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Development and Validation of Piribedil in Tablet Dosage Form by HPLC: A QbD and OFAT Approach

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Piribedil is considered as an antiparkinson agent or dopamine agonist type of medication. A simple, selective, rapid, precise, accurate and economical reverse phase stability indicating HPLC method has been developed and validated as per ICH guidelines for estimation of piribedil (PI) in sustained release tablet dosage form. Xterra RP18 (150 × 4.6 mm, 5 µ) stationary phase by using an isocratic mobile phase consists of pH 2.5 phosphate buffer of 0.05 M and acetonitrile in the ratio of (80:20 v/v) at a flow rate of 1.5 mL/min and column temperature of 50 °C. Piribedil was detected at 240 nm. The chromatographic procedure separated piribedil and its potential degradation products in an overall analysis time of 6 min with piribedil eluting at about 3 min. Piribedil tablets were exposed to thermal, photolytic, acid, base and oxidative stress conditions. The degradation products are well resolved from the piribedil peak indicating the stability indicating the significance of the method. The assay method was found to be linear in the concentration range of 0.024-75 µg/mL with a correlation coefficient of 0.9999. The percentage recovery of assay was found between 100.4 and 100.6. Considerable degradation was found to occur in base hydrolysis, water hydrolysis and oxidation. In the present study a 13.2 % forced degraded sample is used in the robustness parameter for both QbD and OFAT approaches which can produce a robust HPLC method. The proposed method can be used for routine analysis in quality control laboratories for its formulated product.

Keywords: Piribedil, Validation, HPLC, Quality by design (QbD), One factor at a time (OFAT), Stability indicating.

INTRODUCTION

Piribedil (PI) chemically (2-[4-(1,3-benzodioxol-5-ylmethyl)-1-piperazinyl]pyrimidine) (Fig. 1) is a dopamine agonist acting on D2 and D3 central nervous system dopamine receptors [1]. The drug has proved active in patients with Parkinson's diseases, particularly in the control of tremors [2]. Methods for analysis of piribedil in human serum, urine and pharmaceutical dosage form by LC-DAD [3] and its *p*-hydroxylated, catechol and N-oxide metabolites in plasma by high-performance liquid chromatographic (HPLC) [4] are available in the literature. Rohith *et al.* [5] reported a new RP-HPLC method for the determination of piribedil in bulk drug. The aim of this study was to develop a stability indicating assay method for estimation of piribedil in a pharmaceutical dosage form.

For the estimation of piribedil there is no official or draft monograph has been published in any of the pharmacopoeia for compendial applications. There is no reported stability indicating method (SIM) by HPLC for the estimation of piribedil in a pharmaceutical tablet dosage form.

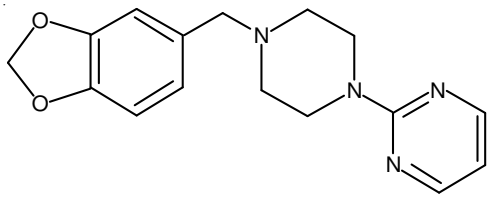

Fig. 1. Chemical structure of piribedil (PI) 2-[4-(1,3-benzodioxol-5-ylmethyl)-1-piperazinyl]pyrimidine

Therefore, it was felt necessary to develop an accurate, rapid, specific and stability-indicating method for the determination of assay of piribedil in tablet dosage form by using quality by design (QbD) principles to ensure the quality of the method throughout the product lifecycle. No interferences

are observed from blank or excipients at piribedil peak retention. The primary advantage of this method is that it is simple and accurate with a shorter run time.

In conventional chromatographic method development, we studied the effect of each factor on the method by varying one factor at a time (OFAT) approach. This procedure generally requires more number of experimental trails and sometimes further method development [6].

The main presumption behind QbD is that quality is 'designed' into the process in a single step by establishing a proper design space with thorough understanding of the quality system parameters [7]. Design space is described as the "interaction of selected factors in multi factorial design to establish a high degree of quality" [8].

The prime objective of this study was to implement both OFAT and QbD approach to develop and validate a HPLC method that could separate degradents in the forced degradation sample (About 13.2 % oxidative degraded sample was taken) of the tablet dosage form with 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 with a suitable degradation data.

Degradation of drug substances between 5 and 20 % has been accepted as reasonable for validation of chromatographic assays [9,10]. Some pharmaceutical scientists think 10 % degradation is optimal for use in analytical validation for small pharmaceutical molecules for which acceptable stability limits of 90 % of label claim is common [11].

A quality by design (QbD) approach to method development uses statistical design of experiments (DoE) to develop a robust method 'design space'. The design space defines the experimental region in which changes to method parameters will not significantly affect the results. This approach builds-in robustness to the method as the method is being developed [12].

EXPERIMENTAL

Piribedil active pharmaceutical ingredient was procured from Ra Chem Pharma Pvt. Ltd. (Hyderabad, India). The tablet dosage form (piribedil retard tablets 50 mg was developed by Epione Labs Pvt Ltd (Hyderabad, India). Acetonitrile of HPLC grade was purchased from Merck, Germany. All analytical reagents like orthophosphoric acid, potassium dihydrogen phosphate, sodium hydroxide, hydrochloric acid, hydrogen peroxide were purchased from Merck, Germany. HPLC grade water was prepared by using the Millipore purification system.

The Shimadzu HPLC instrument (Shimadzu, Kyoto, Japan) equipped with a photodiode array detector was used for stress degradation studies in method validation. Lab solutions software was used for output signal processing. Photo stability exposure studies were carried out in a photo stability chamber (Thermolab, India). Thermal stability studies were performed in a dry air oven (Thermolab, India).

Chromatographic conditions: The chromatographic separation was achieved on an X-Terra RP18 column, 150 × 4.6 mm, 5 µ. The mobile phase composition was pH 2.5 ± 0.05 buffer (6.8 g of potassium dihydrogen phosphate in 1000 mL of water and adjusted to pH 2.5 ± 0.05 with orthophosphoric acid solution) and acetonitrile in the ratio of 80:20 (v/v). The mobile phase was filtered through 0.45 µ membrane filter and sonicate to degas it. The flow rate of the mobile phase was kept at 1.5 mL/min. The column temperature was maintained at 50 °C and the detector wavelength was monitored at 240 nm. The injection volume was 20 µL and the run time was fixed for 6 min. Water and methanol in the ratio of 50:50 (v/v) was used as diluent. All calculations concerning the quantitative analysis were performed with external standardization by measurement of peak areas.

Preparation of standard solution: Weigh accurately working standard equivalent to 50 mg of piribedil into 100 mL volumetric flask, add 70 mL of diluent and dissolve, further make up the volume with diluent. Further dilute 5 mL to 50 mL with diluent.

Preparation of sample solution: Crush to powder 20 tablets, weigh and transfer the tablet powder equivalent to 50 mg of piribedil into 100 mL volumetric flask add 70 mL of diluent, sonicate for 45 min and dilute to volume with diluent. Further filter the solution through 0.45 µm pore size nylon 66-membrane filter. Dilute 5 mL to 50 mL with diluent.

Forced (stress) degradation studies of piribedil tablet dosage form: Stress degradation studies were performed with piribedil tablet dosage form to examine the proposed method regarding its specificity and ability to indicate the stability of piribedil. The forced degradation process was performed under acidic (0.1 M HCl at 60 °C, 1 h), basic (0.1 M NaOH at 60 °C, 1 h), oxidative [hydrogen peroxide (3 %) at room temperature, 6 h] and thermal conditions (dry conditions at 105 °C, 72 h), for water stress study the sample was refluxed with purified water for 2 h at 60 °C. The samples are exposed to humidity for about 7 days at 25 °C and 90 % RH. The light-induced degradation process was performed using UV and visible light (for about 200 W h m^{-2} in UV and for about 1,200 K lux in visible light) in a photostability chamber [13]. The sample solutions prepared under each set of conditions were filtered through nylon 66-membrane of 0.45 µm pore size before dilution. The sample peak purity parameter was assessed for each degradation solution using HPLC-PDA system.

Design of experiments: The design of experiments was performed by using Design-Expert 9.0 software, Full Version (Stat-Ease Inc., USA). For optimization of chromatographic parameters a two level factorial design was used. The flow rate, acetonitrile composition in mobile phase B and column temperature were selected as the three factors and response studied was the resolution between piribedil and its potential degradant in the oxidative stress condition was in the robustness parameter.

Method validation: The defined method for the assay of piribedil tablet dosage form was validated for all parameters like precision, accuracy, robustness, ruggedness, specificity and linearity according to ICH guidelines [14].

System suitability parameter: System suitability parameter is an integral part of the method validation was monitored by applying the following system suitability parameters like tailing factor and theoretical plates. The acceptance criteria set for system suitability parameters were: tailing factor ≤ 2.0 and theoretical plates > 1500. The relative standard deviation

(RSD) for the analyte peak area for the six consecutive injections was < 2.0 % in all cases. The results were found to be within the limits and were presented in Table-1 and Fig. 2 shows the system suitability chromatogram.

TABLE-1
SYSTEM SUITABILITY RESULTS

System suitability parameter	Results	Limits
Relative standard deviation (%)	0.1	< 2.0 for n ≥ 6
USP tailing factor	1.5	≤ 2.0
USP theoretical plates	5522	> 1500

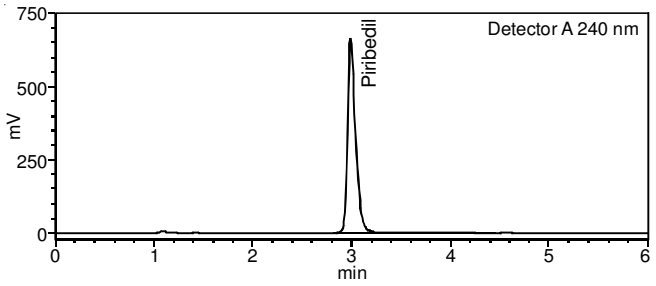

Fig. 2. System suitability chromatogram

Specificity: In the placebo chromatogram there were no peaks observed at the retention times of piribedil and also the degradation studies showed that there was no interference with degradants, peak purity index for the sample solution > 0.990 indicate the method is specific. Fig. 3 shows the blank chromatogram.

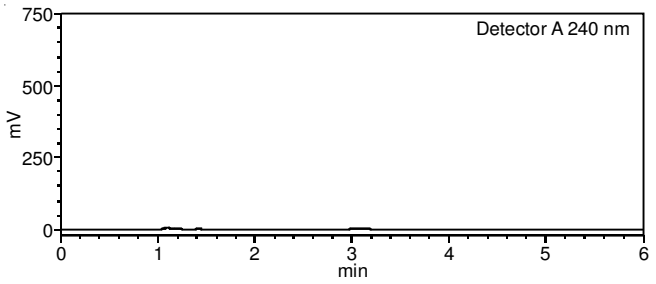

Fig. 3. Blank chromatogram

Precision: The precision of the described test method was estimated by performing six replicate sample preparations and the % relative standard deviation was tabulated in Table-2.

TABLE-2
PRECISION OF THE METHOD

S. No.	Assay (%)	S. No.	Assay (%)
1.	100.2	5.	100.0
2.	100.4	6.	99.6
3.	99.7	Average	100.1
4.	100.6	RSD (%)	0.4

Accuracy: The proposed method accuracy was determined by performing recovery studies at three different levels in triplicate, *i.e.* 50, 100 and 150 % of the target level and calculated the percentage recovery. The results were tabulated in Table-3.

Linearity and range: The linearity of the method was evaluated by measuring standard solutions of the piribedil at different concentrations ranging from LOQ level to 150 % level of the target concentrations. The calibration curves were developed by plotting the peak areas *versus* the concentrations and the regression equation was computed. These results confirm the linear relation between the peak area and concentration (Table-4).

TABLE-3
ACCURACY RESULTS (TRIPLICATE VALUES AT 50, 100 AND 150 % LEVELS)

Accuracy level (%)	Amount added (µg)	Amount recovered (µg)	Recovery (%)[a]
50	141.6	142.1	100.4
100	275.6	277.1	100.5
150	418.6	421.3	100.6

[a]Mean for three determinations

TABLE-4
LINEARITY AND REGRESSION PARAMETERS

Parameter	Value
Linearity range (µg/mL)	0.024-75
Limits of quantification (LOQ, µg/mL)	0.024
Limits of detection (LOD, µg/mL)	0.010
Regression equation:	
Slope (b)	74268
Intercept (a)	36665
Correlation coefficient (r)	0.9999

Limit of detection and limit of quantification: The limit of detection (LOD) and the limit of quantification (LOQ) will expresses the sensitivity of the method and both are estimated by considering the acceptable signal to noise ratios 3:1 and 10:1 respectively (Table-4).

Robustness: The robustness of the method is its capability to remain unaffected by small deliberate changes like mobile phase composition, flow rate and column temperature using standard and an oxidative degraded stress sample of 13.2 % indicates the reliability of a method during normal usage.

Benchtop solution and mobile phase stability: In this parameter both standard and test solutions which were kept on a benchtop for 48 h are analyzed and compared against freshly prepared solutions, no significant change is observed in system suitability parameters and in % piribedil recovery. Hence both the standard and test solutions are stable upto 48 h at room temperature.

For mobile phase stability, the mobile phase was kept on a benchtop for 48 h and analyzed by using freshly prepared standard and test solutions. There was no significant change observed in system suitability parameters and in % piribedil recovery. Hence the mobile phase is stable upto 48 h at room temperature.

Forced (stress) degradation studies: Stress degradation samples were prepared and injected into the HPLC system with a PDA detector. Significant amount of degradation was not observed in photolytic, acid hydrolysis, heat stress, humidity conditions and water hydrolysis. A significant amount of degradation was observed in base stress hydrolysis and oxidative stress hydrolysis. All the peaks due to degradation are well resolved from piribedil peak. Fig. 4 shows the degradation chromatograms of piribedil with the corresponding solvent

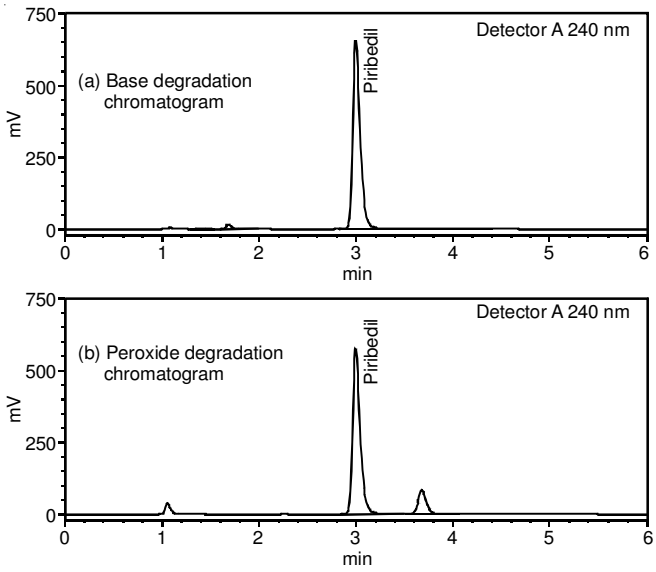

Fig. 4. Chromatograms obtained for forced degradation

as blank. The chromatograms of the stressed samples were evaluated for peak purity of piribedil using Shimadzu Lab solutions software. For all stress degradation samples, the observed peak purity index for the main peak of the drug *i.e.* for piribedil should be not less than 0.990, indicates no interference observed from the degradants in quantifying piribedil in tablet dosage form. Thus, the proposed method considered to be stability-indicating. The summary of the stress degradation studies are given in Table-5.

RESULTS AND DISCUSSION

Method development and optimization of chromatographic conditions: Solubility of the drug plays a major role in analytical method development and then the UV response of the product at varying wavelengths. Piribedil was found to be soluble in a mixture of water and methanol in the ratio of 50:50 (v/v) and the same was used as diluent. A 50 µg/mL solution showed excellent response at 240 nm when it was scanned from 400 to 200 nm in a UV spectrophotometer (Fig. 5).

The HPLC procedure was optimized with an objective to develop a simple stability indicating method. No internal standard was used because no extraction or separation step was involved. Of several solvents and solvent mixtures investigated, using an isocratic mobile phase consists of pH 2.5 phosphate buffer of 0.05 M and acetonitrile in the ratio of (80:20 v/v) at a flow rate of 1.5 mL/min and column temperature of 50 °C is found to be appropriate and able to give satisfactory results.

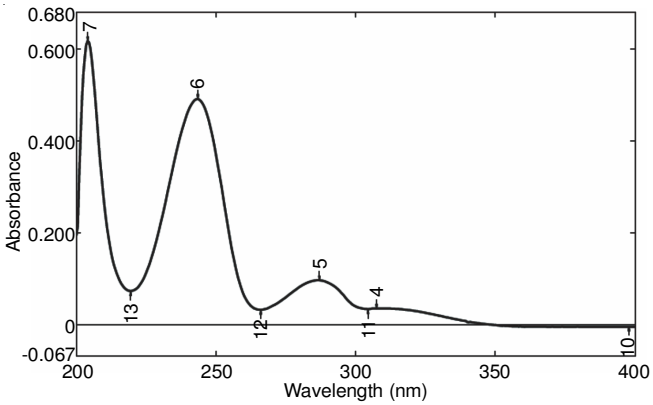

Fig. 5. UV absorption spectra of piribedil

OFAT approach for robustness parameter study in a peroxide degraded sample: In current practice the analytical methods were designed and validated using one-factor-at-a-time (OFAT) approach in which one variable is changed sequentially by keeping other variables constant until a suitable method is produced. This type of development may create an adequate method, but provides a limited understanding of method capabilities and method robustness [15].

In OFAT approach, critical variables such as flow rate and column temperature were varied by keeping one parameters constant in the sample analysis of 13.2 % peroxide degraded sample. Summarized observations are made as per the Table-6. Fig. 6 shows the overlaid chromatogram of all the four runs in the study.

TABLE-6
OFAT APPROACH DATA FOR PIRIBEDIL IN THE ROBUSTNESS PARAMETER STUDY

S. No.	Changed variable	Resolution between Piribedil and its degradant in peroxide stress
Run-1	Flow rate – 1.3 mL/min	4.2
Run-2	Flow rate – 1.7 mL/min	3.8
Run-3	Column Temp. – 45 °C	4.4
Run-4	Column Temp. – 55 °C	3.5

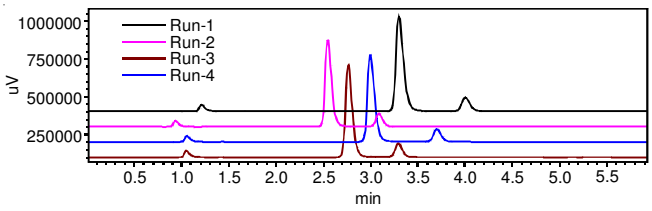

Fig. 6. Overlaid chromatograms obtained for robustness parameter study in OFAT approach

TABLE-5
FORCED DEGRADATION DATA FOR PIRIBEDIL

Stress condition	Assay (%)	Degradation (%)	Mass balance	Peak purity index
Acid (0.1 M HCl at 60 °C, 1 h)	96.9	1.4	98.2	1.000
Base (0.1 M NaOH at 60 °C, 1 h)	95.9	2.3	98.2	1.000
Peroxide (3 % H_2O_2 at 25 °C, 6 h)	85.2	13.2	98.4	1.000
Water (at 60 °C, 2 h)	98.9	1.3	100.2	1.000
Photolytic (for about 200 W h m^{-2} and for about 1,200 K lux)	98.7	0.5	99.2	1.000
Thermal (dry conditions at 105 °C, 72 h)	98.8	1.4	100.2	1.000
Humidity (25 °C and 90 % RH for about 7 days)	97.8	0.5	98.4	1.000

QbD approach for robustness parameter study in a peroxide degraded sample: A quality by design (QbD) approach to method development uses statistical design of experiments (DoE) to develop a robust method 'design space'. The design space defines the experimental region in which changes to method parameters will not significantly affect the results. This approach builds-in robustness to the method as the method is being developed [12].

In present days, analytical method failure is becoming more common especially during method transfer as well as in quality control departments. At this juncture, QbD paradigm is a preferred and recommended strategy to be followed in analytical method development so as to attain regulatory flexibility and reduce OOS, OOT, OOC and OOSC results, high degree of robustness and cost effective analytical method [16].

Design of experiments: A simple fractional factorial design was selected to determine the interactions between the factors selected. The objective was to check the robustness of the proposed method within short time. The number of trials required is 2k-p, where k is the number of factors and p is an arbitrary number. The three factors selected were the acetonitrile composition in mobile phase, flow rate and column temperature based on the method development. For these experiments p = 1, one centre point is selected to add some power to the design and the number of trials was $2^{3-1} + 1 = 5$.

The ranges for the selected factors were fixed during the design of experiments are mentioned in Table-7. The combinations of organic phase variation in mobile phase composition, flow rate and column temperature recommended by the selected design were finally run on the system by analysing the 13.2 % peroxide degraded sample. The observed response such as resolution between piribedil and its degradant was noted and represented in Table-8. Fig. 7 shows the overlaid chromatogram of all the five runs in the study.

TABLE-7
SELECTED FACTORS AND PROPOSED RANGE

Factor selected	Range proposed
Flow rate (mL/min) (F1)	1.3-1.7
Organic phase variation (%) (F2)	80-120
Column temperature (°C) (F3)	45-55
Response	Resolution between Piribedil and its degradant

TABLE-8
TABLE FOR EXPERIMENTAL DESIGN AND THEIR RESPONSES

S. No.	Flow rate (mL/min) (F1)	Organic phase (%) (F2)	Column temperature (°C) (3)	Response[b]
Run-1	1.7	120	55	2.3
Run-2	1.7	80	45	3.3
Run-3	1.3	120	45	2.4
Run-4	1.3	80	55	3.3
Run-5	1.7	100	50	4.2

[b]Response = Resolution between Piribedil and its degradant

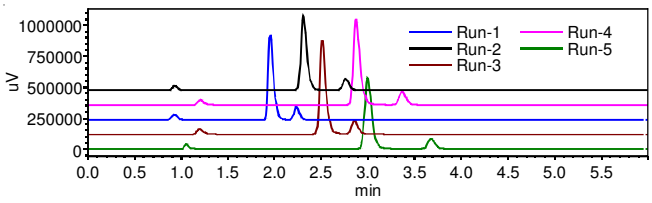

Fig. 7. Overlaid chromatograms obtained for robustness parameter study in QbD approach

The three dimensional response surfaces were also analyzed to anticipate the effects of each parameter and their interaction on the responses. Fig. 8(a-c) shows the effect of selected factors and their interactions on the response. Table-8 shows the optimized values for all of the three factors suggested by the design are given in and Fig. 9 shows the desirability 1. Also, the overlay plot was obtained for the selected responses from the model, which is depicted in Fig. 10.

Conclusion

A simple, specific and reliable isocratic HPLC-DAD method was developed for the estimation of piribedil in their pharmaceutical formulation. The tablet dosage form was subjected to forced degradation by applying several stress conditions. The proposed method was successfully separated all the degradants, estimate the active contents. From an analytical method development point of view, QbD implementation involves the design of robust analytical method based on identification of critical parameters affecting the method performance. The design of experiments examines the key HPLC method components including column temperature, flow and mobile phase solvent ratio. The interrelationships

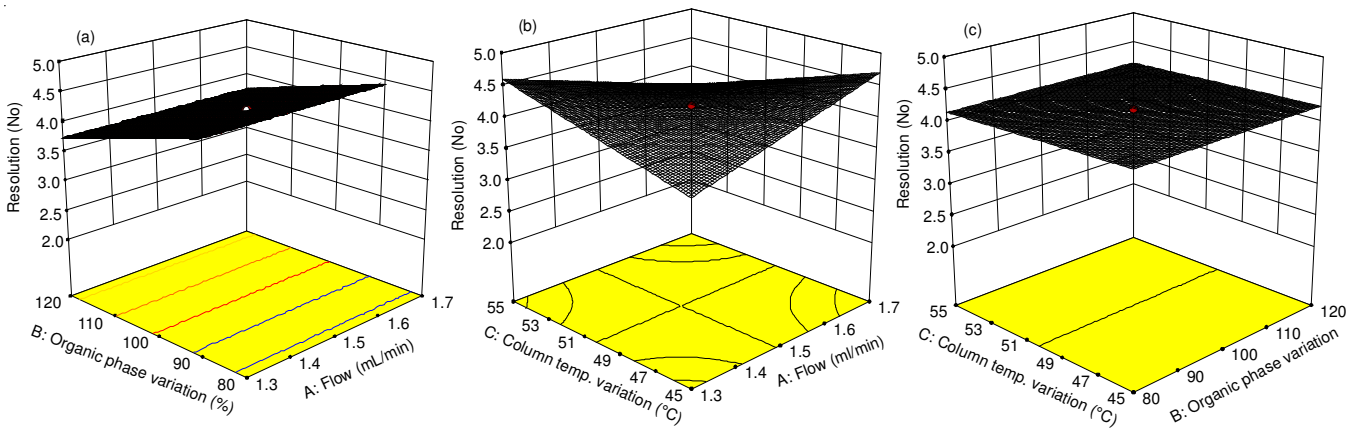

Fig. 8. Effect of factor interactions on response

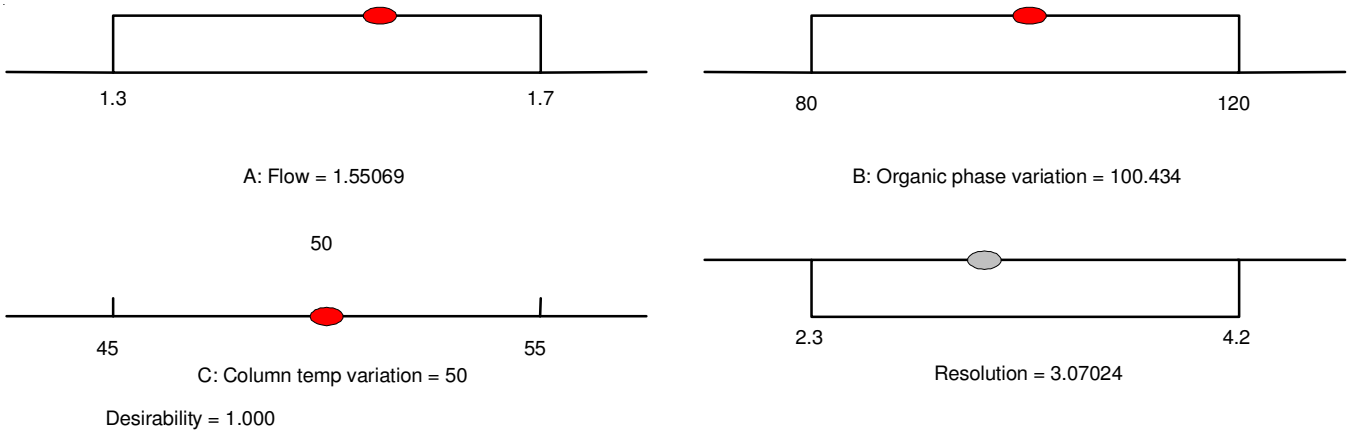

Fig. 9. Desirability study

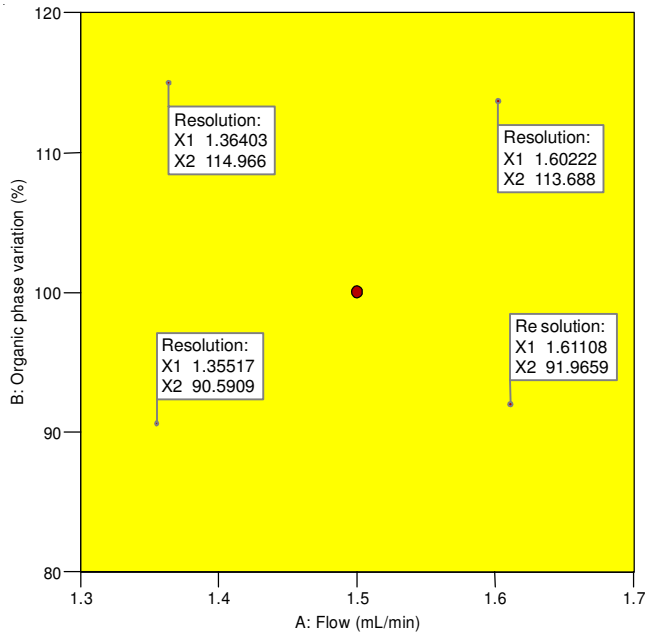

Fig. 10. Overlay plot obtained from the design study

are studied and the preliminary optimized conditions are obtained for each combination. Moreover, this approach ensures robust design of products with fewer problems in development, reduces the number of trials required for post marketing changes and reduction in overall costs of manufacturing resulting in less waste. The proposed method was validated as per ICH guidelines and found to be precise, specific, linear, accurate, rugged and robust with a run time of 6 min is capable to analyze more number of samples in a short period of time. The developed method has shown stability-indicating power, hence the developed method can be adapted to regular quality control analysis.

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