Supplementary Information (SI) for Analytical Methods.
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Supplementary File

Development and Validation of QbD-Integrated Sensitive RP-HPLC Method for quantitation of Fenofibrate: An Application in Quality control

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Table S1: Summary of Reported HPLC Methods for Fenofibrate: Analytical Conditions and Performance Parameters.

#	Mobile Phase	Conc. Range (linearity)	R^2 value	LOD (µg/ml)	LOQ (µg/ml)	RT (Min)	Peak Area	TF	NTP	Reference
1	1 mM ammonium acetate buffer: Acetonitrile (10:90 v/v)	5 –30 µg/mL	0.997	0.14 µg/mL	0.45 µg/mL	6 .15		—	—	[52]
2	0.1 % ortho-phosphoric acid and Methanol (25:75 v/v)	10-50µg/mL	0.998	—	—	7.34		—	—	[56]
3	Acetonitrile and Water (70:30 v/v)	10–60 µg/mL	0.998	0.90 µg/m	2.72 µg/mL	6.89	--	—	—	[57]
4	methanol–water (65:35, v/v)	100–10000 ng/ml	0.999	30 ng/mL	90 ng/mL	1.13		1.48	3845	[58]
5	ACN: Water: TFA (70:30:0.1)	—	—	—	—	6.5		—	—	[12]
6	ACN: DEA buffer (50:50)	20-200 µg/mL	0.996	—	—	5.79		1.05	—	[59]
7	ACN: Water (60:40, pH 3)	87-232µg/ml	0.999	—	—	3.90		1.08	—	[60]
8	MeOH: ACN: Water (70:10:20)	80-400 µg/mL	0.999	—	—	8.10		1.15	—	[61]
9	TEA: ACN (60:40, pH 5.8)	30-70µg/ml	0.999	—	—	2.7	943810	1.58	4578	[62]
10	OPA: Methanol (25:75)	6-80 µg/mL	—	—	—	7.34		1.12	—	[63]

Table S2: Experimental plan of Box-Behnken showing factors with levels

Factors	Level used, actual (Coded)		
	Low (-1)	**Medium (0)**	**High (+1)**
A (pH)	2	2.5	3
B (Flow Rate)	0.7	1.2	1.7
C (Mobile Phase Composition)	65	70	75

Table S3: Surface optimization model (Box –Behnken) with three Critical method parameters viz Mobile Phase Composition, Mobile Phase pH and, Mobile Phase flow rate. And four different response factors such HPLC area, Tailing factor, number of theoretical plate (NTP) and retention time (RT). Total Runs=13

Run		Factors			Response			
Std	Run	A (pH)	B (Flow Rate mL/min.)	C (Mobile Phase Ratio)	R1 (Peak Area)	R2 (Tailing Factor)	R3 (NTP)	R4 (RT min.)
1	11	2.5	1.2	70	1740367	1.124	13344	4.5
2	1	1.5	0.7	70	3822428	1.13	14043	9.14
3	4	1.5	1.7	60	1737444	1.052	19945	10.24
4	6	1.5	1.2	80	1735090	1.22	10356	2.45
5	3	3.5	1.2	70	1238943	1.12	10768	2.96
6	2	3.5	0.7	70	2912120	1.127	17867	6.97
7	5	3.5	1.2	60	1739641	1.052	19616	9.78
8	8	2.5	0.7	60	2900391	1.065	2446	5.207
9	12	2.5	1.2	70	1739795	1.12	13156	4.07
10	7	3.5	1.2	80	1772641	1.22	12066	2.6
11	10	2.5	1.7	70	1552448	1.134	13142	4.61
12	9	2.5	1.7	60	1239159	1.05	16379	7.05
13	13	2.5	1.2	70	1736737	1.129	12841	4.52

Table S4: System suitability analysis of Fenofibrate (n=6)

#	Parameter	Result
1	Average area	1684911
2	% RSD	0.23944
3	Tailing factor	1.12
4	Number of theoretical plates	13344
5	Retention time	4.5 min

Table S5: Linearity, Limit of detection (LOD) and limit of quantification (LOQ)

#	Compound	LOD	LOQ	Curve Equation	Regression Coefficient (R^2)
1	Fenofibrate	0.0438 µg/mL	0.132 µg/mL	y = 61872x + 2921.5	0.999

Table S6: The robustness of developed RP_HPLC method for analysis of Fenofibrate: Variations in flow rate, column oven temperature, mobile phase composition and pH of mobile phase showed little effect on accuracy and other Critical Quality Attributes i.e. NTPs, RT and Tailing Factor of the method.

Robustness				
Parameter	**Number of Theoretical Plates**	**Retention Time (min.)**	**Tailing factor**	**% Recovery of Sample**
Flow rate 1.2±0.2	. 13156±46	4.2. ±0.05	1.05± 0.02	99.6±08
Mobile phase composition (organic phase 70±5)	12066±272	3.96±0.02	1.22±0.6	100.2±1.1
pH (2.5±0.2)	16379±113	4.81±.03	0.85±07	97.7±2.2
Column oven Temperature (40±5^{0}C)	11345±165	4.86±0.01	1.16±0.4	99.2±1.2

Table S7: Formula table for 5 selected gel-matrix sustained release tablet formulations of Fenofibrate. Quantities of optimized formula is given in bold font

#	INGREDIENT	F1	F2	F3	F4	F5
1	Fenofibrate	145	145	**145**	145	145
2	HPMC	160	180	**-**	-	-
3	HPMC K4	120	-	**165**	150	176
4	HPMC K100M	-	120	**155**	170	166
5	PVP K30	75.5	86.5	**65.5**	60.5	65.2
6	MC	12.2	13.2	**11.4**	12.4	11.0
7	Talc	13.3	12.3	**13.4**	11.5	13.2
8	Lactose	160	161	**140.4**	145.6	125.2
9	Xantham gum	144	148	**138.1**	140.3	130.3
10	Microcrystalline cellulose	36	-	**32.2**	30.7	34.1
11	IPA	Q. s	Q. s	**Q. s**	Q. s	Q. s
Total wt. in mg		866	866	**866**	866	866

Table S8: Precompression evaluation of tablet granulation for gel-matrix SR tablet formulation of fenofibrate

#	Tapped density(g/ml)	Bulk density(g/ml)	Angle of repose (0^{0})	Carr's ratio (%)	Harris's ratio	Blend uniformity (%)
F1	0.98	0.39	23..96	28.07	1.34	97.7±1.2
F2	0.91	0.45	24.32	29.04	1.56	99.7±1.8
F3	**0.89**	**0.37**	**22.76**	**30.07**	**1.09**	**98.2±1.1**
F4	1.08	0.42	23.34	31.01	1.31	98.4±1.4
F5	0.97	0.41	24.56	29.11	1.11	99.1±1.9

Table S9: Post-compression characterization of developed gel-matrix sustained release tablets

Formulation	Hardness (kg/cm^2)	Thickness (mm)	Friability	Content uniformity (%)
F1	21	6.1	00.034	98.4±0.99
F2	22	6.0	0.0173	99.2±2.5
F3	**20**	**5.97**	**0.033**	**98.6±0.5**
F4	22	6.3	0.043	99.4±4.4
F5	21	6.0	0.041	99.0±4.5

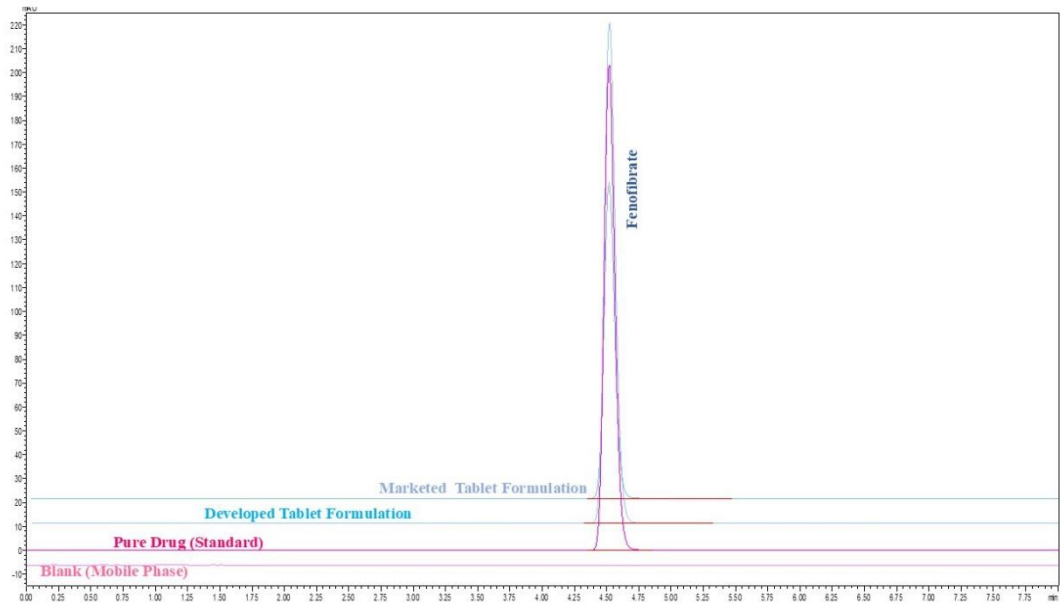


Figure S1: Specificity study of the developed RP-HPLC method: chromatograms of blank (mobile phase), standard (pure drug/fenofibrate), marketed formulation, and Developed tablet formulation (test sample) solutions showing absence of interference at the retention time of fenofibrate.

Figure S2: Peak purity analysis of fenofibrate using PDA detection showing spectral homogeneity across the chromatographic peak

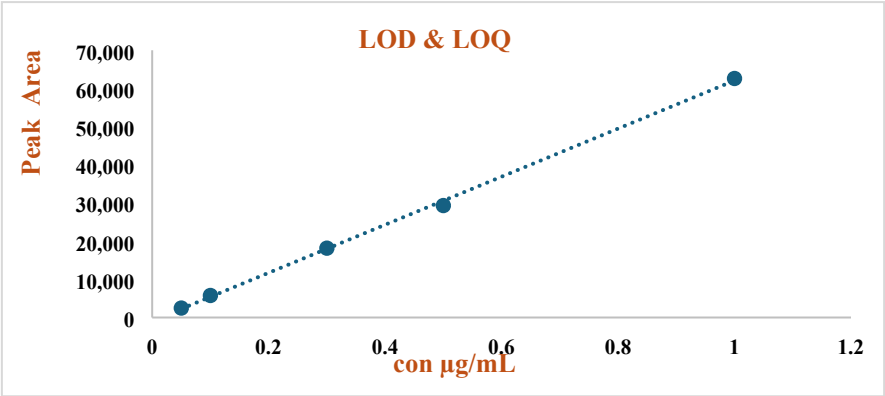


Figure S1/S3: LOD and LOQ of Fenofibrate

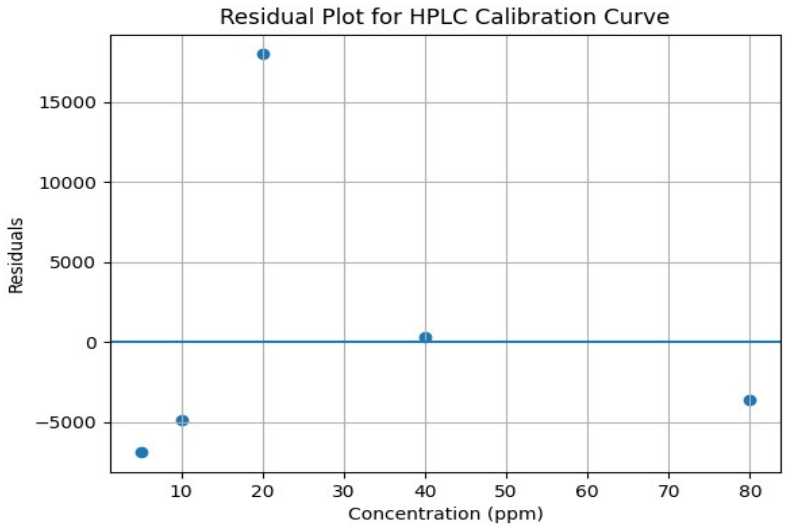
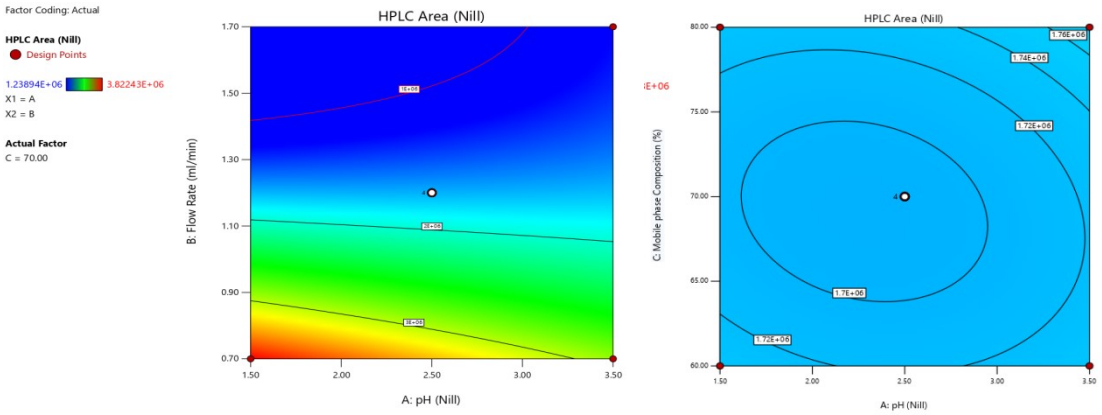
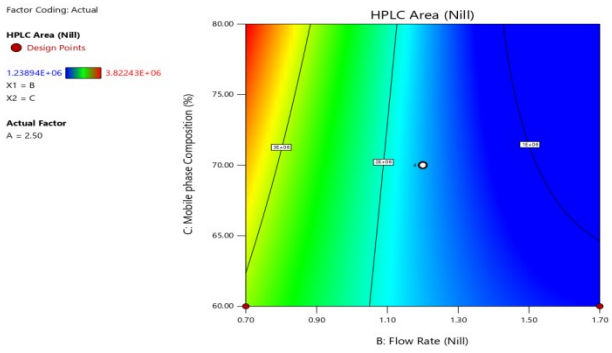


Figure S4: Random distribution of residuals across line Zero indicates that the developed RP-HPLC method for estimation of Fenofibrate shows no bias



3D **3E**



3F

Figure S5(D,E, F): Effect of pH, Flow rate, and mobile phase composition on Peak Area. The plot depicts a decrease in HPLC peak area with an increasing flow rate, while the intermediate pH values provide optimal response (3d.), a curvilinear relationship with optimal response is observed at intermediate levels of the pH and mobile phase composition (3e), and a decrease in peak area at higher flow rates and organic composition (3f). **The mobile phase composition (70:30), flow rate (1.2ml/min), and pH (2.5) are kept constant in d, e, and f scenario respectively.

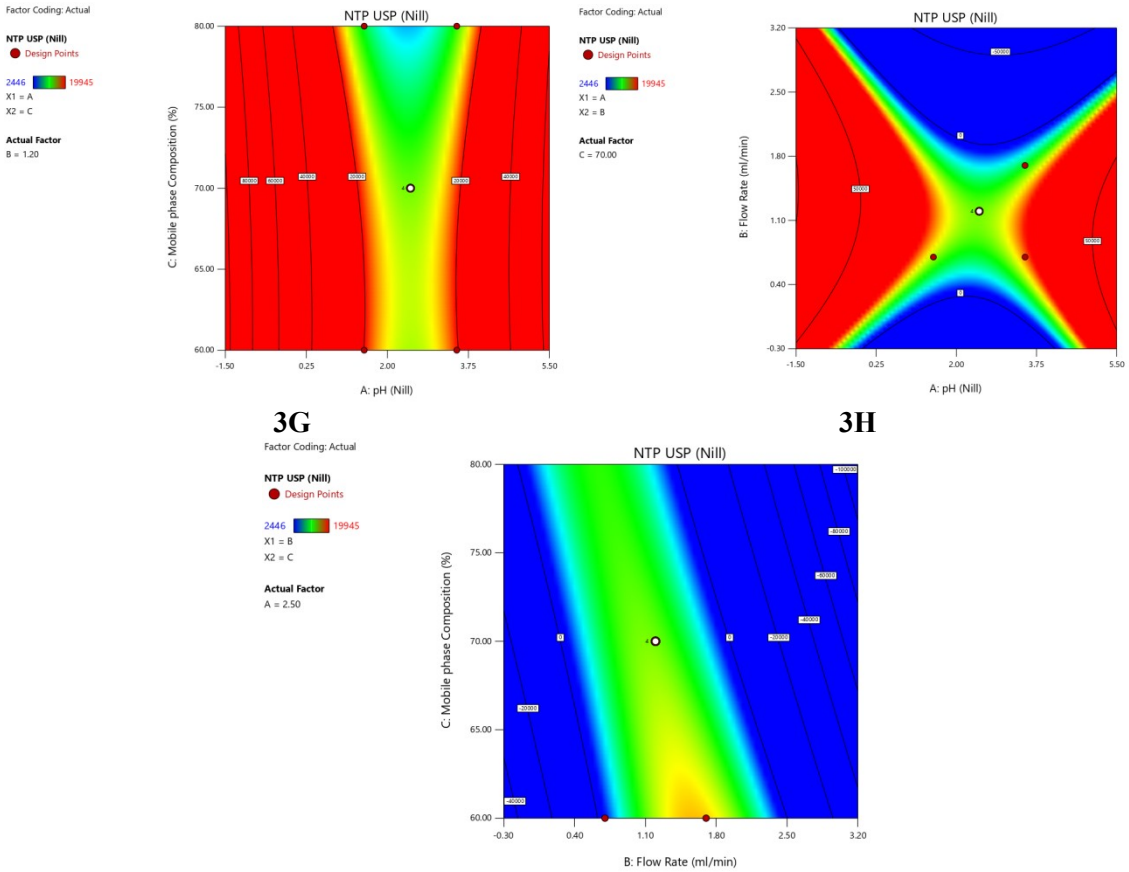
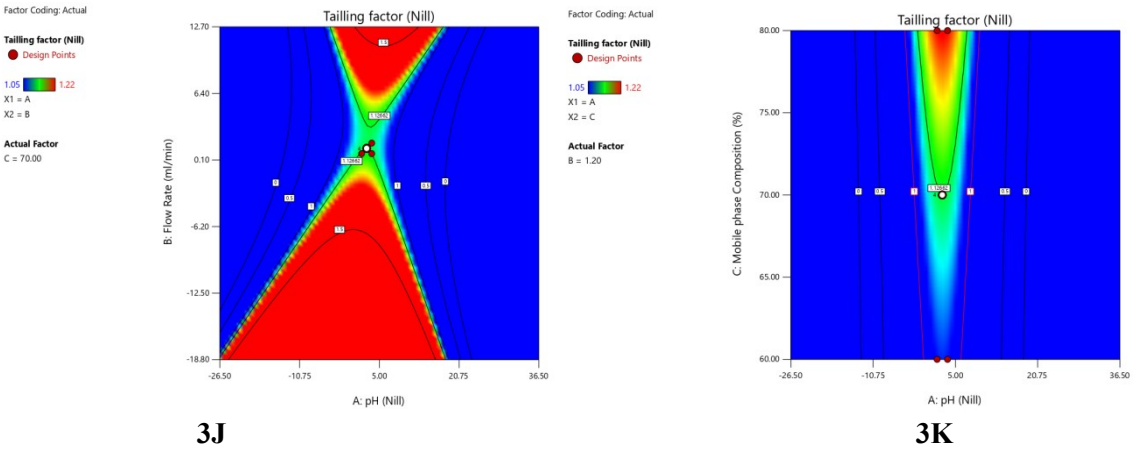
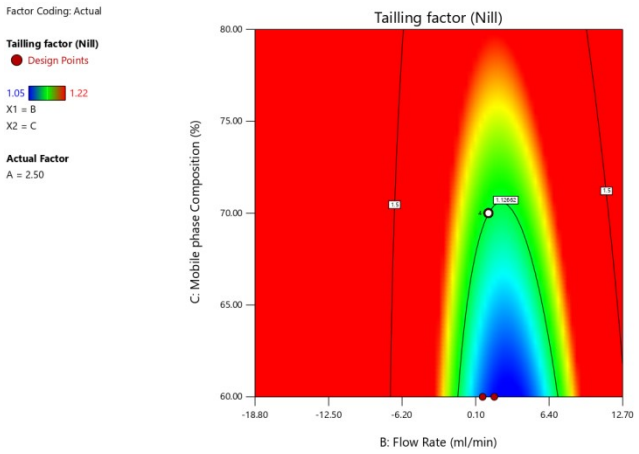


Figure S5 (G, H, I): Effect of pH, Flow rate, and mobile phase composition on number of theoretical plates (NTP). plot depicts a reduced NTP at higher flow rates, while moderate pH values favours improved efficiency (3g), an improved NTP at optimum composition and moderate pH (3h), and a decrease in NTP at higher flow rates and organic phase (3i).***The flow rate (1.2ml/min), mobile phase composition (70:30), and pH (2.5) are kept constant in scenarios of g, h, and i respectively.





3L

Figure S5 (J,K,L): Effect of pH, Flow rate, and mobile phase composition on tailing factor. The plot highlights minimum variation with a slight lowering of tailing at moderate flow rate (3j), an enhanced tailing at higher pH and organic phase composition (3k), and reduced tailing at higher flow rates and moderate composition (3l). ****The mobile phase composition (70:30), pH (2.5), and flow rate (1.2ml/min), are kept constant in j, k, and l scenarios respectively

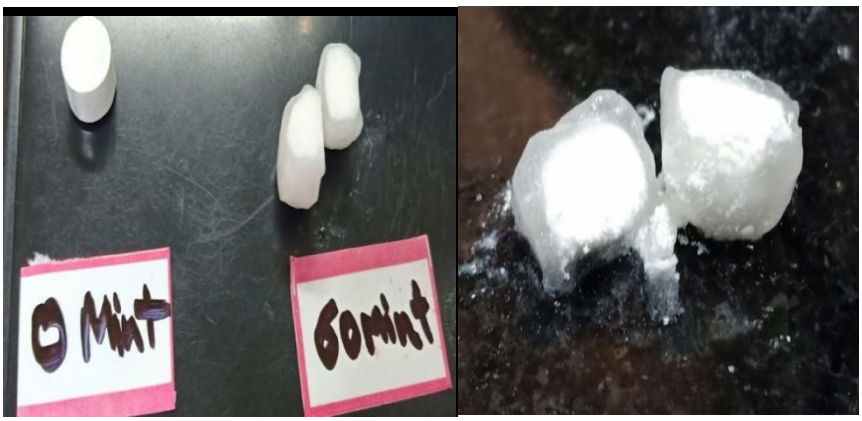


Figure S6: Jelly matrix formation after immersion of developed gel-matrix SR tablet formulation of fenofibrate (F3) in 40% alcohol