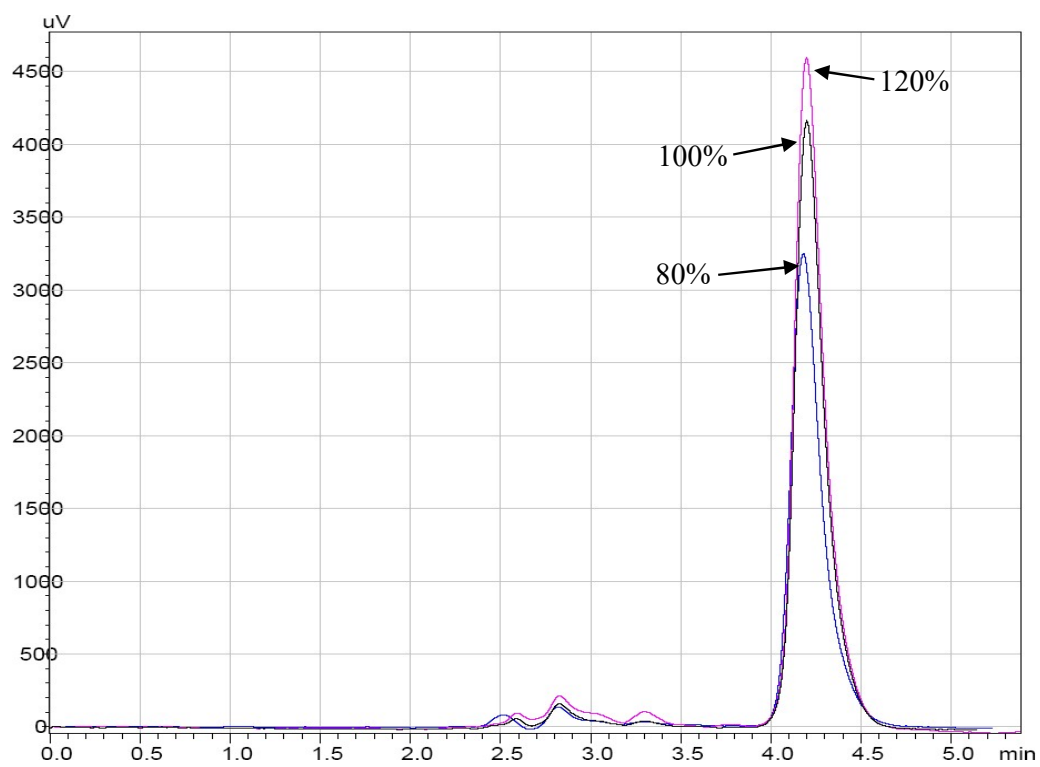
**Figure S15:** Overlay chromatograms of TEL (10-60)  $\mu\text{g/mL}$



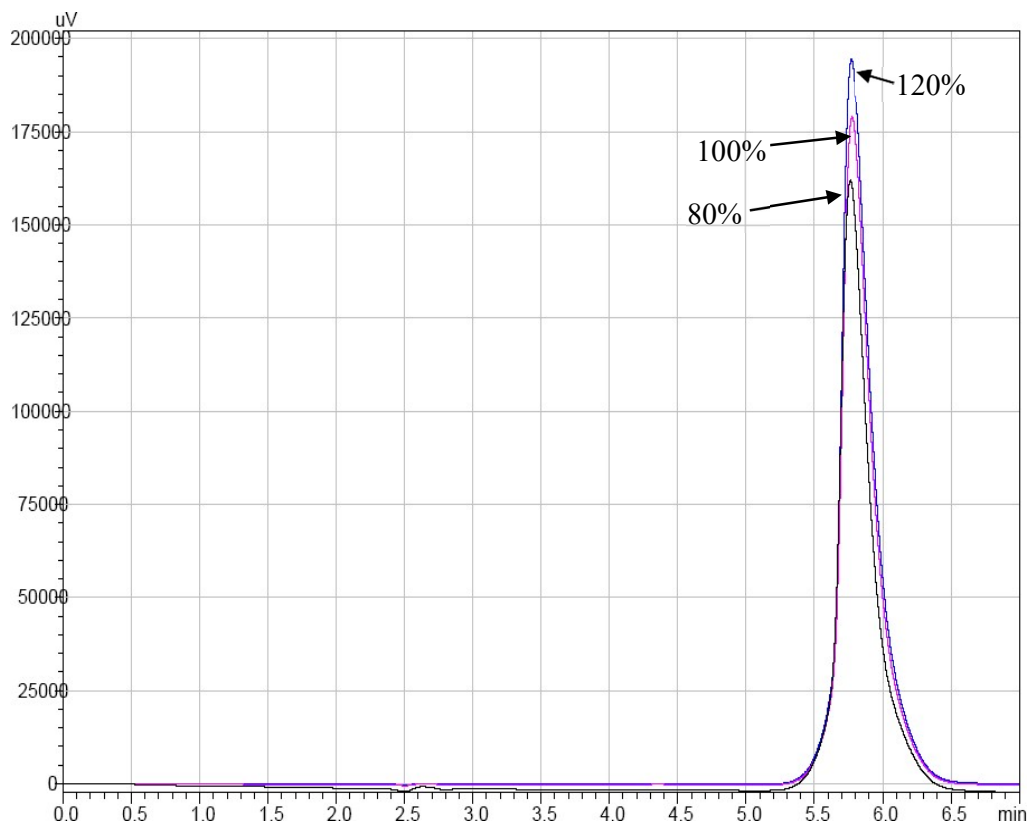
**Figure S16:** Calibration curve of TEL (10-60) µg/mL



**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

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

<b>Drugs</b>	<b>Label Claim (% w/w)</b>	<b>Mean Area</b>	<b>%Assay ± * SD</b>	<b>%RSD</b>	<b>3 Point Purity</b>
<b>CHL</b>	12.5	785061.5	99.32 ± 0.697	0.70	0.999992
<b>MET</b>	25.0	647908.2	99.91 ± 1.189	1.19	0.999960
<b>TEL</b>	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	
<b>Linearity Equation</b>				<b>Linearity Equation</b>				<b>Linearity Equation</b>		
Y = 63018X + 2630.4				Y = 25306X + 15776				Y = 76869X + 16710		
R <sup>2</sup> = 0.9999				R <sup>2</sup> = 0.9998				R <sup>2</sup> = 0.9998		

(\*Mean area of n=6)



**Table S4: Accuracy data for CHL**

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

<b>Level (%)</b>	<b>Sample Conc. (µg/mL)</b>	<b>Std. Conc. (µg/mL)</b>	<b>Total Conc.</b>	<b>Mean Peak Area ± *SD</b>	<b>%RSD</b>	<b>Amount recovered</b>	<b>% Recovery</b>
<b>80</b>	12.5	10	22.5	580327.33 ± 865.07	0.14	22.30	99.15
<b>100</b>	12.5	12.5	25.0	643428.33 ± 1345.76	0.20	24.80	99.21
<b>120</b>	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

<b>Level (%)</b>	<b>Sample conc. (µg/mL)</b>	<b>Std. Conc. (µg/mL)</b>	<b>Total Conc.</b>	<b>Mean Peak Area ± *SD</b>	<b>%RSD</b>	<b>Amount recovered</b>	<b>% Recovery</b>
<b>80</b>	20	16	36	2786457.33 ± 9662.31	0.34	36.03	100.08
<b>100</b>	20	20	40	3079164.33 ± 6400.45	0.20	39.83	99.59
<b>120</b>	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	177045.4	777841.8	1.61	1.09	0.93
			$\pm$	$\pm$	$\pm$			
9.37	18.25	30	3303.666	1941.515	7297.636	0.97	0.90	0.89
			583704	495706.6	2378543			
15.62	31.25	50	5675.538	4503.209	21212.15	0.65	0.62	0.78
			992483.4	811136.8	3850643			
			6486.412	5085.795	$\pm$			
					30347.97			

(\*Mean area of n=3)

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

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

(n=3)

**Table S10:** Robustness data for CHL, MET and TEL

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

(\*Mean area of n=3)