

Advancing Relugolix Analysis: A Comparative Study and AQbD-Driven Method Optimization with Stability Testing

Priyanka Nagar^{1,2}, Arvind Kumar Sharma², Robin Kumar², Chhaya Chauhan³, Rini Singhal³, Minakshi Garg¹

¹Delhi Pharmaceutical Science and Research University, Pushp Vihar, New Delhi, 110017, India.

²Indian Pharmacopoeia Commission, Sector 23, Raj Nagar, Ghaziabad, Uttar Pradesh, 201002, India.

³Raj Kumar Goel Institute of Technology (Pharmacy), 5KM Stone Delhi, Meerut Road, Raj Nagar Extension Road, Ghaziabad, Uttar Pradesh, 201003, India.

Doi: <https://doi.org/10.7324/JAPS.2025.239585>

SUPPLEMENTARY MATERIAL

Table of Contents

Fig. S1 Trial HPLC chromatogram using C8 column

Fig. S2 The Marvin Sketch pKa and solubility graph of relugolix

Fig. S3 Trial HPLC Chromatogram of liquid solvent system by using Acetate buffer pH 7.7 and ACN

Fig. S4 Model graphs showing (a) the effect of all factors on tailing and (b) resolution response

Fig. S5 (a) Peak purity of relugolix (b) Peak purity of amine (c) Peak purity of oxo (d) Peak purity of carboxamide

Fig. S6 Chromatogram showing LOD of amine

Fig. S7 Chromatogram showing LOD of relugolix

Fig. S8 Chromatogram showing LOD of oxo

Fig. S9 Chromatogram showing LOD of carboxamide

Fig. S10 Chromatogram showing LOQ of amine

Fig. S11 Chromatogram showing LOQ of oxo

Fig. S12 Chromatogram showing LOQ of relugolix

Fig. S13 Chromatogram showing LOQ of carboxamide

Fig S14 (a) ^1H NMR Spectra of amine (b) ^{13}C NMR Spectra of amine (c) ^1H NMR Spectra of oxo (d) ^{13}C NMR Spectra of oxo (e) ^1H NMR Spectra of carboxamide (f) ^{13}C NMR Spectra of carboxamide

Fig S15 (a) LC-MS chromatogram of amine (b) LC-MS chromatogram of oxo (c) LC-MS chromatogram of carboxamide

Fig S16 (a) UV Spectra of relugolix (b) UV Spectra of amine (c) UV Spectra of oxo (d) UV Spectra of carboxamide

Table S1 Statistical parameters of the mathematical models obtained by screening **Table S2**

Table representing the results of method precision

Fig. S1 Trial HPLC chromatogram using C8 column

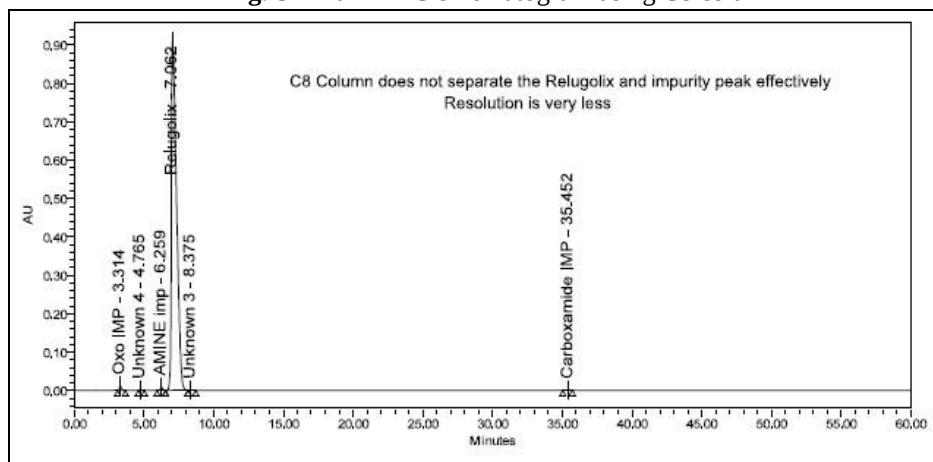


Fig S2 (a) The Marvin Sketch pKa and (b) solubility graph of relugolix

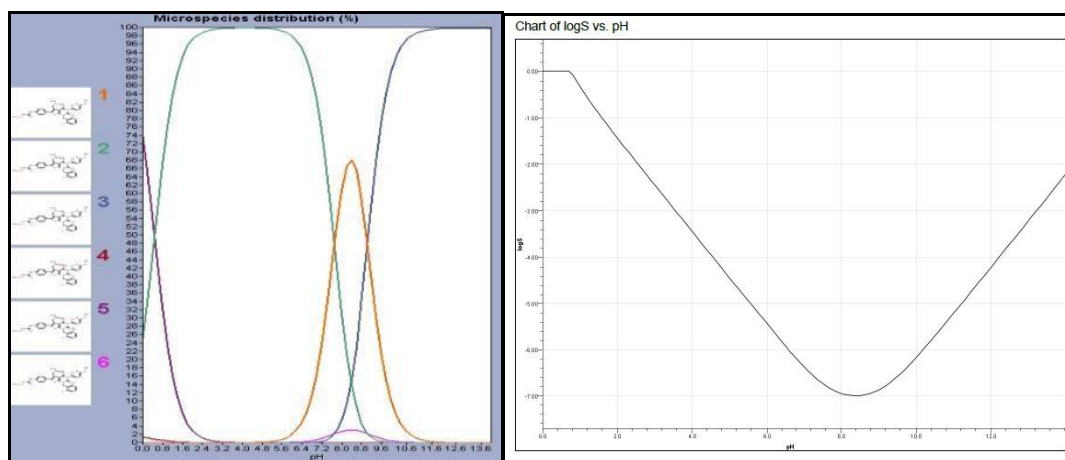


Fig. S3 Trial HPLC Chromatogram of liquid solvent system by using Acetate buffer pH 7.7 and ACN

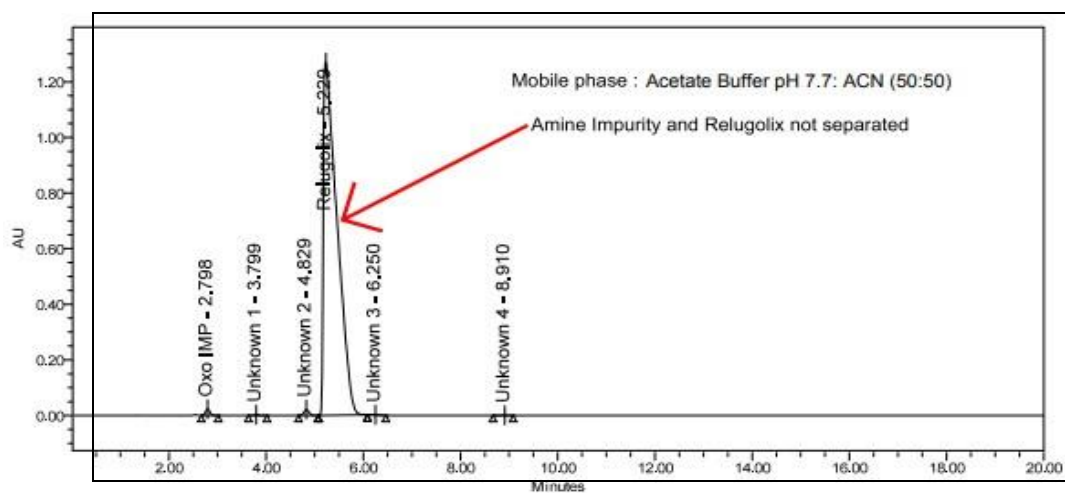


Fig. S4 Model graphs showing (a) the effect of all factors on tailing and (b) resolution response

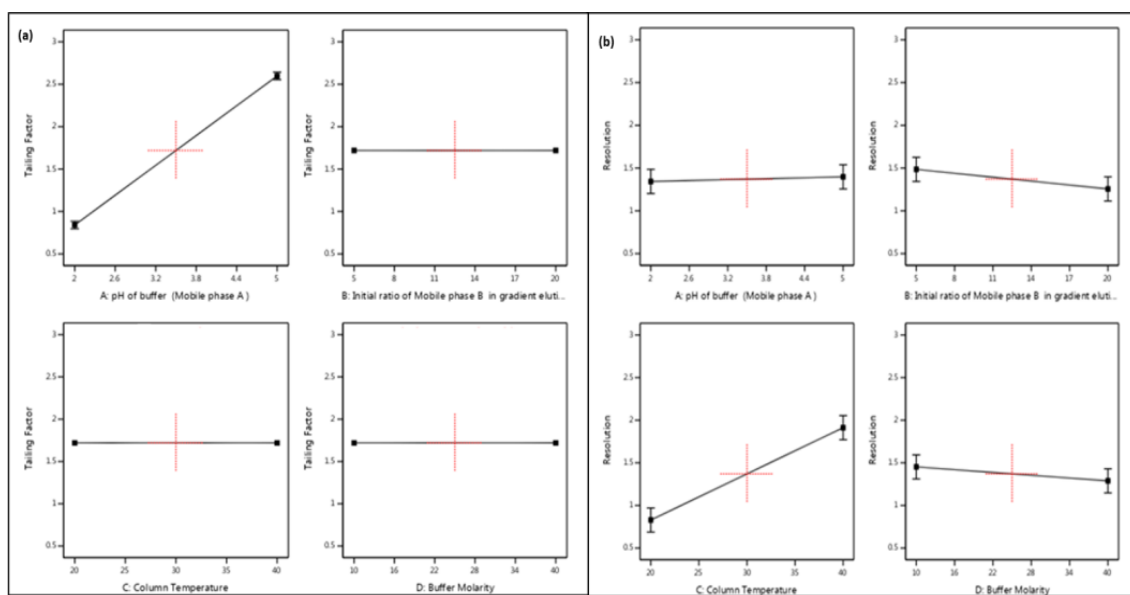


Fig. S5 (a) Peak purity of relugolix (b) Peak purity of amine (c) Peak purity of oxo (d) Peak purity of carboxamide

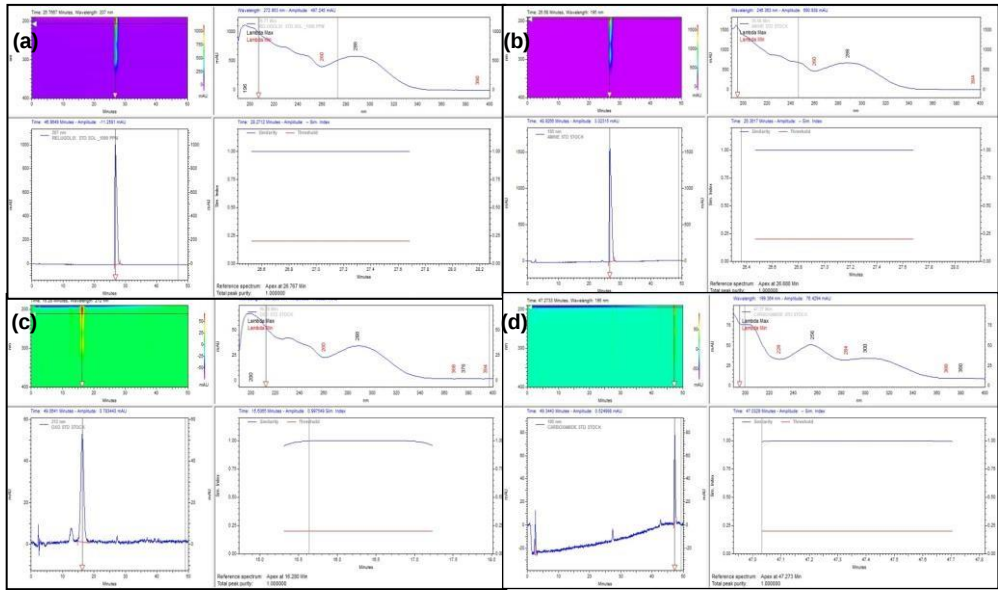


Fig. S6 Chromatogram showing LOD of amine related substance

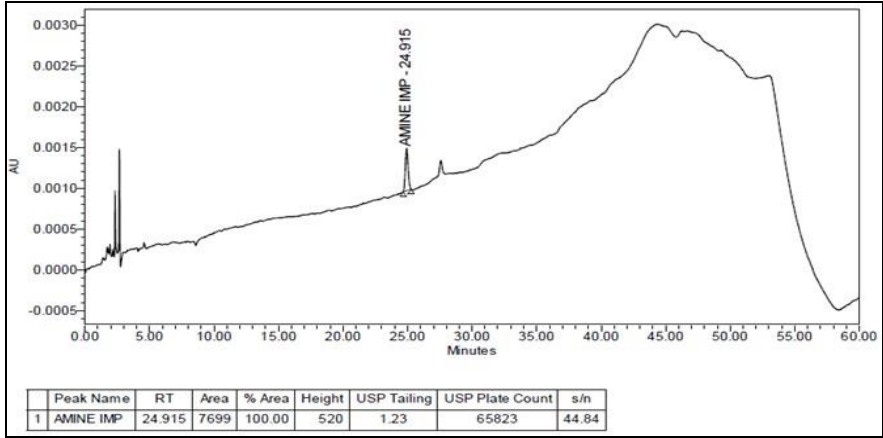


Fig. S7 Chromatogram showing LOD of relugolix

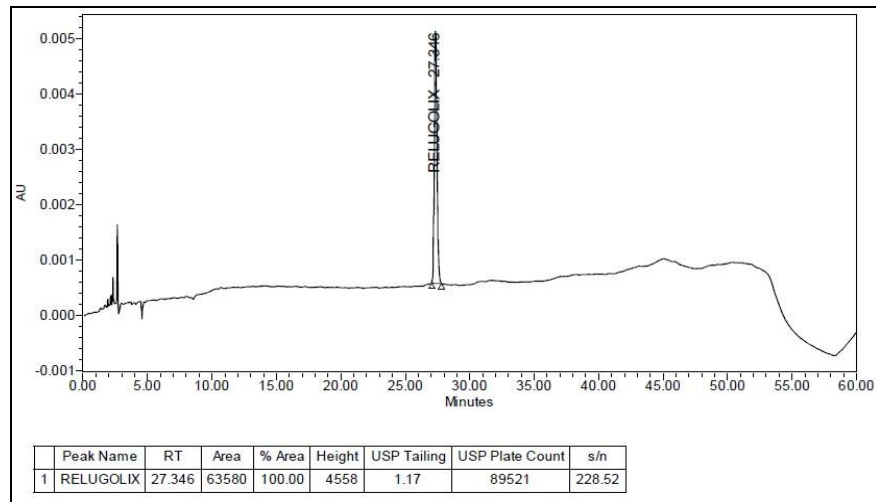


Fig. S8 Chromatogram showing LOD of OXO related substance

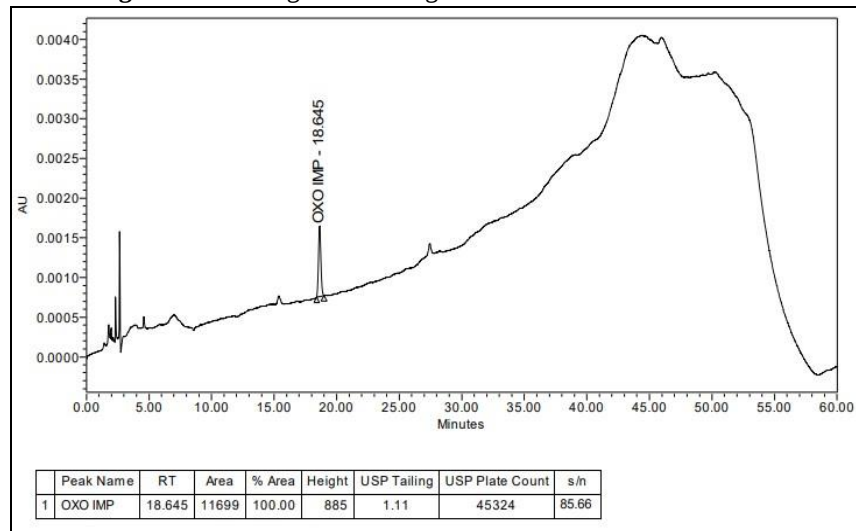


Fig. S9 Chromatogram showing LOD of carboxamide related substance

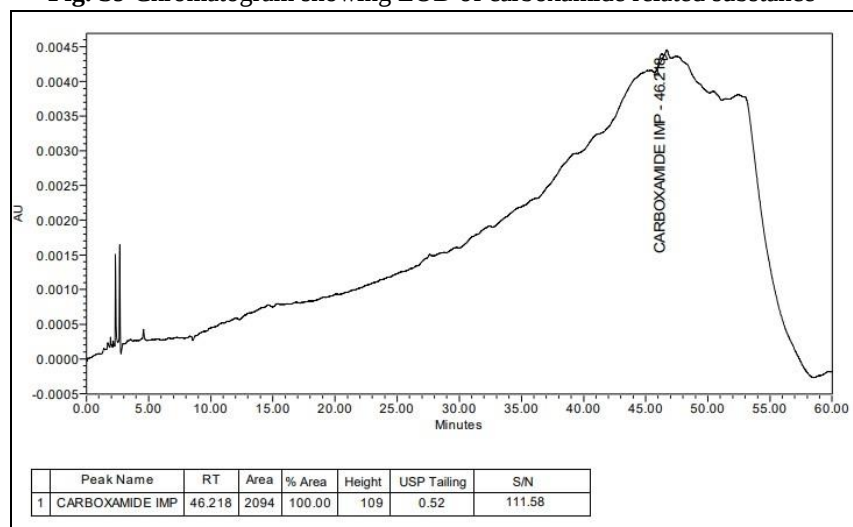


Fig. S10 Chromatogram showing LOQ of amine related substance

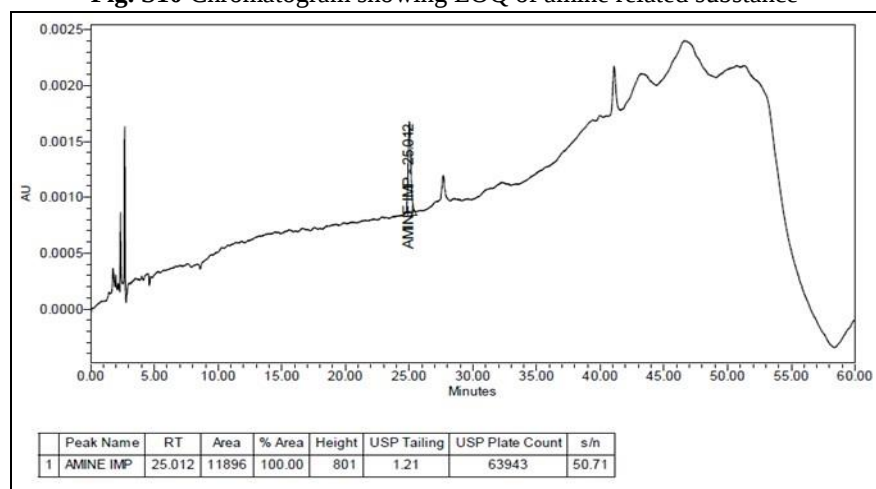


Fig. S11 Chromatogram showing LOQ of OXO related substance

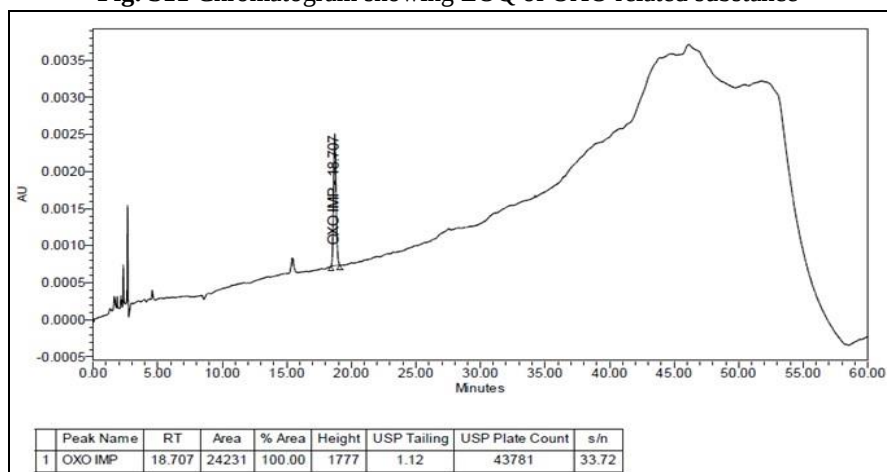


Fig. S12 Chromatogram showing LOQ of relugolix

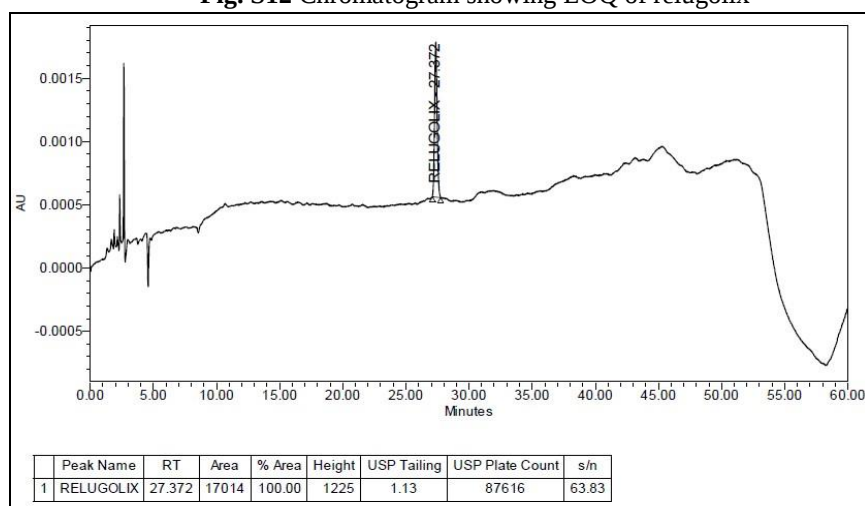


Fig. S13 Chromatogram showing LOQ of carboxamide related substance

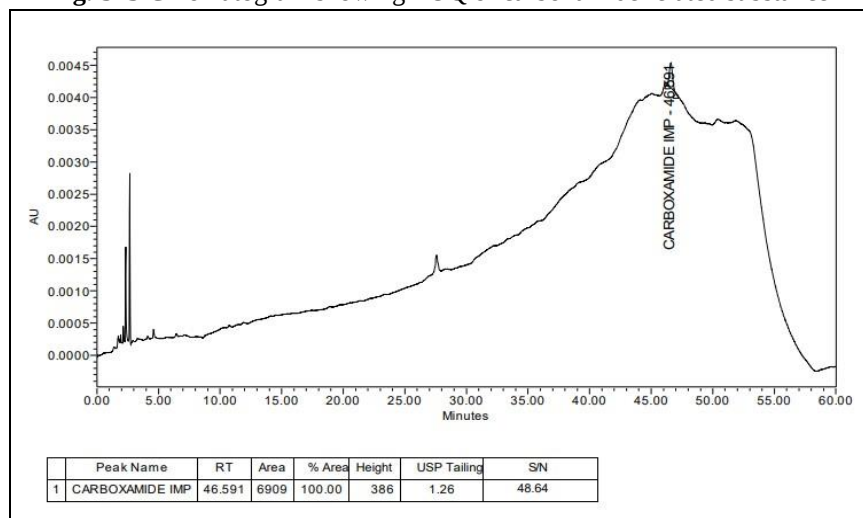


Fig S14 (a) ^1H NMR Spectra of amine (b) ^{13}C NMR Spectra of amine (c) ^1H NMR Spectra of oxo (d) ^{13}C NMR Spectra of oxo (e) ^1H NMR Spectra of carboxamide (f) ^{13}C NMR Spectra of carboxamide

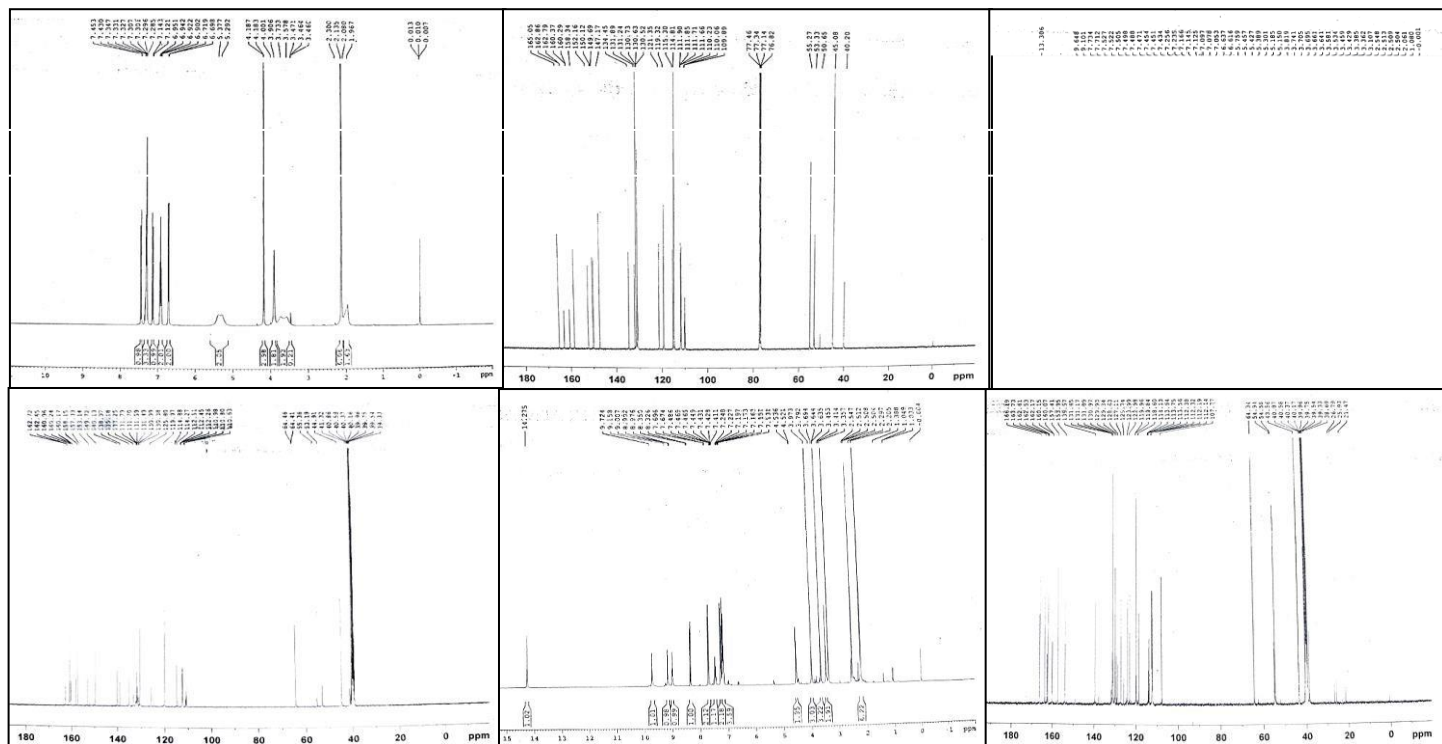


Fig S15 (a) LC-MS chromatogram of amine (b) LC-MS chromatogram of oxo (c) LC-MS chromatogram of carboxamide

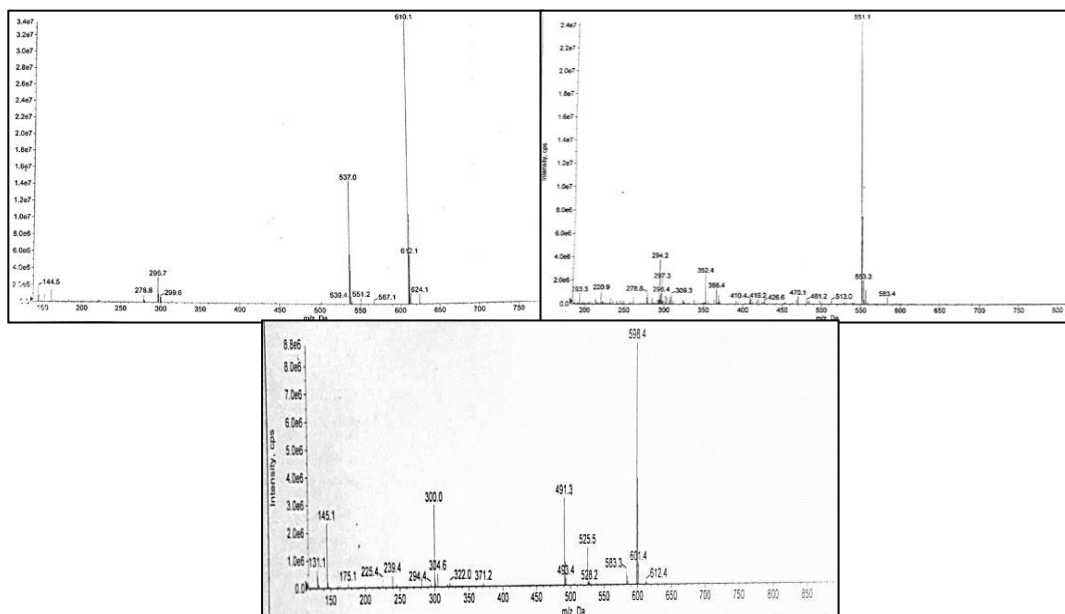


Fig S16 (a) UV Spectra of relugolix (b) UV Spectra of amine (c) UV Spectra of oxo (d) UV Spectra of carboxamide

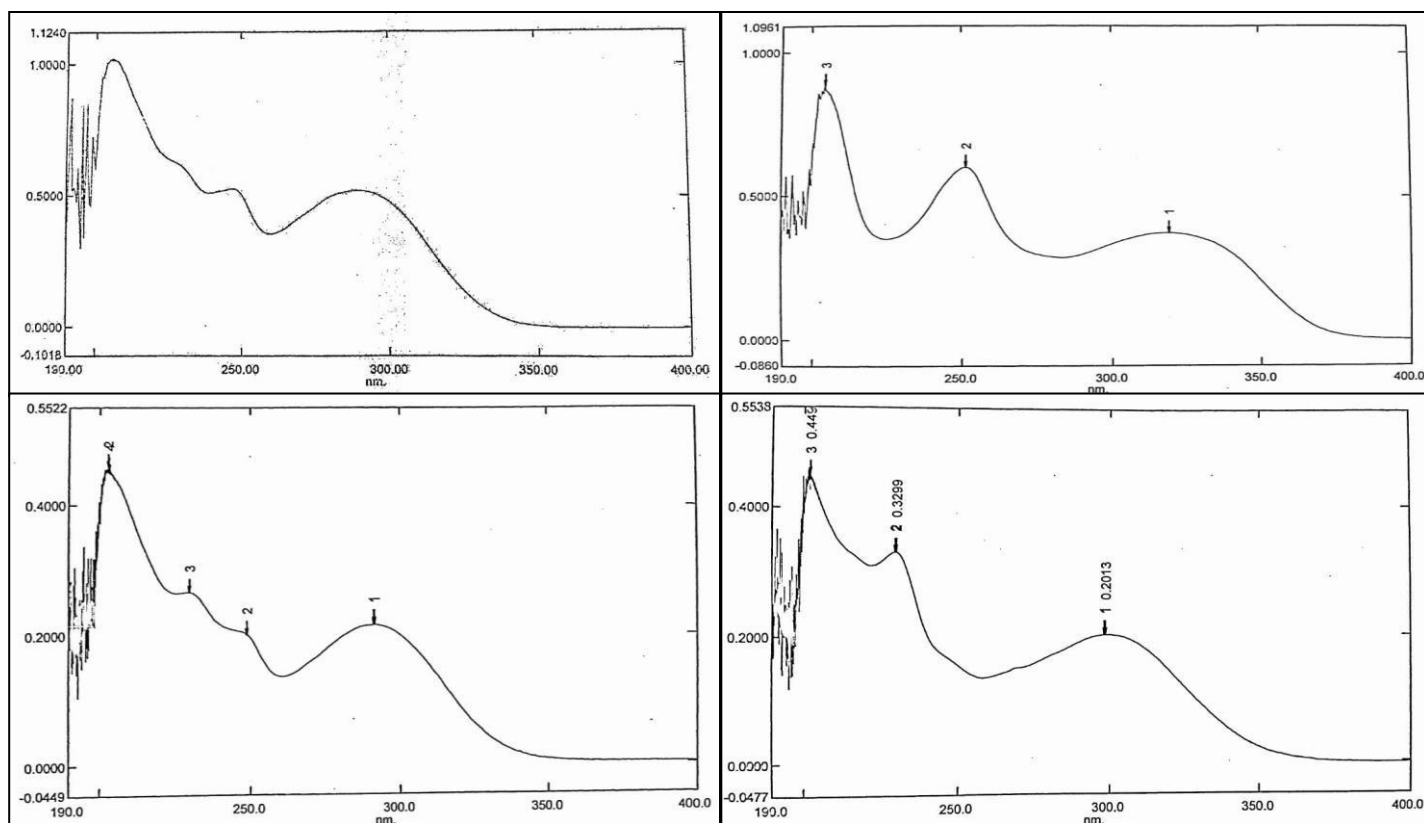


Table S1 Statistical parameters of the mathematical models obtained by screening

Responses	R^2 (coefficient of determination)	R^2 Adjusted (adjusted coefficient of determination)	Predicted R^2	P value (lack of fit)	Model F Value	Lack of fit
Tailing Factor	0.9924	0.9918	0.9901	0.0001	1825.15	Significant
Resolution between relugolix and amine	0.8743	0.8285	0.7340	0.0001	19.12	Significant

Table S2 Table representing the results of method precision

S. No.	Oxo	Amine	Carboxamide	Relugolix	Precision at LOQ			
					Relugolix	Oxo	Amine	Carboxamide
1.	330686	158539	68002	19662	16686	11896	24231	8325
2.	335977	159577	69074	20160	16870	11891	24436	6909.00
3.	335390	158764	69005	19969	16843	12511	24308	7970.00
4.	330437	156874	67982	20165	17014	11873	24648	6896.00
5.	333015	158659	68242	19860	17058	12097	24440	7546.00
6.	331275	157985	69377	19996	16874	12383	24621	7347.00
Average	332796.67	158399.67	68613.67	19968.67	16891	12109	24447	7499
SD	2417.68	906.10	609.75	190.66	132.72	277.64	165.42	572.91
% RSD	0.73	0.57	0.89	0.95	0.79	2.29	0.68	7.64