Pharmaceutica Analytica Acta

Permender R et al., Pharm Anal Acta 2016, 7:6
DOI: 10.4172/2153-2435.1000489

Research Article Open Access

Novel Statistically Designed Qbd Methodology for Quantitative Analysis of Nisoldipine in Pharmaceutical Dosage Forms

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Received date: May 24, 2016; **Accepted date:** Jun 17, 2016; **Published date:** Jun 21, 2016

Abstract

The objective of the study was to develop superlative reproducible spectrophotometric estimation process of Nislodipine via BCG (Bromocresol green) solution using novel statistically Taguchi via factorial design methodology. Primarily, an L_9 design array was experimented to find significant analytical variables and optimize their(s) best levels keeping drug amount constant. Additionally factorial i.e. Box-Behnken design (BBD) of better factors (X, Y & Z) levels at positive, null and negative spaces responses (R=absorbance) was studied to found better to best level. Moreover, polynomial quadratic equations for two and three dimensional models were designed to predict the best significant independent levels. The selected variable responses were statistically evaluated using ANOVA and showed correlation coefficient (r^2=0.999) followed by good Beer's (5-40 µg/ml) range at maximum absorption wavelength (420 nm) and was validated according to ICH guidelines. This robust novel statistically designed strategy can be considered as quality by design (QbD) quantitative methodology for Nisoldipine estimation in pharmaceutical's.

Keywords: Nisoldipine, BCG, Array, BBD, Models, Quantitative analysis, Validation

Inroduction

Nisoldipine [(±) 3-isobutyl-5-methyl-1, 4-dihydro-2, 6-dimethyl-4-(2-nitrophenyl) pyridine-3, 5-dicarboxylate] is a 1, 4-dihydropyridine calcium channel blocker (Figure 1a) [1]. It acts primarily on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive conformation. By inhibiting the influx of calcium in smooth muscle cells, it prevents calcium-dependent smooth muscle contraction and subsequent vasoconstriction and may be used alone or in combination with other agents in the management of hypertension. It exists physically as yellow crystalline substance, practically insoluble in water but soluble in ethanol, methanol [2].

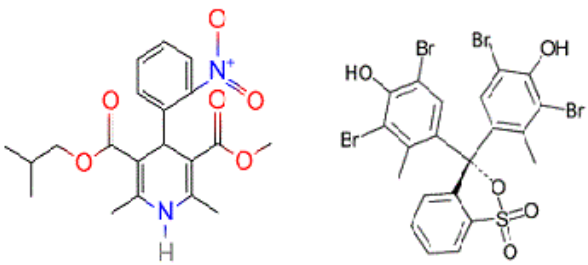

Figure 1: a) Nisoldipine b)Bromocresol Green.

Literature survey reveals the analytical methods developed for determination of Nisoldipine in human plasma by voltammetry [3-5], polarography [6] and UV [7-10]. Its pharmacokinetic properties and determination of impurities are reported by RP-HPLC [11-12] and HPLC [13];

But none of the method used statistically quality by design (QbD) methodology, till date.

In this method, a yellow-crystalline drug (Nisoldipine) in the presence of a reducing agent condensed with bromocresol green (Figure 1b) in acidic pH to form a complex [10]. The yellow colored ion-pair complex showed maximum absorption at the wavelength of 420 nm that obeys the Beer's law in the concentration range of 5-40 µg/ml.

So, this research was an attempt to develop a novel statistical "Quality by Design" (QbD Taguchi & Factorial [14-16]) approaches for antihypertensive agent via best quantitative validated [17-18] estimation. All generated preliminary trails of Taguchi array were performed experimentally, and then obtained data responses were interpreted to find key variables (volume of BCG solution, pH & organic solvent) and their(s) considerable levels by observing the responses (absorbance). The responses "Signal to Noise" found "better" levels of significant variables which were performed experimentally according to Box-Behnken design (BBD) runs and ANOVA analysis was used to derive polynomial equations of variables responses. Thus, variables "better to best" level response interactive effects were studied (two factors at a time) to found "best" models (spaces) and validated, further to find the finest level. The designed preeminent fitted spaces of significant variables would be considering as QbD superlative quantitative estimation analysis of Nisoldipine in pharmaceuticals forms.

Materials & Method

The model drug (Nisoldipine) gift sample and others analytical grade chemicals & reagents (BCG, HCl and methanol) were obtained from Orchid Pharma, Chennai and procured from Central Drug House Ltd. & S. D. Finechem Ltd, Mumbai respectively. For performing preliminary array trails, the analytical balance (A/GR-200, Anakek Services, Mumbai), pH meter (Aminco, Swastika, Ambala) and double beam UV-Visible (JASCO UV-600 series) spectrophotometer at fixed λ_{max} 420 nm was used to record the absorbance for quantitative estimation.

Methods

The model drug sample (Nisoldipine; 10 mg) was taken & dissolved in methanol to acquire (100 µg/ml) stock solution for desired concentration. A numerous set of experimental concentrations were prepared along with triaryl-methane dyes i.e. bromocresol green (BCG) and pH-buffer (HCl) solution, by appropriate dilution of the stock solution using methanol.

Screening study

A quality statistical (Mini-Tab® Software L_9; N1-N9) design array of independent variables trials A, B & C; BCG (3 to 7ml); Buffer (pH 3-5) & methanol (20 to 40ml) respectively were conducted experimentally at constant drug concentration & observed their responses (Y=absorbance against organic blank) at λ_{max} 420nm. All generated variable combinations individual or together obtained and responses calculated by "Signal to Noise" (S/N) values are shown in Table 1. As well, the S/N ratio fitness for each array responses were studied to find "better" level ranges which were used further to predict the "better to best" level compositions.

Orthogonal array at numerous spaces										
Independent Factors	**Actual**				**Units**	**Coded**	**Spaces**			
	BCG	3	5	7	ml	A	1	2	3	
	Buffer	3	4	5	pH	B	1	2	3	
	Methanol	20	30	40	ml	C	1	2	3	
L_9 array responses										
Analysis	**BCG (ml)**	**Buffer (pH)**	**Methanol (ml)**	**Y Absorbance**		**S/N**		**Lack of Fitness**	**Desirability 1=100%**	**"Better to Best" predicted level**
Codes	A	B	C	Actual	Predicted	Actual	Predicted			
N1	3	3	20	0.449	0.4486	6.955	6.961	-0.006	-1.016	
N2	3	4	30	0.447	0.448	6.994	6.974	0.02	0.980*	Smaller
N3	3	5	40	0.441	0.4403	7.111	7.125	-0.014	-1.014	
N4	5	3	30	0.438	0.4373	7.17	7.185	-0.015	-1.015	is
N5	5	4	40	0.431	0.4307	7.31	7.316	-0.006	-1.01	
N6	5	5	20	0.424	0.425	7.453	7.433	0.02	0.980*	better
N7	7	3	40	0.42	0.421	7.535	7.515	0.02	0.980*	
N8	7	4	20	0.417	0.4163	7.597	7.611	-0.015	-1.015	(A5B3C30; A2B1C2)
N9	7	5	30	0.415	0.4147	7.639	7.645	-0.006	-1.006	

Table 1: Generated Array Trials at Diverse Levels.

Box-Behnken strategy

A statistical factorial design methodology was used to found-out the significant factor's optimum level. The significant factors "better to best" levels were optimized by response surface design (RSM) and also the equations of designed models were generated. The optimum levels of significant variables (X, Y & Z) were fitted to model experimental response and predicted values at negative to positive spaces. The dimensional model plots demonstrated interaction effects individually and significant variables at same time on the response to find optimized level.

Factor Level's Coded

	Negative	Null	Positive
Actual	-1	0	1
X= BCG-reagent (ml)	4.5	5	5.5
Y= Buffer (pH) solution (ml)	2.5	3	3.5
Z= Methanol (ml)	25	30	35
Dependant variables Constraints			
R = Absorbance $0.397 \leq Y_1 \geq 0.477$			
Design runs results			
Independent Response (R)			

Batches	X	Y	Z	Observed	Predicted
BBD-N1	0	0	0	0.436	0.44
BBD-N2	0	0	0	0.436	0.44
BBD-N3	0	1	1	0.415	0.41
BBD-N4	0	0	0	0.435	0.436
BBD-N5	0	-1	-1	0.441	0.44
BBD-N6	-1	0	1	0.427	0.428
BBD-N7	1	0	-1	0.441	0.44
BBD-N8	1	-1	0	0.477	0.48
BBD-N9	-1	-1	0	0.428	0.43
BBD-N10	-1	0	-1	0.397	0.401
BBD-N11	1	0	1	0.425	0.424
BBD-N12	0	0	0	0.436	0.44
BBD-N13	0	0	0	0.436	0.44
BBD-N14	1	1	0	0.413	0.414
BBD-N15	0	-1	1	0.451	0.45
BBD-N16	-1	1	0	0.424	0.42
BBD-N17	0	1	-1	0.411	0.41
Regression analysis results					

Response	R^2	Adjusted R^2	Predicted R^2	SD	% CV
Absorbance	0.9994	0.9985	0.9919	0.007	0.16

Table 2: Box Behnken design of independent factor & levels at constant drug (10ug/ml).

QbD method

As per best optimized levels, the required model drug aliquots were taken and volume of bromocresol green (BCG) via finest hydrogen ion concentration (pH) were added. Also further required concentration was obtained by diluting with methanol and measuring the absorbance at λ_{max} 420 nm against the blank. Then, calibration curve was constructed (absorbance vs µg/ml) to found optimized linearity with well fitted coefficient of correlation value. Moreover, percentage recovery and intra/inter-day precision analysis were performed to found the relative standard deviation.

Result and Discussion

L9 array

To develop a better quality methodology, the designed array trails were performed to measure response (absorbance) of significant independent factors (Table 1). The response results were analyzed by drawing "Signal to Noise" (S/N) ratio plot of significant factor at each level. The plots (Figure 2) demonstrated and suggested factors level "Smaller is better", B was most significant while A & C were moderate & least effective respectively on response which were also confirmed by S/N ratios fitness (observed vs interpreted) study. Additionally, during fitness studied array coded N2, N6 & N7 were found most S/N fitted at each level's (98%) and highlighted in Table 2. Though, the "Smaller is better" predicted quality levels A2B1C2 (A5B3C30) can considered for another Design of experiment methodology to study "better to best" level effects and to develop best space quantitative method.

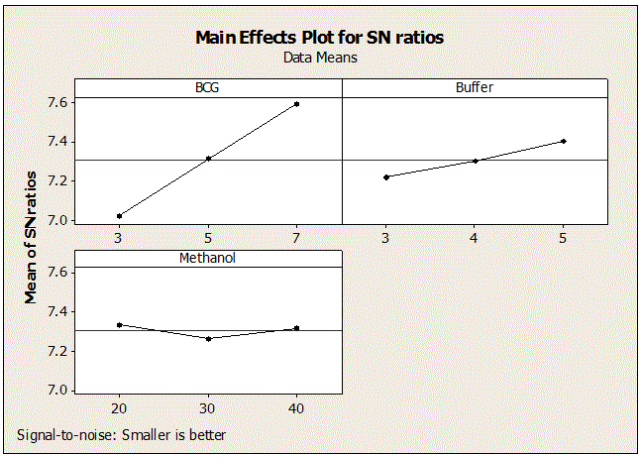

Figure 2: Graphical illustration of independent factor's "Smaller is better" levels effect on responses (in term of S/N values).

Design & analysis

Another, Box-Behnken Design (BBD) methodology was used to predict better (A5B3C30; Table 1) and to design significant independent diverse "better to best" (BBD-N1 to 17) level's at negative, null and positive spaces run and to perform experimentally in constant conditions. A quadratic second-order polynomial derived equation of significant variables response at "better to best" level was generated. The proposed equation was $R=0.44 + 0.01X - 0.017Y + 0.0035Z - 0.015XY - 0.012XZ - 0.0015YZ - 0.00365X^2 + 0.00335Y^2 - 0.00965Z^2$; where, R is absorbance, X is BCG-reagent, Y is Buffer (pH) and Z is methanol. The model responses had adequate precision (151.442) with predicted R-square (0.9919) being better fitted with adjusted R-square (0.9985). As well, there is only 0.01% chance of having a large noise which implied that lack of fitness of (F-value 4.17) model is non-significant. The best fitted model analysis (F-value 1196)

demonstrated significant terms (X, Y, Z, XY, XZ, YZ, X^2, Y^2, Z2) at negative space of Y (with level of X & Z along with null space) had a more pronounced interactive (Figure 3 and 4 showed the independent factor of levels on response) effect via good correlation coefficient. There-fore, "better to best" levels of variables (X, Y & Z where-as, Y= negative= pH 3 and X & Z= null= 5 & 30 ml respectively) have targeted space as comparison to others. Numerical prediction checkpoint spaces were further used for "best" quantitative determination methodology.

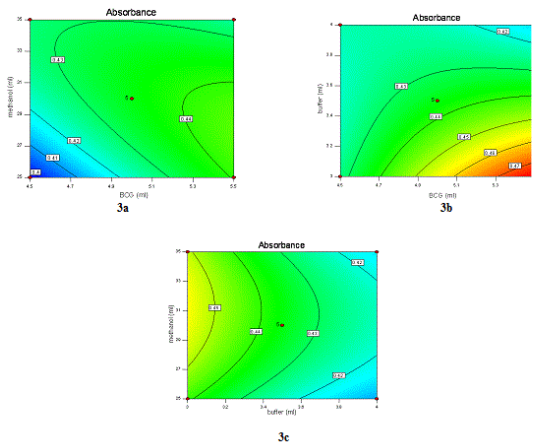

Figure 3: Two dimensional plots shown interactive effect of: 3a) BCG & Buffer; 3b) BCG & methanol; 3c) Buffer & methanol on response (absorbance).

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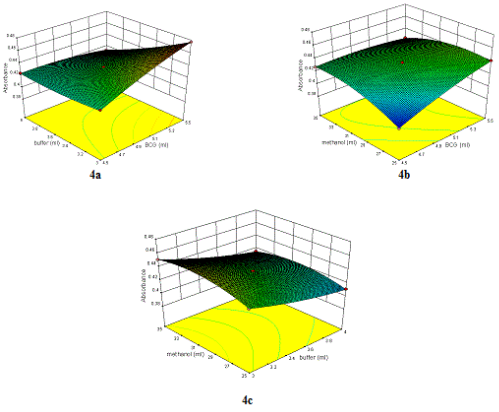

Figure 4: Three dimensional plots shown interactive effect of: 4a) BCG & Buffer; 4b) BCG & methanol; 4c) Buffer & methanol on response (absorbance).

Quality by design determination

The designed models best fitness targeted level was used to predict variables optimized spaces based on the numerical checkpoint (BN-1 to 14; Table 3). All variables combinations quantitative responses were obtained experimentally to get optimized [B0N (3 or 8 or 13)] "best" fitted space (actual vs predicted; Figure 5) which to be considered as quality by design (QbD) preeminent model for Nisoldipine determination in dosage forms.

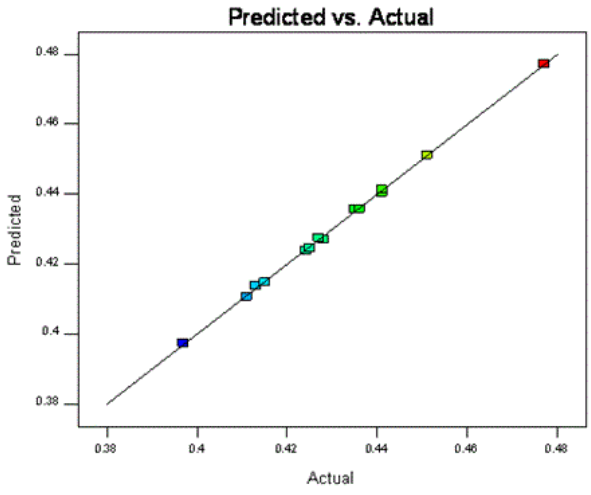

Figure 5: Best fitted space (actual vs predicted) responses plot.

Coded	BCG	Buffer	Methanol	Absorbance	Fitness (1=100%)	
	(X in ml)	(Y= pH)	(Z in ml)	(R)	Desirability	Best

BN-1	5.01		3			30	0.456		0.994			
BN-2	5.01		3.01			30	0.456		0.991			
BN-3	5		3.01			30	0.456		0.998			B0N3BEST
BN-4	5		3.04			30	0.454		0.985			
BN-5	4.95		3			30	0.454		0.971			
BN-6	4.5		3.03			30	0.427		0.987			
BN-7	4.73		3			30	0.441		0.833			
BN-8	5		3			30	0.456		0.999			B0N8
BN-9	5		3			29.9	0.456		0.993			
BN-10	5		3			29.75	0.456		0.983			
BN-11	5		3			30.15	0.456		0.99			
BN-12	4.97		3			30	0.455		0.983			
BN-13	4.51		3			30	0.428		0.995			B0N13
Model	A	B	C	AB	AC	BC	A2	B2	C2	Lack of Fit		
Prob. > F	<0.0001					<0.0033	<0.0001			<0.101		
	Significant					to			Non-significant			

Table 3: Numerical Checkpoint spaces prediction of finest as best levels of factors and their results.

Brand	Labeled quantity	quantity found a	% Labeled quantity	% RSD
Sular	40.0 mg	39.56	98.9	± 0.021

Table 4: Results of estimation of Nisoldipine in dosage forms.

Method for Dosage Form

For the quantitative analysis, Nisoldipine formulation was procured from the market weighed and powdered. The drug powdered was accurately weighed (10 mg equivalent), transferred to volumetric flasks and dissolved in 25 ml methanol and then sonicated for 10 minutes and finally methanol was added to make-up the volume up to 100 ml. The solution was filtered and the sample solutions were prepared from stock solution by appropriate dilutions to get desired concentrations with-in the Beer's law range limits and measured the absorbance (at λmax 420nm) against blank.

Validation

Linearity was determined by preparing different concentrations of sample solution (20 µg mL-1). The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by assaying samples, at the same concentration and during the same day. The intermediate precision was studied by comparing the assays on different days (3 days). Three (B0N3, B0N8 & B0N13) sample solutions ranging 16, 20 & 24 µg mL-1 were prepared and analyzed. The accuracy of the method was determined as recovery from 20 µg mL-1 standard solution spiked with 80, 100 and 120% extra Nisoldipine. Specificity was determined by observing that the placebo samples were free from any interfering substances. Placebo samples were prepared by dissolving expected ingredients other then drugs in equal proportions and then assayed in order to verify that none of the excipients of the tablets interfered with the quantity of drugs. Limit of detection (LOD) & Limit of Quantification were calculated against blank. All solutions were prepared & used in triplicate.

Conclusion

Nisoldipine can be determined in pharmaceutical tablets based on reaction with BCG reagent in acidic conditions. The results obtained confirm the optimization and suitability of the proposed method for the precise analysis of Nisoldipine in quality control laboratories. The Taguchi design array and response surface methodology can be effectively applied for analysis of pharmaceuticals.

Conflict of Interest

No conflict of interest.

Acknowledgement

The authors are thankful to Orchid Pharma, Chennai for providing model drug gift sample. The research facility provided by IKG Punjab

Technical University, Kapurthala and Chandigarh College of Pharmacy; Landran India is gratefully acknowledged.

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