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QbD approach for analytical method development of anti-pschotic drug

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ABSTRACT

The scientific way to develop a simple and robust analytical HPLC method for the critical separationsis QbD approach. Quality-by-design (QbD) is a systematic approach to product or process development, which begins with predefined objectives, and uses science and risk management approaches to gainproduct and process understanding and ultimately process control. The concept of QbD canbe extended to analytical methods. A simple Analytical method was developed and used to identify and quantify simultaneously the two active pharmaceutical ingredients Resperidone and Benzoic acid. In the present work, three independent factors were used such as flow rate (A), wavelength (B) and pH of buffer (C). Totally 27 experimental runs were suggested by the software for analyzing the interaction of each level on formulation characters and the peak area (R1), tailing factor (5%) (R2) and number of theoretical plate USP (NTP) (R3) were considered as response factors (dependent factors). The significance of independent factors was determined using Fisher's statistical test for Analysis of the Variance (ANOVA) model that was estimated. Waters Xterra C-18 column (150 mm × 4.6 mm, 5 µm pore size), column was the most suitable one since it produced symmetrical peaks with better resolution. The UV detector response of RIS and BA was studied and the best wavelength was found to be 275 nm showing highest sensitivity of both compounds. The method was validated for specificity, reproducibility, accuracy, linearity, robustness and solution stability and can be used for the assessment of quality of drug product in development and stability samples of the marketed oral solution. The target degradation for the stability indicating ability of the assay method was tried in the present study and there was no any interfering peaks found due to degradation products.

Keywords: Analytical Method Development, HPLC, QbD approach, DOE, Design Expert

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INTRODUCTION

Risperidone is the most frequently used atypical antipsychotic drug for a treatment of schizophrenia, bipolar disease and behavioral disorders. According to the WHO the mental disorders/diseases are at 3rd rank in the world as per the survey done by WHO in the 2010 for the DALY index (Disability Adjusted Life Years) [1-3]. Risperidone is a white to almost white powder. It is practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in ethanol, and dissolves in dilute acid solutions. Risperidone exhibits polymorphism. Risperidone is a selective monoaminergic antagonist with unique properties. It has a high affinity for both serotonergic 5-HT2 and dopaminergic D2 receptors. Risperidone binds also to alpha1-adrenergic receptors and, with lower affinity, to H1-histaminergic and alpha2-adrenergic receptors. Risperidone has no affinity for cholinergic receptors. Although risperidone is a potent D2 antagonist, that is considered to improve the positive symptoms of schizophrenia, it causes less depression of motor activity and induction of catalepsy than classical antipsychotics. Balanced central serotonin and dopamine antagonism may reduce extrapyramidal side effect liability and extend the therapeutic activity to the negative and affective symptoms of schizophrenia [4].

Analytic method development and validation are key elements of any pharmaceutical development program. An HPLC analysis method is developed to identify, quantity or purifying compounds of interest. This technical brief will focus on development and validation activities as applied to drug products. Effective method development ensures that laboratory resources are optimized, while methods meet the objectives required at each stage of drug development. Method validation, required by regulatory agencies at certain stages of the drug approval process, is defined as the "process of demonstrating that analytical procedures are suitable for their intended use"[5-7].

Chromatographic method development can be an time consuming and subjective process. As companies accelerate drug development programs and candidate compounds move through this process, fast and robust HPLC method development becomes increasingly important. Most method development is done using a manual, one-factor-at-a-time (OFAT) process where the approach is to vary one system parameter at a time and examine the resultant performance. This procedure is continued until no further improvement is obtained, at which time another parameter is selected for study [8-14]. These separations are often sub-optimal in terms of resolution, tailing, retention time and lack robustness. This can be particularly problematic when preparative chromatography is required to purify milligram to gram amounts of product, as compounds that appear to be well resolved at the analytical scale, may no longer separate efficiently when scaled up, necessitating either further method development or additional product purification steps. This process can be improved by applying a Quality-by-Design (QbD) strategy that develops analytical LC methods to meet performance requirements using sound statistical experimentation principles that accurately quantify system behavior and then scale these up for preparative separations. (1, 2, 3, 4) This can be done using a software based Design Of Experiments (DOE) applications that relies on multivariate modeling to automatically predict and generate optimized analytical HPLC methods that can be transferred to preparative HPLC systems and rapidly scaled up, significantly increasing productivity[15-22].

The primary objective of this study was to implement QbD approach to develop and validate an RP-HPLC method and to establish and in-depth understanding of the method and build in the quality during the method development to ensure optimum method performance over the lifetime of the product.

MATERIALS AND METHODS

Instrumentation

HPLC system consisted of Agilent Technologies, US, 1260 Infinity Quaternary LC System model connected to VWD UV detector, column oven and auto sampler (Agilent Technologies, US) was used in this study. Chromatograms were recorded by a computer and treated with the aid of Chrome Eleon. A Watters (Xttera) C18 column (250 mm · 4.6 mm id, 5 µm pore size) was used to perform the separation.

Milli-Q water was produced with a water purification system, Millipore Corporation (Billerica, MA, USA).

The mobile phase was filtered through the Millipore glass filter (Millipore filter cellulose nitrate grided with 0.22 µm size) assembly attached with vacuum pump. The mobile phase was sonicated with Ultrasonic Cleaner – 15L, PCI Analytics Pvt. Ltd, Bhandup, West Mumbai.

The pH of mobile phase or buffer was measured with Eutech Instruments pH 510 using a glass electrode Van London Co., USA.

Chemical and solvents:

Risperidone USP (RIS) was provided by Janssen Pharmaceutica Ltd. Wallingstown, Little Island, Ireland. Benzoic Acid (BA) was provided from Panreca Quimica SLV, Belgium. Methanol and acetonitrile were of Analytical Grade and purchased from Finar chemicals pvt ltd company (Gujarat India). Pottasium Dihydrogen Phosphate (KH2PO4) purchased from SDF chemicals, Ltd. Orthophosphoric acid was of analytical grade and procured from Rankem Chemicals Ltd. Mili-Q (0.22µ) water was used for preparing mobile phase and other solutions. Pharmaceutical finished dosage forms utilized in the present work was provided by Janssen Pharmaceutical Ltd. Wallingstown, Little Island, Ireland. Product was marketed as RISPERDAL® 1mg/ml oral solution.

Chromatographic Conditions:

Separation was achieved on Waters Xterra C-18 column (150 mm × 4.6 mm, 5 µm pore size) maintained ambient column oven temperature and sampler temperature. Isocratic elution with methanol: water (50:50% v/v) mobile

phase at the flow rate of 0.8 ml/min was carried out. The detection was monitored at 275 nm and injection volume was 10 µl. The peak purity was checked with the DAD detector.

Standard stock solutions:
Stock solutions of 1 mg/ml and 2 mg/ml of RIS and BA respectively were prepared by dissolving them in acetonitrile and water. Standard calibration solutions were prepared by dilution of the stock solutions using the diluent. These solutions were considered at seven different levels which were 25%, 50%, 100%, 150% and 200% of the test concentration[6]. For RIS and BA mixtures, standard solutions of RIS and BA containing a constant concentration of 0.2 mg/ml and 0.4 mg/ml respectively (internal standard) were prepared in diluent by maintaining the concentrations in the range of 0.05 mg/ml to 0.4 mg/ml and 0.1 mg/ml to 0.8 mg/ml respectively. The calibration curves for RIS and BA mixtures were constructed by plotting the peak area against the drug concentration. The diluent is prepared by adding water and acetonitrile (80:20 v/v).

Selection of detection wavelength:
The detection wavelength was selected by scanning the 10 µg/ml concentration solution of Risperidone and Benzoic Acid in the mobile phase in UV spectrophotometer and maximum absorption was selected as 275 nm.

Selection of mobile phase:
The pure drug of Risperidone and Benzoic Acid were injected into the HPLC system and run in different mobile phase system. Different mobile phases like acetonitrile, methanol, water and different pH buffer are tried. It was concluded that Potassium phosphate buffer of pH- 3.00 and methanol gives satisfactory results which passes the ICH guideline i.e. ICH Q2 (R1). Hence finalized mobile phase is Methanol and potassium phosphate buffer of pH- 3.00 (50:50).

Sample preparations:
The label claim of the marketed preparation is 1 mg/ml and 2 mg/ml of RIS and BA respectively. 10 ml of marketed preparation was transferred into a 50ml volumetric flask and made up volume with diluent this is the test solution concentration.

Preparation of Phosphate buffer:
Potassium dihydrogen phosphate (2.72 g) was dissolved in 1000 mL of Mili-Q water (filtered through 0.22µm size filters) and pH 3.0 was adjusted with orthophosphoric acid. It was filtered through 0.45 µm nylon membrane filter and degassed. It was used for the preparation of mobile phase.

System suitability test (SST) / Specificity and formulation analysis:
The specificity of method was established by preparing placebo solution by optimized method for assay of the samples using equivalent weight of the placebo with marketed preparation. Chromatogram of the placebo was not showing any interference at the retention time of Risperidone and Benzoic Acid (Figure). The SST ensures the validity of the analytical procedure as well as confirms the resolution between different peaks of interest.

All critical parameters tested met the acceptance criteria on all days. Adequate resolution between the RIS and BA peaks ensured the specificity of the method. The system suitability assessment for the analytical HPLC method established instrument performance parameters such as peak area, % R.S.D., column efficiency (N) and USP tailing factor (Tf) for both the analytes. Three different pH of Potassium Dihydrogen Phosphate such as 2.9, 3.0 and 3.1 were studied and optimized with DOE. This pH was used in the study for best retention of acidic drug in a reversed-phase system. The mobile phase was selected in 25:25:50 ratio of Acetonitrile: Methanol: Phosphate buffer (pH-3.00). The 10 µl injection volume was used in the study. The UV detector was used for detection of drug in the samples. As like pH, three different wavelengths such as 270, 275 and 280 nm were studied and one wavelength was selected based on DOE. Similarly, the effect of flow rate (i.e. 0.6, 0.8 and 1.0 mL/min) was studied and based on DOE, the final flow rate was selected. Most of the formulation studies involve only one variant at a time by keeping others as constant. With the help of full factorial design investigators can study the effect of all the factors by varied simultaneously. The factorial design helps to study the effects caused by independent factors and interactions between those self-governing factors [18]. In the present work, three independent factors were used such as flow rate (A), wavelength (B) and pH of buffer (C). Three factorial levels were used in the study and were coded as _1, 0 and +1 for low, medium and high, respectively [19-22]. Totally 27 experimental runs were suggested by the software for analyzing the interaction of each level on formulation characters and the peak area (R1), tailing factor (5%) (R2) and

number of theoretical plate USP (NTP) (R3) were considered as response factors (dependent factors). Tables 1 and 2 show the factors chosen and different factor level settings. The significance of independent factors was determined using Fisher's statistical test for Analysis of the Variance (ANOVA) model that was estimated.

Table 1: 3^3 Factorial design with upper, middle& lower limits of all factors Statistical Optimization technique

3 Factors	3 Levels		
	Low (-1)	Middle (1)	High (+1)
Flow Rate	0.6	0.8	1.0
pH of Solvent	2.9	3.0	3.1
Column Temperature	20 °C	30	30 °C

The polynomial equation for the experimental design with three factors is given below:

$$R = \beta o + \beta 1\, A + \beta 2\, B + \beta 3\, C + \beta 2\, AB + \beta 2\, AC + \beta 2\, BC + \beta 2\, (A * A) + \beta 2\, (B * B) + \beta 2\, (C * C)$$

where, R is the response, b is the regression coefficients and A, B and C represent flow rate, wavelength and pH of buffer, respectively.

Table 2: 3^3 Factorial design with high & low level factor statistical sequence optimization

Run No.	Replicates	Flow Rate	pH of Solvent	Column Temperature (°c)
1	3	0.6	2.9	20
2	16	1	2.9	20
3	19	0.6	3.1	20
4	12	1	3.1	20
5	6	0.6	2.9	30
6	4	1	2.9	30
7	15	0.6	3.1	30
8	11	1	3.1	30
9	10	0.46	3	25
10	8	1.13	3	25
11	2	0.8	2.83	25
12	20	0.8	3.16	25
13	18	0.8	3	16.59
14	17	0.8	3	33.40
15	1	0.8	3	25
16	13	0.8	3	25
17	9	0.8	3	25
18	5	0.8	3	25
19	14	0.8	3	25
20	7	0.8	3	25

RESULTS AND DISCUSSION

Method optimization

Waters Xterra C-18 column (150 mm × 4.6 mm, 5 µm pore size), column was the most suitable one since it produced symmetrical peaks with better resolution. The UV detector response of RIS and BA was studied and the best wavelength was found to be 275 nm showing highest sensitivity of both compounds. Several modifications in the mobile phase composition were made in order to study the possibilities of changing the selectivity of the chromatographic system.

These modifications included the change of the type and ratio of the organic modifier, flow rate, temperature and stability of RIS and BA was also studied. Initially no peaks were observed when acetonitrile and phosphate buffer in different ratios were utilized, at temperature of 30°C and 1.0 ml/min flow rate on a C8 column. So acetonitrile was replaced by methanol, at that time both drugs again didn't show peaks. Hence the C8 column was replaced by C18 column methanol and phosphate buffer (pH- 3.0) 50:50, peaks of both drugs were observed, but with less resolution and with peak broadening effect for RIS and BA at temperature of 30°C. Then ratio of buffer and methanol was changed to 40:60, peaks of both drugs were observed with good resolution without peak broadening, tailing, fronting and with good sensitivity as well, at 35°C temperature and flow rate of 1.0 ml/min. The effect of flow rate on the separation of peaks was studied by varying the flow rate from 0.5 to 1.3 ml/ min; a flow rate of 0.8 ml/min was optimal for good separation and resolution of peaks in a reasonable time as shown in Fig. 2 shows the chromatogram

for a working standard mixture of RIS and BA, respectively. System suitability parameters with peak purity data are given in Table 3.

Table 3: System suitability parameters of both drugs (RIS and BA)

Sr. No.	Parameters	Risperidone	Benzoic Acid
1	**Retention Time**	3.045	4.162
2	**Resolution USP**	NA	4.28
3	**Theoretical Plates USP**	2025	4381
4	**Tailing Factor USP**	1.16	1.09

Method validation

The method was validated according to ICH guidelines. The following validation characteristics were addressed: linearity, range, accuracy, precision, specificity, and robustness. Specificity of the method was determined by analyzing samples containing a mixture of the drug product and excipients. All chromatograms were examined to determine RIS & BA.

Formulation analysis and system suitability/ Specificity

The assay for the marketed oral solution was established with present chromatographic condition developed and it was found to be more accurate and reliable. The average drug content was found to be 100.9 % for RIS, 100 % for BA. With % RSD for RIS and BA was 0.1 and 0.1 %.

Linearity and range

Linearity was determined for RIS and BA in the range of 25% - 200% of test solution concentration. The correlation coefficient ('r2') values were >0.998 (n = 6) indicating an excellent correlation between peak areas and analyte concentrations. (Table 4 with Graph shown in Fig 1 and 2)

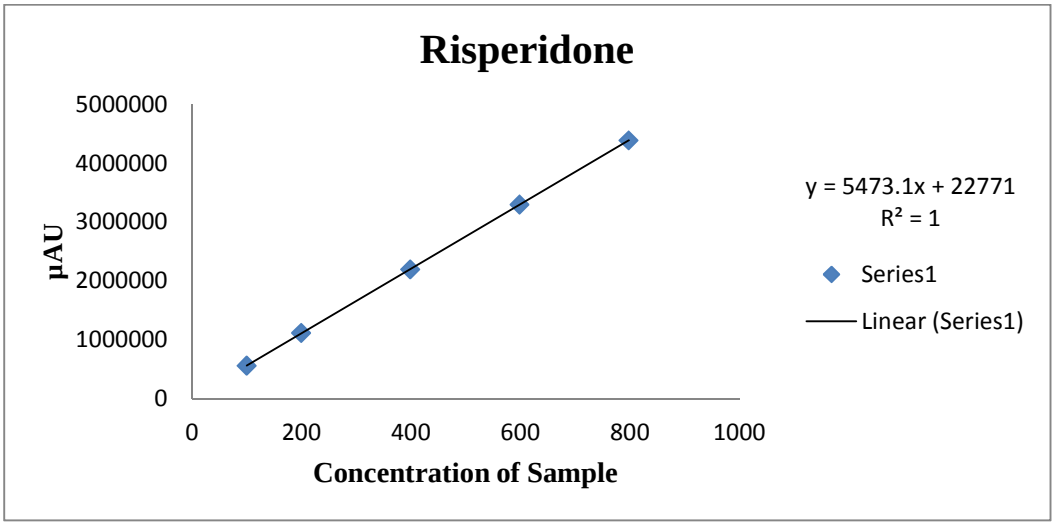

Fig 1: Linearity Graph of Risperidone

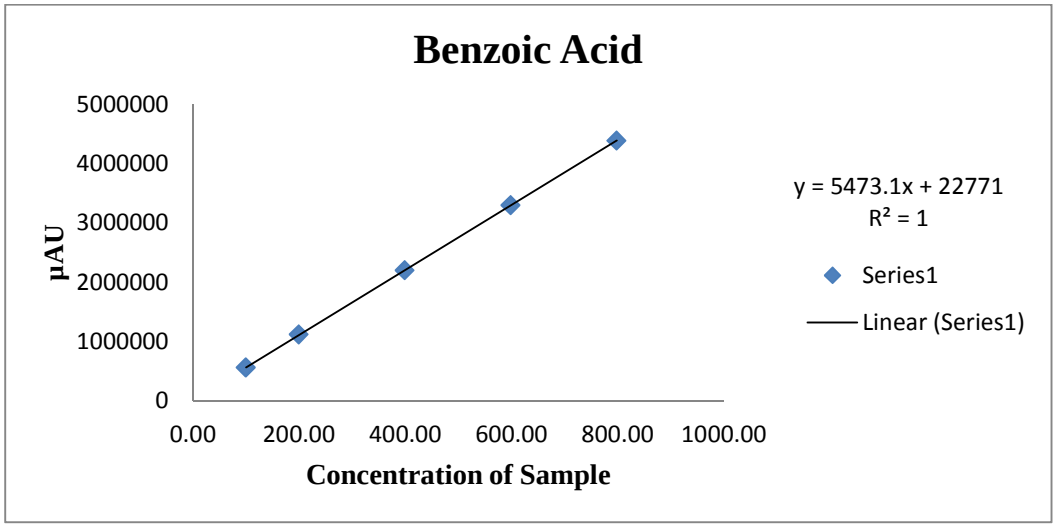

Fig 2: Linearity Graph of Benzoic Acid

Accuracy (% Recovery)

The mean percentage recoveries obtained were 101.3%, 100% for RIS and BA respectively. The developed method was found to be accurate as the mean percentage recoveries obtained for RIS and BA were found to be within limit as recommended by ICH guidelines. The developed method was found to be accurate as the % RSD values for accuracy studies were <2% (Table 4), as recommended by ICH guidelines.

Table No. 4: Result of validation parameters

Sr. No.	Parameters	Risperidone	Benzoic Acid
1	**Accuracy / % Recovery**	101.3	100
2	**Precision (% RSD)**	100.9 (0.1%)	100 (0.1%)
3	**% Assay**	100.6	100
4	**Linearity (r^2 value)**	1.000	1.000

Method Precision

The system precision was demonstrated by preparing the standard solution at test concentration and injected repeatedly for six times. The % RSD for repeatability of sample preparation is 0.12 % and 0.07 % for RIS and BA respectively. The precision is satisfactory and the % RSD is not more than 2.0% as per ICH guidelines. The results are shown in Table 4.

Robustness and solution stability studies

The % Assay and % RSD was found to be in range 100 ± 1.5% and <2, respectively. It indicates that method follow specification of ICH guideline. Results of the stability studies were in the range of 99.5 - 101.5%. Stability as described in method development under experimental section was studied. Results of the stability studies were within the acceptable limit (98 - 102%).

Forced Degradation

As per ICH guidelines, the target degradation below 0.5% should be there for the stability indicating ability of the assay method and the same was tried in the present study. No interfering peaks were found due to degradation products at the drugs Rt's.

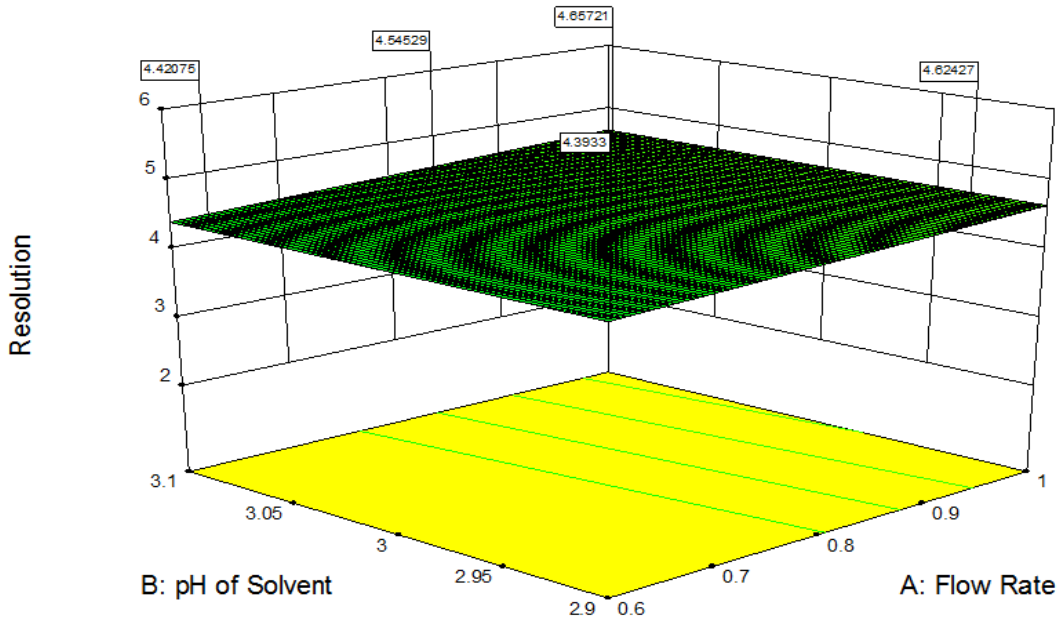

Fig. 3 - Effect of Flow rate and pH of Solvent at medium level

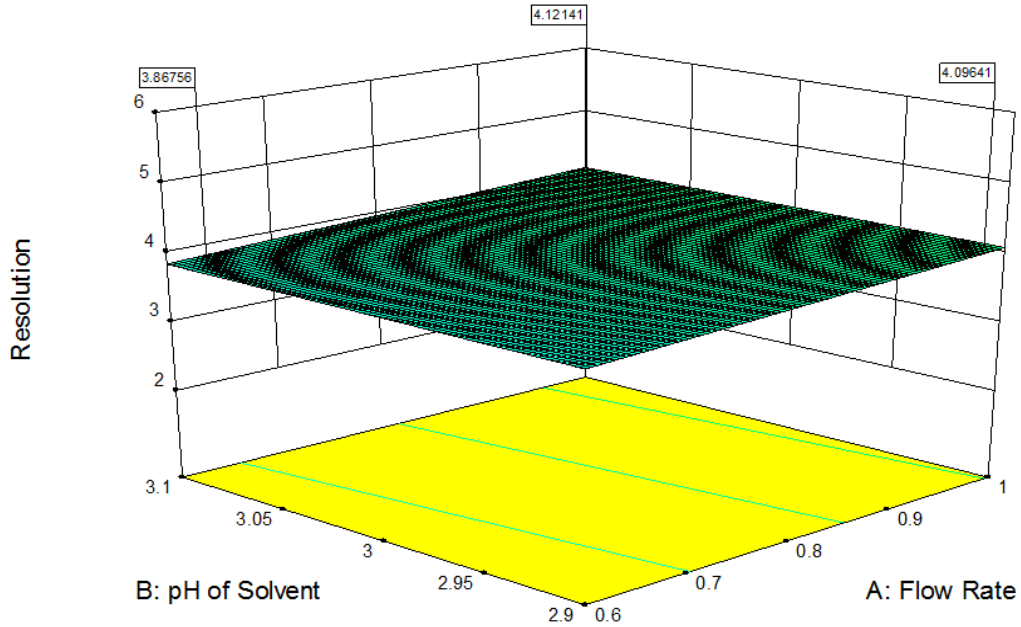

Fig. 4 - Effect of Flow rate and pH of Solvent at low level

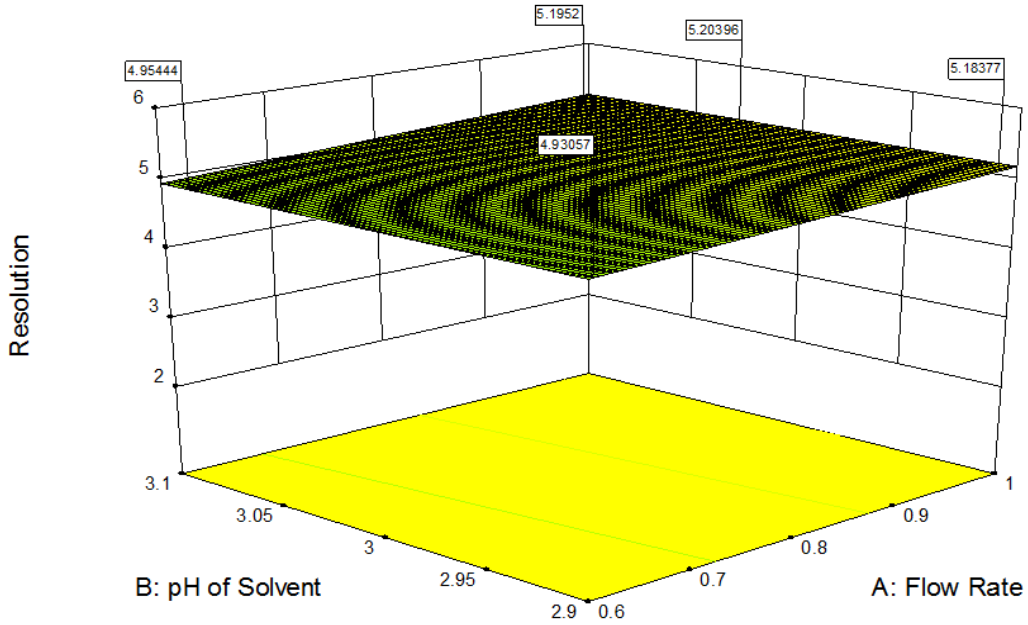

Fig. 5 - Effect of Flow rate and pH of Solvent at high level

CONCLUSION

A robust method for degradation of Risperidone and Benzoic was developed using a Quality by Design approch on an Design-Expert® Software, Version 9. Three independent factors were used such as flow rate (A), wavelength (B) and pH of buffer (C). Totally 27 experimental runs were suggested by the software for analyzing the interaction of each level on formulation characters and the peak area (R1), tailing factor (5%) (R2) and number of theoretical plate USP (NTP) (R3) were considered as response factors (dependent factors). The method was validated according to ICH guidelines. Specificity of the method was determined by analyzing samples containing a mixture of the drug product and excipients. The assay for the marketed oral solution was established with present chromatographic condition developed and it was found to be more accurate and reliable. The % Assay and % RSD was found to be in range 100 ± 1.5% and <2, respectively. It indicates that method follow specification of ICH guideline. Results of the stability studies were in the range of 99.5 - 101.5%. As per ICH guidelines, the target degradation for the stability indicating ability of the assay method was tried in the present study. No interfering peaks were found due to degradation products at the drugs Rt's. Design Expert was able to automatically predict and test speed and resolution optimized analytical methods that separated all the drug peaks. Analytical to prep scale-up of the drug peaks was successful with sufficient resolution of the critical peak pairs to ensure that maximum recovery of pure fractions would be possible.

ABBREVIATIONS
BA- Benzoic Acid
RIS- Risperidone
HPLC- High Performance Liquid Chromatography
ICH- International Conference of Harmonization
DAD-Diode Array Detector
KH2PO4 -Pottasium Dihydrogen Phosphate
SST-System Suitability Test
Tf- Tailing Factor
R.S.D.- Relative Standard Deviation

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