Supporting Information

A New Stability Indicating HPLC Method with QDa and PDA Detector for The Determination of Process and Degradation Impurities of Ivabradine Including Separation of Diastereomeric N-Oxides

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Supporting Information

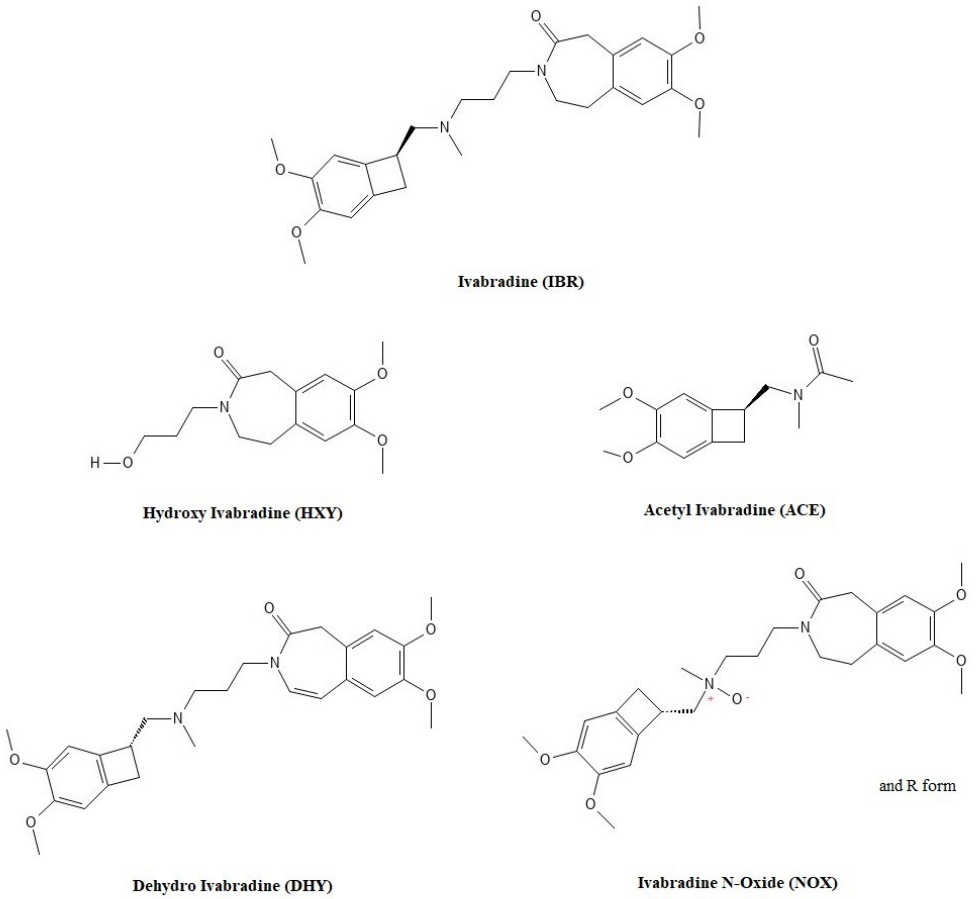

Fig. S1 Molecule structures of IBR, HXY, ACE, DHY and NOX

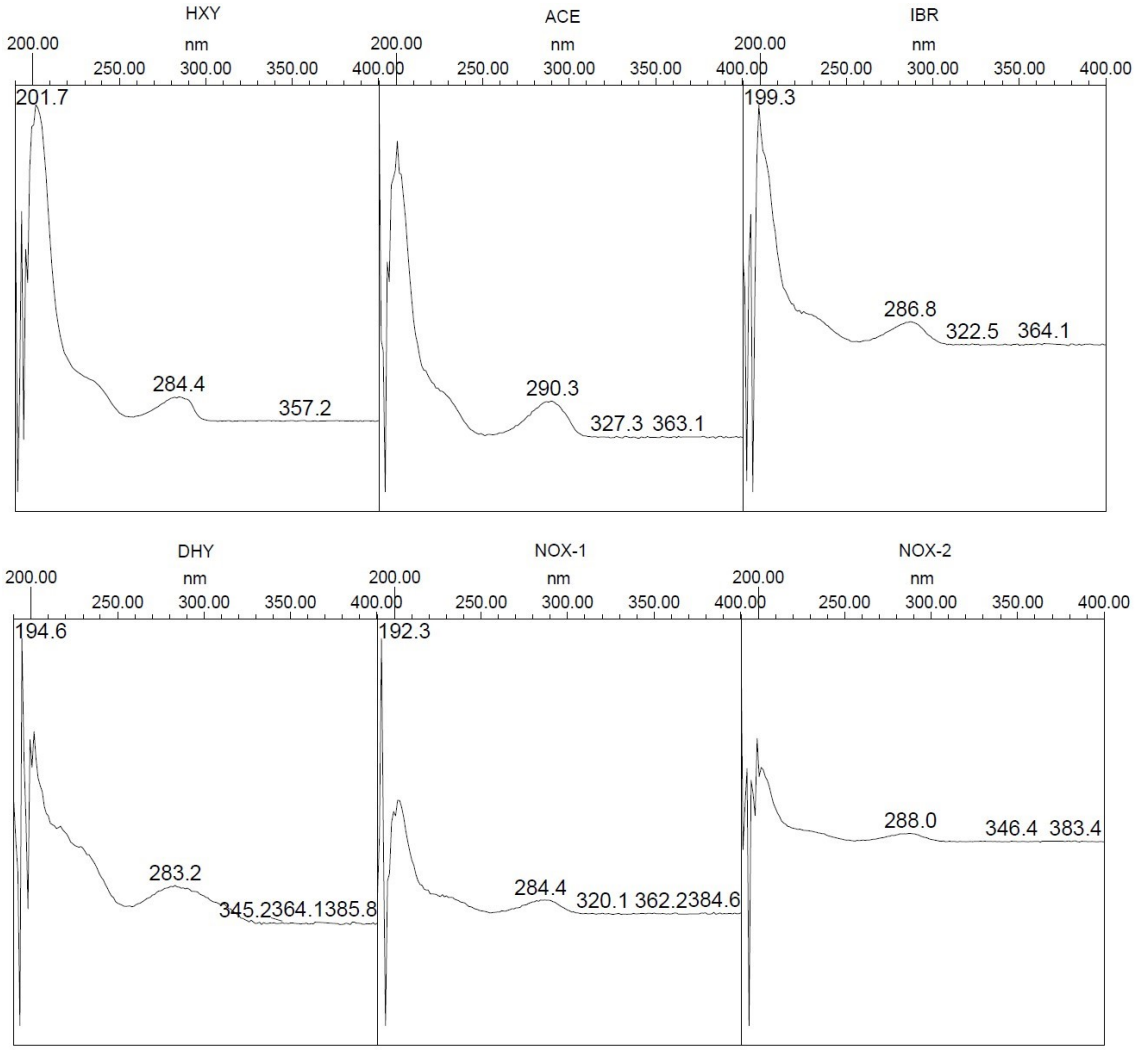

Fig. S2 UV spectra of HXY, ACE, IBR, DHY, NOX-1 and NOX-2

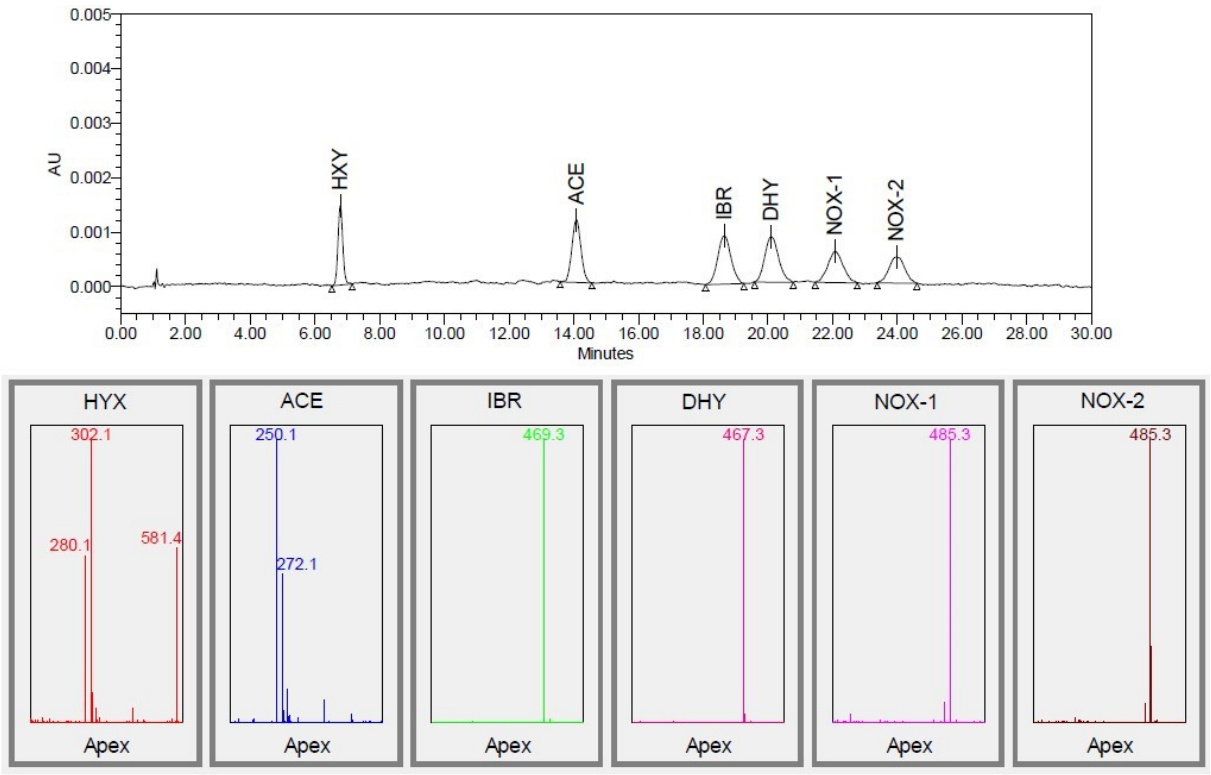

Fig. S3 Chromatogram of standard solution at 285 nm and M+ data of substances

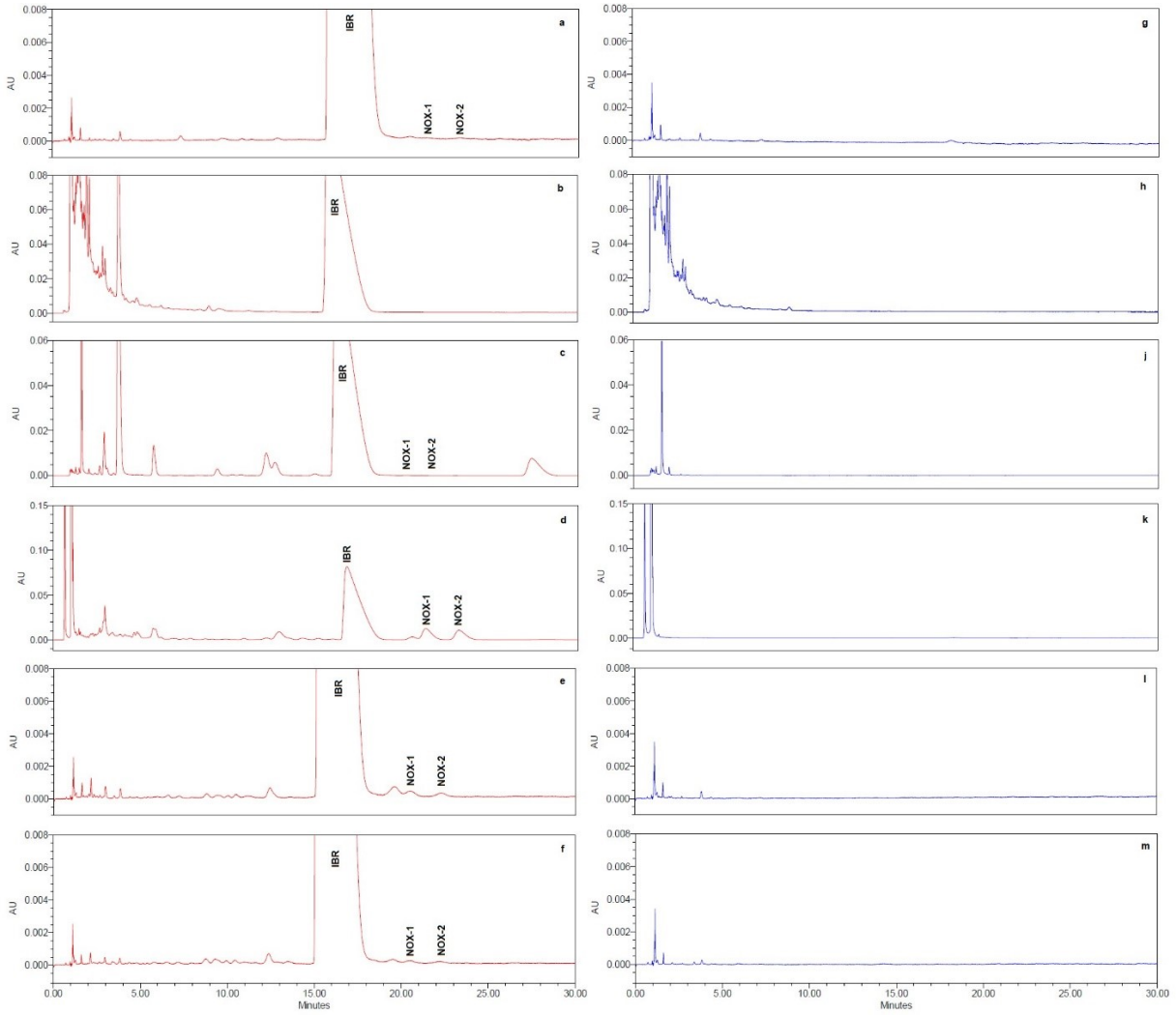

Fig. S4 Chromatograms of IBR 7.5 mg Film Coated Tablet and its placebo tablets with/without degradation

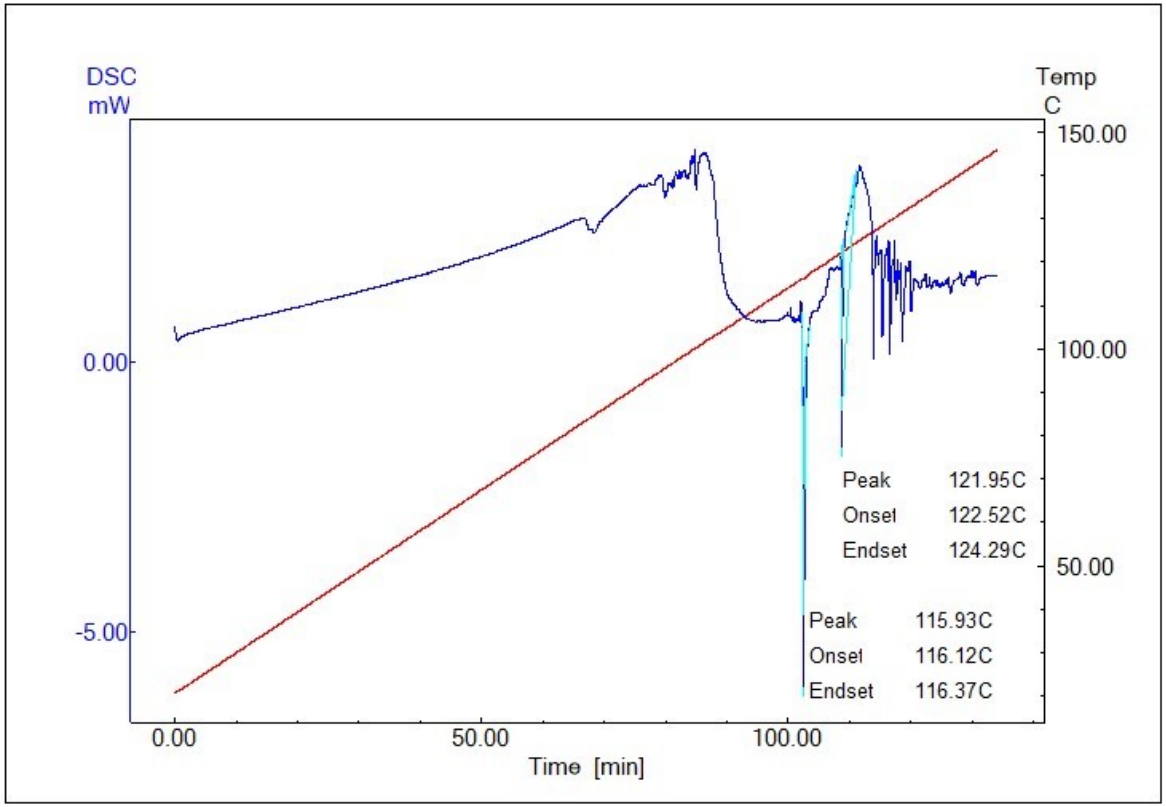

Fig. S5 DSC thermogram of synthesized yellowish cyrstals

Table S1 Degradants and conditions for degradation studies

Type	Degradant	Condition	Degradant to finish reaction
Alkali Degradation	3 N NaOH (2.5 ml)	70 °C - 6 hours	3 N HCl (2.5 ml)
Acidic Degradation	3 N HCl (2.5 ml)	70 °C - 6 hours	3 N NaOH (2.5 ml)
Oxidative Degradation	30% H_2O_2 (2.5 ml)	70 °C - 6 hours	-
Thermal Degradation*	Temperature	70 °C – 7 days	-
Photodegradation*	UV Light	1.2 million lux hours – 7 days	-

*They represent solid-state degradations, because sample tablets, placebo tablets and IBR active ingredient were directly subjected to the two during 7 days.

Table S2 Detailed degradation impurity results (%) of IBR 7.5 mg Film Coated Tablet with M+ base peak data (m/z)

RRT	a	b	c	d	e	f	m/z	RRT	a	b	c	d	e	f	m/z
0.085	-	-	0.048	-	-	-	421.21	**0.375***	-	-	-	-	-	-	280.33
0.089	-	-	-	0.026	-	-	309.28	**0.386**	-	-	-	0.068	-	-	551.34
0.112	-	-	-	0.021	-	-	279.25	**0.397**	-	-	-	0.077	-	-	535.40
0.120	-	-	-	0.050	-	-	256.97	**0.422**	-	-	-	0.068	-	-	503.36
0.121	-	-	-	-	0.030	-	363.19	**0.446**	-	-	-	0.166	-	-	535.37
0.123	-	-	-	0.140	-	-	208.16	**0.494**	-	-	-	0.105	-	-	501.38
0.128	-	-	-	0.150	-	-	298.25	**0.498**	-	-	-	0.076	0.033	0.038	485.27
0.136	-	-	0.023	-	-	-	479.27	**0.532**	-	-	0.395	-	-	-	487.31
0.136	-	-	-	0.038	-	-	293.21	**0.539**	0.022	0.260	-	0.151	0.029	0.042	455.30
0.151	-	-	-	0.169	-	-	505.33	**0.581**	-	-	0.046	0.180	-	-	485.32

0.151	-	-	0.172		-	-	393.25	**0.593**	-	-	0.052	-	0.024	0.022	485.33
0.164	-	-	-	0.230	-	-	423.30	**0.636**	-	0.097	-	-	0.021	-	455.31
0.166	-	-	1.007	-	-	-	487.30	**0.691**	-	-	1.846	-	-	-	469.32
0.168	-	-	-	1.496	-	-	293.25	**0.692**	-	-	-	0.299	-	-	501.37
0.168	-	-	-	-	0.026	-	293.16	**0.733**	-	-	-	2.571	-	-	501.35
0.174	-	-	0.164	-	-	-	487.32	**0.717**	0.020	0.031	-	-	0.117	0.082	483.30
0.192	-	-	-	0.323	-	-	409.30	**0.719**	-	-	1.133	-	-	-	469.29
0.196	-	-	0.036	-	-	-	493.28	**0.763**	-	-	-	-	-	0.018	501.31
0.213	-	8.175	15.574	-	-	-	487.29	**0.777***	-	-	-	-	-	-	250.30
0.218	-	-	-	0.160	-	-	505.32	**0.808**	-	-	-	0.314	-	-	501.37
0.233	-	-	-	0.141	-	-	503.33	**0.849**	-	-	0.125	-	-	-	487.34
0.237	-	-	-	0.070	-	-	503.29	**0.859**	-	-	-	0.212	-	-	499.30
0.249	-	-	-	0.060	-	-	433.27	**0.907**	-	-	-	0.131	-	-	485.32
0.263	-	-	-	0.294	-	-	409.28	**1.081***	-	-	-	-	-	-	467.57
0.273	-	-	-	0.617	-	-	503.38	**1.155**	0.019	-	-	0.500	0.178	0.037	483.27
0.325	-	-	-	0.852	-	-	487.29	**1.187***	0.011	-	0.014	3.632	0.101	0.028	485.28
0.326	-	-	1.251	-	-	-	523.33	**1.284***	0.022	-	0.011	3.819	0.072	0.030	485.29
0.332	-	-	-	0.729	-	-	487.31	**1.551**	-	-	3.326	-	-	-	505.34
0.350	-	-	-	0.079	-	-	505.37	**1.598**	-	-	-	0.194	-	-	455.36

** RRT 0.375 – HXY; RRT 0.777 – ACE; RRT 1.081 – DHY; RRT 1.187 – NOX-1; RRT 1.284 – NOX-2*

Table S3 Solubility study of diastereomeric N-Oxides

Solvent	Presence of ppt	NOX-1		NOX-2		Source of ppt
		Expected Result	Experimental Result	Expected Result	Experimental Result	
Methanol	-	942.95	942.96	994.88	994.90	-
Distilled Water	+	1000.34	1000.34	1078.52	1063.03	NOX-2
Ethyl Acetate	+	897.96	897.97	956.49	942.17	NOX-2
Dichloromethane	+	898.16	886.32	956.49	930.58	Both
Tetrahydrofuran	+	831.98	7.97	890.55	8.45	Both

Table S4 Physical properties of diastereomeric N-Oxides

Solubility Results (mg ml^{-1})			
	NOX-1	**NOX-2**	**Descriptive Term**
Methanol	> 942.95	> 994.88	freely soluble
Distilled Water	> 1000.34	1063.03	freely soluble
Ethyl Acetate	> 897.96	942.17	freely soluble
Dichloromethane	886.32	930.58	freely soluble
Tetrahydrofuran	7.97	8.45	slightly soluble
Other Physical Properties			
	NOX-1	**NOX-2**	
Specific Rotation, $[\alpha]_D^{25}$ (in methanol)	+ 22.4 ^{o}	+ 64.7 ^{o}	
Melting Point	Two sharp peaks were obtained at 115.93 ^{o}C and 121.95 ^{o}C		

Table S5 Results of specificity parameter

Solution Type	Standard Solution [a]		Standard Solutions [b]		Sample Solution		Sample + Impurity Spike Solution		Active Ingredient + Placebo + Impurity Spike Solution	
Component	**A**	**T**	**A**	**T**	**A**	**T**	**A**	**T**	**A**	**T**
HXY	2.304	2.731	2.347	2.609	-	-	2.621	3.066	2.653	3.266
ACE	3.831	4.586	4.210	4.541	-	-	4.352	5.093	4.032	4.608
IBR	4.469	5.098	5.492	5.778	0.069	0.257	0.069	0.245	4.707	5.586
DHY	3.647	3.901	3.635	4.376	-	-	2.302	3.152	3.142	3.778
NOX-1	6.747	7.698	7.912	9.633	-	-	7.153	7.477	7.348	8.743
NOX-2	7.342	8.423	9.414	10.062	-	-	7.39	8.576	8.253	9.299

[A] Angle ; [T] Threshold

[a] It consisted of HXY, ACE, IBR, DHY, NOX-1 and NOX-2

[b] They represent standard solutions of only one component (HXY, ACE, IBR, DHY and NOX Standard Solutions

Table S6 Summary results of system suitability, linearity and range, LOQ and LOD, accuracy and precision parameters

	HXY	ACE	IBR	DHY	NOX-1	NOX-2
System Suitability						
Retention Time (RT)	6.78 ±0.002	14.08 ±0.002	18.66 ±0.01	20.09 ±0.01	22.07 ±0.04	23.97 ±0.01
RRT	0.36 ±0.0001	0.76 ±0.0003	1.00	1.08 ±0.001	1.18 ±0.002	1.28 ±0.001
RSD (%)	0.96	0.87	0.99	0.75	1.42	1.44
Plate Count	9632	9713	9916	10239	10767	10866
Tailing Factor	1.08	1.07	1.07	1.05	1.03	1.00
Resolution	-	18.50	7.20	1.90	2.41	2.10
Linearity and Range						
Linear Range (µg ml^{-1})						
LOQ%	0.098	0.119	0.171	0.163	0.674	0.553
150%	2.256	2.234	2.250	2.257	2.268	2.268
Regression Equation (Y = mx + b)						
Slope (m)	9673772.7	15258654.5	13706488.9	17127556.4	4071051.4	5278666.7
Intercept (b)	55.78	-2.61	489.96	-283.01	147.31	215.94
Regression Coefficient (R)	1.0000	0.9999	1.0000	0.9997	0.9999	0.9996
R^2 value	0.9999	0.9997	0.9999	0.9993	0.9998	0.9993
Y-intercept (at 100% level)	0.38	-0.01	0.48	-1.14	2.32	2.56
LOQ and LOD						
LOQ (µg ml^{-1})	0.098	0.119	0.171	0.163	0.674	0.553
LOQ (%)	0.013	0.016	0.023	0.022	0.089	0.073
RSD (%)	2.40	0.89	2.29	1.33	1.48	1.89
S/N	10.23	10.08	10.11	10.29	10.04	10.30
LOD (µg ml^{-1})	0.032	0.039	0.056	0.054	0.222	0.183
LOD (%)	0.004	0.005	0.008	0.007	0.029	0.024
S/N	3.02	3.21	3.12	3.01	3.30	3.06
Accuracy						
Average Recovery (± SD)	102.44 ±0.86	102.13 ±0.80	100.07 ±1.32	100.67 ±1.53	99.26 ±2.49	99.00 ±1.98
RSD (%)	0.84	0.79	1.32	1.52	2.51	2.00
Precision						
RSD (%)						
Intraday	0.90	0.54	1.18	0.94	0.97	0.51
Interday	0.67	0.91	0.71	1.33	1.31	0.81
F-Test						

F	1.81	2.88	2.72	2.05	1.78	2.52
$F_{critical\ two\text{-}tailed}$	5.05	5.05	5.05	5.05	5.05	5.05
t-Test						
$t_{statistic}$	0.87	0.49	0.48	1.12	0.76	0.23
$t_{critical\ two\text{-}tailed}$	2.26	2.31	2.31	2.26	2.26	2.31