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Quantification of aripiprazole and lamotrigine using chemometrics, spectrophotometric and RP-HPLC methods: Development and validation approach

Krishna Jadav and Rajashree Mashru

Abstract
The present research work describes simple, sensitive, accurate, rapid, precise and economic UV Spectrophotometric, Chemometric assisted and RP-HPLC methods for simultaneous estimation of Aripiprazole and Lamotrigine in bulk and synthetic mixture. UV Spectrophotometric methods applied are: (i) Absorption Correction Method (ii) First Derivative Zero Crossing Point Method (iii) Chemometrics assisted UV spectrophotometric methods. The RP-HPLC method applied by QbD approach and Force Degradation study are carried out by RP-HPLC Method. The Proposed methods have been validated as per ICH guideline and successfully applied to the simultaneous estimation of Aripiprazole and Lamotrigine in their Laboratory Synthetic Mixture.

Keywords: Aripiprazole, lamotrigine, spectrophotometric method, chemometrics method, RP-HPLC

Introduction
In combination of Aripiprazole and Lamotrigine was studied under clinical trial phase IV and it was proved that demonstrated a delay in time to depressive relapses and tended to prolong the time to manic/mixed relapses. So, this combination may provide a synergistic treatment effect in preventing depressive relapses for this subpopulation in mixed-episode patients. The combination of aripiprazole and lamotrigine demonstrated a safe and adequate tolerability profile. Marketed formulation of this combination is not available and the analytical study was carried out in laboratory synthetic mixture [1-2].
The literature survey revealed that there are several analytical methods reported for ARP either individually like spectrophotometric method, RP-HPLC or in combination with other drugs. For LMG spectrophotometric method, RP-HPLC method, several analytical methods reported for simultaneous determination of these drug individually or with other drug in pharmaceutical formulation. Present work evidently describes simple, rapid, accurate and precise UV Spectrophotometric Method, Chemometrics assisted method, RP-HPLC Method by QbD approach and Force Degradation study. For simultaneous estimation of ARP and LMG was validated as per ICH guideline [3-10].

Experimental
Instrumentation
Spectrophotometric measurements were performed on a Shimadzu 1700 double beam UV–VIS spectrophotometer with a fix slit width of 1 nm coupled with Shimadzu UV PC software (UV probe) version 2.10. Chemometrics is done by Design expert 7.0, Matlab r2018b, (for CLS and ILS), Unscrambler X 10.5.1 (for PCR and PLS) and Microsoft office. HPLC was performed on isocratic Shimadzu (Shimadzu Corporation, Kyoto, Japan) chromatographic system equipped with Shimadzu LC-20AT pump and Shimadzu SPD-20AV UV/VIS absorbance detector Data acquisition and integration was performed using Spinchrome software (Spincho biotech, Vadodara).

Chemicals and Reagents
Gift samples of standard Active Pharmaceutical Ingredients- Aripiprazole and Lamotrigine were provided by Zydus Pharmaceutical Pvt. Ltd., India. Analytical grade all chemicals and reagents used for the study was supplied from Research-Lab fine chem industries, Mumbai.

1) Classical UV spectrophotometric methods for simultaneous estimation of aripiprazole and lamotrigine preparation of standard stock solution

10mg of ARP and LMG were separately weighed accurately and transferred into two 10 ml volumetric flasks. Methanol was added into the volumetric flasks to dissolve the standards and finally volume was made up to the mark with Methanol to obtain standard solutions of ARP (1000 µg/ml) and LMG (1000 µg/ml) respectively.

Preparation of calibration curve of standard ARP and LMG

From working standard solution of ARP (100 µg/ml), aliquots of 0.10 ml, 0.15 ml, 0.20 ml, 0.25 ml, 0.30 ml and 0.35 ml were withdrawn and transferred to 10ml volumetric flasks. Volume was made up to the mark with Methanol to produce 1.0 µg/ml, 1.5 µg/ml, 2.0 µg/ml, 2.5 µg/ml, 3.0 µg/ml and 3.5 µg/ml of ARP respectively. From the working standard solution of LMG (100µg/ml), aliquots of 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, 3.0 ml and 3.5 ml were transferred to 10ml volumetric flasks and volume was made up to the mark with Methanol to produce 10 µg/ml, 15 µg/ml, 20 µg/ml, 25 µg/ml, 30 µg/ml and 35 µg/ml of LMG respectively.

Method A: Absorption correction method

Absorption correction method is modification of simultaneous equation method. λmax of Aripiprazole (255 nm), absorbance of Lamotrigine was there. But at the λmax of Lamotrigine (307 nm), Aripiprazole showed negligible absorption. So, for this combination, absorbance correction method was developed.

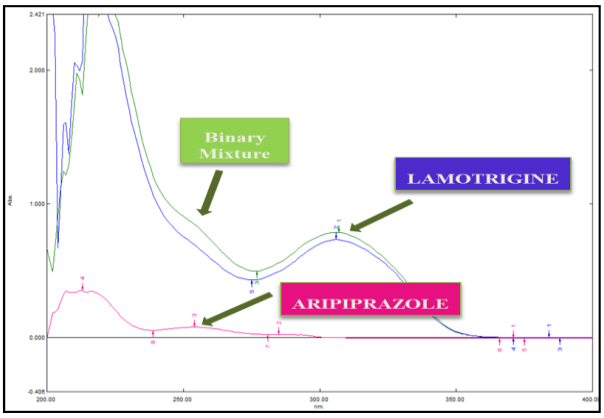

Fig 1: Overlain UV spectra of ARP, LMG and binary mixture

Method B: First derivative zero crossing point method

The absorption spectra of the solutions of ARP and LMG were recorded in the range of 200 nm to 400 nm and were stored in the memory of the instrument and transformed to first derivative with Δλ = 10 nm and scaling factor = 1. Figure 2 shows that at 254 nm, ARP shows zero crossing point and hence LMG can be determined while at 275 nm, LMG shows zero crossing point and hence ARP can be determined.

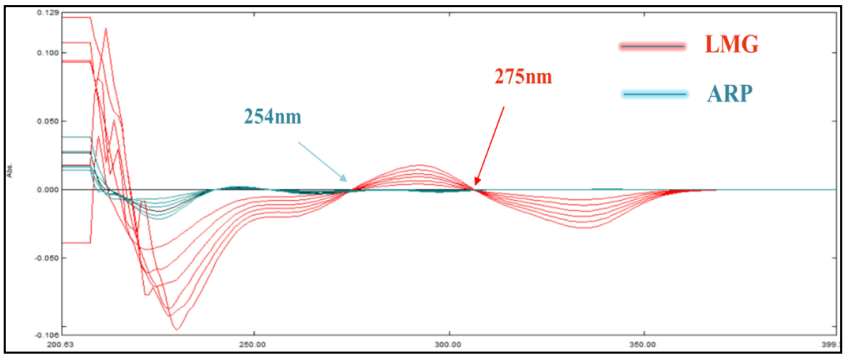

Fig 2: overlain first derivative spectra of ARP and LMG with their zero crossing points

2) Chemometrics assisted UV spectrophotometric methods for simultaneous estimation of aripiprazole and lamotrigine

Chemometric methods are one kind of multivariate analysis i.e. considering more than one variable at a time. When applied to UV spectrophotometry, many wavelengths are taken as variable and absorbance at each wavelength is considered. Least square approach involves mathematical modelling by which the square of residual (Difference between actual and predicted concentration) is minimized to lowest level. Four different Chemometric methods are used which are:

1. Classical least squares
2. Inverse least squares
3. Principal component regression
4. Partial least squares or projection to latent structures

Chemometric methods are one kind of multivariate analysis i.e. considering more than one variable at a time. Here, we are considering absorbance at 16 different wavelengths (230 to 275 nm with interval of 3.0 nm) – 16 variables in contrast to other univariate methods described earlier where absorbance at only one wavelength is considered.

Preparation of standard solutions

Stock standard Solution, 1 mg/ml and 1 mg/ml in methanol, of pure sample of ARP and LMG were freshly prepared by individually weighing of 25 mg in 100 ml volumetric flask diluted with methanol respectively for both drugs. The stock standard solution was diluted appropriately with the methanol to get a working standard solution of 100 µg/ml solution for both ARP and LMG respectively.

Construction of calibration set

Total 26 binary mixture standards with required concentrations (As shown in concentration matrix for calibration) were prepared from working standard solutions.

Construction of validation set

Total 10 binary mixture standards with required concentrations (As shown in Table 1) were prepared from working solutions.

Table 1: Concentration matrix for Calibration and Validation

Calibration Set					
No. of mixtures	conc. (µg/ml)		No. of mixtures	conc. (µg/ml)	
	ARP	LMG		ARP	LMG
1	2.0	20	14	2.5	20
2	1.0	30	15	1.0	35
3	2.0	25	16	3.0	30
4	1.5	15	17	1.5	25
5	3.0	20	18	3.5	15
6	1.0	25	19	3.0	10
7	2.5	25	20	2.5	15
8	1.5	35	21	1.5	10
9	3.5	25	22	1.5	20
10	3.0	35	23	3.5	10
11	3.0	25	24	1.0	15
12	3.5	35	25	3.5	20
13	2.5	35	26	3.0	15

Validation Set					
No. of mixtures	conc. (µg/ml)		No. of mixtures	conc. (µg/ml)	
	ARP	LMG		ARP	LMG
1	2.0	15	6	2.5	30
2	2.0	35	7	1.5	30
3	1.0	20	8	1.0	10
4	2.5	10	9	2.0	10
5	2.0	30	10	3.5	30

Producing absorbance matrix A

Absorbance matrix A was produced by measuring absorbance at 16 wavelengths in the spectrum region between the 230 nm to 275 nm at 3 nm wavelength interval. This region was selected because it contained most relevant information about both the drugs. All four methods are developed.

3) Implementation of box-behnken experimental design for development and validation of RP-HPLC method for simultaneous estimation of lamotrigine and aripiprazole

By considering the current regulatory requirement for an analytical method development, a RP-HPLC method for simultaneous analysis of Aripiprazole and Lamotrigine in bulk and synthetic mixture has been optimized using analytical QbD approach. A simple, rapid, accurate and precise isocratic HPLC method was developed for simultaneous estimation of Aripiprazole and Lamotrigine by QbD approach using Box-Behnken Design.

Selection of detection wavelength

Working Standard solutions of LMG and ARP were scanned between 200-400 nm in UV-visible spectrophotometer and showed good sensitivity at 225nm as shown in Figure 3 which was selected as the analytical wavelength.

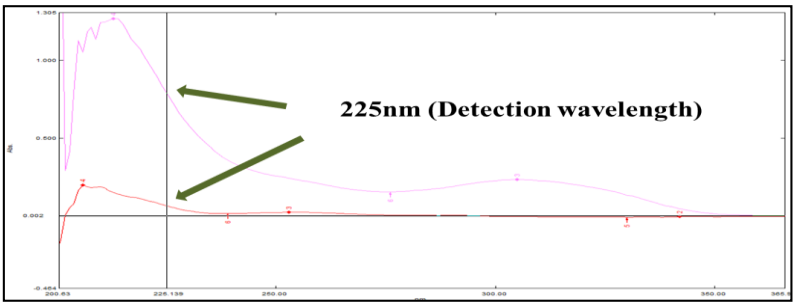

Fig 3: Selection of wavelength for detection ARP (Red) and LMG (Pink)

Quality by design approach in analytical method development

Table 2: CQAs

Critical Quality Attributes		
1	Retention Time	Marker of the separation ability of compound
2	No. of plates	Indicator of the mobile phase suitability and method performance
3	Tailing factor	Indicator of method efficiency
4	Resolution	Quantitative measure of how well two peaks can be differentiated

- **Primary hazard analysis**

Table 3: Critical Quality Attributes (CQAs) - TAILING FACTOR

Parameters	Severity		Probability		Risk Priority Number (RPN)	
	LMG	ARP	LMG	ARP	LMG	ARP
pH	2	1	1	2	2	2
%Organic	3	2	2	3	6	6
Buffer Strength	2	1	2	2	4	2
Flow rate	2	3	2	2	6	4
Wavelength	1	2	1	1	1	2

Table 4: Critical Quality Attributes (CQAs) – THEORITICAL PLATES

Parameters	Severity		Probability		Risk Priority Number (RPN)	
	LMG	ARP	LMG	ARP	LMG	ARP
pH	2	2	2	2	4	4
%Organic	2	3	2	2	4	6
B. Strength	1	2	2	2	2	4
Flow rate	1	3	3	2	3	6
Wavelength	1	2	2	2	2	4

Table 5: Critical Quality Attributes (CQAs) – RETENTION TIME

Parameters	Severity		Probability		Risk Priority Number (RPN)	
	LMG	ARP	LMG	ARP	LMG	ARP
pH	3	2	1	2	3	4
%Organic	2	3	2	3	4	9
B. Strength	1	2	3	2	3	4
Flow rate	3	2	1	2	3	4
Wavelength	1	1	1	2	1	2

Table 6: Critical Quality Attributes (CQAs) – RESOLUTION

Parameters	Severity		Probability		Risk Priority Number (RPN)	
	LMG	ARP	LMG	ARP	LMG	ARP
pH	1	3	2	1	2	3
%Organic	3	3	3	3	9	9
B. Strength	2	2	1	2	2	4
Flow rate	2	1	2	3	4	3
Wavelength	1	2	2	1	2	2

Table 7: CNX Approach

Control variables	Column stationary phase, Organic modifier type, Buffer type, Wavelength
Noise variables	pH meter calibration, instrument calibration (detector, pump, injector, tubing's), material purity
Experimental variables	pH of buffer, buffer ratio, injection volume, flow rate

Table 8: Risk Estimation Matrix

Parameters	Tailing Factor		Theoretical plates		Retention Time		Resolution	
	LMG	ARP	LMG	ARP	LMG	ARP	LMG	ARP
pH								
%Organic								
B. Strength								
Flow rate								
Wavelength								

Severe Moderate Low

Table 9: Initial Trials

Mobile phase	Ratio	Lamotrigine		Aripiprazole	
		RT (min)	Peak Characteristics	RT (min)	Peak characteristics
Methanol: Water	50:50	2.58	Broad	6.31	Distorted
ACN: Water	50:50	1.90	Broad and asymmetric	7.96	Asymmetric
ACN: Water	70:30	2.305	Sharp	4.23	Very close
Water: MeOH: ACN	50:30:20	3.47	tailing	6.29	Broad and Asymmetric
Phosphate Buffer pH3.0: ACN	50:50	3.24	Slightly broad	4.26	Very close
Phosphate Buffer pH 3.0: ACN	70:30	3.63	Slightly fronting	15.37	Late elution
Phosphate Buffer pH 5.0: ACN	60:40	3.602	Sharp and symmetric	5.96	Broad
Phosphate Buffer pH4.0: ACN	50:50	3.58	Good peak	7.24	Slightly fronting was observed
Phosphate Buffer pH 3.5: ACN	60:40	3.5	Slightly broad	12.024	Late elution
Phosphate Buffer pH 3.5: ACN	65:45	3.58	Sharp and symmetric	13.96	Late elution and slightly tailing

Parameter screened like pH, % Organic, Buffer Strength, Flow rate, Wavelength. Screening, optimization and validation of ARP and LMG were performed successfully based upon the above data.

4) Force degradation study by RP-HPLC method for simultaneous estimation of lamotrigine and aripiprazole

In order to determine whether the developed analytical method was stability indicating, active pharmaceutical ingredient (API) and synthetic formulation of LMG and ARP were degraded under various stressed conditions to conduct forced degradation studies. All the degradation studies were followed by the percentage recovery of the drug.

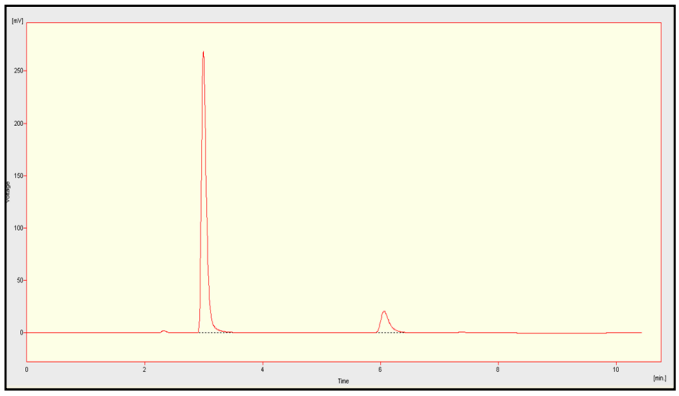

Fig 4: Control chromatogram of LMG and ARP

Table 10: Forced Degradation study

Stressor Condition	Stressor Concentration	Stressor Temperature	Stressor Time
Acidic	1N HCl	80°C	6hrs
Basic	1N NaOH	80°C	6hrs
Oxidation	1%H_2O_2	RT	4hrs
Dry heat stability	---	80°C	14 Days
Photostability	5382 LUX and 144UW/cm2	---	14 Days

Preparation of Degradation sample of synthetic mixture

1ml of prepared synthetic mixture was withdrawn accurately and transferred to a 10 ml volumetric flask containing suitable stressor. Aliquot of 1 ml was withdrawn accurately and neutralized if required, finally the volume being made up to 10 ml using Mobile phase. For Photochemical, Dry heat and Thermal-Humidity induced degradation, suitable amount of synthetic mixture was spread uniformly and subjected to stressor treatment as specified. Appropriate dilutions of the degradation samples were then subject to analysis.

Results and Discussion

1) Classical UV spectrophotometric methods for simultaneous estimation of Aripiprazole and Lamotrigine

Method validation

Developed spectrophotometric methods for the simultaneous estimation of ARP and LMG were validated according to ICH Q2 (R1) guidelines.

i) Linearity and Range

Table 11: Linearity parameter for ARP and LMG

Parameters	Absorption correction method		First derivative ZCP method	
	ARP	LMG	ARP	LMG
Analytical wavelength(nm)	255	307	275	254
Linearity range (µg/ml)	1.0-3.5	10-35	1.0-3.5	10-35
Regression equation	y = 0.2892x - 0.0649	y = 0.0258x - 0.0275	y= -0.0006x + 9E-06	y= -0.0006x + 0.0004
Correlation co-efficient	0.9996	1.0000	0.9997	0.9996
Slope	0.2892	0.0258	0.0006	0.0006
Std.error on slope	0.0063	0.0017	0.0189	0.2084
Intercept	0.0649	0.0275	9E-06	0.0004
Std.error in intercept	0.0072	0.0020	0.0216	0.2335
Confidence interval (95%) on intercept	0.085 to 0.044	0.033 to 0.021	0.0420 to 0.0779	0.0553 to 1.351
LOD	0.3356	0.9614	0.3581	1.0853
LOQ	1.0171	2.9133	2.9217	8.8538

ii) Precision

Table 12: Result of Precision Study

Analytical Method	Analytes Drug	Intraday precision		Interday precision	
		SD	%RSD	SD	%RSD
Absorption Correction Method	ARP	0.00127	1.455	0.00112	1.121
	LMG	0.00380	0.538	0.00498	0.764
First Derivative ZCP Method	ARP	0.000022	1.233	0.000030	1.644
	LMG	0.000214	1.379	0.00022	1.519

iii) Accuracy

Table 13: Result of Recovery Study

Method	% Spiking	Conc ACTUAL (µg/ml)		Conc ADDED (µg/ml)		Conc RECOVER* (µg/ml)		% RECOVERY ± SD*	
		ARP	LMG	ARP	LMG	ARP	LMG	ARP	LMG
A	80	1.0	10	0.8	8	0.78	8.16	98.60 ± 0.0054	102.12 ± 0.0066
	100	1.0	10	1.0	10	0.98	10.24	98.04 ± 0.0032	102.43 ± 0.0245
	120	1.0	10	1.2	12	1.19	12.41	99.99 ± 0.0027	103.46 ± 0.0248
B	80	1.0	10	0.8	8	0.81	8.14	101.38 ± 0.000020	101.80 ± 0.00044
	100	1.0	10	1.0	10	0.98	10.41	98.333 ± 0.000055	104.11 ± 0.00052
	120	1.0	10	1.2	12	1.23	12.33	102.77 ± 0.000060	102.84 ± 0.00053

Applicability of the developed UV spectrophotometric methods

Table 14: Analysis of synthetic mixture

Mixture composition: - ARP: LMG (10mg: 100mg)		
Method	**ARP* ± SD**	**LMG* ± SD**
Absorption Correction Method	98.29% ± 0.024	98.67% ± 0.237
First Derivative ZCP Method	101.57% ± 0.059	102.84% ± 0.045

2) Chemometrics assisted UV spectrophotometric methods for simultaneous estimation of aripiprazole and lamotrigine
i) Classical least squares and 2. Inverse least squares

Table 15: K-matrix and P-matrix

Wavelength(nm)	K-Matrix		P-Matrix(shown in transposed form)	
	ARP	LMG	ARP	LMG
230	0.0285	0.0142	36.4797	-344.8
233	0.0264	0.0185	-304.2773	391.02
236	0.0249	0.0242	194.6799	100.91
239	0.0244	0.031	-43.849	-399
242	0.0248	0.0385	-26.3124	297.54
245	0.0259	0.0462	65.846	-179.1
248	0.0277	0.0533	19.4665	181.35
251	0.0298	0.0582	175.2738	-0.13
254	0.0325	0.0603	-113.9534	-187
257	0.0356	0.0589	5.2175	256.32
260	0.0384	0.054	-290.7914	-263.1
263	0.0404	0.0461	92.2709	116.95
269	0.0415	0.0365	323.7915	92.037
272	0.0415	0.0276	-374.1333	-92.84
275	0.041	0.0207	-23.7624	-196.9

3. Principle component regression

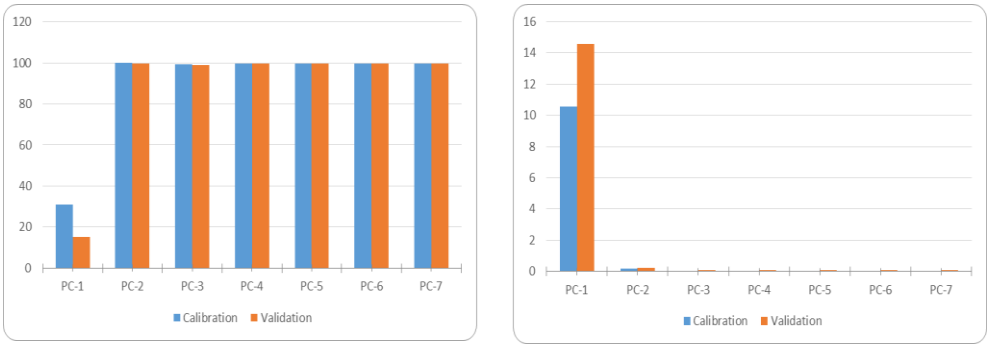

Fig 5: Effect of number of PCs on explained Y variance and residual Y variance

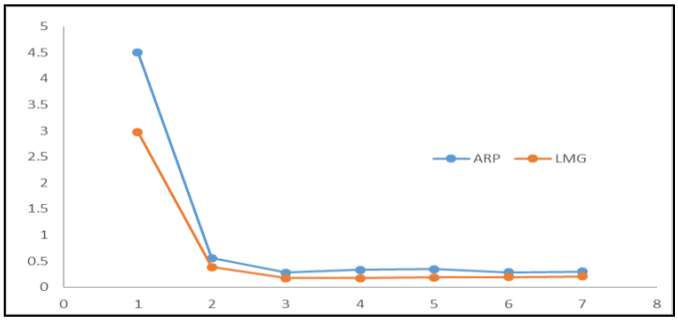

Fig 6: Effect of number of PCs on RMSEP

4. Partial least squares or projection to latent structures

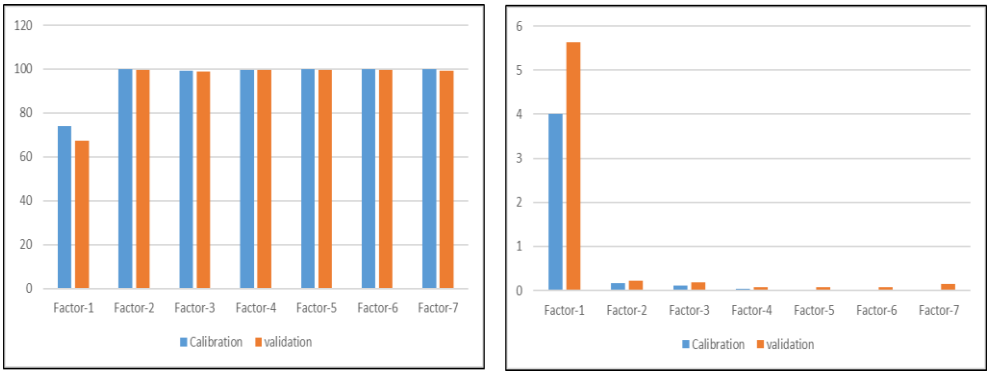

Fig 7: Effect of number of PCs on explained Y variance and residual Y variance

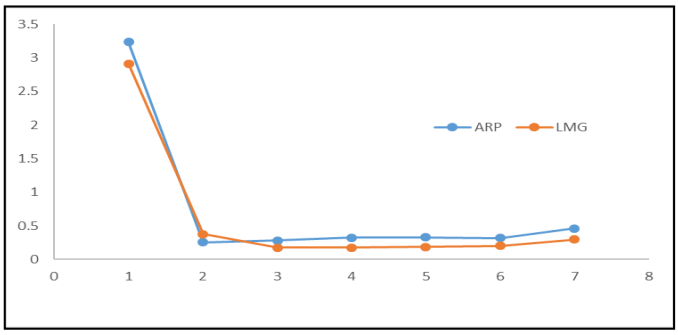

Fig 8: Effect of number of factors on RMSEP

Table 16: RMSEP for ARP and LMG by Chemometric Methods

Drug	RMSEP			
	CLS	ILS	PCR	PLS
ARP	0.05766	0.03462	0.0210145	0.0210145
LMG	0.19697	0.19804	0.0234038	0.0238237

Applicability of the developed chemometric methods

Table 17: Analysis of Synthetic mixture

Method	CLS	ILS	PCR	PLS
ARP	99.96±0.269	99.84±1.390	100.02±0.0031	100.02±0.0031
LMG	100.01±0.0269	99.45±1.390	100.19±0.0309	100.19±0.0309

3) Implementation of box-behnken experimental design for development and validation of RP-HPLC method for simultaneous estimation of lamotrigine and aripiprazole

2- level fractional factorial: Quality by design approach for screening stage

A design layout using fractional factorial design was generated by Design Expert 7.0.0, 5 factors which influence the method parameters were screened for their significance on the analytical method. Variables studied were pH, Flow rate, %organic, B. Strength and Detection wavelength. A Fractional factorial screening design was applied to investigate the significance of these 5 factors.7 responses were studied, namely Retention time of LMG and ARP, Theoretical plates of LMG and ARP and Resolution, Symmetry factor for LMG and ARP.

Table 18: Runs for the Screening Design

Std	Run	F1: pH	F2: %organic	F3: B. Strength	F4: Flow Rate	F5: Wave Length	R1: RT1	R2: RT2	R3: TP1	R4: TP2	R5: TF1	R6: TF2	R7: Resolu Tion
22	1	4	40	20	0.8	230	4.51	12.1	3015	4387	0.67	1.27	14.3
11	2	3	60	10	1.2	225	2.25	3.08	4039	4362	1.16	1.5	5.10
17	3	3	40	10	0.8	230	4.27	12.3	2903	4367	0.79	1.56	15.2
14	4	4	40	20	1.2	225	3.0	8.16	2544	3325	0.65	1.02	12.8
25	5	3	40	10	1.2	230	2.86	8.33	2432	3261	0.69	1.28	13.4
12	6	4	60	10	1.2	225	2.44	3.71	4083	4520	1.14	1.53	6.81
24	7	4	60	20	0.8	230	3.38	4.61	5563	5481	0.95	1.60	5.74
30	8	4	40	20	1.2	230	3.00	8.17	2550	3540	0.65	1.09	13.1
3	9	3	60	10	0.8	225	3.35	4.60	5834	5714	1.48	1.47	5.98
28	10	4	60	10	1.2	230	2.41	3.54	3994	4830	1.22	1.36	6.33
9	11	3	40	10	1.2	225	2.94	8.41	2700	3911	0.78	1.41	14.3
18	12	4	40	10	0.8	230	4.35	13.8	2710	4267	0.69	1.47	16.1
32	13	4	60	20	1.2	230	2.37	3.7	3112	4114	1.06	1.5	4.82
20	14	4	60	10	0.8	230	3.58	5.17	5528	5338	1.30	1.62	6.71
13	15	3	40	20	1.2	225	2.68	7.02	2136	3404	0.53	0.99	12.1
2	16	4	40	10	0.8	225	4.95	16.4	2808	4201	0.74	1.75	16.5
15	17	3	60	20	1.2	225	2.17	2.9	3757	4095	0.92	1.35	4.53
29	18	3	40	20	1.2	230	2.68	7.02	2136	3752	0.54	1.08	12.6
16	19	4	60	20	1.2	225	2.36	3.25	2727	4064	1.03	1.5	4.59
6	20	4	40	20	0.8	225	4.47	12.0	3066	4134	0.68	1.43	14.1
5	21	3	40	20	0.8	225	3.98	10.2	2523	4611	0.59	1.34	13.6
7	22	3	60	20	0.8	225	3.23	4.31	5780	5527	1.0	1.27	5.41
4	23	4	60	10	0.8	225	3.55	4.98	4858	3552	1.21	1.69	5.31
10	24	4	40	10	1.2	225	3.27	10.3	2423	5612	1.02	1.36	17.3
19	25	3	60	10	0.8	230	3.34	4.58	6205	5665	1.5	1.56	5.99
27	26	3	60	10	1.2	230	2.25	3.08	4039	4362	1.16	1.46	5.10
21	27	3	40	20	0.8	230	3.97	10.2	2427	4569	0.57	1.12	13.5

31	28	3	60	20	1.2	230	2.17	2.90	3757	4373	1.0	1.37	4.63
23	29	3	60	20	0.8	230	3.26	4.38	5888	4724	0.92	0.69	5.28
1	30	3	40	10	0.8	225	4.26	12.2	2885	4391	0.76	1.63	15.2
8	31	4	60	20	0.8	225	3.5	4.75	3303	5563	0.83	1.41	5.04
26	32	4	40	10	1.2	230	3.23	10.2	2468	5019	0.95	1.44	16.7

The diagrammatic outputs of fractional factorial design by Pareto charts

From the Pareto charts, it could be concluded that % organic ratio is most critical factor for Retention time, Asymmetry, theoretical plates and Resolution. Flow rate is also showing significant effect on RT and Resolution. pH is also showing effect on RT, Asymmetry and Resolution. Buffer concentration and Wavelength show effect on Theoretical plates.

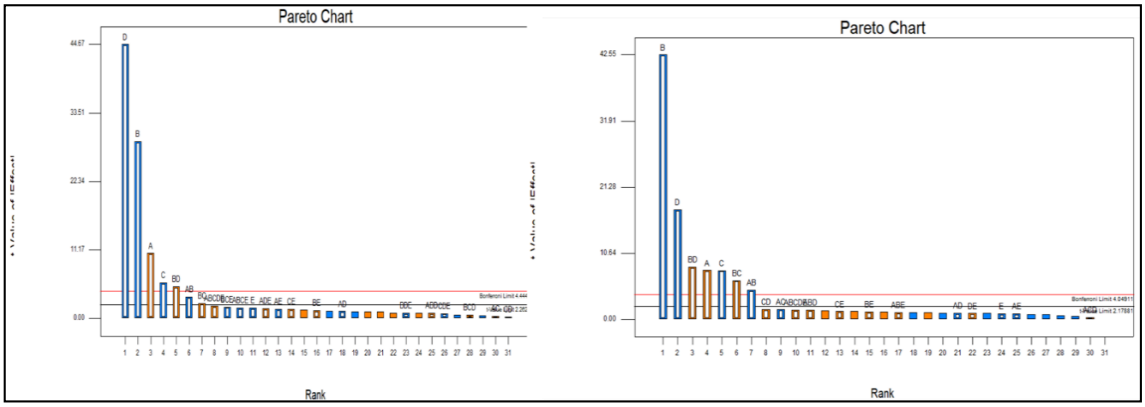

Fig 9: Pareto Chart for Retention Time

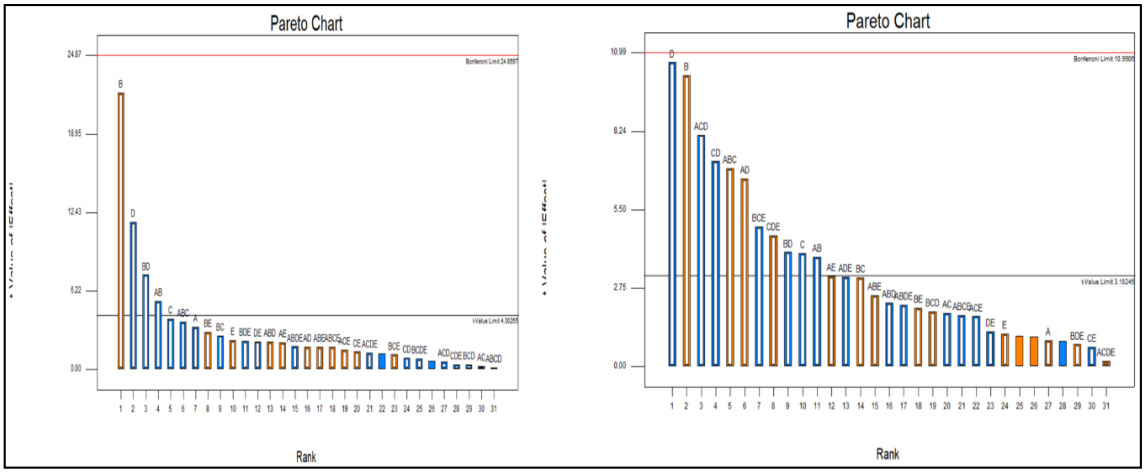

Fig 10: Pareto Chart f or Theoretical Plates

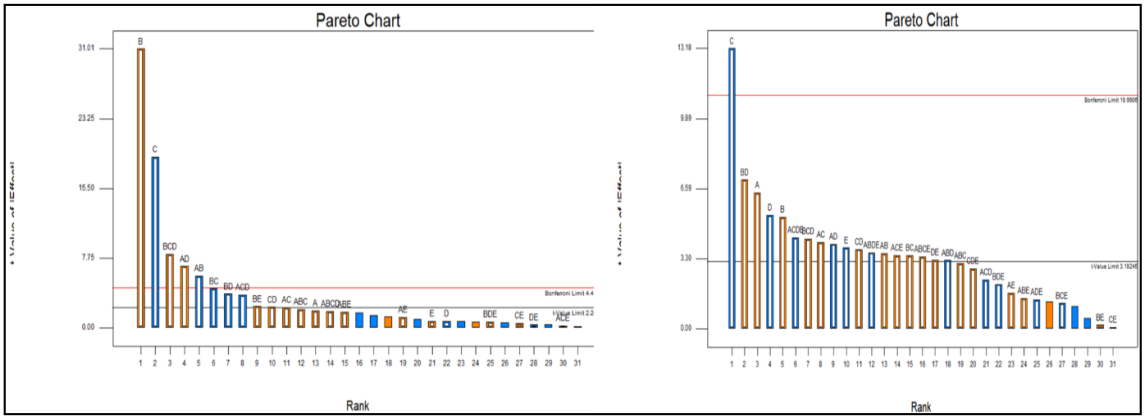

Fig 11: Pareto Chart for Tailing factor

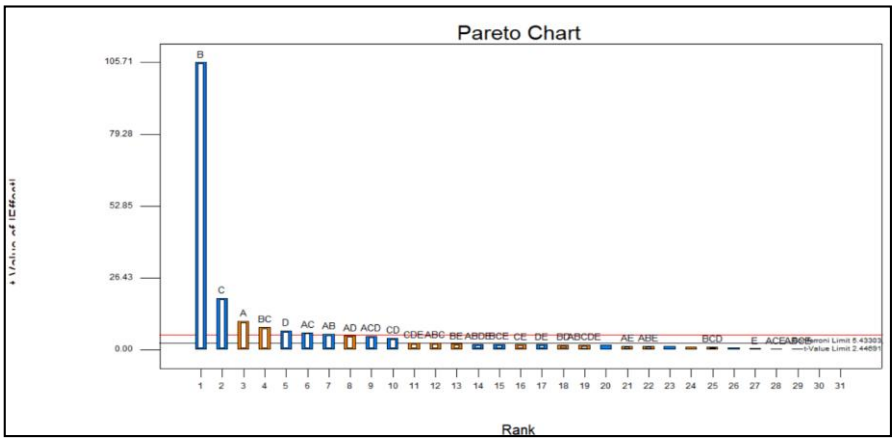

Fig 12: Pareto Chart for Resolution

Conclusion of: 2-level fractional factorial design

Four factors pH, %aqueous, Buffer concentration and Flow rate found to be most significant overall affecting almost all the responses involved. Hence, these 4 factors were selected for the next stage of Optimization in QbD by applying Box-Behnken design.

Box-behnken design for RP-HPLC method optimization

Table 19: BBD Trials

Std	Run	F1: pH	F2: %organic	F3: B. strength	F4: Flow Rate	R1: RT1	R2: RT2	R3: TP1	R4: TP2	R5: TF1	R6: TF2	R7: Resolution
12	1	4	50	15	1.2	2.55	4.46	3174	2759	1.00	1.97	7.34
11	2	3	50	15	1.2	2.27	3.54	3524	2964	1.00	1.54	6.19
10	3	4	50	15	0.8	4.62	9.94	4619	3893	1.09	2.32	11.7
2	4	4	40	15	1.0	3.53	9.49	2588	3019	0.79	2.76	12.3
19	5	3	50	20	1.0	2.77	4.59	3754	4045	0.76	1.45	7.74
6	6	3.5	50	20	0.8	3.67	6.46	4002	3800	0.87	1.64	8.57
29	7	3.5	50	15	1.0	2.87	4.84	3789	3595	0.97	1.69	7.72
20	8	4	50	20	1.0	2.99	5.01	4975	5013	1.03	1.17	7.06
21	9	3.5	40	15	0.8	3.33	4.47	6168	4517	1.28	3.84	5.22
1	10	3	40	15	1.0	2.79	4.94	4048	1906	0.97	1.66	6.86
17	11	3	50	10	1.0	2.89	5.01	3833	3595	0.91	1.66	8.14
4	12	4	60	15	1.0	3.11	5.17	4709	3476	1.05	1.81	7.78
18	13	4	50	10	1.0	3.02	5.27	3934	3489	1.07	2.08	8.21
13	14	3.5	40	10	1.0	3.65	11.7	2373	3312	0.86	2.34	14.5
14	15	3.5	60	10	1.0	2.78	3.9	6195	6964	1.39	1.29	6.79
3	16	3	60	15	1.0	2.87	4.35	3789	3500	1.00	1.09	6.16
23	17	3.5	40	15	1.2	2.83	7.71	2376	3505	0.64	1.36	12.9
8	18	3.5	50	20	1.2	2.48	4.39	3003	2673	0.88	1.60	7.34
28	19	3.5	50	15	1.0	2.87	4.84	3789	3595	0.97	2.04	7.72
9	20	3	50	15	0.8	3.48	5.79	3978	4090	1.06	1.65	7.95
26	21	3.5	50	15	1.0	2.87	4.84	3569	3600	0.97	1.82	7.65
16	22	3.5	60	20	1.0	2.67	3.49	6753	5953	1.26	1.57	5.27
27	23	3.5	50	15	1.0	2.87	4.84	3789	3482	0.95	1.80	7.66
24	24	3.5	60	15	1.2	2.52	3.27	5985	5936	1.2	1.43	5.03
5	25	3.5	50	10	0.8	3.75	6.49	4186	4059	0.98	1.85	8.58
25	26	3.5	50	15	1.0	2.87	4.84	3789	3605	0.95	1.88	7.74
15	27	3.5	40	20	1.0	3.33	8.86	2513	3550	0.71	1.69	12.8
7	28	3.5	50	10	1.2	2.52	4.39	3312	3295	1.00	1.76	7.76
22	29	3.5	60	15	0.8	3.99	5.97	6125	2462	0.90	1.00	5.8

Analysis responses obtained from BBD design

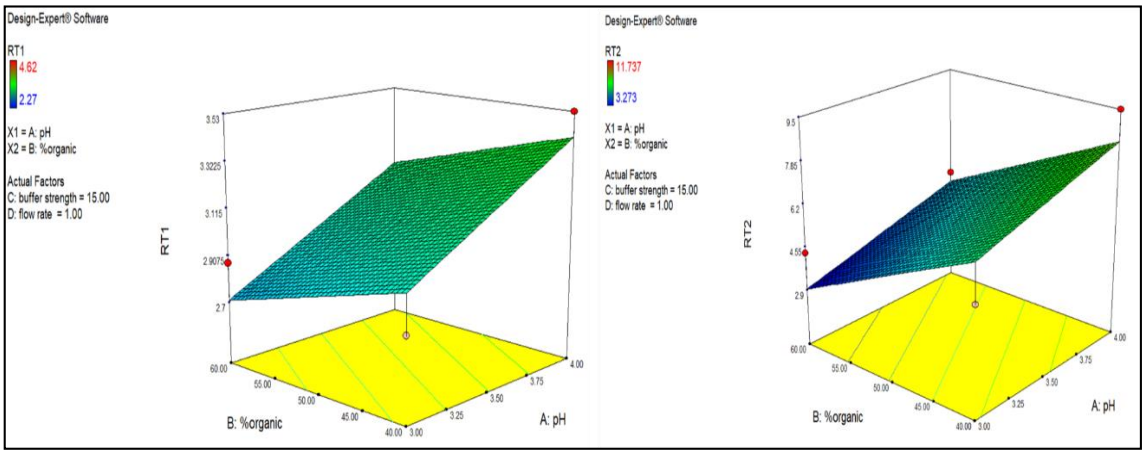

Fig 13: 3D Contour Plots of Retention Time

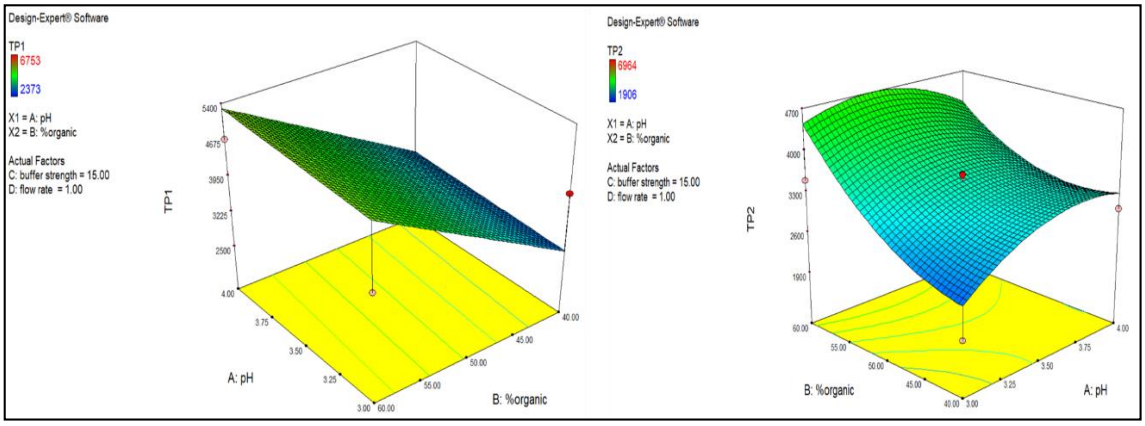

Fig 14: 3D Contour Plots of Theoretical Plates

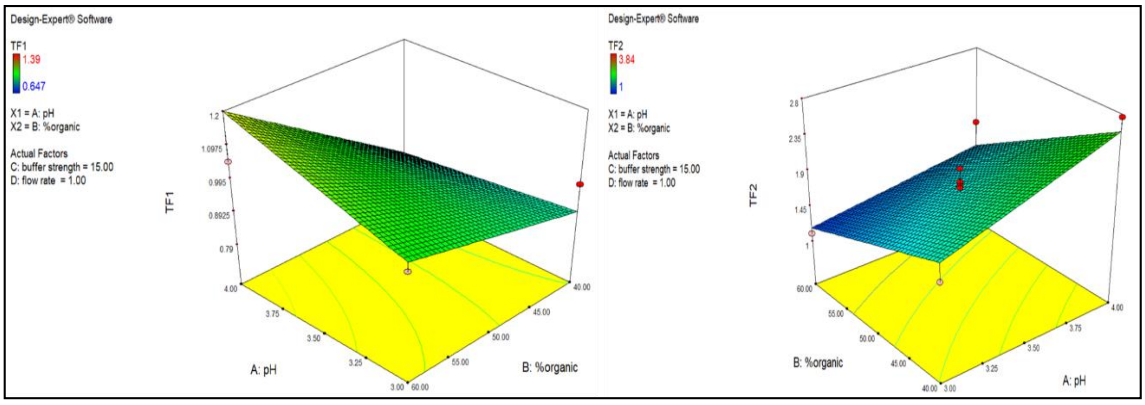

Fig 15: 3D Contour Plots of Tailing Factor

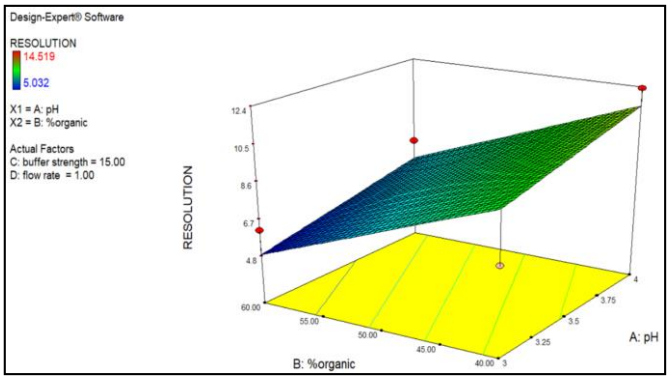

Fig 16: 3D Contour Plots of Resolution

Method obtained after optimization of design

Figure shown below displays the desirability plot for the optimized solution. The red region in it indicates Desirability of 1 (maximum desirability).

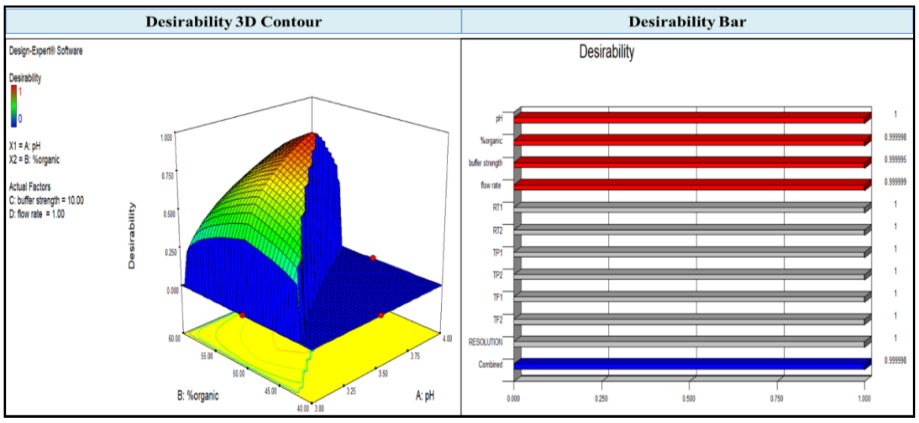

Fig 17: Desirability 3D Contour and Bar for optimization of method

Design space of developed method

The multidimensional combination and interaction of input variables (e.g. material attributes) and process parameters that have been demonstrated to provide assurance of quality is called design space. The overlay plot displays the design space. Yellow region shows that varying the experimental variables in this region, the method remains robust. Grey areas indicate non-robust region.

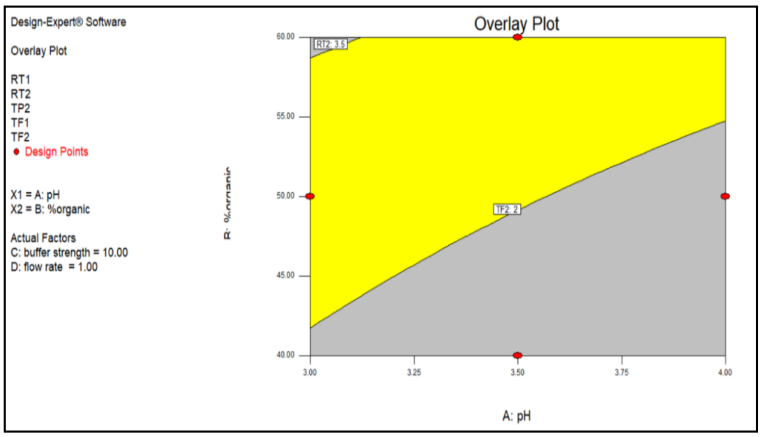

Fig 18: Overlay plot (Indicating Design space)

Table 20: Optimized Method Parameters

Method parameter	Optimized value
Column	CHROMATOPAK, Peerless C18 column, (Column dimensions: 250 mm x 4.6 mm,5 µm)
Mobile phase	Acetonitrile: Potassium Dihydrogen Phosphate Buffer pH 3.5 = 50:50
Flow rate	1.0 ml/min
Retention time	3. 015± 0.016 min for LMG and 6.043 ± 0.067 min for ARP
Detection wavelength	225nm
Temperature	Ambient

Method Validation

Developed RP-HPLC method was validated according to ICH Q2 (R1) guidelines.

1) Linearity

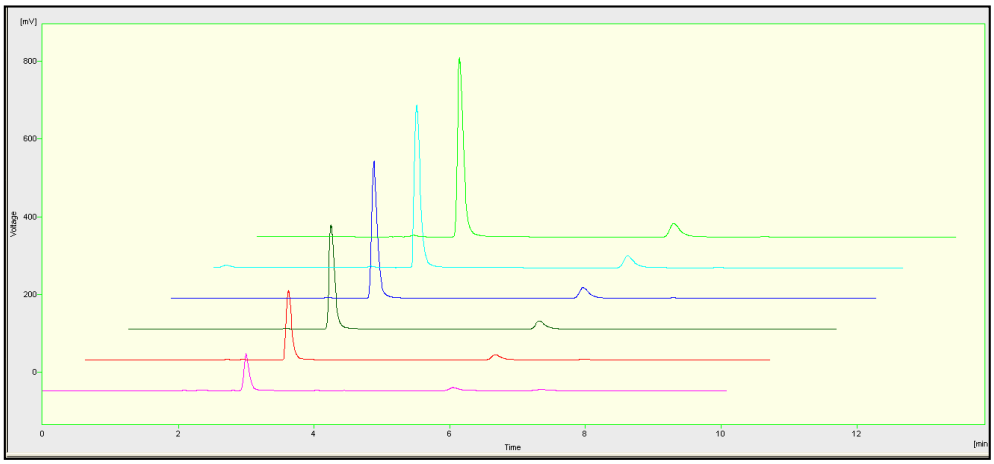

Fig 19: Overlain chromatogram of LMG (10-60 µg/ml) and ARP (1.0-6.0 µg/ml)

Table 21: Linearity parameters for LMG and ARP

Parameters	LMG	ARP
Analytical wavelength(nm)	225	225
Linearity range (µg/ml)	10-60	1.0-6.0
Regression equation	y = 50.191x + 55.491	y = 64.226x + 15.529
Correlation co-efficient	0.9966	0.9988
Slope	50.191	64.226
Std.error on slope	61.450	4.59
Intercept	55.491	15.529
Std.error in intercept	57.206	4.279
Confidence interval (95%) on intercept	-103.34 to 214.23	3.648 to 27.410

2) Precision

Table 22: Precision Study of LMG and ARP

	LMG			ARP		
Precision	**Conc (µg/ml)**	**Mean Area±SD, n=3**	**%RSD**	**Conc (µg/ml)**	**Mean Area±SD, n=3**	**%RSD**
Intraday	30	1582.92±2.795	0.1765	3.0	214.89±0.988	0.4600
	40	2144.93±3.922	0.1828	4.0	277.118±1.93	0.6987
	50	2594.92±3.86	0.1490	5.0	338.029±1.65	0.4891
Interday	30	1583.191±3.320	0.2097	3.0	214.079±0.625	0.2920
	40	2144.063±4.809	0.2243	4.0	274.872±1.460	0.5312
	50	2595.085±4.301	0.1657	5.0	338.653±1.078	0.3185

3) Accuracy

Table 23: Accuracy study of LMG and ARP for synthetic mixture

% Spiking	Concentration Actual (µg/ml)		Concentration Added (µg/ml)		Concentration Recovered (µg/ml)		%Recovery ± SD	
	LMG	ARP	LMG	ARP	LMG	ARP	LMG	ARP
80	10	1.0	8.00	0.8	7.9855	0.790	99.819	98.837
100	10	1.0	10.0	1.0	10.176	1.016	101.76	101.25
120	10	1.0	12.0	1.2	11.957	1.191	99.643	99.256

4) Robustness

Table 24: Robustness of HPLC method

Sr. No.	Factors		Peak Area (mV.s)	
			LMG	ARP
A	pH	3.4	2155.782	270.863
		3.5	2145.93	275.249
		3.6	2140.542	276.123
		MEAN± SD	2147.418±7.728	274.078±2.818
		%RSD	0.3598	1.028
B	Flow Rate	0.9	2142.015	275.475
		1.0	2144.648	277.856
		1.1	2152.637	280.460

		MEAN± SD	2146.433±5.531	277.93±2.493
		%RSD	0.2577	0.8971
C	Wave length	224	2150.724	280.842
		225	2146.391	275.458
		226	2143.762	274.021
		MEAN± SD	2146.959±3.515	276.774±3.595
		%RSD	0.1637	1.2991
D	%Organic	48	2141.045	273.794
		50	2146.852	275.456
		52	2155.495	278.964
		MEAN± SD	2147.797±7.271	276.071±2.639
		%RSD	0.3385	0.9560

5) System suitability

System suitability testing was carried out on freshly prepared standard solution (n=6) of LMG and ARP.

Table 25: Results of system suitability parameters

Parameters	Data Obtained	
	Lamotrigine	Aripiprazole
Retention Time ± SD	3.015 ± 0.00388	6.043 ± 0.03684
Theoretical Plate ± SD	3952.96 ± 87.674	4197.25 ± 118.356
Tailing Factor ± SD	1.39033 ± 0.0511	1.95233 ± 0.04933
Resolution ± SD	9.9951 ± 0.5436	

Applicability of proposed method

Table 26: Assay Results

Drug	Label claim	% Assay (Avg ± SD); n=6	%RSD
LMG	100mg	99.89±0.320	0.32046
ARP	10mg	99.16±0.875	0.88256

4) Force degradation study by RP-HPLC method for simultaneous estimation of lamotrigine and aripiprazole

Acid- induced degradation

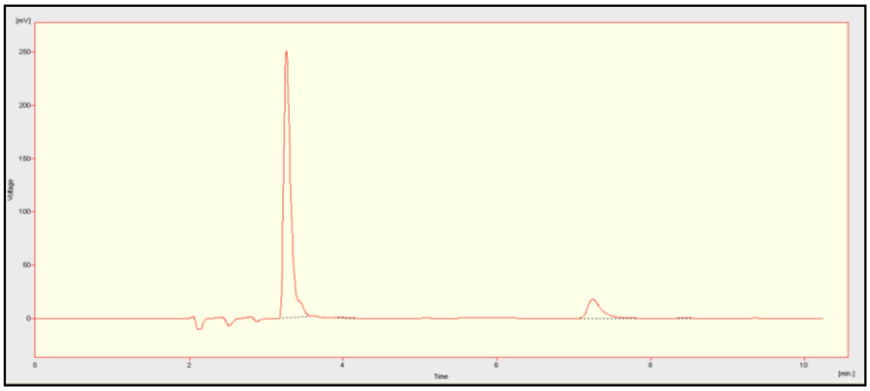

Fig 20: Acid degradation of mixture LMG and ARP (6hr)

Base- induced degradation

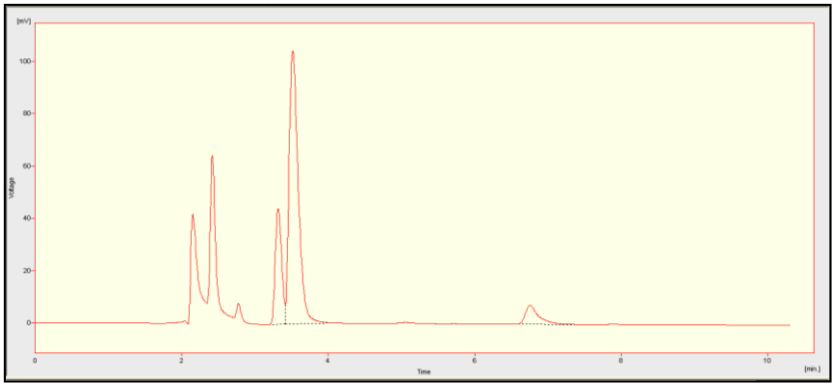

Fig 21: Base (1N NaOH) degradation of Mixture LMG and ARP (6hr)

Peroxide induced degradation (Oxidative Hydrolysis)

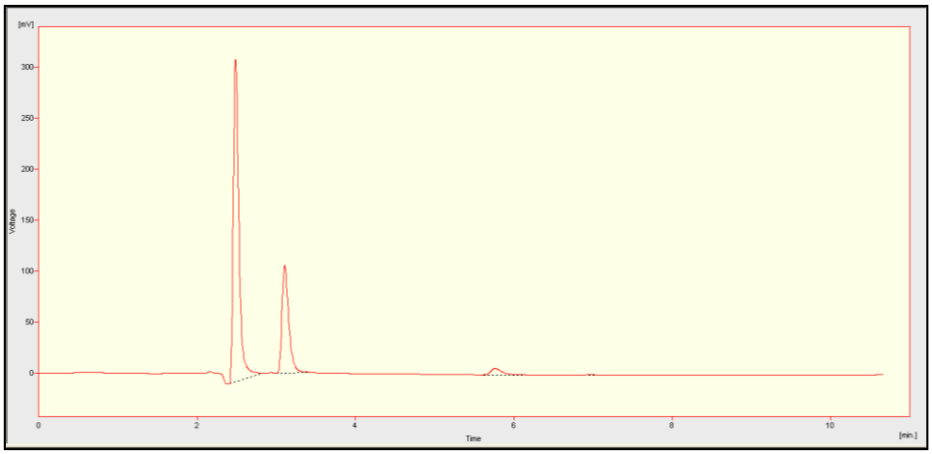

Fig 22: Oxidative Hydrolysis of Mixture of LMG and (intensity of the peaks remains consistent with all the time intervals-4hrs)

Dry heat induced degradation

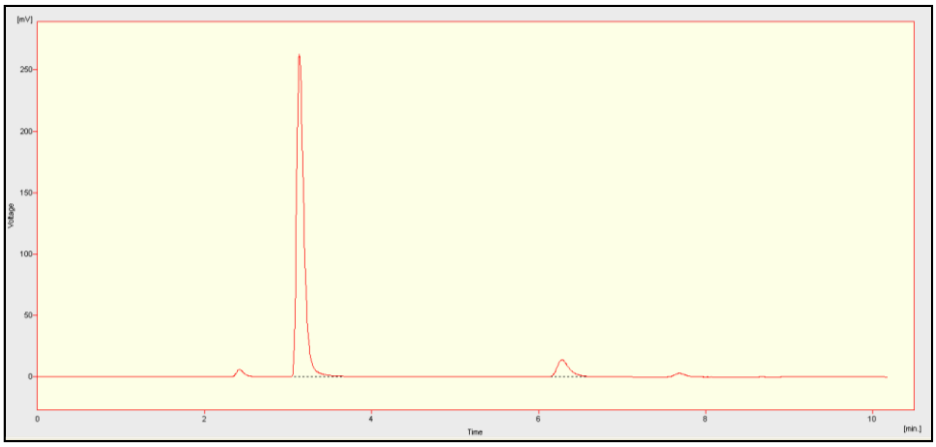

Fig 22: Dry heat studies at 80°C of LMG and ARP after 14days (intensity of the peaks remain consistent with all the time intervals)

Photochemical Degradation

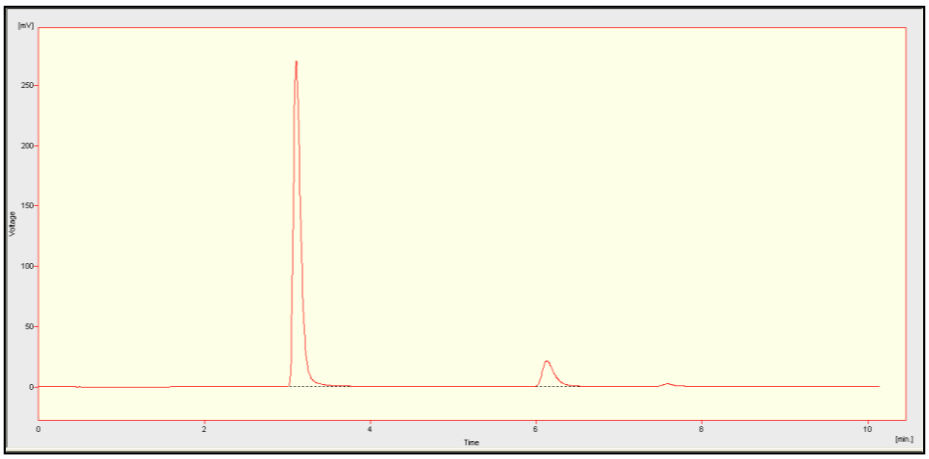

Fig 23: Photo stability studies of LMG and ARP after 14days. (Intensity of the peaks remains consistent with all the time intervals)

Table 27: Result of Force Degradation Study

Stressor Type	Stressor concentration	Stressor Time	%Degradation	
			LMG	ARP
Acidic	1N HCl at 80°C	6hrs	7.065%	6.18%
Basic	1N NaOH at 80°C	6hrs	42.16%	56.81%
Oxidation	1%H_2O_2 at RT	4hrs	4.78%	8.94%
Dry heat Stability	80°C	14 Days	1.20%	33.22%
Photostability	5382 LUX and 144UW/cm2	14 Days	6.68%	4.20%

Conclusion

The Developed UV Spectrophotometric methods, Chemometric Assisted methods and RP-HPLC method by QbD approach was simple, rapid, accurate, precise and robust. The proposed all methods were successfully validated according to ICH Q2 (R1) guidelines. The sample recovery was in good agreement with the composition of synthetic mixture, suggested non-interference of additives in its estimation. Hence, the developed all methods could be successfully applied for estimation of Lamotrigine and Aripiprazole in routine analysis.

References

1. Carlson BX, Ketter TA, Sun W, Timko K, McQuade RD, Sanchez R, *et al*. Aripiprazole in combination with lamotrigine for the long-term treatment of patients with bipolar I disorder (manic or mixed): a randomized, multicentre, double-blind study (CN138-392). 2012; 14:41-53.
2. Bristol-Myers Squibb. A Phase IV Study of the Safety and Efficacy of Aripiprazole in Combination with Lamotrigine in the Long-Term Maintenance Treatment of Patients with Bipolar I Disorder with A Recent Manic or Mixed Episode; Otsuka Pharmaceutical Development & Commercialization, Inc.; ClinicalTrials.gov Identifier: NCT00277212, 2013.
3. Haaland DM, Thomas EV. Partial Least-Squares Methods for Spectral Analyses. Anal Chem. 1988; 60(11):1193-1202.
4. Dinc E. Spectral analysis of benazepril hydrochloride and hydrochlorothiazide in pharmaceutical formulations by three chemometric techniques. Anal. Lett. 2002; 35(6):1021-1039.
5. Sandeep K, Induri M, Sudhakar M. Validated Spectrophotometric Quantification of Aripiprazole in Pharmaceutical Formulations by Using Multivariate Technique. 2013; 3(2):469-472.
6. Mohanr A, Ghosh SK. Development and Validation of UV/Visible Spectrophotometric Method for the Estimation of Lamotrigine in Bulk and Pharmaceutical Formulations. 2013; 2(11):1246-1251.
7. Bais S, Chandewar A, Kakani A, Singhvi I, Shirsat M. Development and Validation of a Stability-Indicating HPLC Method for Analysis of Lamotrigine in Bulk Drug and Formulations. 2013; 2(3):334-353.
8. Atodaria E, Mashru R. Development and Validation of some Classical and Novel Spectrophotometric Methods and RP-HPLC Method by QbD Approach for Simultaneous Estimation of Aripiprazole and Clozapine in Synthetic Mixture. 2018; 7(10):521-557.
9. ICH Q2 (R1). Validation of analytical procedure, Text and methodology, Geneva, International conference on Harmonization, 2005.
10. ICH Q1 (R2). International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, Topic Q1: Stability Testing of Pharmaceuticals (Q1A to Q1E), 2009.