Research Article

# Analytical quality risk management and DoE based development of robust chromatographic method for simultaneous estimation of tizanidine hydrochloride and nimesulide in their combined pharmaceutical dosage forms

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**Abstract**
Analytical quality risk management (ICH Q9 guideline) concept based robust high performance thin layer chromatographic method has been developed with help of design of experiment (DoE) tool for simultaneous estimation of tizanidine hydrochloride and nimesulide in their combined pharmaceutical dosage forms. Risk identification and assessment were done with brainstorming process with help of ishikawa diagram and experimental results based risk factor priority number (RPN). Critical risk factors which having RPN number more than sixty were further analysed for their criticality in method development by DoE based Taguchi screening design. From seven critical risk factors, volume of methanol in mobile phase and migration distance were identified as highly risky factors for development of HPTLC method. DoE based central composite design was applied for risk factors analysis and mitigation by optimisation of identified high risk factors. After implementation of risk minimisation operable design region and control strategy were set for development of robust HPTLC method for simultaneous estimation of tizanidine hydrochloride and nimesulide. Developed analytical method was validated for specificity, linearity, accuracy, precision and LOD-LOQ as per ICH guideline Q2R1. Validated HPTLC method was applied for assay of combined marketed pharmaceutical dosage forms of both drugs and results were found in good agreement with labelled claim of dosage forms.

**Keywords** Analytical quality risk management · Taguchi design · DoE · Central composite design · Method operable design region (MODR)

## 1 Introduction

Tizanidine 5-chloro-4-(2-imidazolin-2-ylamino)-2,1,3-benzothiadiazole (Fig. 1a) is $\alpha_2$-adrenergic agonist and centrally acting myotonolytic skeletal muscle relaxant with a chemical structure unrelated to other muscle relaxants. It reduces spasticity by increasing presynaptic inhibition of motor neurons. The effects of Tizanidine are greatest on polysynaptic pathways. The overall effect of these actions is thought to reduce facilitation of spinal motor neurons. It also reduces increased muscle tone associated with spasticity in patients with multiple sclerosis or spinal cord injury [1–5].

Nimesulide, 4-nitro-2-phenoxymethane sulphonanilide (Fig. 1b) is a non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. It's approved for the treatment of acute pain, the symptomatic treatment of osteoarthritis and primary dysmenorrhoea in adolescents. It has a multifactorial mode of action and is characterized by a fast onset of action [1–5].

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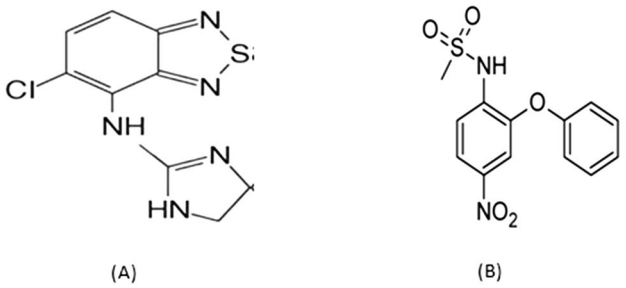


**Fig. 1** Chemical structure of **a** Tizanidine hydrochloride **b** Nimesulide

The literature review described HPTLC, HPLC, radioimmunoassay and UV–Visible spectrophotometric method for determination of tizanidine hydrochloride individually and combination with other drugs in their pharmaceutical dosage form. The literature review described HPTLC, HPLC, gas chromatography (GC), potentiometric titration in non-aqueous media and UV–Visible spectrophotometric method for determination of nimesulide individually and combination with other drugs in their pharmaceutical dosage form. The literature review also described spectrophotometry and HPLC methods for simultaneous estimation of tizanidine Hydrochloride and nimesulide in combined dosage form [6–12]. But, there was no reported HPTLC method for simultaneous estimation of tizanidine hydrochloride and nimesulide in their combined dosage form using quality by design approach. HPTLC method is simple, less solvent consuming and less time consuming as compared with other chromatographic method. Generally in literature implementation of quality by design in analytical method is not properly followed. Implementation of quality by design approach should be based on sound science and quality risk management. Quality risk management part was always missing in development of analytical method by quality by design. Quality risk management as per ICH Q9 guideline is regulatory requirement for development of analytical method. So, it was thought of interest to develop and validate HPTLC method for simultaneous estimation of Tizanidine Hydrochloride and Nimesulide in their combined dosage form based on concepts of quality risk management and design of experiment to provide correct roadmap for implementation of quality by design approach.

## 2 Experimental

### 2.1 Instrumentation

The HPTLC system (Camag Switzerland) consisting of Linomat V semiautomatic spotting device, TLC Scanner IV (Camag Muttenz, Switzerland), twin-trough developing chamber (10 × 10 cm), UV cabinet with dual wavelength UV lamps, winCATS software, syringe (100 µL capacity, Hamilton) were used for chromatographic study. Electronic analytical balance (Shimadzu AUX-220) was used for all the weighing purpose.

### 2.2 Chemicals and reagents

Tizanidine Hydrochloride was kindly supplied as a gift sample by Sun Pharma Ltd, Bharuch, Gujarat, India and Nimesulide was kindly supplied by Yarrow Chem. product, Wadala (E), Mumbai, India. All chemicals and reagents AR grade were used and purchased from s. d. Fine-Chem Limited, Mumbai, India. Tablet containing tizanidine hydrochloride 2 mg and nimesulide 100 mg were procured from the local market.

### 2.3 Preparation of working standard solution

The combined working standard solution was prepared by mixing of 1 mL of tizanidine hydrochloride standard stock solution (50 µg/mL) and 1 mL of Nimesulide standard stock solution (1000 µg/mL) into 10 mL volumetric flask and diluted up to mark with methanol to get a solution having strength 5 µg/mL of Tizanidine Hydrochloride 100 µg/mL of Nimesulide. Similarly remaining working standard solutions were prepared by diluting appropriate volume of standard stock solutions of both drugs with methanol to get combined working standard solution having strength of 10, 15, 20, 25 µg/mL of Tizanidine Hydrochloride 200, 300, 400, 500 µg/mL of Nimesulide.

### 2.4 Risk identification and assessment

Implementation of quality risk management approach started with brain storming process for identification of potential critical risk factors for development of HPTLC method. Potential critical risk factors were identified, categorised and set in fish bone diagram (Fig. 2). Risk assessment was performed by giving risk score to each risk factor

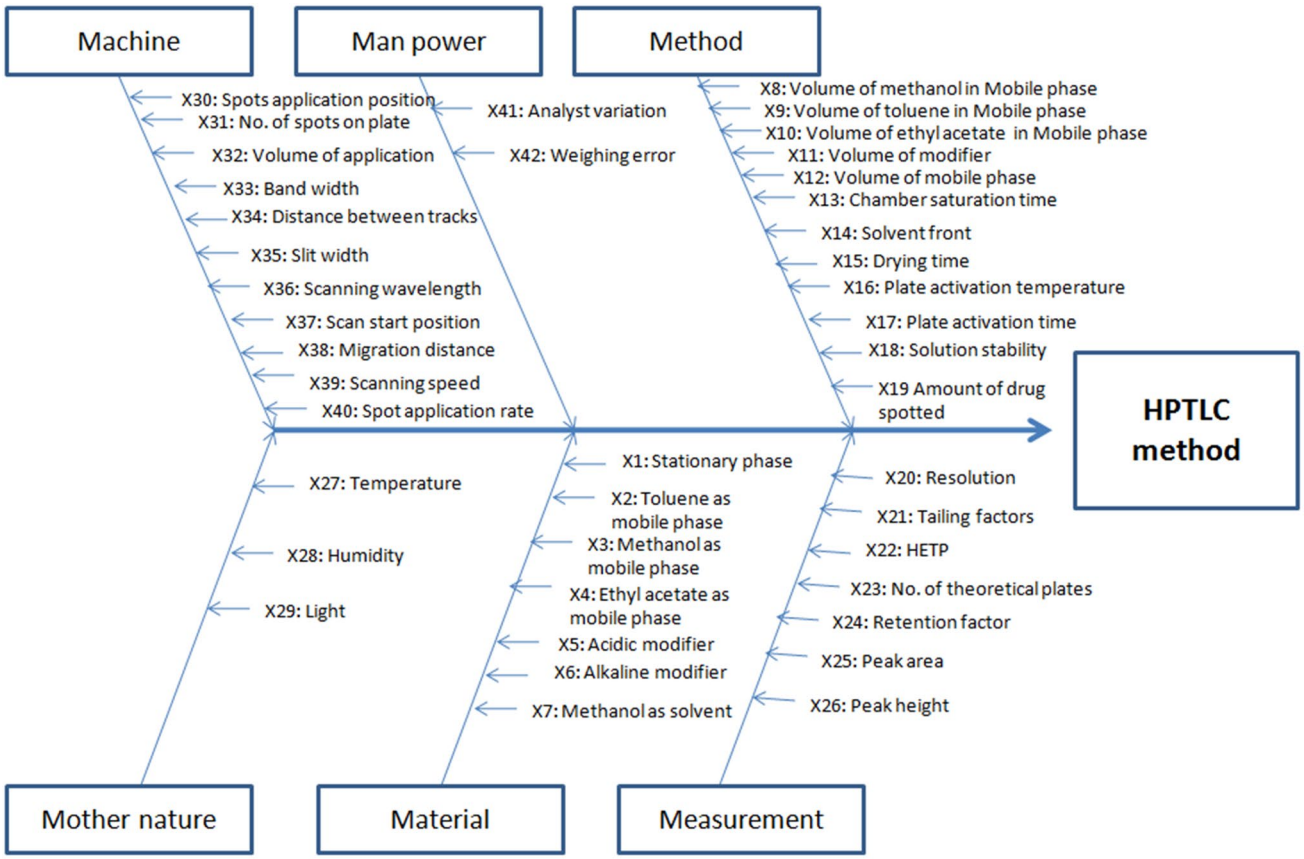


**Fig. 2** Risk factors identification by Ishikawa diagram

on base of its occurrence (O), severity (S) and detectibility (D) in development of HPTLC method (Table). Risk score was given on base of factors having very low (02), low (04), medium (06), high (08), very high (10) impact on development stage of HPTLC method. Risk priority number (RPN) was calculated by multiplication of occurrence, severity and detectibility. Minimum and maximum risk priority number was found 8 and 200 respectively. RPN more than 60 was set as limit for identification of critical risk factors to be considered for risk analysis in development of HPTLC method (Figure). Risk factors categorised under mother nature like temperature and humidity having RPN more than 60 were controlled by air conditioning during development of HPTLC method. From measurement category, resolution and tailing factors of two drugs having RPN more than 60 were selected as mode of measurement for further risk analysis.

## 2.5 DoE based risk factors analysis by Taguchi screening design

From identified potential critical risk factors, seven risk factors were identified as potentially critical by risk assessment for development of HPTLC method. High level (+ 1) and low level (− 1) value of each risk factors was identified by preliminary experimental trials (Table 1). From DoE based screening methods, Taguchi screening method was selected which required minimum experimental run for risk analysis of seven risk factors on development of HPTLC method using resolution and tailing factors of both drugs as responses. Experimental runs were performed in laboratory and responses measured. Measured responses were entered in design expert software 10 (trial version) against respective experimental run and risk analysis performed using ANOVA and pareto chart.

## 2.6 Critical risk factors analysis by DoE based central composite design

From seven potentially critical factors, after screening design study only two risk factors volume of methanol in mobile phase composition and migration distance were found critical risk factors for development of HPTLC method. To establish the relationship between critical risk factors and resolution of two drugs, DoE based central composite design was selected for critical risk factors

**Table 1** Design metrics for Taguchi screening design

| Exp. Run | Factor 1<br>Volume of methanol in mobile phase (mL) | Factor 2<br>B: Sat. time (min) | Factor 3<br>C: Drying time (min) | Factor 4<br>D: Volume of mobile phase (mL) | Factor5<br>E: Band width (mm) | Factor 6<br>F: Slit dimension (mm × mm) | Factor 7<br>G: Migration distance (mm) | Response<br>Resolution |
|---|---|---|---|---|---|---|---|---|
| 1 | 3 | 15 | 30 | 8 | 8 | 4 × 0.3 | 80 | 1.2 |
| 2 | 1 | 45 | 30 | 10 | 8 | 4 × 0.3 | 70 | 0.6 |
| 3 | 1 | 45 | 30 | 8 | 6 | 6 × 0.3 | 80 | 0.8 |
| 4 | 3 | 45 | 15 | 10 | 6 | 4 × 0.3 | 80 | 1.7 |
| 5 | 3 | 15 | 30 | 10 | 6 | 6 × 0.3 | 70 | 2.5 |
| 6 | 1 | 15 | 15 | 10 | 8 | 6 × 0.3 | 80 | 0.7 |
| 7 | 1 | 15 | 15 | 8 | 6 | 4 × 0.3 | 70 | 1 |
| 8 | 3 | 45 | 15 | 8 | 8 | 6 × 0.3 | 70 | 3.6 |

analysis. Experimental runs suggested by design expert software were performed in laboratory and resolutions of two drugs measured. Resolutions were added in software against respective experimental run and response surface analysis was performed with help of ANOVA and contour plots.

## 2.7 Risk mitigation and development of MODR

The critical effects of risk factors volume of mobile phase and migration distance on resolution of two drugs for development of HPTLC method were optimised for resolution more than 1.5. After the mitigation of risk factors effect, method operable design region (MODR) was determined from the overlaid plot for development of HPTLC method which gives resolution of both drugs more than 1.5 with compact and sharp bands. Mathematical model suggested for MODR was validated by performing suggested experimental trials in laboratory for verification of risk mitigation.

## 2.8 Control strategy and optimised chromatographic conditions

From method operable design region for resolution more than 1.5, control strategy was set for development of HPTLC method for simultaneous estimation of tizanidine hydrochloride and nimesulide in their pharmaceutical dosage forms. As per the control strategy of HPTLC method optimised chromatographic conditions were as follow: Chromatographic separation was performed on 10 × 10 cm aluminium backed plates precoated with 250 µm layer of silica gel 60 $F_{254}$ (E. Merk, Darmstadt, Germany). The TLC plate was pre-washed with methanol and activated at 110 °C for 20 min prior to spotting. The samples were spotted on TLC plate 15 mm from the bottom edge by Linomat V semi-automatic spotter using following parameters: band width, 6 mm; track distance, 11.6 mm; application rate, 0.1 µL/s. The TLC plate was developed in twin through chamber using toluene: methanol (8:2, v/v) as mobile phase chamber saturation time, 30 min; migration distance, 75 mm. The TLC plate was dried, scanned and analysed by TLC Scanner IV and WinCATS software using following parameters: slit dimension, 4 × 0.30 mm; scanning speed, 20 mm/sec; detection wavelength, 316 nm.

## 2.9 Procedure for calibration curve

From each combined working standard solutions (5, 10, 15, 20 and 25 µg/mL of tizanidine hydrochloride and 100, 200, 300, 400, and 500 µg/mL), 5µL were spotted on same TLC plate. The TLC plate was developed, dried and analysed as described under chromatographic conditions. Calibration curve was obtained by plotting peak area against respective concentration of both drugs.

## 2.10 Method validation

As per the ICH guideline Q2R1, developed method was validated for specificity, linearity, precision, accuracy, LOD and LOQ. Specificity of HPTLC method is ascertained by comparing Rf values and insitu absorbance-reflectance UV spectrum of sample tizanidine hydrochloride and nimesulide with that of standards of both drugs. Linearity was confirmed by repeating procedure on calibration curve five times. Precision study was performed in term of repeatability of sample measurement, repeatability of sample application, intraday and interday precision study as per guideline. Accuracy study was performed by standard addition at level of 80, 100 and 120% in preanalysed sample of both drugs. LOD and LOQ of both drugs were calculated using mathematical equations given in ICH

guideline using linearity data. Robustness study was done by applying minor deliberate variations in mobile phase composition, mobile phase volume, saturation time and scanning wavelength.

### 2.11 Assay of combined marketed formulations

The twenty tablets were weighed and finely powdered. The tablet powder equivalent to 10 mg tizanidine hydrochloride was accurately weighed and transferred into a 100 mL volumetric flask, 50 mL of methanol was added and the solution was sonicated for 10 min and diluted up to 100 mL with methanol and filtered through Whatman filter No. 41. From resulting solution, 0.5 mL of was transferred into a 10 mL volumetric flask and diluted up to mark with methanol. From this solution, 15 µL was spotted on a TLC plate. The TLC plate was developed, dried and analysed as described under chromatographic conditions. Amount of tizanidine hydrochloride and nimesulide from marketed formulation was calculated.

## 3 Results and discussion

### 3.1 Preliminary trials for HPTLC method development

For separation of tizanidine hydrochloride and nimesulide, number of polar and non polar organic solvent have been tried for mobile phase composition using silica gel G $F_{254}$ as stationary phase. Toluene and methanol were found effective solvent for better separation of two drugs with compact spot. Both drugs are soluble in methanol hence methanol was considered as significant solvent for mobile phase composition for further optimization by design of experimentation and quality risk management.

### 3.2 Risk identification and assessment

As per the ICH Q9 guideline, quality risk management started with risk identification followed by risk assessment and analysis. The risk identification was performed by brain storming process, literature review and preliminary experimental trials. Identified risk factors were hierarchically organised in ishikawa diagram in six different categories (Fig. 2). Risk assessment was performed by giving score to each risk factor according to their occurrence (O), severity (S) and detectibility (D) on by experimental trial and prior knowledge. If detectibility and severity of risk factor were very high to very low for loss of resolution in HPTLC method development, risk factors scored with 10 to 2 respectively by preliminary experimental trials. If detectibility of risk factor uncertain scored with 10 and certain risk factors scored with 2. Risk priority number was calculated by multiplying risk score of severity, detectibility and occurrence for each risk factor. The range of risk factor priority number was found 8–200. For identification of probable critical risk factors for HPTLC method development, the RPN limit was set 60 (Fig. 3). The risk factors having RPN above 60 were identified as probably critical risk factors need to be analysed for their criticality in method development. The risk factors categorised under mother nature were found probably critical risk factors which are uncontrollable so these risk factors were fixed by applying air conditioning system during method development. Seven risk factors were found probably critical for development of HPTLC method for estimation of both drugs.

### 3.3 DoE based risk factors analysis by Taguchi screening design

Seven identified probably critical risk factors by risk assessment process were further analysed for their criticality by DoE based Taguchi screening design which required eight experimental runs (Table 1). The levels (high + 1 and low − 1) of each risk factor for experimental run were identified by preliminary experimental runs. Measured resolutions were entered in design metrics given by design exert software (trial version) against respective experimental run and analysed for criticality level. As per the ANOVA table (Table 2) the probability value for selected model for screening design was found 0.0248 which is less than 0.05 that indicates model is significant for analysis of factor criticality in method development. The model F-value of 15.19 implies the model is significant and there is only 2.48% chance that F-value could occur large due to noise. The probability values for F ratio of volume of methanol and migration distance were found 0.0093 and 0.0440 respectively which showed critical effect of risk factors in method development. Other factors having probability for F ratio were found more than 0.05 for 95% confidence interval that indicates remaining factors are not having significant effect on resolution of two drugs. As per perato chart, the bar lines of volume of methanol and migration distance were found above critical line while bar lines of remaining risk factors were found below critical line that indicates volume of methanol and migration distance having critical risk in development of HPTLC method. In pareto chart (Fig. 4), orange coloured bar line of methanol volume showed positive effect and blue coloured bar line showed negative effect of migration distance on resolution of two drugs. From screening design it was concluded that volume of methanol and migration distance need to be controlled for mitigation of risk in development of HPTLC method.

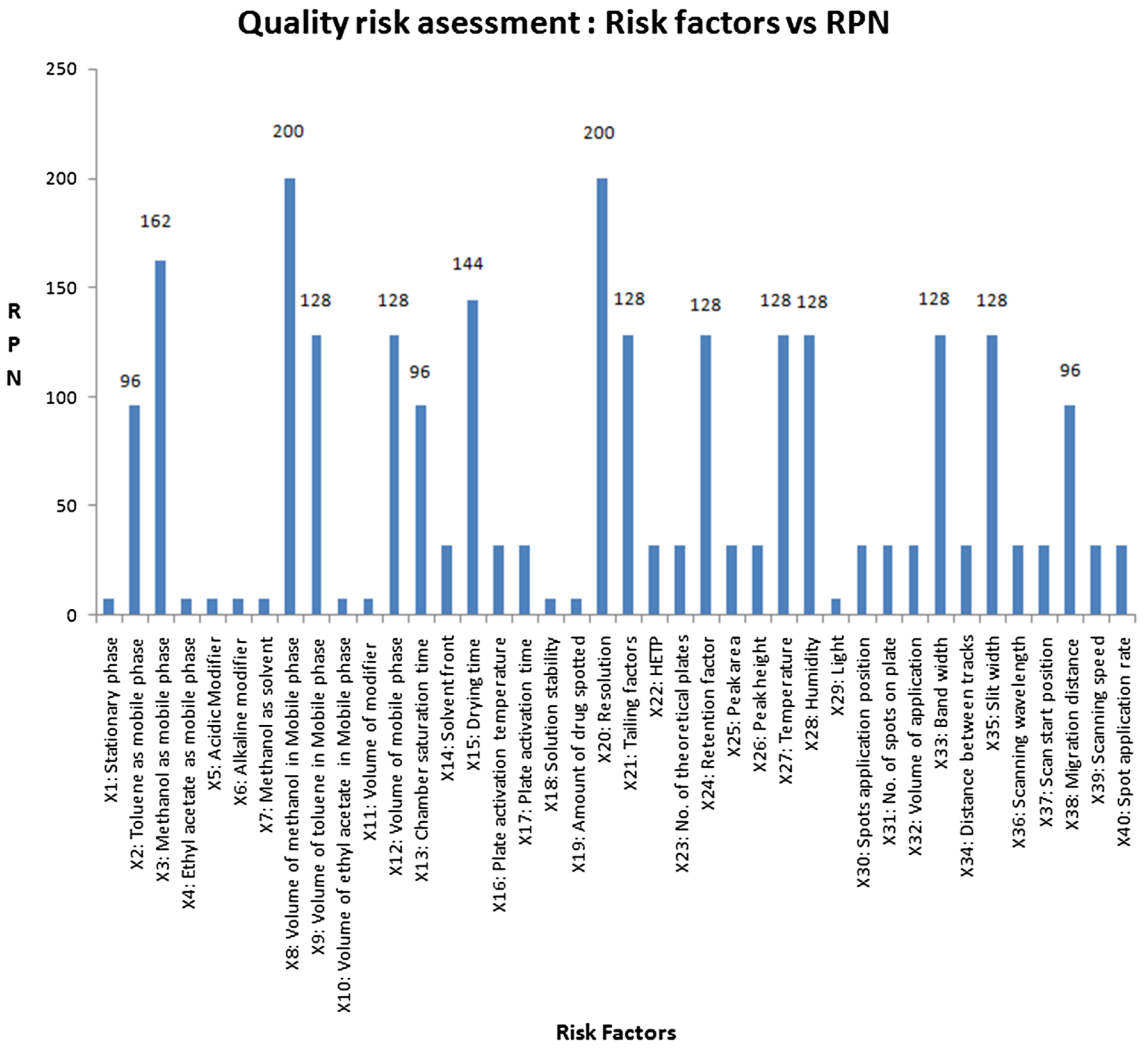


**Fig. 3** Risk assessment graph of RPN versus function of risk factors

**Table 2** ANOVA table for Taguchi screening design

| Source | Sum of squares | df | Mean square | F value | *P*-value Prob > F |
|---|---|---|---|---|---|
| Analysis of variance table [Partial sum of squares–Type III] | | | | | |
| Model | 7.37 | 4 | 1.84 | 15.19 | 0.0248 |
| *A-Volume of methanol in mobile phase* | *4.35* | *1* | *4.35* | *35.89* | *0.0093* |
| *C-Drying time* | *0.45* | *1* | *0.45* | *3.72* | *0.1493* |
| *F-Slit dimension* | *1.20* | *1* | *1.20* | *9.91* | *0.0514* |
| *G-Migration distance* | *1.36* | *1* | *1.36* | *11.23* | *0.0440* |
| Residual | 0.36 | 3 | 0.12 | | |
| Cor Total | 7.73 | 7 | | | |

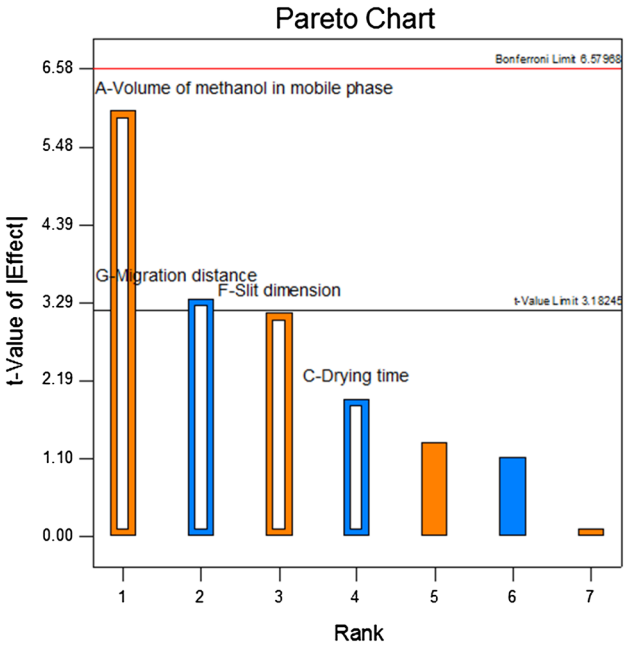


**Fig. 4** Pareto chart for Taguchi screening design

## 3.4 Critical risk factors analysis by DoE based central composite design

From risk analysis by Taguchi design, volume of methanol in mobile phase and migration distance were found critical factors out of seven identified probable risk factors for HPTLC method development. Critically identified risk factors need to be optimized to mitigate their risk for HPTLC method development which was performed by DoE based central composite design. Central composite design was selected as response surface methodology to establish relationship between identified critical risk factors and resolution of drugs. Central composite design had suggested four axial, four factorial and five central points in design metrics to be performed in laboratory. All thirteen experimental runs were performed in laboratory and measured resolutions were entered in design expert software against respective experimental run in design metrics for statistical analysis (Table 3). Central composite design had suggested quadratic model (Fig. 5) with adjusted R-squared value and predicted R-squared value 0.9725 and 0.9146 with *P* value of 0.0400 which indicates quadratic model is significant for response surface analysis. Lack of fit *P*-value was found 0.1735 which showed insignificant lack of fit value for selected quadratic model. From data analysis by ANOVA test, model F-value was found 85.80 which indicated selected model is significant and only 0.01% chance that F-value could occur large due to noise. The p-values

**Table 3** Design metrics for Central composite design

| Run | Space type | Factor 1 A: Volume of methanol (mL) | Factor 2 B: Migration distance (mm) | Response Resolution |
|---|---|---|---|---|
| 1 | Factorial | −1.000 | −1.000 | 0.6 |
| 2 | Center | 0.000 | 0.000 | 3.5 |
| 3 | Center | 0.000 | 0.000 | 3.9 |
| 4 | Axial | 0.000 | −1.414 | 2.5 |
| 5 | Factorial | −1.000 | 1.000 | 0.9 |
| 6 | Center | 0.000 | 0.000 | 4.2 |
| 7 | Axial | −1.414 | 0.000 | 0.5 |
| 8 | Axial | 1.414 | 0.000 | 10 |
| 9 | Factorial | 1.000 | −1.000 | 7.7 |
| 10 | Factorial | 1.000 | 1.000 | 8.5 |
| 11 | Center | 0.000 | 0.000 | 4.1 |
| 12 | Center | 0.000 | 0.000 | 4.5 |
| 13 | Axial | 0.000 | 1.414 | 5.2 |

for F-ratio of main effect of two critical risk factors and quadratic effect of volume of methanol in mobile phase were found less than 0.05 for 95% confidence interval which showed main effect of two factors and quadratic effect of were found significant for selected model. The *P*-values for interaction of two factors and quadratic effect of migration distance were found more than 0.05 showed their insignificant contribution for HPTLC method development. Model R-squared, adjusted R-squared value and predicted R-squared value were found 0.9839, 0.9725 and 0.9146 respectively which showed predicted R-squared value is in reasonable agreement with adjusted R-squared with difference of less than 0.2 indicate good prediction power of selected model. Adequate precision value of model was found 29.651 which is greater than 4 showed model can be used to navigate the design space. All significant terms in model equation having positive sign indicates their positive effect on resolution of both drugs (Table 4).

Full model mathematical equation was found as follow:

$$\begin{aligned}\text{Resolution} = {} & 4.04 + 3.52 * \text{volume of methanol} \\ & + 0.61 * \text{migration distance} + 0.12 \\ & * \text{volume of methanol} * \text{migration distance} \\ & + 0.57 * \text{volume of methanol}^2 \\ & - 0.13 * \text{migration distance}^2\end{aligned} \quad (1)$$

Reduced model was found as follow:

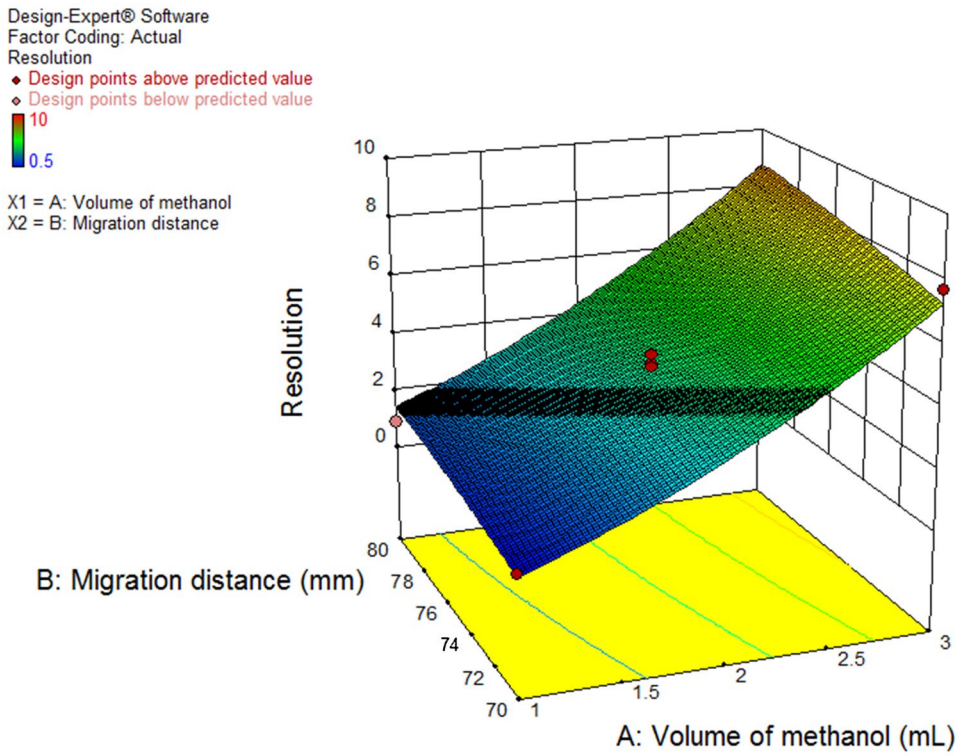


**Fig. 5** 3D contour plot for response surface analysis by central composite design

$$\text{Resolution} = 4.04 + 3.52 * \text{volume of methanol} + 0.61 * \text{migration distance} + 0.57 * \text{volume of methanol}^2 \quad (2)$$

### 3.5 Risk mitigation and development of MODR

Risk mitigation was done by optimization of volume of methanol in mobile phase composition and migration distance by central composite design. Volume of methanol in mobile phase and migration distance were optimised for navigation of design space with resolution of both drugs more than 1.5. Different solutions were suggested by design expert software for different combination of volume of methanol in mobile phase composition and migration distance with desirability value of 1 for resolution of two drugs more than 1.5. From suggested combinations of critical risk factors some were selected to perform in laboratory for validation of model prediction power. Selected combinations of mobile phase composition and migration distance were tried in laboratory and resolution measured. Experimental resolution was compared with predicted resolution of model suggested by design expert software for % variation value. % variation values for all experiment were found less than 2 that indicated good prediction power of model and risk mitigation is successfully done for HPTLC method development. Hence MODR suggested by model (Fig. 6) can be used for setting of control strategy for HPTLC method for simultaneous estimation of tizanidine hydrochloride and nimesulide.

### 3.6 Control strategy and optimised chromatographic conditions

After risk mitigation and MODR navigation, operating values of all risk factors were set for control strategy in development of HPTLC method for simultaneous estimation of tizanidine hydrochloride and nimesulide. As per the control strategy if volume of methanol in mobile phase composition varied from 1.3 to 2.5 mL with migration distance value of 70 to 80 mm in experimentation of HPTLC method, the resolution of both drugs were found always more than 1.5 with symmetrical shape of peak of both drugs. From set of control strategy, for HPTLC method development purpose, volume methanol was kept 2 mL with toluene volume of 8 mL and 75 mm of migration distance. Both drugs were separated well with desirable resolution and compact peak with Rf values of 0.28 and 0.60 for Tizanidine Hydrochloride and Nimesulide respectively (Fig. 7). Both spots were scanned from 200 to 700 nm to obtain in situ UV reflectance spectrum. The overlain UV

**Table 4** ANOVA table for central composite design

| Source | Sum of squares | df | Mean square | F value | *P*-value Prob > F | |
|---|---|---|---|---|---|---|
| Analysis of variance table [Partial sum of squares—Type III] | | | | | | |
| Model | 104.61 | 5 | 20.92 | 85.80 | < 0.0001 | Significant |
| *A-Volume of methanol* | *98.95* | *1* | *98.95* | *405.77* | *< 0.0001* | |
| *B-Migration distance* | *3.02* | *1* | *3.02* | *12.40* | *0.0097* | |
| *AB* | *0.063* | *1* | *0.063* | *0.26* | *0.6282* | |
| $A^2$ | *2.29* | *1* | *2.29* | *9.39* | *0.0182* | |
| $B^2$ | *0.11* | *1* | *0.11* | *0.45* | *0.5217* | |
| Residual | 1.71 | 7 | 0.24 | | | |
| *Lack of fit* | *1.15* | *3* | *0.38* | *2.79* | *0.1735* | Not significant |
| *Pure error* | *0.55* | *4* | *0.14* | | | |
| Cor Total | 106.32 | 12 | | | | |
| R-Squared | 0.9839 | | | | | |
| Adjusted R-Squared | 0.9725 | | | | | |
| Predicted R-Squared | 0.9146 | | | | | |
| Adequate Precision | 29.651 | | | | | |

spectra of tizanidine hydrochloride and nimesulide indicate that both drugs were showed reasonable absorbance at 316 nm wavelength. So, 316 nm was selected as wavelength for simultaneous estimation of tizanidine hydrochloride and nimesulide.

### 3.7 Calibration curve for tizanidine hydrochloride and nimesulide

A good linear relationship over the concentration range 25–125 ng per spot for tizanidine hydrochloride and concentration range 500–2500 ng per spot for nimesulide was observed. The correlation of coefficient was found to be 0.9970 for tizanidine hydrochloride and 0.9950 for nimesulide. The 3D chromatogram of calibration curve for tizanidine hydrochloride and nimesulide is shown in Fig. 8.

### 3.8 Method validation

The peak purity of both drugs were assessed by comparing UV absorbance-reflectance spectra of both drugs from marketed formulations with that of standard drugs at peak start, peak apex and peak end positions of the spot and correlation coefficient values were found more than 0.9990. The peak areas of tizanidine hydrochloride and nimesulide were linearly increased in concentration range of 25–125 and 500–2500 ng/spot with correlation coefficient value of 0.9983 and 0.9945 respectively. The % RSD for repeatability of peak area measurement was found to be 0.11 and 0.24 for tizanidine hydrochloride and nimesulide, respectively. The % RSD for repeatability of sample application was found to be 0.89 and 0.42 for tizanidine hydrochloride and nimesulide, respectively. The % RSD values of intraday precision was found to be 1.56–1.96% for tizanidine hydrochloride and 1.24–1.89% for nimesulide. The % RSD values of interday precision was found to be 1.79–2.03% for tizanidine Hydrochloride and 1.67–1.84% for nimesulide. The % recovery by standard addition method was found 98.20–100.12% for tizanidine hydrochloride and 97.90–101.49% for nimesulide. The LOD was found to be 2.56 ng/spot for tizanidine hydrochloride and 67 ng/spot for nimesulide. The LOQ was found to be 7.78 ng/spot for tizanidine hydrochloride and 201 ng/spot for nimesulide. The summary of validation parameter is depicted in Table 5.

### 3.9 Assay of combined marketed formulations

The spots at $R_f$ 0.28 (for tizanidine Hydrochloride) and 0.60 (for nimesulide) were observed in the

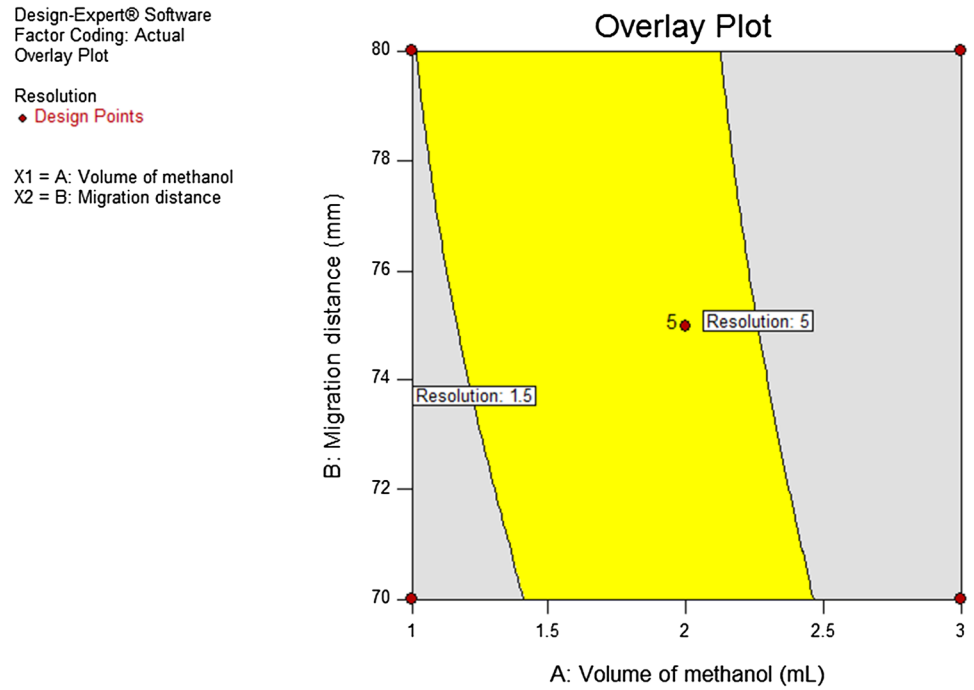


**Fig. 6** Method Operable Design Region (MODR) for HPTLC method

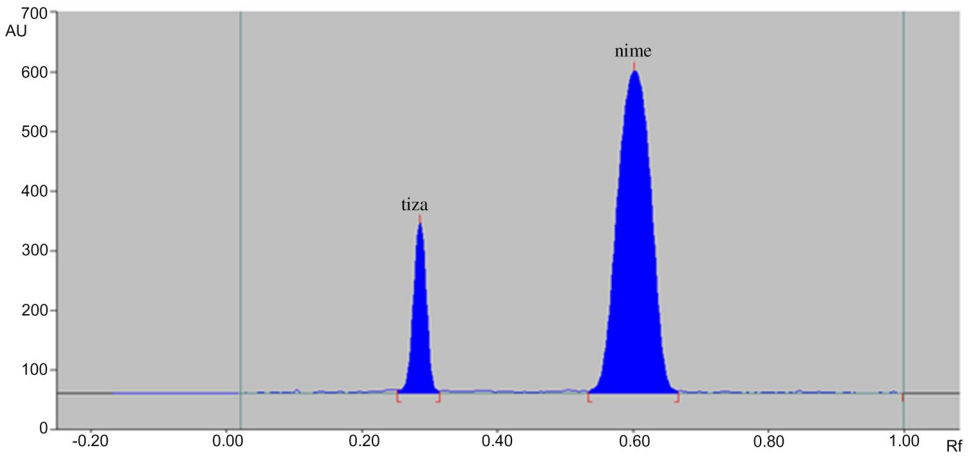


**Fig. 7** Chromatogram of tizanidine hydrochloride and nimesulide

chromatogram of the drug sample from marketed formulation. The drug content was found to be 98.56% ± 0.55 and 102.40% ± 0.50 for tizanidine hydrochloride and nimesulide, respectively. There was no additional peak observed except tizanidine hydrochloride and nimesulide that indicate no interference of excipients in estimation of tizanidine hydrochloride and nimesulide in their pharmaceutical dosage form.

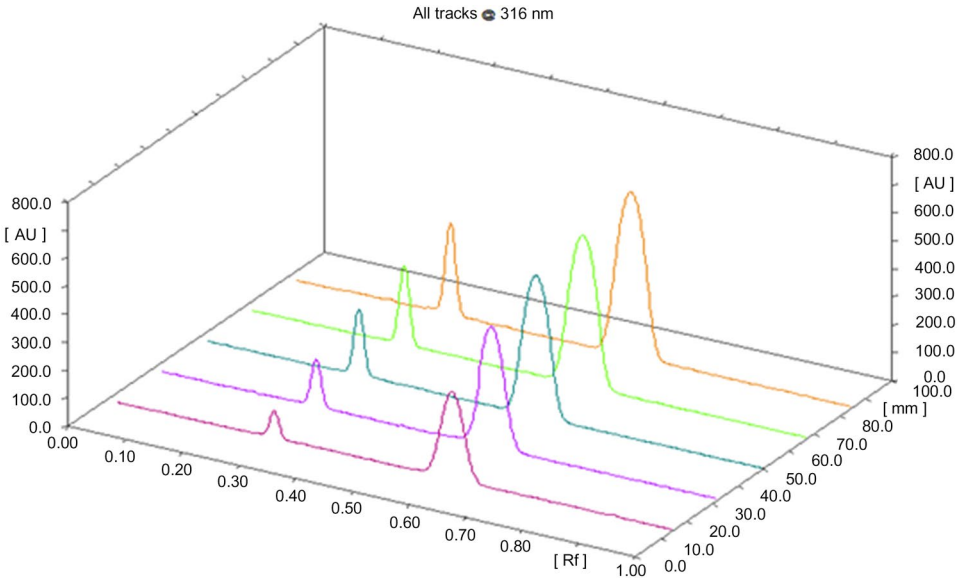


**Fig. 8** 3D chromatogram for linearity and range of two drugs

**Table 5** Summary of validation parameter

| Sr. no | Parameters | Results | |
|---|---|---|---|
| | | Tizanidine Hydrochloride | Nimesulide |
| 1 | Linearity Range | 25–125 (ng per spot) | 500–2500 (ng per spot) |
| 2 | Correlation coefficient | 0.9983 | 0.9945 |
| 3 | Limit of detection | 2.56 ng/spot | 67 ng/spot |
| 4 | Limit of quantification | 7.78 ng/spot | 201 ng/spot |
| 5 | Accuracy(% Recovery) | 98.20–100.12% | 97.90–101.49% |
| 6 | Precision (% RSD) | | |
| | Repeatability sample application | 0.89 | 0.42 |
| | Repeatability peak measurement | 0.11 | 0.24 |
| | Intraday precision | 1.56–1.96 | 1.24–1.82 |
| | Interday precision | 1.79–2.03 | 1.67–1.84 |
| 7 | Specificity | Specific | Specific |

## 4 Conclusion

Quality risk management and design of experiment based HPTLC method has been developed for simultaneous estimation of tizanidine hydrochloride and nimesulide in their tablet dosage form. Risk identification has been done with help of ishikawa diagram and more than thirty risk factors were identified for development of HPTLC method. By risk assessment seven risk factors were found probably critical having RPN more than sixty. Risk analysis was performed by DoE based Taguchi screening design which showed volume of methanol in mobile phase and migration distance were found critical with help of statistical analysis. Critical risk factors were further analysed for risk mitigation by DoE based central composite design and MODR navigation done by optimisation of HPTLC method giving resolution of two drugs with more than 1.5. Control strategy was set for HPTLC method and risk mitigation was verified by model validation. Developed HPTLC method gave resolution more than 1.5, proper peak shape with tailing factor in range of 0.9 to 1.2, capacity factors value in range of 1 to 5, selectivity factor value 1 to 20 and Rf values in range of 0.2 to 0.8 as per the acceptance criteria for chromatographic method. The developed method was validated for specificity study and peak of each drug was found pure. In each precision study, % RSD was found less than 2 which indicated method is precise. % recovery of drug was found in range of 98–102 which indicates method is accurate. LOD and LOQ were found nanogram level which indicates method is sensitive. Developed and validated

HPTLC method was applied for assay of tizanidine hydrochloride and nimesulide in their tablet and results were found in good agreement with labelled claim of tablet. Hence, HPTLC method is in compliance with regulatory requirements as per ICH guideline Q8, Q9 and Q2 R1 and can be applied for quality control of pharmaceutical dosage forms of nimesulide and tizanidine hydrochloride in pharmaceutical industry.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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