

QUALITY BY DESIGN-ASSISTED DEVELOPMENT AND VALIDATION OF A ROBUST RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF PRAMIPEXOLE AND RASAGILINE IN A SYNTHETIC MIXTURE

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ABSTRACT

Background: Simultaneous estimation of low-dose, extended-release combinations in pharmaceutical preparations requires highly robust, sensitive, and validated analytical methods. This study presents a systematic Quality by Design (QbD)-assisted reversed-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of Pramipexole (PRA) and Rasagiline (RAS) in a synthetic mixture.

Methods: A three-level factorial design utilizing Design-Expert software was employed to evaluate the impact of critical chromatographic factors-specifically the proportion of acetonitrile, flow rate, and column temperature-on analytical target profile responses including retention times, tailing factors, and resolution. Chromatographic separation was achieved on a Zorbax SB C18 column (250 × 4.6 mm, 5 μm) using a mobile phase consisting of acetonitrile and 0.1% ortho-phosphoric acid in water at an optimized flow rate of 0.5 mL/min and a detection wavelength of 210 nm.

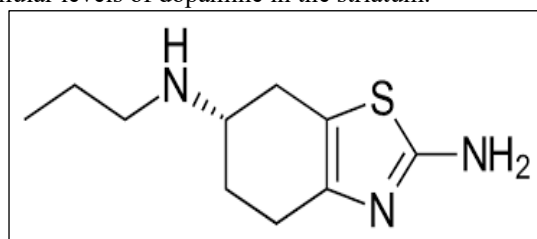
Results: Under optimized conditions, sharp, well-resolved peaks were obtained with retention times of 5.48 min for Pramipexole and 11.15 min for Rasagiline, and a resolution of 15.52. The method was validated in accordance with International Council for Harmonisation (ICH) guidelines, showing excellent linearity ($R^2 > 0.999$), high accuracy (mean recovery between 99.51% and 99.83%), and precision (RSD < 2%). Furthermore, forced degradation studies under acid, base, oxidative, and thermal stress conditions demonstrated the stability-indicating power of the method, effectively resolving intact drugs from degradation products.

Conclusion: The developed QbD-assisted RP-HPLC method is reliable, accurate, and suitable for routine quality control and stability testing of Pramipexole and Rasagiline pharmaceutical formulations.

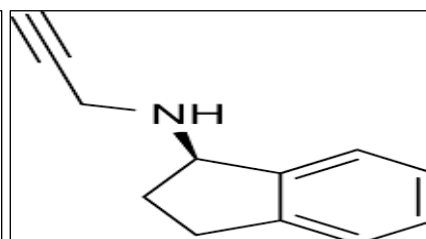
Keywords: *Quality by Design (QbD); RP-HPLC; Pramipexole; Rasagiline; Method validation; Forced degradation*

INTRODUCTION

Pramipexole is a (6S)-N6-propyl-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine. It is a drug used to treat the symptoms of Parkinson's Disease (PD). It is a non-ergot dopamine agonist drug that is efficacious in treating various Parkinson's symptoms such as tremor, rigidity, and bradykinesia. Pramipexole shows specificity and strong activity at the D2 subfamily of dopamine receptors in vitro, binding selectively and D2 receptors and showing a preference for the D3 receptor subtype rather than other subtypes. Rasagiline is a (1R)-N-(prop-2-yn-1-yl)-2,3-dihydro-1H-inden-1-amine. Rasagiline is an indane that consists of 1-aminoindane bearing an N-propargyl substituent. A selective, irreversible monoamine oxidase-B inhibitor. It has a role as an EC 1.4.3.4 (monoamine oxidase) inhibitor and a neuroprotective agent. MAO-B inhibitory activity, which causes an increase in extracellular levels of dopamine in the striatum.



Structure of Pramipexole



Structure of Rasagiline

OBJECTIVE

The combination is a low-dose, extended-release therapy (known as P2B001) to treat early Parkinson's disease with improved motor control and fewer side effects. The adoption of the Quality by Design (QbD) approach aligns

with the latest regulatory standards in method development, which focus on minimizing risks to quality. Therefore, the goal was to develop a QbD-based reverse phase high-performance liquid chromatographic (RP-HPLC) method for the combination in its synthetic mixture. There are no reported methods by QbD for both combination as a synthetic mixture. So, the motivation is to develop, validate and study the forced degradation of the combination.

EXPERIMENTAL WORK

Method development by HPLC

Preparation of standard stock solution

Preparation of standard stock solution of PRA

- Master Stock Solution: Accurately weighed 60 mg PRA dissolved in 100 ml acetonitrile ($600 \mu\text{g}.\text{ml}^{-1}$). Withdraw 1.0 ml from above Solution and make up to 10 ml with methyl acetonitrile ($60 \mu\text{g}.\text{ml}^{-1}$).
- Standard Solution: Withdraw 1.0 ml from Master Stock Solution and make up to 10 ml with acetonitrile ($6.0 \mu\text{g}.\text{ml}^{-1}$).

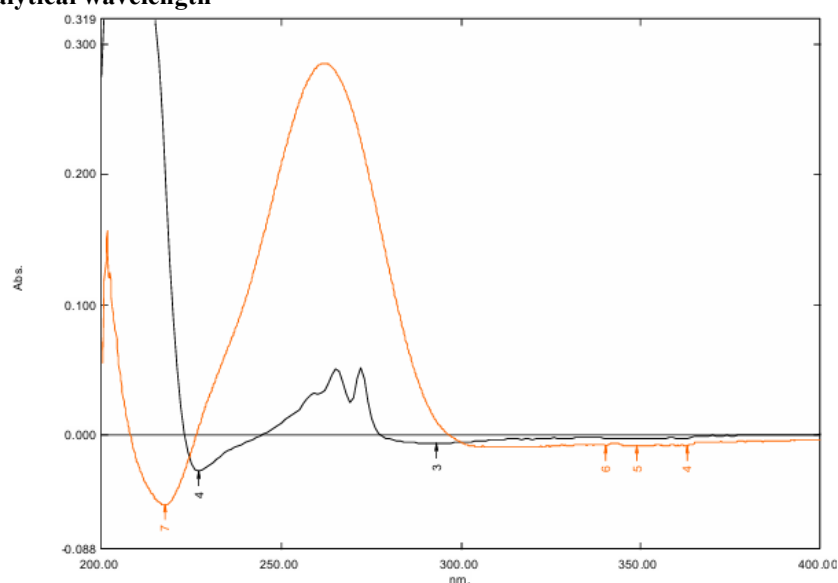
Preparation of standard stock solution of RAS

- Master Stock Solution: Accurately weighed 75 mg RAS dissolved in 100 ml acetonitrile ($750 \mu\text{g}.\text{ml}^{-1}$). Withdraw 1.0 ml from above Solution and make up to 10 ml with acetonitrile ($75 \mu\text{g}.\text{ml}^{-1}$).
- Standard Solution: Withdraw 1.0 ml from Master Stock Solution and make up to 10 ml with acetonitrile ($7.5 \mu\text{g}.\text{ml}^{-1}$).

Preparation of standard stock solution of mixture

- Master Stock Solution: Weigh accurately about 60 mg of PRA and 75 mg of RAS and transfer into a 100 ml volumetric flask. Make up the volume of the flask to the mark with acetonitrile. ($600 \mu\text{g}/\text{ml}$ PRA + $750 \mu\text{g}/\text{ml}$ RAS). Transfer 1.0 ml of this solution to 10 ml volumetric flask, and make up the volume with acetonitrile, resulting solution gives $60 \mu\text{g}/\text{ml}$ PRA + $75 \mu\text{g}/\text{ml}$ RAS.
- Standard Solution: 1.0 ml from Stock Solution and make up to 10 mL with acetonitrile (PRA+RAS = $6.0+7.5 \mu\text{g}.\text{ml}^{-1}$).

Selection of analytical wavelength



UV Spectra of PRA $10 \mu\text{g}.\text{ml}^{-1}$ and RAS $10 \mu\text{g}.\text{ml}^{-1}$ were recorded and overlapped. Iso absorptive point was found at 226 nm, but absorbance of both the drug was significantly lower on this point. It was observed that, significant absorbance observed at 210 nm, it was selected as analytical wavelength for further trials.

Selected factors based on Trial and Error for generation of design (Design Space)

- **Factor 1:** Proportion of Acetonitrile
- **Factor 2:** Flow rate of mobile phase
- **Factor 3:** Temperature

Effect of all the parameters will be checked on (Analytical Target Profile)

- Resolution
- Retention Time
- Tailing Factor

By taking above 3 factors in consideration, 3 Level Factorial design has been created by Design Expert 10 software.

Design Summary

File Version: 10.0.1.0

Study Type: Response Surface

Subtype: Randomized

Design Type: 3 Level Factorial

Runs: 27

Design Model: Quadratic

Blocks: No Blocks

Build Time (ms): 5.00

Factor	Name	Units	Type	Subtype	Minimum	Maximum	Code	Values	Mean
A	Proportion of CAN	ml	Numeric	Continuous	20	30	- 1.000 = 20	1.000 = 30	25
B	Flow Rate	ml/min	Numeric	Continuous	0.5	0.7	- 1.000 = 0.5	1.000 = 0.7	0.6
C	Temperature	°C	Numeric	Continuous	20	30	- 1.000 = 20	1.000 = 30	25

AQbD Design data set for (PRA+RAS = 6+7.5 µg/ml)

Run	Factor 1 Proportion of CAN	Factor 2 Flow Rate	Factor 3 Temperature	Rt of PRA	Rt of RAS	Tf of PRA	Tf of RAS	Resolution
1	25	0.6	20	3.59	7.24	1.88	1.48	11.77
2	20	0.5	30	5.07	10.23	1.91	1.5	14.48
3	30	0.5	30	2.74	5.51	1.71	1.34	8.59
4	20	0.7	30	3.62	7.31	1.85	1.46	12.59
5	25	0.7	25	2.93	5.91	1.8	1.43	10.24
6	25	0.5	20	4.31	8.69	1.91	1.5	12.71
7	25	0.6	25	3.42	6.9	1.83	1.45	11.77
8	20	0.6	20	4.67	9.42	1.98	1.56	14.71
9	20	0.7	20	4	8.07	1.95	1.54	13.77
10	30	0.7	20	2.16	4.35	1.75	1.39	7.89
11	20	0.6	30	4.24	8.52	1.88	1.48	13.53
12	25	0.7	30	2.79	5.62	1.75	1.39	9.65
13	20	0.5	20	5.6	11.3	1.98	1.58	15.65
14	30	0.7	25	2.06	4.14	1.7	1.35	7.3
15	30	0.6	30	2.28	4.59	1.68	1.33	7.65
16	30	0.6	25	2.4	4.83	1.73	1.37	8.24
17	30	0.7	30	1.95	3.94	1.65	1.31	6.71
18	25	0.5	30	3.9	7.86	1.81	1.42	11.53
19	20	0.6	25	4.45	8.97	1.93	1.52	14.12
20	25	0.5	25	4.1	8.28	1.86	1.46	12.12
21	30	0.5	20	3.02	6.08	1.81	1.43	9.77
22	20	0.7	25	3.81	7.69	1.9	1.5	13.18
23	25	0.7	20	3.08	6.21	1.85	1.46	10.83
24	20	0.5	25	5.34	10.76	1.96	1.54	15.07
25	30	0.5	25	2.88	5.8	1.76	1.38	9.18
26	30	0.6	20	2.52	5.07	1.78	1.41	8.83
27	25	0.6	30	3.25	6.55	1.77	1.42	10.59

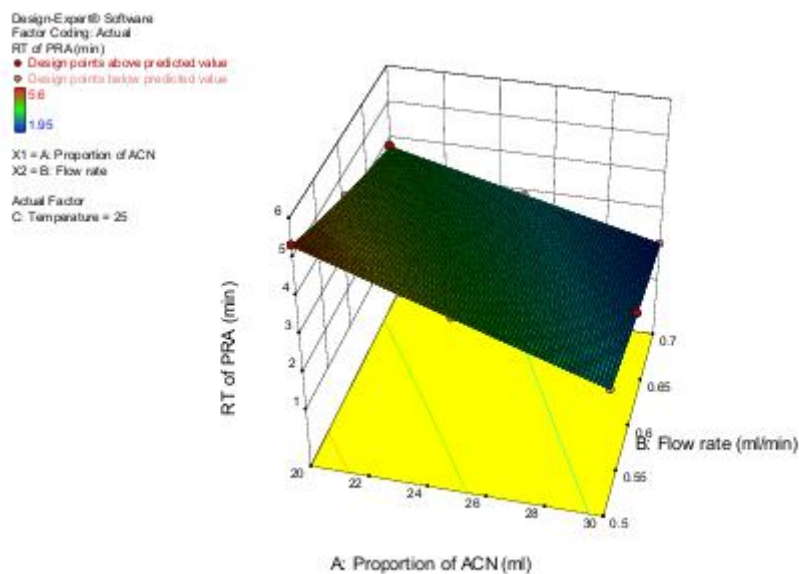


Fig 1 3D surface plot for response 1

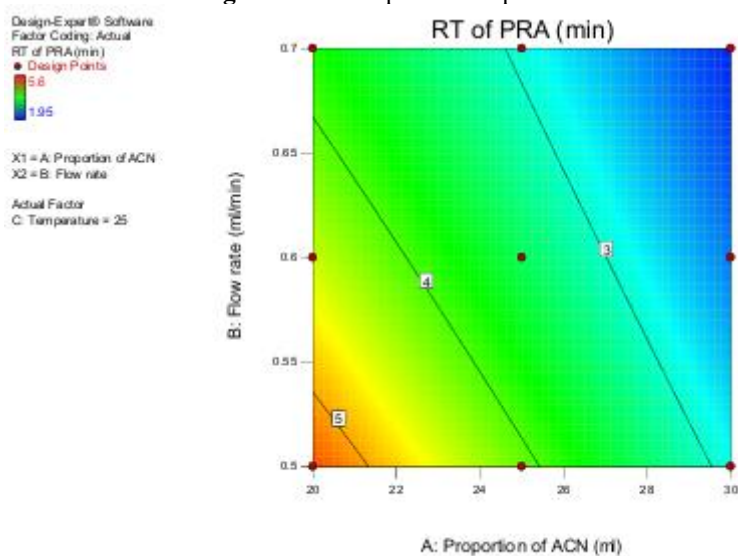


Fig 2: Contour plot of response 1

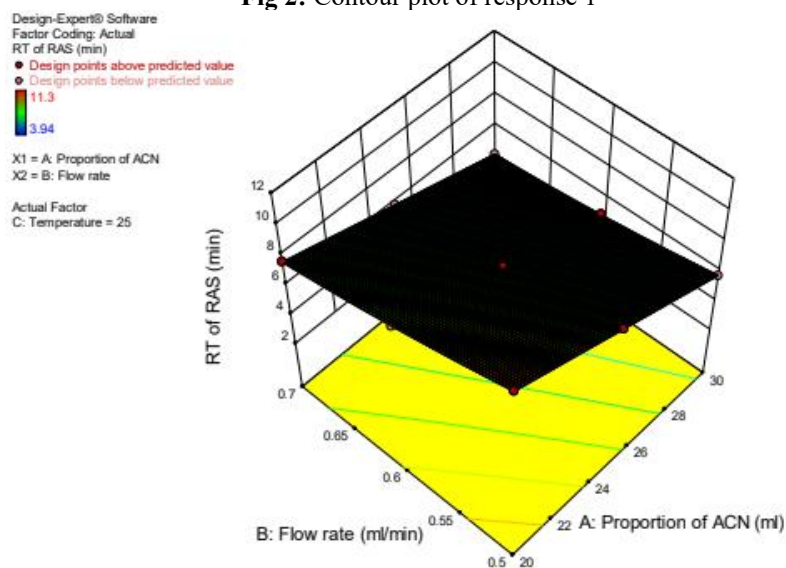


Fig 3: 3D surface plot for response 2

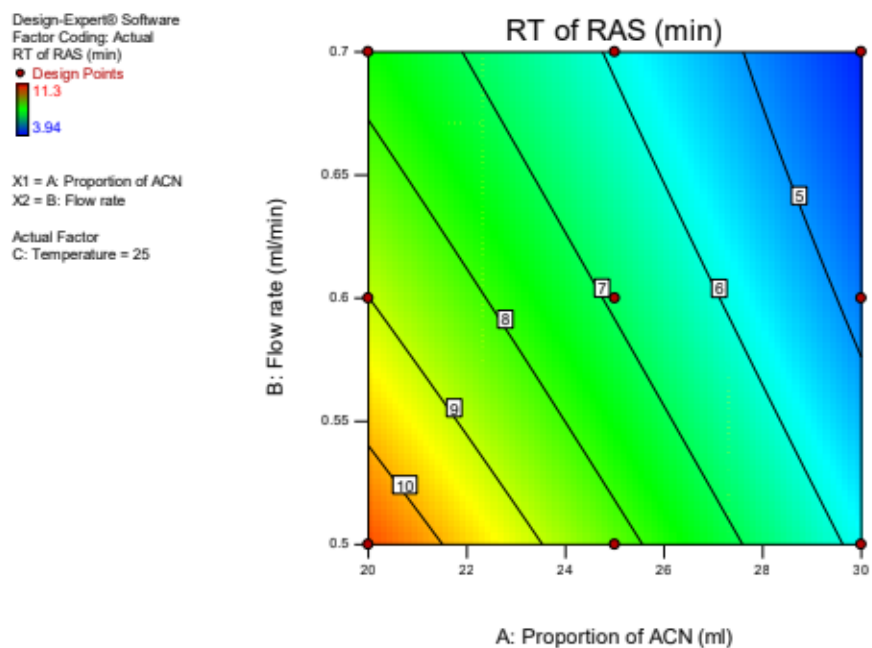


Fig 4: Contour plot of response 2

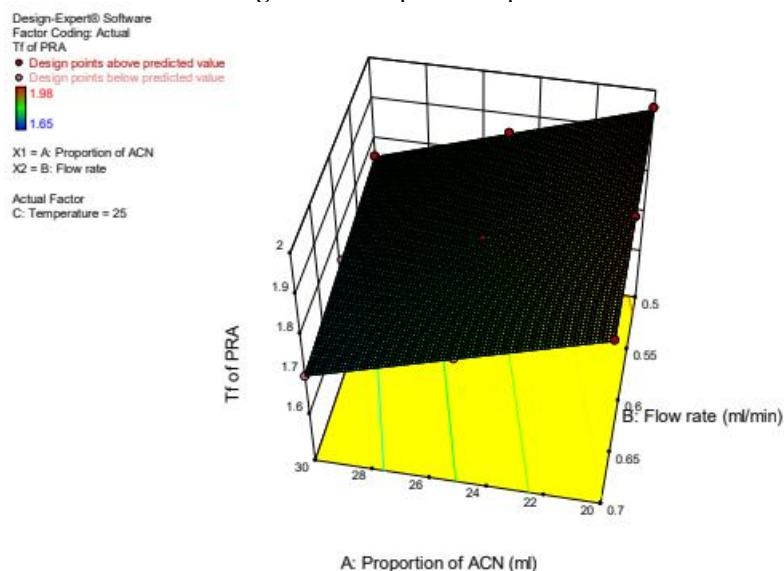


Fig 5: 3D surface plot for response 3

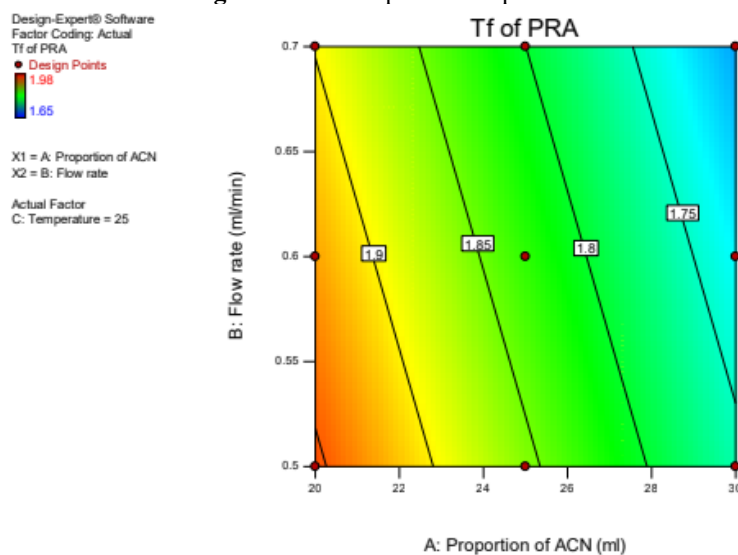


Fig 6: Contour plot of response 3

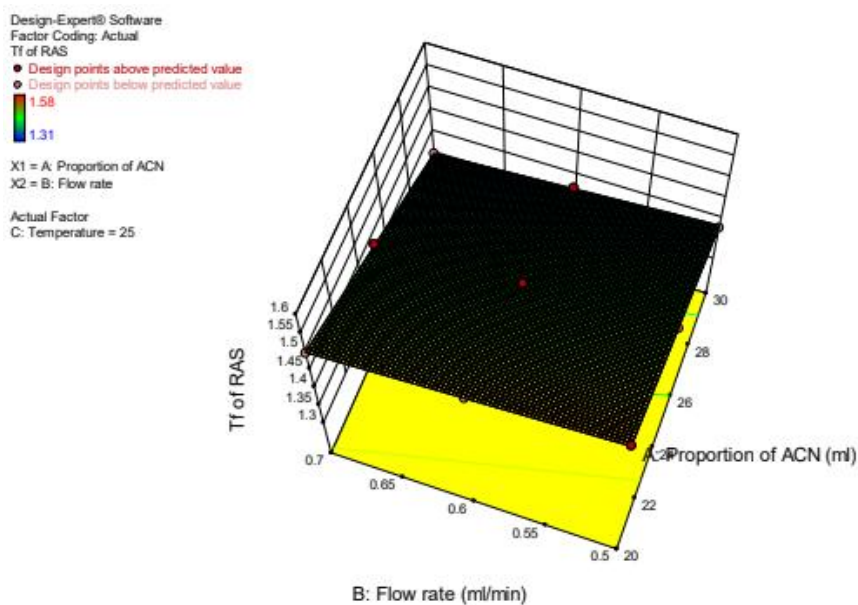


Fig 7: 3D surface plot for response 4

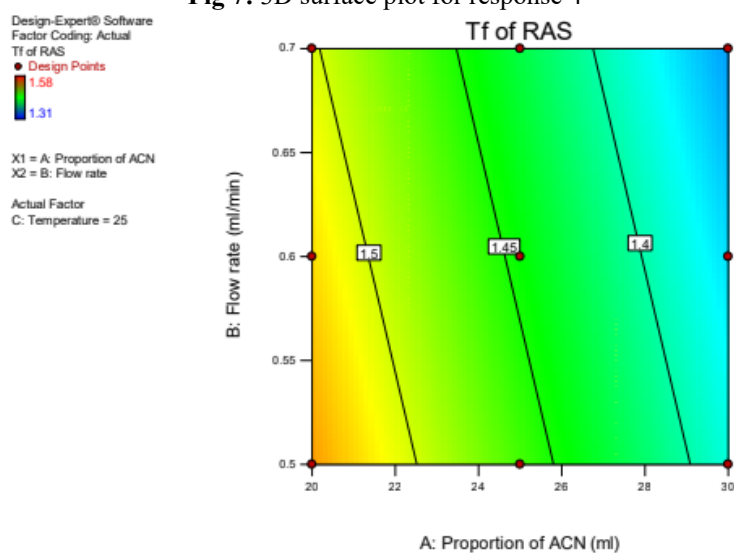


Fig 8: Contour plot for response 4

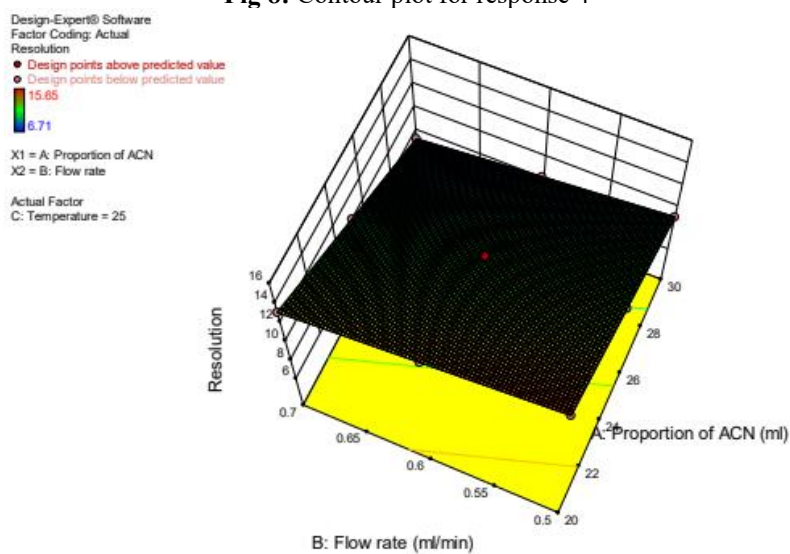


Fig 9: 3D surface plot for response 5

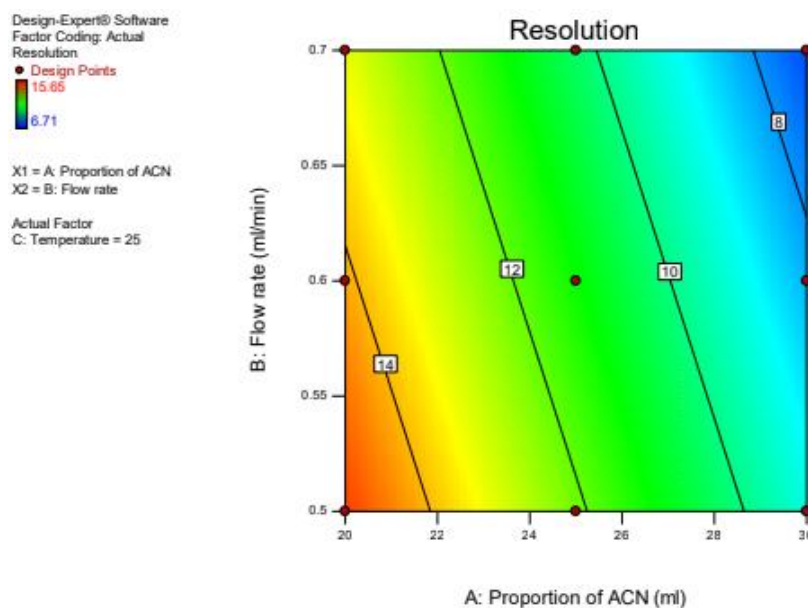


Fig 10: Contour plot for response 5

Response 1: Rt of PRA

Final Equation in terms of coded factors:

$$\text{Rt of PRA} = +3.42 - 1.04 * A - 0.59 * B - 0.17 * C + 0.18 * AB + 0.051 * AC + 0.028 * BC + 3.889\text{E-}003 * A^2 + 0.096 * B^2 + 5.556\text{E-}004 * C^2$$

Response 2: Rt of RAS

Final Equation in terms of coded factors:

$$\text{Rt of RAS} = +6.90 - 2.11 * A - 1.18 * B - 0.35 * C + 0.35 * AB + 0.11 * AC + 0.059 * BC + 3.333\text{E-}003 * A^2 + 0.20 * B^2 + 0.000 * C^2$$

Response 3: Tf of PRA

Final equation in terms of coded factors:

Full model:

$$\text{Tf of PRA} = +1.83 - 0.098 * A - 0.028 * B - 0.049 * C$$

Response 4: Tf of RAS

Final equation in terms of coded factors:

$$\text{Tf of RAS} = +1.44 - 0.076 * A - 0.018 * B - 0.039 * C$$

Response 5: Resolution

Final equation in terms of coded factors:

$$\text{Resolution} = +11.20 - 2.94 * A - 0.94 * B - 0.59 * C$$

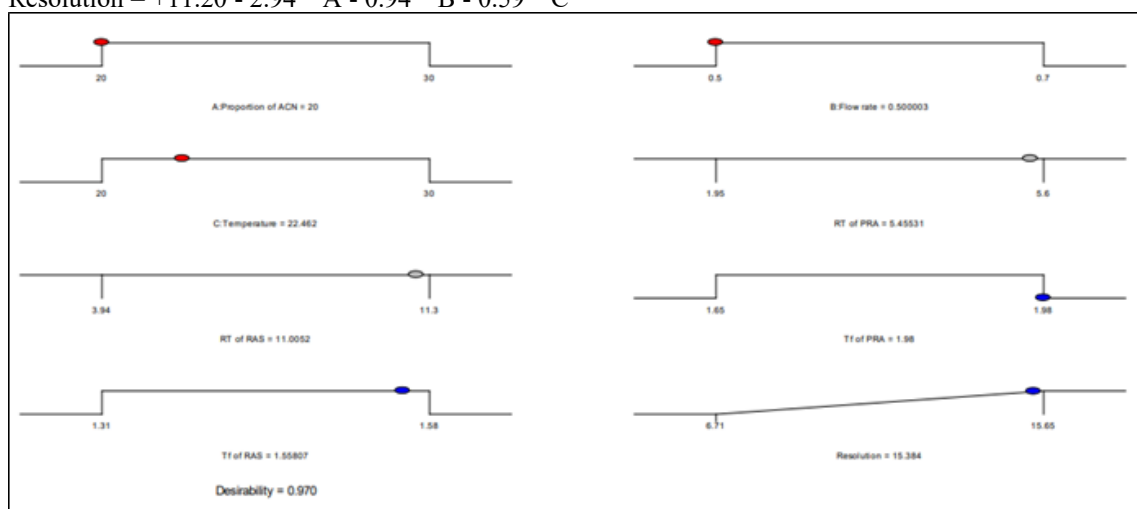


Fig 11: Prediction of Dependent and Independent variables by Design Expert Software

Chromatographic Condition after optimization by Design Expert Software

Mobile Phase	Acetonitrile (20 v/v) - optimized 0.1% OPA in water (80 v/v)
Temperature	22 °C - optimized
Selection of stationary phase	Zorbax SB C18, 250*4.6 mm, 5 µm
Flow Rate	0.5 ml/min - optimized

Injection volume	10 µl
Analytical wavelength	210 nm

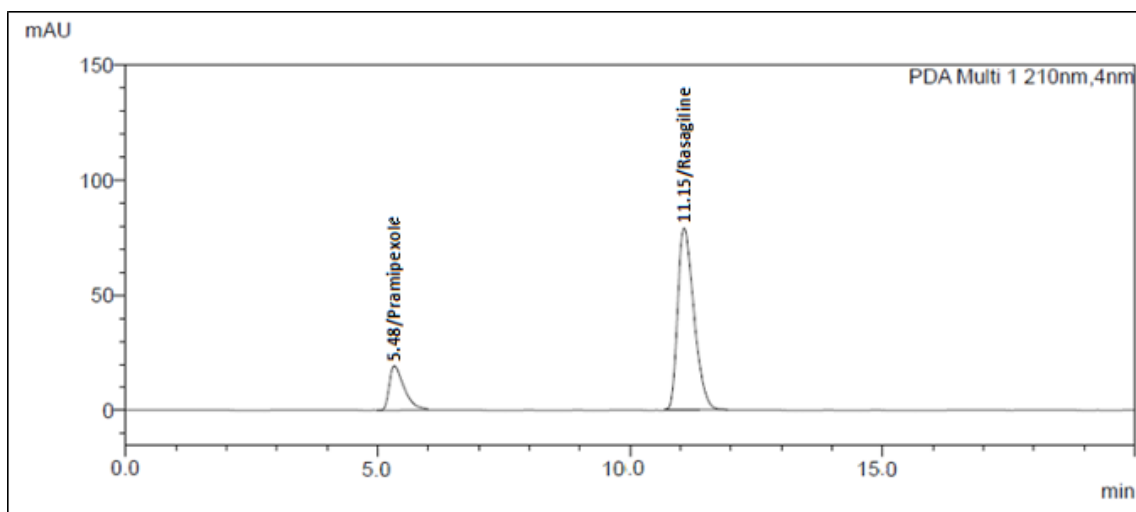


Fig 12: Chromatogram under optimized condition after QbD

Parameter	Predicted value	Actual Value
Retention time of PRA	5.45	5.48
Retention time of RAS	11.00	11.15
Tailing factor of PRA	1.98	1.87
Tailing Factor of RAS	1.55	1.48
Resolution	15.38	15.52

System Suitability Parameters

Solution of PRA+RAS (6.0+7.5 µg/ml) was injected 3 times for determination of System suitability parameters which includes Retention time (Rt), Tailing factor (Tf), Resolution (Rs) and number of theoretical plates. System suitability parameters for selected concentration were determined by RSD.

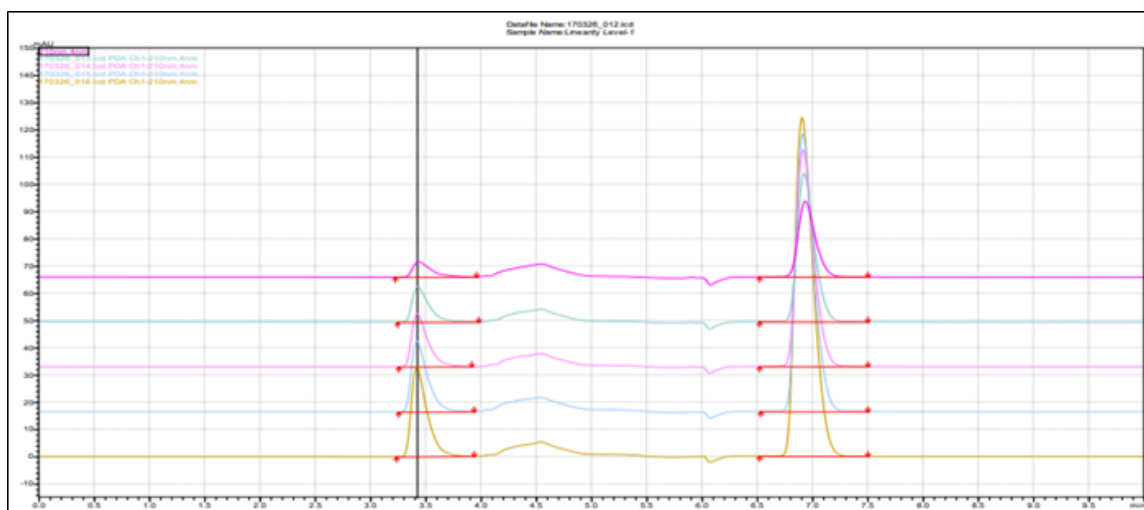
Retention Time			Tailing Factor			Theoretical Plates			Resolution	
	PRA	RAS		PRA	RAS		PRA	RAS		
1	5.48	11.15		1.87	1.48		8745	11263		15.52
2	5.49	11.21		1.84	1.45		8856	11524		15.47
3	5.51	11.26		1.81	1.47		8691	11366		15.56
Mean	5.49	11.21	Mean	1.84	1.47	Mean	8764.00	11384.33	Mean	15.52
SD	0.02	0.06	SD	0.03	0.02	SD	84.12	131.46	SD	0.05
RSD	0.28	0.49	RSD	1.63	1.04	RSD	0.96	1.15	RSD	0.29

Analytical Method Validation

1. Linearity and range

Preparation of Solution for linearity studies:

Weigh accurately about 60 mg of PRA, 75 mg of RAS and transfer into a 100 ml volumetric flask. Make up the volume of the flask to the mark with Methanol. (600 µg/ml PRA + 750 µg/ml RAS). Transfer 1.0 ml of this solution to 10 ml volumetric flask, and make up the volume with methanol, resulting solution gives 60 µg/ml PRA + 75 µg/ml RAS. Various aliquots were prepared from this solution for linearity.



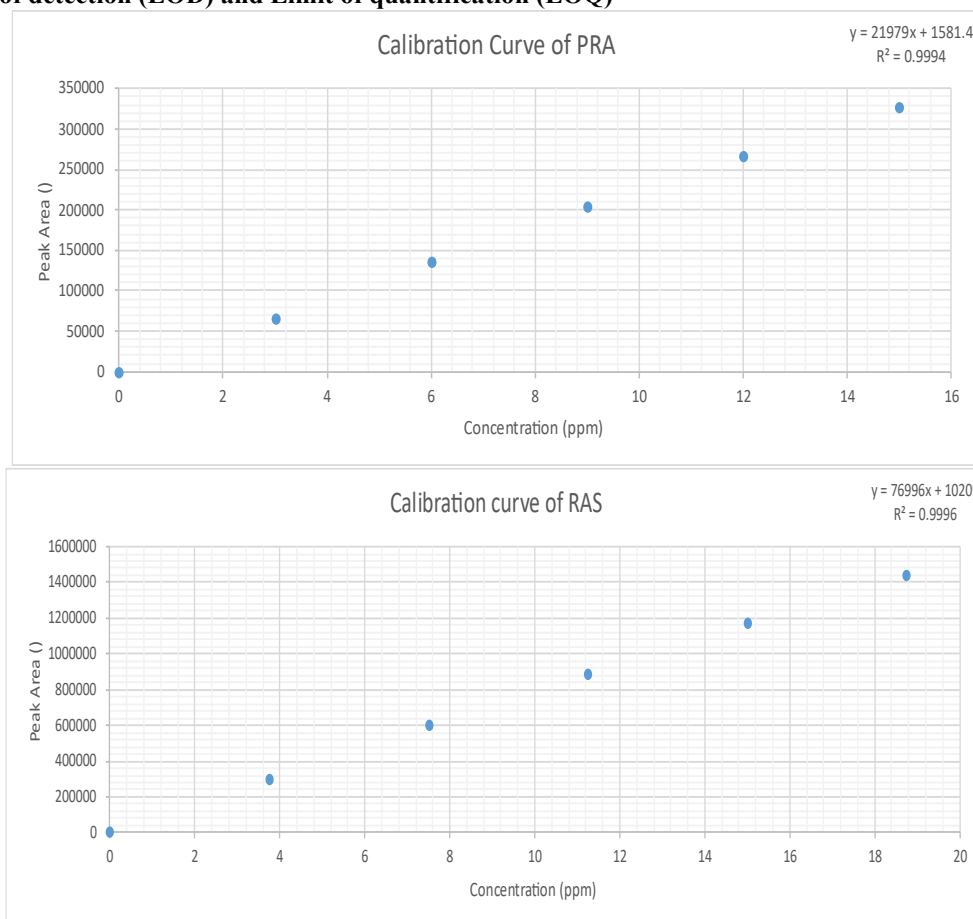
Chromatogram of Linearity Overlay

2. Repeatability

Prepared standard working solution of mixtures having concentration of PRA (3.0 to 15.0 µg/ml) and RAS (3.75 to 18.75 µg/ml) were injected at volume of 20 µL into column by employing optimized chromatographic conditions. Each standard mixture was injected 5 time and peak area was monitored. Each concentration was monitored for repeatability by RSD.

LINEARITY AND REPEATABILITY											
PRA						RAS					
Concentration	3	6	9	12	15	Concentration	3.75	7.5	11.25	15	18.75
Mean	65600.8	135590.6	203429.4	266425	327496.2	Mean	297685	598022.8	886340.4	1170337.6	1439884.8
SD	994.28	1807.21	2435.71	2716.71	2936.13	SD	4353.12	7515.39	9914.54	10413.28	9932.51
RSD	1.52	1.33	1.20	1.02	0.90	RSD	1.46	1.26	1.12	0.89	0.69

3. Limit of detection (LOD) and Limit of quantification (LOQ)



LOD and LOQ

	PRA	RAS
LOD	0.185328	0.089105
LOQ	0.5616	0.270014

4. Accuracy

Accuracy of the analytical method has been performed by spiking of placebo with the standard. Spiking of the sample was performed at 50, 100 and 150 % of the target concentration.

Preparation of sample solution: As per procedure of assay.

Preparation of standard solution: As per procedure of linearity.

Each solution was chromatographed for 3 time and area obtained was subjected to statistical analysis to get idea about mean % recovery.

	PRA					
	50%		100%		150%	
	Amount of drug recovered	% Recovery	Amount of drug recovered	% Recovery	Amount of drug recovered	% Recovery
Mean	2.99	99.67	5.99	99.83	8.98	99.81
SD	0.05	1.53	0.10	1.64	0.10	1.16

	RAS					
	50%		100%		150%	
	Amount of drug recovered	% Recovery	Amount of drug recovered	% Recovery	Amount of drug recovered	% Recovery
Mean	3.74	99.73	7.46	99.51	11.20	99.56
SD	0.05	1.33	0.07	0.87	0.12	1.03

5. Intraday Precision and Interday Precision

- Method precision was determined by performing intraday and interday precision.
- Mixture that represents overall range (PRA+RAS = 3+3.75, 9+11.25 and 15+18.75 µg/ml) were analyzed on same day at different time interval for intraday precision.
- Mixture that represents overall range (PRA+RAS = 3+3.75, 9+11.25 and 15+18.75 µg/ml) were analyzed on different days for interday precision.

	Intraday Precision					
	PRA			RAS		
	3	9	15	3.75	11.25	18.75
MEAN	65834.00	202073.67	328205.67	294197.67	887580.67	1442919.33
SD	996.92	2398.03	2991.89	4265.79	9849.01	10207.07
RSD	1.51	1.19	0.91	1.45	1.11	0.71

	Inter-day Precision					
	PRA			RAS		
	3	9	15	3.75	11.25	18.75
MEAN	65691.00	202781.00	327680.33	294621.67	887101.67	1443185.67
SD	1025.78	2488.03	3160.47	4427.68	10329.30	10916.49
RSD	1.56	1.23	0.96	1.50	1.16	0.76

6. ASSAY

Sample Preparation: Weigh placebo (as mentioned in accuracy, 60 mg of PRA and 75 mg of RAS, and transfer into a 100 ml volumetric flask. Make up the volume of the flask to the mark with Methanol. (600 µg/ml PRA + 750 µg/ml RAS). Sonicate this solution for 5 minutes and filter it. Transfer 1.0 ml of this filtrate to 10 ml volumetric flask, and make up the volume with methanol, resulting solution gives 60 µg/ml PRA + 75 µg/ml RAS.

Test solution: withdraw 1ml from above solution in 10 mL volumetric flask; make up the volume with methyl alcohol. (6.0 µg/ml PRA + 7.5 µg/ml RAS)

PRA			RAS		
	Amount Found	%Assay		Amount Found	%Assay
Mean	6.00	99.94	Mean	7.47	99.64
SD	0.08	1.39	SD	0.05	0.67
RSD	1.39	1.39	RSD	0.67	0.67

FORCED DEGRADATION STUDIES

1. Acid hydrolysis

Solution A: Weigh accurately about 60 mg of PRA, 75 mg of RAS and transfer into a 100 ml volumetric flask. Make up the volume of the flask to the mark with Methanol. (600 µg/ml PRA + 750 µg/ml RAS). Transfer 1.0 ml of this solution to 10 ml volumetric flask, and make up the volume with methanol, resulting solution gives 60 µg/ml PRA + 75 µg/ml RAS.

STEP 1: From the above solution A, take 1.0 mL and transfer it to a 10 ml volumetric flask and add 5 ml of 0.5N HCl. Heat the flask at 70 Degrees on Hot plate for 1 Hr. After the heating cool down the solution and neutralize the contents with 1N KOH or NaOH. Make up the volume up to mark with mobile phase if necessary. (Filter the solution if precipitates are observed). (PRA+RAS = 6+7.5 µg.ml⁻¹)

STEP 2: Inject 10 µL of the above solution and observe the chromatogram.

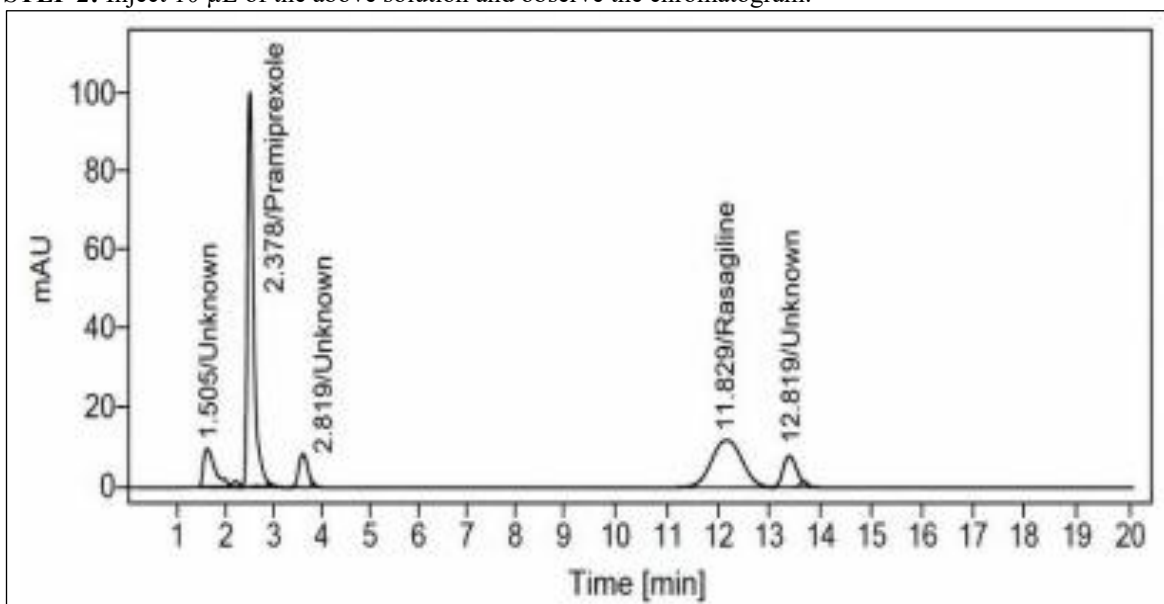


Fig 13: Chromatogram of Acid Hydrolysis (Treated sample): With 0.1N HCl and refluxed for 1 hr at 60°C

Sr. No	Drug	Area		% Degradation
		Zero Hour	Treated	
1	Pramipexole	135746	120162	11.48
2	Rasagiline	594622	533911	10.21

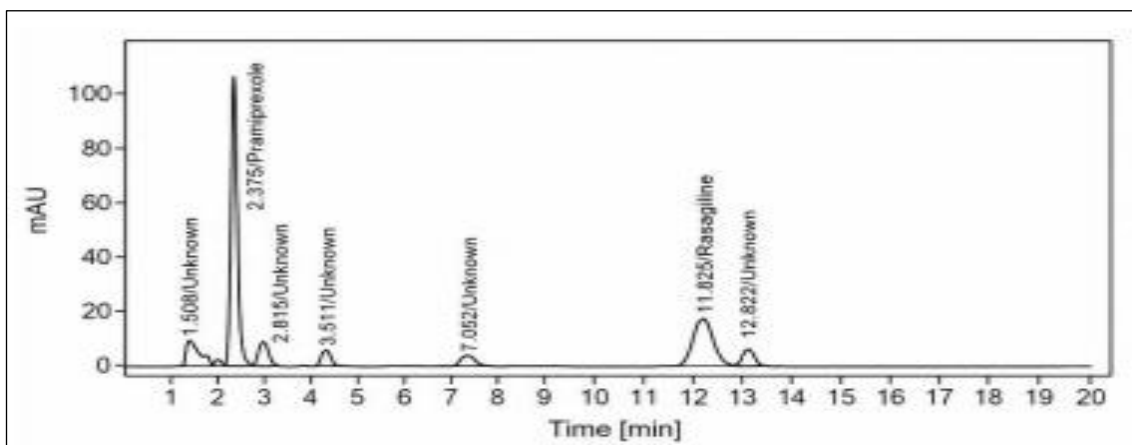


Fig 14: Chromatogram of Acid Hydrolysis (Treated sample): With 0.5N HCl and refluxed for 30 min. at 70°C

Sr. No	Drug	Area		% Degradation
		Zero Hour	Treated	
1	Pramipexole	135746	112371	18.75
2	Rasagiline	594622	499839	16.99

2. Base Hydrolysis

Solution A: Weigh accurately about 60 mg of PRA, 75 mg of RAS and transfer into a 100 ml volumetric flask. Make up the volume of the flask to the mark with Methanol. (600 µg/ml PRA + 750 µg/ml RAS). Transfer 1.0 ml of this solution to 10 ml volumetric flask, and make up the volume with methanol, resulting solution gives 60 µg/ml PRA + 75 µg/ml RAS.

STEP 1: From the above solution A, take 1.0 mL and transfer it to a 10 ml volumetric flask and add 5 ml of 0.5N

NaOH. Heat the flask at 70 Degrees on Hot plate for 1 Hr. After the heating cool down the solution and neutralize the contents with 1N HCl. Make up the volume up to mark with mobile phase if necessary. (Filter the solution, if precipitates are observed). (PRA+RAS = 6+7.5 µg.ml-1)

STEP 2: Inject 10 µL of the above solution and observe the chromatogram

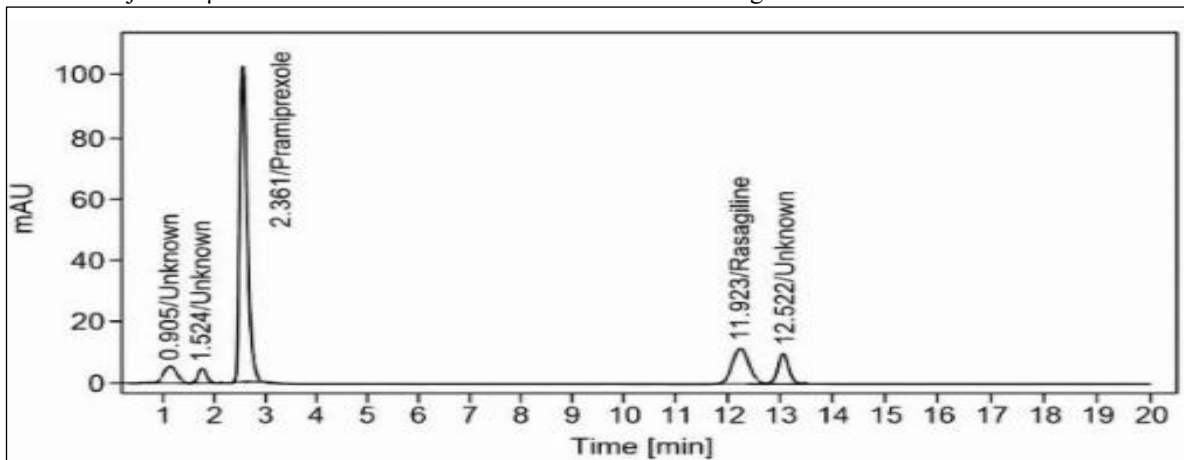


Fig 17: Chromatogram of Base Hydrolysis (Treated sample): With 0.1N NaOH and refluxed for 1 hr at 60°C

Sr. No	Drug	Area		% Degradation
		Zero Hour	Treated	
1	Pramipexole	135463	120210	11.26
2	Rasagiline	594708	514125	13.55

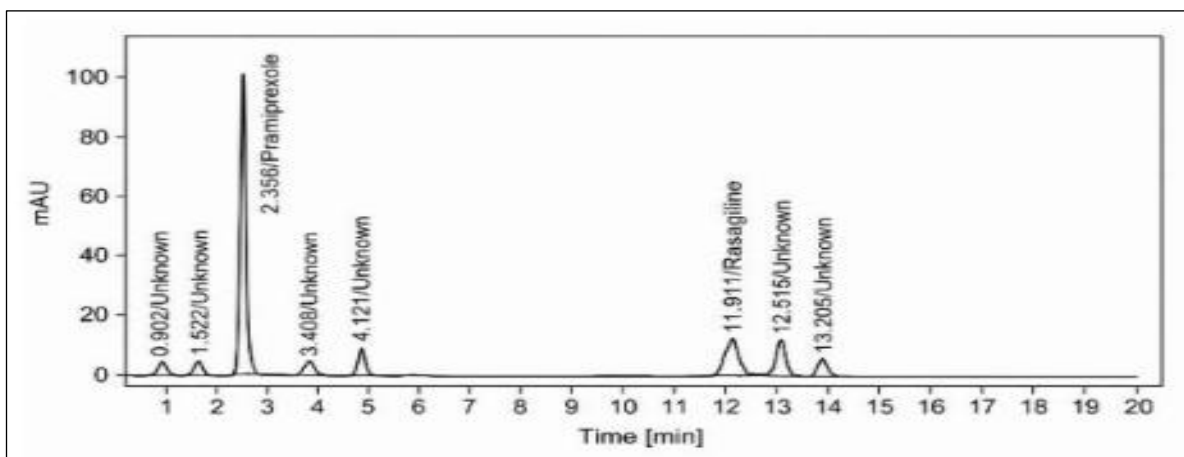


Fig 18: Chromatogram of Base Hydrolysis (Treated sample): With 0.5N NaOH and refluxed for 30 min. at 70°C

Sr. No	Drug	Area		% Degradation
		Zero Hour	Treated	
1	Pramipexole	135463	110037	18.96
2	Rasagiline	594708	480286	19.54

3. Oxidative Stress

Solution A: Weigh accurately about 60 mg of PRA, 75 mg of RAS and transfer into a 100 ml volumetric flask. Make up the volume of the flask to the mark with Methanol. (600 µg/ml PRA + 750 µg/ml RAS). Transfer 1.0 ml of this solution to 10 ml volumetric flask, and make up the volume with methanol, resulting solution gives 60 µg/ml PRA + 75 µg/ml RAS.

STEP 1: From the above solution A, take 1.0 mL and transfer it to a 10 ml volumetric flask and add 5 ml of 3% Hydrogen peroxide. Heat the flask at 70 Degrees on Hot plate for 1 Hr. After the heating cool down the solution, dilute the contents up to mark with mobile phase if necessary. (Filter the solution, if precipitates are observed). (PRA+RAS = 6+7.5 µg.ml-1)

STEP 2: Inject 10 µL of the above solution and observe the chromatogram

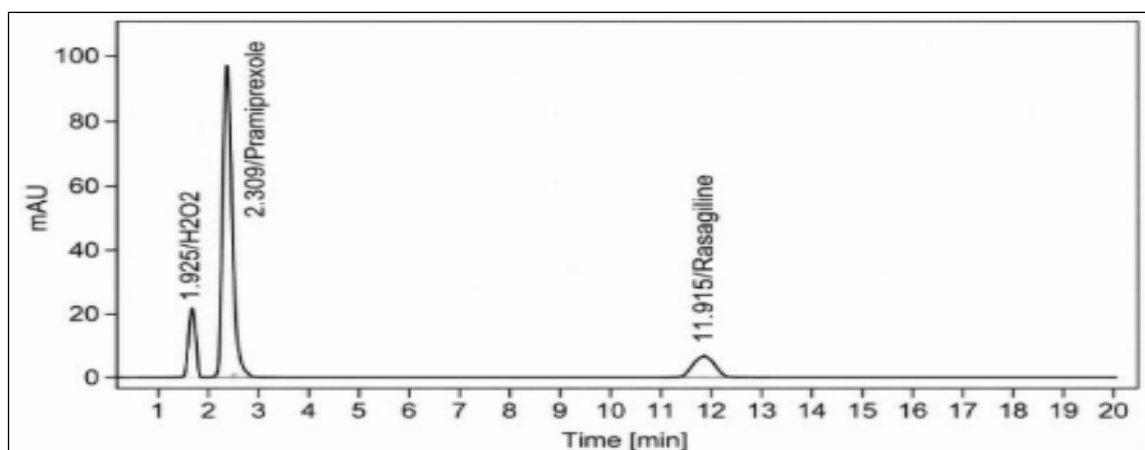


Fig 21: Chromatogram of Oxidative Stress (Treated sample): With 1% H₂O₂ and refluxed for 1 hr at 60°C

Sr. No	Drug	Area		% Degradation
		Zero Hour	Treated	
1	Pramipexole	135866	122580	9.58
2	Rasagiline	594882	535275	10.02

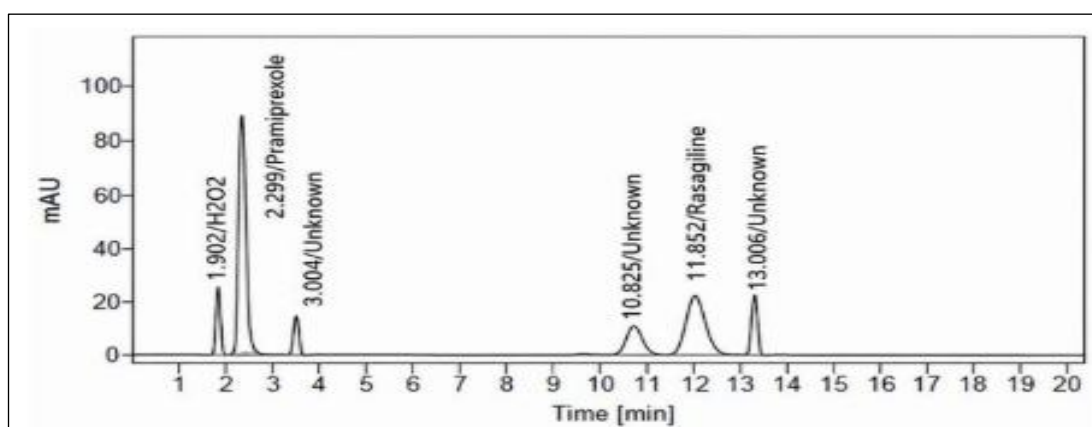


Fig 22: Chromatogram of Oxidative Stress (Treated sample): Exposed with 3% H₂O₂ at 70°C for 1 hr

Sr. No	Drug	Area		% Degradation
		Zero Hour	Treated	
1	Pramipexole	135866	120581	11.25
2	Rasagiline	594882	521295	12.37

4. Thermal Stress

STEP 1: Weigh accurately about 60 mg of PRA and 75 mg of RAS into a petri plate and keep it under hot air oven at 70 degrees for 3 hours. After exposure remove the plate and rinse the contents with methanol in to a 100 ml volumetric flask. Make up the volume to mark with methanol. Further take 1 ml from the above solution in a 10 ml volumetric flask and dilute the contents with mobile phase. (PRA+RAS = 6+7.5 µg.ml⁻¹)

STEP 2: Inject 10 µL of the above solution and observe the chromatogram.

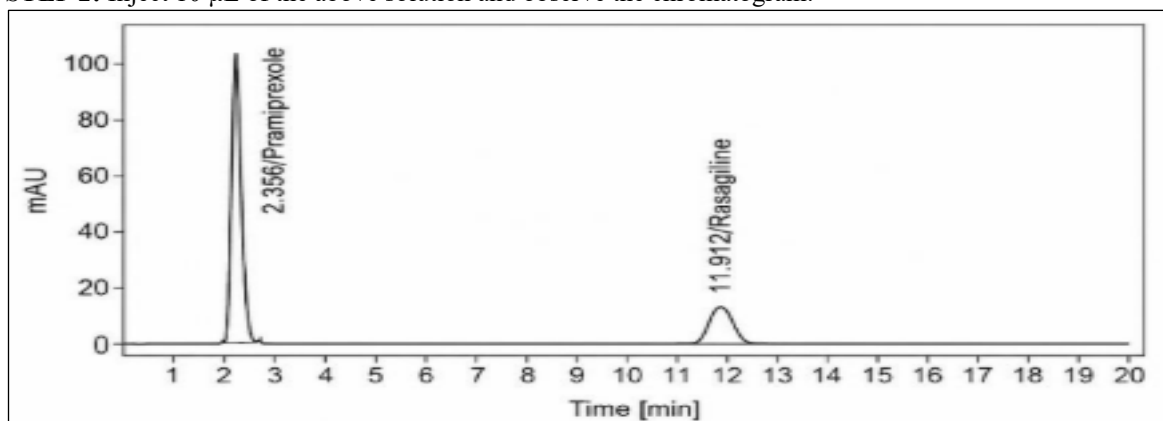


Fig 25: Chromatogram of Thermal stress (Treated sample) for 70°C for 1 Hr

Sr. No	Drug	Area		% Degradation
		Zero Hour	Treated	

1	Pramipexole	135894	132347	2.61
2	Rasagiline	594911	573851	3.54

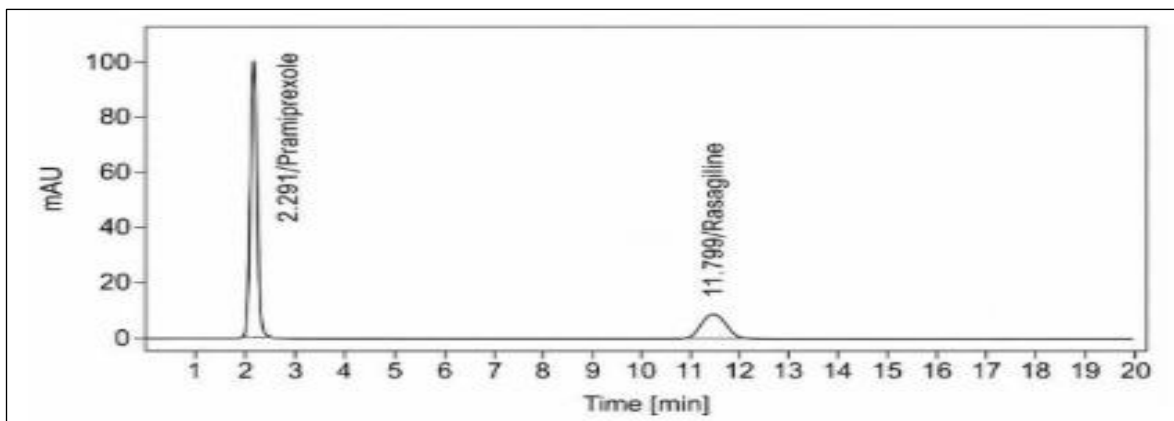


Fig 26: Chromatogram of Thermal stress (Treated sample) for 70°C for 2 Hrs

Sr. No	Drug	Area		% Degradation
		Zero Hour	Treated	
1	Pramipexole	135894	123786	8.91
2	Rasagiline	594911	538038	9.56

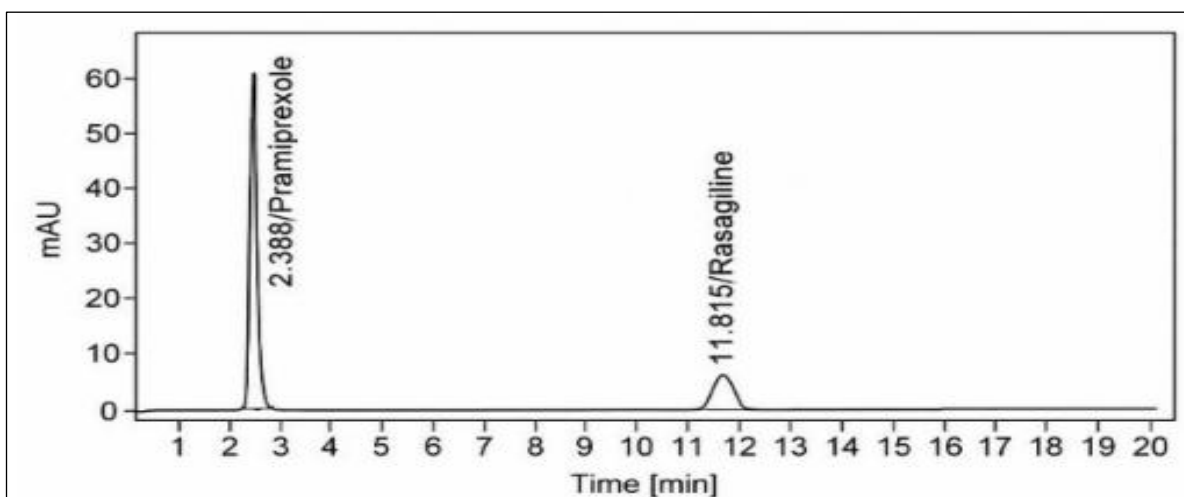


Fig 27: Chromatogram of Thermal stress (Treated sample) for 70°C for 3 Hrs

Sr. No	Drug	Area		% Degradation
		Zero Hour	Treated	
1	Pramipexole	135894	116108	14.56
2	Rasagiline	594911	500796	15.82

CONCLUSION

In this study, a robust, systematic, and eco-efficient RP-HPLC method was successfully developed and validated for the simultaneous estimation of Pramipexole (PRA) and Rasagiline (RAS) in synthetic mixtures. By integrating a **Quality by Design (QbD)** approach via a three-level factorial design using Design-Expert software, the critical chromatographic parameters-including the proportion of acetonitrile, flow rate, and column temperature-were rigorously optimized to achieve excellent resolution and peak symmetry within a short run time.

The method validation, performed in strict accordance with **ICH guidelines**, confirmed that the developed assay is linear, accurate, precise, and robust across the specified ranges. Furthermore, forced degradation studies demonstrated the stability-indicating capability of the method, effectively resolving the intact drug peaks from their respective degradation products generated under acid, base, oxidative, and thermal stress conditions.

Consequently, this QbD-assisted RP-HPLC method can be reliably employed for routine quality control analysis, stability testing, and regulatory evaluation of Pramipexole and Rasagiline pharmaceutical formulations in industrial and research settings.

ACKNOWLEDGMENTS

The authors express their gratitude to LJ University and the LJ Department of Pharmaceutical Sciences for providing the necessary research facilities and infrastructure to complete this study.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest regarding the publication of this paper

Author Contributions

- **Revati Mudaliar:** Conceptualization, methodology, software handling, formal analysis, investigation, data curation, writing - original draft preparation.
- **Dilip Maheshwari:** Conceptualization, supervision, resources, validation, writing - review and editing, project administration.

Nomenclature & Abbreviations

Abbreviation	Expanded Term
ACN	Acetonitrile
DoE	Design of Experiments
ICH	International Council for Harmonisation
LOD	Limit of Detection
LOQ	Limit of Quantification
OPA	Ortho-phosphoric acid
PRA	Pramipexole
QbD	Quality by Design
RAS	Rasagiline
RSD	Relative Standard Deviation
Rt	Retention time
RP-HPLC	Reversed-Phase High-Performance Liquid Chromatography
Tf	Tailing factor
UV	Ultraviolet

REFERENCES

1. International Council for Harmonisation (ICH). ICH Harmonised Tripartite Guideline: Validation of Analytical Procedures: Text and Methodology Q2(R1). Geneva: ICH; 2005. Available from: <https://www.ich.org/page/quality-guidelines>
2. Shaikh MI, Raval AM. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Metformin Hydrochloride, Linagliptin and Dapagliflozin in Synthetic Mixture. Asian Journal of Pharmaceutical Research and Development. 2026 Jun 20;14(3):18-30.
3. Pandey P, Badawy SI. A quality by design approach to scale-up of high-shear wet granulation process. Handbook of Pharmaceutical Wet Granulation. 2026 Jan 1:601-32.
4. Dubey J, Jariwala KJ, Srivastava BK, Koundinya J, Rajyaguru B, Vaidya F. A Quality by Design Approach to Development and Validation of a Laser-based Analytical Method for Particle Size Distribution of Dapagliflozin Propanediol. Journal of Advances in Medical and Pharmaceutical Sciences. 2026 Feb 4;28(1):15-38.
5. International Council for Harmonisation (ICH). ICH Harmonised Guideline: Analytical Procedure Development Q14 and Validation of Analytical Procedures Q2(R2). Geneva: ICH; 2023. Available from: <https://www.ich.org/page/quality-guidelines>
6. Joshi SY, Detholia KK, Raval AM, Dharu NR, Rathod SR, Kushkiwala AM. Development and validation of a stability-indicating RP-HPLC method for quantitative estimation of sunitinib malate in pharmaceutical dosage form. Int J Drug Deliv Technol. 2026;16(12s):418-26.
7. Goswami V, Shukla R, Patel B, Suthar A, Patel P, Raval AM. HPTLC Method Development and Validation for Simultaneous Estimation of Rifaximin and Metronidazole Benzoate in Combined Tablet Dosage Form. Int J Drug Deliv Technol. 2026;16(46s):257-65.
8. Zhou H, Li D, Yu J, Gu Z, Wei H. Meta-analysis and pharmacoeconomic study of rasagiline versus selegiline in the treatment of Parkinson's disease. Annals of Medicine. 2026 Dec 31;58(1):2711475.
9. Patel AB, Patel MN, Patel MN, Patel MK, Prajapati MF, Patel MR, Raval AM. Analytical Method Development and Validation of Withaferin A and Piperine in Capsule Dosage Form. Journal of Animal Environment. 2026 Aug 1:239-44.
10. Sangshetti JN, Deshpande M, Zaheer Z, Shinde DB, Arote R. Quality by design approach: Regulatory need. Arabian Journal of chemistry. 2017 May 1;10:S3412-25.
11. Thakor AD, Dharajiya RM, Shaikh MZ, Raval AM. A review on neuropharmacology: mechanisms, drug classes, and clinical applications. Asian Journal of Pharmaceutical Research and Development. 2026 Feb 15;14(01):114-21.
12. Huang J, Kaul G, Cai C, Chatlapalli R, Hernandez-Abad P, Ghosh K, Nagi A. Quality by design case study: an integrated multivariate approach to drug product and process development. International journal of pharmaceutics. 2009 Dec 1;382(1-2):23-32.
13. Wu B, Wang W, Zheng Z, Cao S, Wang X, Hu W, Lin B, Zhang W. Pharmacokinetics and bioequivalence of rasagiline mesylate tablets in healthy Chinese subjects under fasting and fed conditions: an open, randomized, single-dose, four-period crossover clinical trial. BMC Pharmacology and Toxicology. 2026 Aug 28.

14. Singh S, Bakshi M. Guidance on conduct of stress tests to determine inherent stability of drugs. *Pharm Technol.* 2000;24(6):1-14.
15. Swartz ME, Krull IS. *Handbook of Analytical Validation.* Boca Raton: CRC Press; 2012. DOI: 10.1201/b11463
16. Snyder LR, Kirkland JJ, Dolan JW. *Introduction to Modern Liquid Chromatography.* 3rd ed. Hoboken: John Wiley & Sons; 2010. DOI: 10.1002/9780470508183
17. Khandai M, Panda SK, Sahoo DK, Zankhwala FM, Raval MA, Suthar MA, Gupta MA, Khade MS. Artificial Intelligence in Precision Drug Delivery: From Machine Learning to Intelligent Nanomedicine. *International Journal of Artificial Intelligence and Machine Learning.* 2026 Sep 14;6(10s):56-68.
18. Design-Expert Software. Version 10. Stat-Ease Inc., Minneapolis, MN, USA. Available from: <https://www.stat-ease.com>
19. Patel N, Raval AM. Gastro-retentive drug delivery system: A review. *Int J Pharm Sci.* 2026;4(1):734-42.
20. Mashru R, et al. Development and validation of chemometric assisted methods and stability indicating RP-HPLC method for simultaneous estimation of rasagiline mesylate and pramipexole in synthetic mixture. *Acta Sci Pharm Sci.* 2019;3(8):154-68.
21. Dave V, Patel PU. Analytical QbD Assisted RP-HPLC Method Development and Validation for Estimation of Anti-diabetic Drug Remogliflozin in Bulk and its Pharmaceutical Dosage form. *Research Journal of Pharmacy and Technology.* 2025;18(7):3244-9.
22. Raval MA, Suthar MA, Durani MB, Thakar MN, Zankhwala MF, Kushkiwala MA, Rathod MS. Smart Co-Processed Excipient Platforms: A Novel Strategy for Multifunctional Optimization of Ibuprofen Tablet Formulations. *practice.* 2025;1:2.
23. Ventorp F, Asp M, Olsson S, Lindahl J, Möller S, Svensson M, Ängeby F, Pravdinske A, Tjernberg J, Schrey S, Ståhl D. Efficacy and target engagement of dopamine agonist pramipexole for anhedonic depression: a randomized placebo-controlled trial. *Nature Medicine.* 2026 Jun 12:1-9.
24. Gupta A, Sharma A, Devi G, Srujani C, Viswanath A, Madhuri D, Shaik M. QbD-Assisted RP-HPLC Method Development and Validation for the Quantification of Sotagliflozin With LC-MS/MS Characterization of Its Degradation Products and Greenness Assessment Using AGREE and MoGAPI. *Biomedical Chromatography.* 2026 Oct;40(10):e70597.
25. Beg S, Hasnain MS, Rahman M, Swain S. Introduction to quality by design (QbD): fundamentals, principles, and applications. In *Pharmaceutical quality by design* 2019 Jan 1 (pp. 1-17). Academic Press.
26. Rao BV, Sankar DG. Stability indicating RP-HPLC assay method for pramipexole dihydrochloride in bulk drug and pharmaceutical dosage form. *World J Pharm Sci.* 2012;1(1):30-41.
27. Kushkiwala AM, Zankhwala FM, Patel MD, Raval AM. Flurbiprofen loaded Ethosomal gel: Design, optimization, and anti-inflammatory activity. *International Journal of Research and Analytical Reviews.* 2024;11(4):709-20.
28. Ermer J, Miller JH, editors. *Method Validation in Pharmaceutical Analysis: A Guide to Best Practice.* Weinheim: Wiley-VCH; 2005. DOI: 10.1002/3527604677
29. Raval AM, Rana T, Joshi SY, Buch S, Arora B, Patel VS. Artificial intelligence in pharmacy and healthcare: applications in drug discovery, precision medicine, clinical practice, and future perspectives. *Asian Journal of Pharmaceutical Research and Development.* 2026 Apr 15;14(2):62-9.
30. Chandragirivar PC, Banu A, Raval AM, Kusuma R, Srinidhi G, Yashwanth HB, Suheel A. Improved Dissolution Performance of Fenoprofen Calcium Using PEG 6000 Solid Dispersions: Preparation by Fusion Method and Physicochemical Characterization. *Int J Drug Deliv Technol.* 2026;16(36s):771-81.
31. Kumar PA, Mohammed N, Chandragirivar PC, Raval AM. Design Space Exploration Of Trazodone Nanocrystals Via DoE: Formulation And In Vitro-In Vivo Assessment. *Int J Drug Deliv Technol.* 2026;16(42s):1358-68.
32. Olanow CW, Kieburtz K, Leinonen M, Elmer L, Giladi N, Hauser RA, Klepiskaya OS, Kreitzman DL, Lew MF, Russell DS, Kadosh S. A randomized trial of a low-dose Rasagiline and Pramipexole combination (P2B001) in early Parkinson's disease. *Movement Disorders.* 2017 May;32(5):783-9.
33. Falwariya R, Jethva T, Raval AM, Lokhande D. Comprehensive review: Microneedle patches-A painless revolution in transdermal drug delivery. *World J Pharm Med Res.* 2026;12(1):199-207.
34. Ravikkumar VR, Tajuddin TE, Elakkiya SM, Zankhwala MF, Lavhate MR, KL K. Optimization of *Saccharomyces cerevisiae*-Mediated Biotransformation Using Box-Behnken Design for Enhanced Flavonoid Profile: Impact on Luteolin, Quercetin, Kaempferol, Isorhamnetin, and Apigenin Content. *Cuestiones de Fisioterapia.* 2025 Jan 4;54(2):336-49.
35. Mevada J, Patel K, Raval AM. Role of pharmacovigilance in drug safety monitoring. *World J Pharm Med Res.* 2025;11(11):235-40.
36. Madhavaram S, Jays J. Integration of analytical quality by design green and white analytical chemistry in RP-HPLC method development. *Discover Chemistry.* 2026 Jul 31;3(1):431.
37. Pathak VK, Chandra P, Singh B. Quality by design based analytical (RP-HPLC) method development and validation of coenzyme q10 in single and combination with clomiphen citrate: An extensive review. *Letters in Drug Design & Discovery.* 2026 Jan 6:100233.

38. Ghazy FH, El-Bagary RI, Fahim SH, Mahmoud ST. A Box-Behnken design-assisted RP-HPLC method development and optimization for simultaneous determination of four antiviral drugs in pharmaceutical dosage forms. *Scientific Reports*. 2026 May 11;16(1):14733.
39. Velhal SS, Chaudhari BP. Quality by design-based RP-HPLC methods for pharmaceutical analytical estimation: A review. *Talanta*. 2026 Jul 1:130236.
40. Bukke SP, Kusuma PK, Bhadru B, Shanmugam ST, Kumarachari RK, Narapureddy BR, Onohuean H. RP-HPLC method development, validation, and greenness profile evaluation: Concurrent quantification of telmisartan and atorvastatin calcium in combined pharmaceutical formulation. *Acta Chromatographica*. 2026 May 29;38(2):352-65.
41. Sahu R, Kapadia R. Analytical Method Development and Validation of Donepezil HCl by RP-HPLC. *Research Journal of Pharmacy and Technology*. 2026 Jul 1;19(7):3007-12.
42. Dhule OS, Chagediya V, Rajurkar VG. A Stability-Indicating RP-HPLC Method for The Development and Validation of APD Quantification in Cream Formulation. *International Journal of Scientific Research and Technology*. 2026 Jul 14;3(07):347-60.
43. Kalaria DR, Singhal M, Patravale V, Merino V, Kalia YN. Simultaneous controlled iontophoretic delivery of pramipexole and rasagiline in vitro and in vivo: transdermal polypharmacy to treat Parkinson's disease. *European Journal of Pharmaceutics and Biopharmaceutics*. 2018 Jun 1;127:204-12.