

Development of a quality by design based hybrid RP-HPLC method for Glimepiride: Bioanalytical and industrial applications

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Supplementary material

Design-Expert® Software
Factor Coding: Actual
Original Scale
Overlay Plot

HPLC Area
HPLC Hight
Number of Theoretical Plate
● Design Points

X1 = A: Flow Rate
X2 = B: Mobile Phase pH

Actual Factor
C: Column Oven Temp. = 40.00

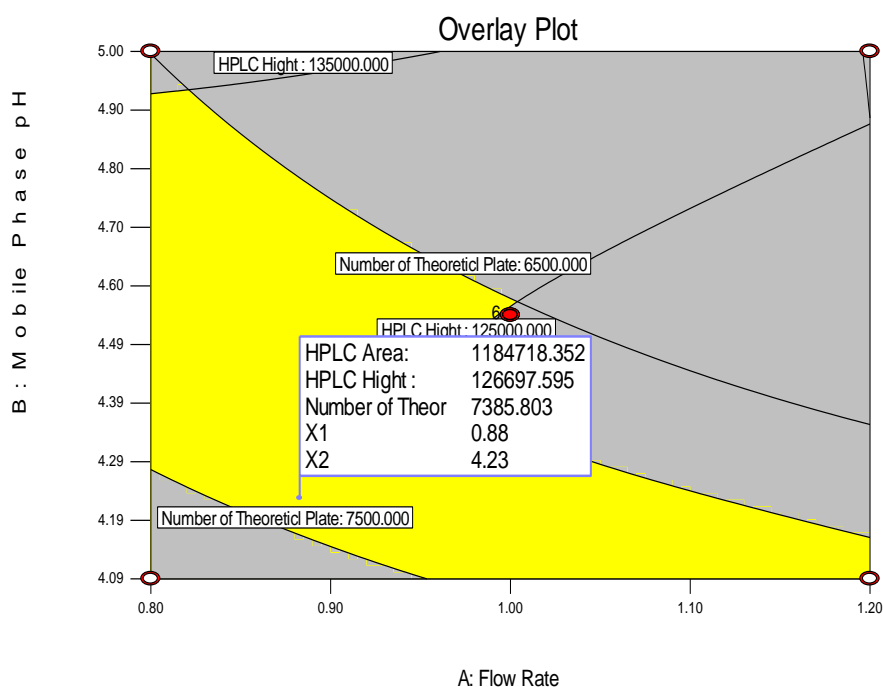


Figure S1: Graph (B) displays an overlay plot of predicted results for the chosen method with a flow rate (X1) of 0.88 mL/minute, mobile phase pH (X2) of 4.80, and an actual factor C of 40.2°C for the column oven temperature. The projected response factor is presented in the center of the flag, featuring HPLC area, height, and the number of theoretical plates.

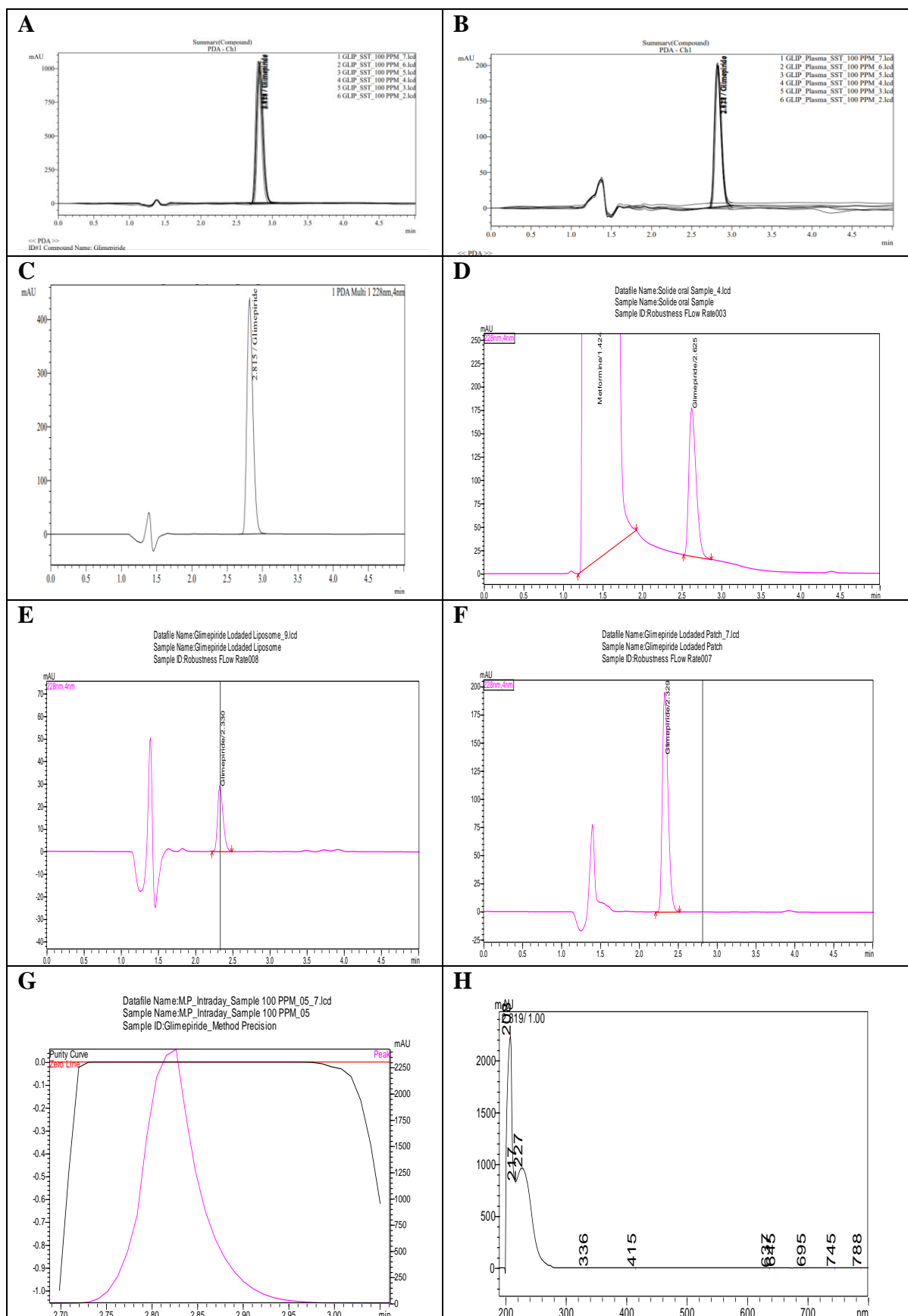


Figure S2: Here's a representative chromatogram of various dosage forms: (A) System suitability chromatogram of glimepiride in diluent media. (B) System suitability chromatogram of glimepiride in rat plasma. (C) Standard chromatogram with pure glimepiride. (D) Marketed

tablet containing metformin and glimepiride. (E) Liposomal formulation bearing glimepiride. (F) Transdermal patch loaded with glimepiride as the active drug. (G) Peak purity graph of glimepiride, showing a purity angle less than the purity threshold. (H) HPLC PDA-detector graph between 200 to 800 nm with the lambda max (λ) set at 227 nm.

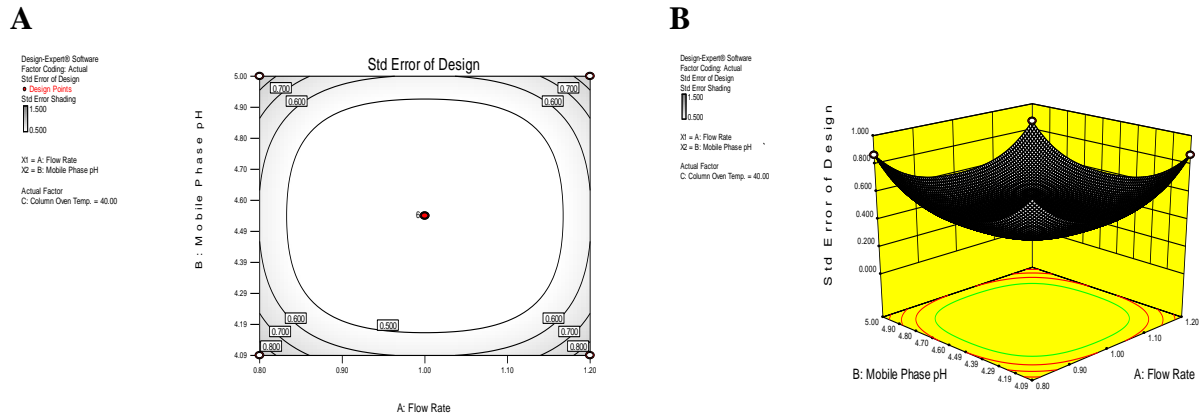




Figure S3 Box–Behnken designs standard error experiment design, the directed graph in (A) counter view and (B) 3 D view. The experiment was designed at three levels of factor X1- flow rat X2- mobile phase pH and actual factor C: column oven temperature.

A	B
 <p style="text-align: center;">CSIR- Central Drug Research Institute Pharmaceutics Division (PCS-004)</p> <p style="text-align: center;">Instrument Method Report</p> <p style="text-align: center;">Method</p> <p><<Header>> Generated : 19-08-2020 18:18:30 GeneratedBy : abhiram kumar Modified : 19-08-2020 18:18:31 ModifiedBy : abhiram kumar</p> <p><<System Controller>> Model : CBM-20Alite Event1 : On Event2 : On Sample Load Timing : Automatic Sample Loading Overlap Time : 0.00 min</p> <p><<Data Acquisition>> LC Stop Time : 10.00 min PDA Detector Name : PDA PDA Sampling Frequency : 1.5625 Hz PDA Start Time : 0.00 min PDA End Time : 10.00 min PDA Time Constant : 0.640 sec</p> <p><<Pump>> Mode : Isocratic flow Pump A : LC-20AD Pump B : LC-20AD Flow/Pressure : Flow Pump A Flow : 0.5000 mL/min Pump B Flow : 0.5000 mL/min Pump A PressMax : 35.0 MPa Pump A PressMin : 0.0 MPa Pump B PressMax : 35.0 MPa Pump B PressMin : 0.0 MPa</p>	 <p style="text-align: center;">CSIR- Central Drug Research Institute Pharmaceutics Division (PCS-004)</p> <p style="text-align: center;">Instrument Method Report</p> <p style="text-align: center;">Method</p> <p><<Header>> Generated : 26-08-2020 19:04:58 GeneratedBy : abhiram kumar Modified : 26-08-2020 19:04:58 ModifiedBy : abhiram kumar</p> <p><<System Controller>> Model : CBM-20Alite Event1 : On Event2 : On Sample Load Timing : Off Sample Loading Overlap Time : 0.00 min</p> <p><<Data Acquisition>> LC Stop Time : 5.00 min PDA Detector Name : PDA PDA Sampling Frequency : 1.5625 Hz PDA Start Time : 0.00 min PDA End Time : 5.00 min PDA Time Constant : 0.640 sec</p> <p><<Pump>> Mode : Isocratic flow Pump A : LC-20AD Pump B : LC-20AD Flow/Pressure : Flow Pump A Flow : 0.5280 mL/min Pump B Flow : 0.3520 mL/min Pump A PressMax : 35.0 MPa Pump A PressMin : 0.0 MPa Pump B PressMax : 35.0 MPa Pump B PressMin : 0.0 MPa</p>
C	

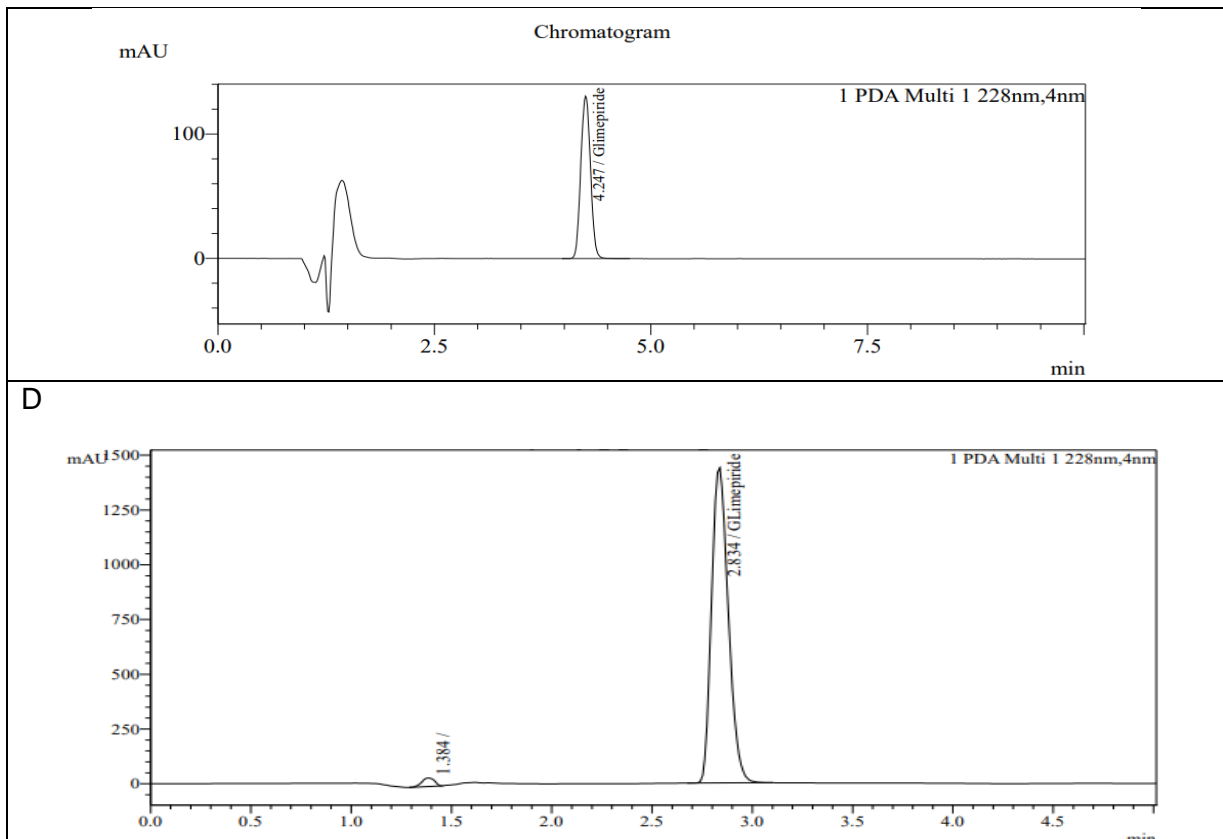


Figure S4: The depicted HPLC instrument methods and chromatograms provide meaningful results. (A) represents the standard conventional method with a flow rate of 1 mL/min and a retention time of 10 minutes. (B) shows the optimized HPLC method using the QbD (Quality by Design) approach, with a flow rate of 0.88 mL/min. The impact of the optimized method is illustrated in the HPLC chromatograms, showing differences in analyte retention time and overall runtime. (C) displays the chromatogram recorded using the conventional method, while (D) represents the chromatogram from the QbD-based method. In conclusion, the optimized method demonstrated better chromatographic performance and reduced runtime, making it more cost-effective for industrial-scale applications.

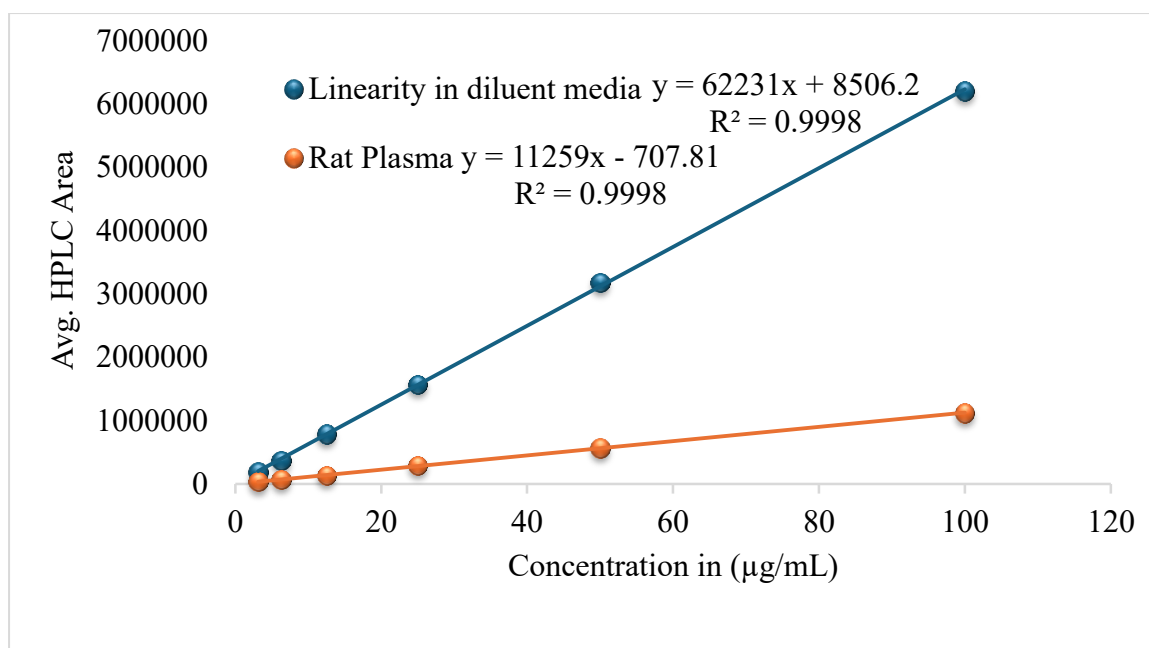


Figure S5 The linearity of the glimepiride graph was confirmed across a concentration range of 3.12 to 100 µg/mL using six different concentrations. The experiment was replicated three times (n=3) for both the diluent media and rat plasma. Consequently, the coefficient of determination (R^2) was found to be 0.999 for both sample types.

Table

Table S1: (a) Box-Behnken surface model for optimization of response factor: flow rate, column oven temperature, and mobile phase pH, (b) System suitability of glimepiride in diluent media and mouse plasma.

(a) Box-Behnken (Factors)					
Parameters	Standard	Robust %	±	High +	Low -
Flow Rate (mL/min)	1	20	0.2	1.2	0.8
Column Oven Temperature (°C)	40	20	8	48	32
Mobile Phase pH	4.55	10	0.455	5.005	4.095
(b) System Suitability					
SST Parameters	USP Limits		Diluent Media	Mouse Plasma	
USP(NTP), n=6	NLT 2000		27812	30191	
Tailing Factor (n=6)	NMT 2		1.233	1.264	
% RSD (N=6)	NMT 2		0.165	0.480	
Retention time (RT)	± 10 % of main peak		2.8	2.8	

NLT: Not less than; NMT: Not more than,