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QbD in development and validation of a rapid RP-HPLC method for determination of Trospium chloride in bulk drug

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Abstract

The present study describes a simple, accurate, precise and cost effective reverse phase high-performance liquid chromatographic (RP-HPLC) method for determination of Trospium chloride in bulk.The systematic approach, one of the parts of QbD was use for the analytical method development. Chromatographic separation was carried out with C18 column; different mobile phases were tried starting with methanol and water. The separation was carried on Grace C-18 column (4.6×250 mm, 5-µm particle size) with mobile phase of 1mM pot. Dihydrogen phosphate buffer (pH-5.1): Acetonitrile (70:30 v/v). Peak was obtained at retention time of 5.56 min flow rate of 1 ml/min. Detection was done using UV detector at 216 nm. Optimization was done by response surface methodology, applying a three level Box Behnken design with three centre points. Three factors selected were flow rate, wavelength and ACN concentration in mobile phase. The optimized chromatographic method was validated according to the International Conference on Harmonization (ICH) Q2 (R1) guidelines for linearity, range, accuracy, precision and robustness.

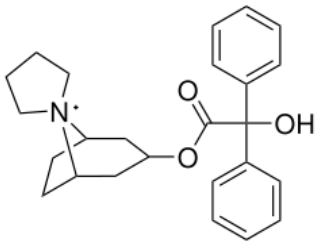
Key words: RP-HPLC; QbD; Acetonitrile (ACN); Trospium Chloride.

Introduction

Quality by Design (QbD) is, “a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management.” QbD has become an important concept

for the pharmaceutical industry that is further defined in the International Conference on Harmonisation (ICH) guidance on pharmaceutical development. This approach ensures a very high likelihood of method success during the product lifecycle. The scientific understanding gained during the method development process can be used to devise method control elements and to manage the risks identified. Thus, the validation which is usually performed after method development will serve the purpose of confirming method performance as opposed to identifying potential problem areas. [1]

Trospium Chloride [7, 11]



Trospium Chloride, Chemically is

3- (2-hydroxyl-2, 2-diphenylacetoxy) Spiro [bicycle [3.2.1] octane-8, 1'pyrrolidin]-1'-ium chloride.

Trospium Chloride is an antispasmodic, antimuscarinic agent. It is used in the treatment of overactive bladder with urge incontinence. It is also used as anticholinergic compound. It acts as a direct antagonist at muscarinic acetylcholine.

Trospium chloride is a white crystalline powder having melting point 268^0 C. It is soluble in Acetonitrile and water.

The aim of this work is to use QbD as an approach for development of an analytical RP-HPLC method for estimation of trospium chloride in bulk and also validate the method as per ICH guidelines.

Material and method for Trospium Chloride

Materials

Reagents and chemicals

Reference standard of Trospium Chloride was obtained from Glenmark pharmaceutical limited Mumbai. HPLC grade methanols, Acetonitrile, acetic acid of Merck were used. All aqueous solutions were prepared with HPLC grade ready water obtained in-house, Milli-Q water purification system (Millipore, USA).

Instrumentation

- HPLC analysis was carried out using a Jasco HPLC 2080 model chromatograph (Japan) equipped with a PU-2080 isocratic delivery system (pump), Jasco UV-2075 plus detector, the analytical column was a Grace smart reverse phaseC-18 column (4.6 × 250 mm, 5 µm particle size). Data acquisition and processing was performed using JASCO BORWIN software.
- Milli-Q water purification system (Millipore, USA).
- UV visible spectrophotometer (Double Beam), JASCO 630V and wavelength range of 200 to 400nm.

a) Trospium Chloride sample preparation

Trospium Chloride stock solution for optimization of experiments was prepared by accurately weighing 10mg of Trospium Chloride and dissolving in 100ml Methanol to yield a final concentration of 100µg/ml Trospium Chloride. From above stock solution 10µg/ml sample was prepared for analysis.

b) Mobile phase preparation

1mM potassium Dihydrogen phosphate buffer solution was prepared by dissolving 5.04 g disodium hydrogen phosphate and 3.01 g of potassium Dihydrogen phosphate in sufficient water to produce 1000 ml. adjust the pH 5.1 with glacial acetic acid. Buffer solution was degassed with sonicator and filtered prior to use for HPLC analysis.

c) Wavelength selection for analysis

Appropriate dilutions of Trospium Chloride were prepared and samples were scanned using UV spectrophotometer in the range of 200nm to 400nm. An absorbance maximum was obtained at 216nm.

d) Analytical target profile

"QbD is systematic approach to product, process design and development."[4]. Hence it began with determination of goal or method intent. In emphasis given on the product and process understanding [3]. Here method intent was to develop HPLC method of Trospium Chloride which is robust, accurate, precise and USP tailing less than 2, number of theoretical period as per requirement and short analysis time i.e. less than 10 min. as per QbD norms a robust method should be developed with help of visualized a design space.

e) Instrument Qualification

Analytical procedures in pharmaceutical analysis are subjected to highly formalized validation procedures in order to demonstrate that they are suitable for the intended use. As a consequence, prior to method validation it is necessary to assure that the equipment or analytical test system itself is adequately design, maintained, calibrated and tested. These tests are called as analytical instrument qualification (AIQ). Qualification phases for analytical instrument are

- Design qualification
- Installation qualification
- Operational qualification
- Performance qualification

Here in HPLC system are "of the shelf" equipment, design qualification may be disregarded here. Installation qualification establishes that the instrument is received as designed and that it is properly installed. As far as practical experimentation is considered only operational qualification and performance qualification combine parameters were done as reported by L.Kaminski et al. [6]

Precision of injection volume

It was determined by comparing peak area received with fixed 20µl injection and calibrated dosage loop tolerance limit set was <1%RSD.

Injection carryover

Injection carry over was determined by running a blank test directly after an analysis and measuring possible absorption there should not be any peak from previous analysis.

Flow rate accuracy

It was determine by measuring the volumetric flow rate of mobile phase through the column over a previously set period of time 1.0ml/min for 10 min, 2.0 ml/min for 5 min, 2.5 ml/min for 10 min. RSD should be <1% or tolerance limit is ±3%.

Flow rate precision

A flow rate precision was determined by measuring the RSD of retention times. Limit set was <1% RSD.

Wavelength accuracy

It was done by scanning the compound with known specific maxima. Tolerance limit is specific maxima ±2nm.

Linearity of detector

Linearity of detector was determined by injecting increasing concentration of test substance and tolerance limit set was $R^2 \geq 0.999$.

f) Risk assessment

It is commonly understood that risk is defined as the combination of probability of occurrence of harm and severity of that harm. Risk assessment helps to increase quality of method or process. Also it is determine for effect of input variable on method or process. From risk assessment one can recognise critical attributes that are going to affect final quality of product. A risk assessment is helpful for effective communication between FDA and industry, research/development, and manufacturing and among multiple manufacturing sites within company. Various tools for risk assessment are [2],

Ishikawa or fishbone diagram,

Failure mode effect analysis (FMEA),

Pareto analysis.

g) Initial Chromatographic condition

Chromatographic separation was carried out with C18 column; different mobile phases were tried starting with methanol and water. The separation was carried on Grace C-18

column (4.6×250 mm, 5-µm particle size) with mobile phase of 1mM pot. Dihydrogen phosphate buffer (pH-5.1): Acetonitrile (70:30 v/v) degassed in a sonicator for 10 min and filtered through 0.45µ membrane filter before use. Peak was obtained at retention time of 5.56 min flow rate of 1 ml/min, prior to the injection of drug solution: column was equilibrated with mobile phase flowing through the system. Detection was done using UV detector at 216 nm. Further changes were done according to optimization model. pH was change by using acetic acid.

h) Method design

Optimization was done by response surface methodology, applying a three level Box Behnken design with three centre points **(table 1)**. Three factors selected were flow rate, wavelength and ACN concentration in mobile phase. Evaluation of main factor, their interaction and quadric effect on peak USP tailing factor were done. Injection volume of 20µl, column oven temperature 25^0C was kept constant as their effect on tailing was less significant. Experiments were conducted by making triplicate injections (total 51 runs) of standard Trospium Chloride solution and the average of USP tailing was analysed using Design Expert 8 software.**(table 2)** Application of multivariate regression analysis resulted in a fitted full quadrate model for the average responses for peak USP tailing given by the equation 1

$$Y= \beta_0+\beta_1X_1+\beta_2 X_2+ \beta_3 X_3+ \beta_{11} X_1^2+ \beta_{22} X_2^2+ \beta_{33} X_3^2 + \beta_{12} X_1 X_2+ \beta_{13} X_1X_3+ \beta_{23} X_2X_3$$

Where Y is the response, β_0 is the arithmetic mean response. B_1 β_2 and β_3 are regression coefficients of the factor X_1, X_2 and X_3 respectively. B_{11}, β_{22} β_{33} are squared coefficients β_{12}, β_{13} and β_{23} are interaction coefficients. [8, 9]

Table 1: Chromatographic factors and response variable for Box Behnken experimental design.

Chromatographic Condition	Low	Level used Centre	High
Flow rate (X_1)	0.8	1	1.2

ACN Conc. (X_2)	20	30	40
Wavelength (X_3)	215	216	217

Table 2: Box Behnken method used for Trospium Chloride optimization

(Where '+' indicate the high value, '-'indicates lower value and '0' is the centre)

Run	Coded (X_1,X_2, X_3)	Flow Rate (ml/min)	ACN Conc. (%)	Wavelength (nm)
1	+0+	1.2	30	217
2	-0+	0.8	30	217
3	000	1	30	216
4	000	1	30	216
5	++0	1.2	40	216
6	0++	1	40	217
7	-0-	0.8	30	215
8	+0-	1.2	30	215
9	0+-	1	40	215
10	+-0	1.2	20	216
11	000	1	30	216
12	-+0	0.8	40	216
13	0-+	1	20	217
14	000	1	30	216
15	--0	0.8	20	216
16	0--	1	20	215
17	000	1	30	216

i) Critical Quality Attribute (CQA)

From software generated results the critical factors which affect the tailing and capacity factor were determined. Factor such as flow rate, Acetonitrile concentration in mobile

phase and wavelength were found to be critical. Selection of stationary phase was also critical parameter. The nature of the drug is more retentive on C-18 than C-8.

j) Method validation

The optimized chromatographic method was validated according to the International Conference on Harmonization (ICH) Q2 (R1) [5] guidelines for linearity, range, accuracy, precision and robustness. For system suitability, standard solution of 100µg/ml of Trospium Chloride was prepared by diluting and mixing drug with methanol. Six replicate injection of the system standard solution were analysed before sample analysis. The acceptance criteria for Trospium Chloride were less than 2% relative standard deviation (RSD)for peak area, retention time, symmetry USP tailing factor less than 2 and number of theoretical plates greater than 2000 for all peaks.

Linearity

As per ICH guidelines the linearity of analytical procedure is its ability (within in a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in sample. Standard calibration curves were prepared with five different concentrations by making serial volume to volume dilution of stock solution with methanol, over the range of 10, 20, 30, 40 and 50µg/ml. Three replicate injections of each concentration were made to determine the linearity of Trospium Chloride over the concentration range. Linear concentration curves of peak area versus drug concentration were plotted using linear least squares regression and evaluated for linearity.

Accuracy and precision

According to ICH Q2 guidelines accuracy of analytical procedure is the closeness of agreement between a reference or true value and value obtained while precision is usually reported as the per cent relative standard concentration standard deviation of a set of responses [5]. Accuracy and precision of the method were evaluated for Trospium Chloride drug substance by analysing standard samples prepared daily from stock solution. Three replicate of each low (10µg/ml), intermediate (30µg/ml), high (50µg/ml) standard were analysed daily over three days as a part of validation and quality control.

Accuracy and precision were determined by analysing the mean, standard deviation and relative standard deviation of the peak areas and their resultant concentrations. An acceptance criterion for precision is that the RSD of the standards should not be more than 2.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate change in method parameter and provide an indication of its reliability during normal usage [5]. There should be reliability of an analysis with respect to deliberate variations in method parameter such as flow rate (±0.1ml/min), pH (±0.1 units), mobile phase proportion.

Result and discussion for Trospium Chloride

Preliminary studies

Different mobile phases were tried starting with methanol and water. The separation was carried on Grace C-18 column (4.6×250 mm, 5-µm particle size) with mobile phase of 1mM pot. Dihydrogen phosphate buffer (pH-5.1): Acetonitrile (70:30 v/v) peak was obtained at retention time of 5.56 min, flow rate of 1 ml/min, at 216nm wavelength. Further optimization was done by carrying runs as by Box-Behnken design.

Instrument Qualification

Instrument qualification was done by considering combine parameters for operational qualification and performance qualification as in **(Table 3)**

Table 3: Result of instrument qualification in term of OQ & PQ

Module	Parameter	Findings	Limits
Injector	Precision of injector volume	RSD : 0.6	<1% RSD
	Injection carryover	No carryover	No carryover
Solvent delivery system	Flow rate accuracy	Expected volume +/-8%	Expected volume +/- 3%
	Flow rate precision	RSD: 0.8	<1%RSD

Detector	Wavelength accuracy Linearity of detector response	Specific maxima +1nm R^2=0.999	Specific maxima +/-2nm $R^2 \geq 0.999$

Method design

Box Behnken

Multivariate regression analysis was applied and fitted full quadratic model was obtained for the USP tailing factor of peak. Factor considered here are flow rate, ACN conc., and wavelength. Regression analysis and p-values obtained from software generated report are given in **(Table 4)**

Table 4: Regression coefficients and associated probability values (p-value) for USP tailing of Trospium chloride

Term	Coefficient	p-value
Intercept	1.31	<0.0001
Flow rate	0.000	
ACN conc.	0.11	<0.0001
Wavelength	0.000	
Flow rate x ACN conc.	0.000	
Flow rate x Wavelength	0.000	
ACN conc. x Wavelength	0.000	
Flow rate x Flow rate	0.020	<0.0001
ACN conc. x ACN conc.	0.030	<0.0001
Wavelength x Wavelength	0.020	<0.0001

Analysis of variance (ANOVA) was perform to study the significance of the factors and interaction terms on the response i.e. USP tailing of the peak, p-value simply provide the cut-off beyond which we assert that the findings are 'statistically significant' by convention, it is p<0.05[10]. A value of Probe > F was found to be less than 0.05, hence model was found to be

significant for prediction of response. Entire model was fitted well for optimization. Also a lack fit was significant.

All factors found were Significant (p-value <0.0001). Three of the factors were found to affect the peak response from their respective coefficients. None of the above is showing inverse relationship with tailing. Response surface and contour plot were studied to visualize effect of factor and their interaction so as to develop design space for robust method 3D graph are given below in **Fig.1 (A.B.C)**

From the graph some facts about effect of the factors and their interaction on the response can be found. Curvatures in the contour plot show nonlinear relationship between factors. From **(Fig.1A)** showing effect of flow rate and ACN conc.(where wavelength is constant at 216nm), it can be observed that flow rate does not show effect on tailing but as ACN conc. crosses 30% it shows that tailing factor exceed the limit.

When ACN conc. was kept constant. Flow rate and wavelength was studied. **(Fig.1B)** it was found that at flow rate more than 1 ml it shows increase in tailing and wavelength is not showing much effect.

When flow rate was kept constant at 1 ml and wavelength and ACN concentration was studied. Wavelength shows decrease in tailing. And ACN conc. more than 30 % shows increase in tailing. **(Fig.1C**) .From the three of diagrams it can be concluded that flow rate should be 1 ml, ACN concentration has lesser effect on tailing but at higher concentration tailing was found to be affected.

To obtain optimum set of condition to achieve desired goal composite desirability parameters were applied. Response was set to minimum tailing below target value of 1.31.

Optimum condition having desirability was chosen from obtained runs i.e. flow rate of 1 ml, ACN concentration of 20% and wavelength at 215 nm **(Fig.2**).Set of conditions were analysed to compare predicted response with actual response. Six replicates of 10µg/ml of solution at above specified conditions were taken. Difference in the response was not more than 3%.

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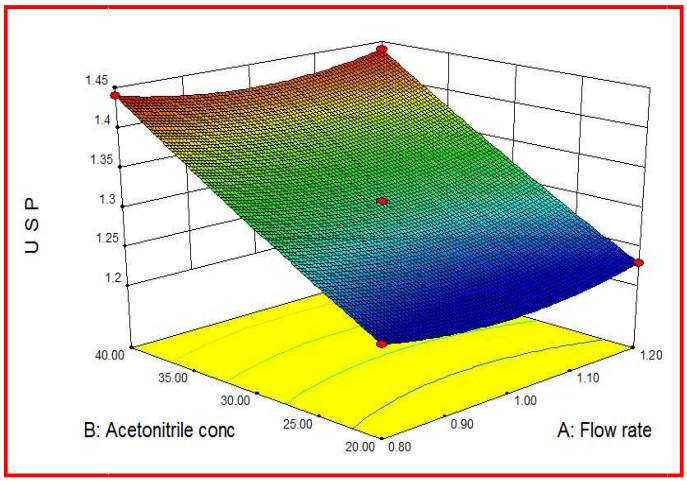
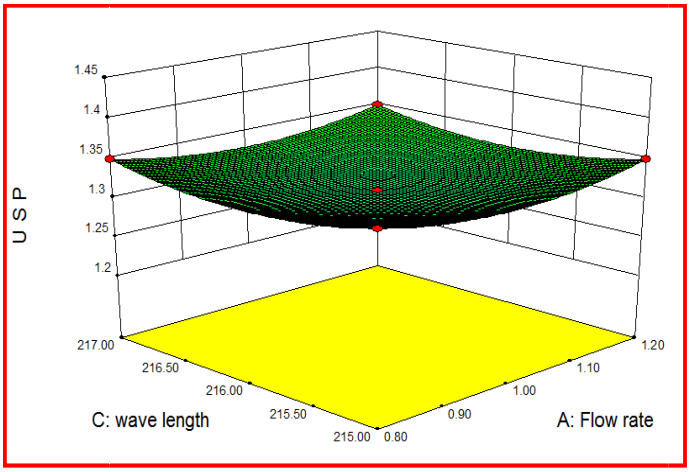


[A]



[B]

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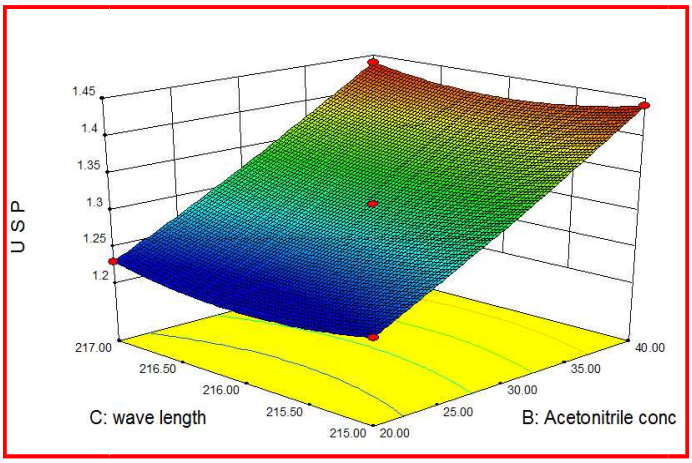

[C]

Figure 1 : Response surface (3D) and contour plots showing the effects of flow rate, ACN conc. and wavelength on USP tailing factor of Trospium Chloride

A) Effect of flow rate and ACN conc. B) Effect of flow rate and wavelength. C) Effect of ACN conc. and wavelength

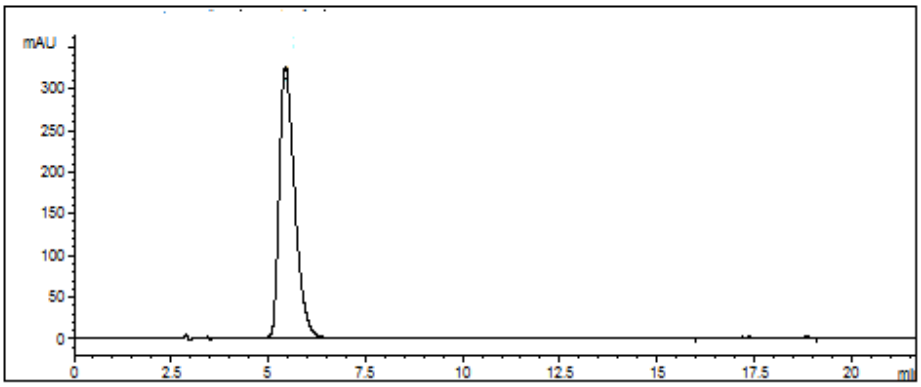

Figure 2: Chromatogram of Trospium Chloride

Method Validation

Method validation was done according to the ICH guideline Q2 [5]. Results were within the specified limit. Method was found to be accurate, precise and robust. Validation results are given below in **(Table 5).**

Table 5: Validation of method in term of linearity, precision and accuracy of Trospium Chloride

Validation parameter	Result	Acceptance criteria
Linearity (10-50 ug/ml)	Coefficient of Correlation-0.999	Coefficient of Correlation0>0.999
Accuracy	Recovery-99.5%	Recovery 98-102%
Precision		
Repeatability	RSD: 0.331%	RSD less than 2%

Linearity

A set of six solution of Trospium chloride at concentration ranging from 10-50 ug/ml were prepared. Each sample was analysed in triplicate, calibration curve was constructed by plotting the peak area verses the concentration using linear regression analysis. The correlation coefficient was found to be 0.999 **(Table 6) (Fig.3).**

Table 6: Linearity of Trospium chloride

Standard Concentration (µg/ml)	Peak area of Trospium chloride
10	2553
20	4315
30	6404
40	8357
50	10208
Regression equation	y= 193.52x+561.8
Regression coefficient	0.999

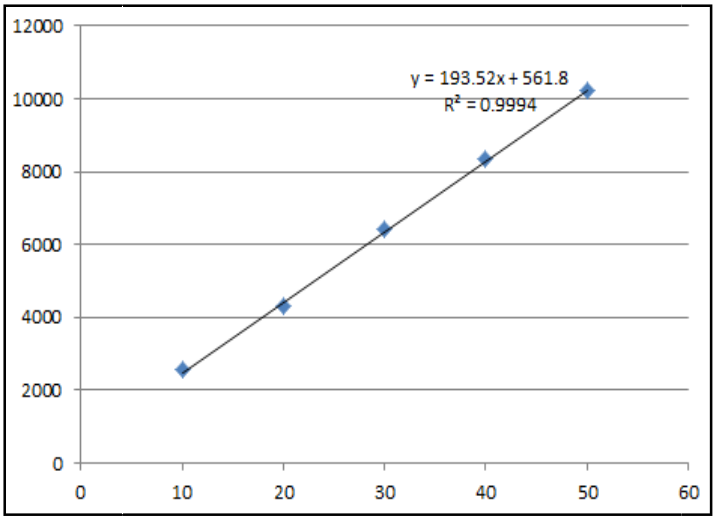

Figure 3: Linearity plot of Trospium Chloride

Repeatability

Repeatability was determined by running six replicates of samples and evaluating the average and %RSD for sample by comparing peak area. **(Table 7) .**

Table 7: Repeatability of Trospium Chloride

Sr. No.	Concentration (ug/ml)	Peak Area
1	10	2553
2	10	2558
3	10	2547
4	10	2548
5	10	2533
6	10	2545
Average		2547.3
%RSD		0.331

Conclusion

The Quality by Design approach has been successfully used to develop HPLC method for trospium chloride API. Systematic approach was utilized to develop an efficient and robust method which includes beginning with determination of target profile characteristics, instrument qualification, risk assessment, design of experiment and validation.

Three factors that were determined to significantly affect the peaks were then analysed to determine their interactions and quadratic effects with the least possible runs by using Box-Behnken model in conjunction with response surface methodology. Response surface diagrams and contour plots were studied for coming to conclusion which factor are affecting response and their limits were recorded.

A desirability function was applied to determine the optimum conditions. Optimum conditions were obtained; the one with higher desirability was selected. Replicates of run having optimized condition were taken to confirm the predicted response with actual response.

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References

1. Yan Li, Gerald J Terfloth, Alireza S Kord, A systematic approach to RP-HPLC method development in a pharmaceutical QbD environment. http://Americanpharmaceuticalreview.com. last accessed on 2012.
2. Borman P, Schweitzer M, Pohl M, Hanna-Brown M, Nethercote P, Hansen G, Smith K, Larew J. Implication and opportunities of applying QbD principles to analytical measurements. Pharm Technology, 2010,34, 52-59.
3. Arnum PA. 2007. A FDA perspective on Quality by Design. Pharmaceutical technology sourcing and management, http://www.pharmtech.com/pharmtech/article/article Last accessed on.2012.

4. ICH Harmonised tripartite guideline pharmaceutical development Q8 (R2). www.ich.org. last accessed on.2013.
5. ICH Harmonised tripartite guideline validation of analytical procedure: text and methodology Q8 (R2). www.ich.org. last accessed on.2013.
6. Kaminski L, Degenhardt M, Ermer J, Feubner C, Fritzn H, Peter L, Bernd R, Martin T, Hermann W. Efficient and economic HPLC performance qualification. J Pharm Biomed Anal. 2010, 51, 557-564.
7. Chatwal GR and Anand SK. Instrumental methods of chemical analysis, 5th edition, Mumbai:Himalaya Publishing House; 2007,2,149-2.150.
8. Awotwe-Otoo D, Agarabi C, Faustino PJ, Habib MJ, Lee S, Khan MA, Shah RB. Application of Quality b y Design elements for the development and optimization of an analytical method for protamine sulphate. J Pharm Biomed Anal. 2012,25,61-67.
9. Torrealday N, Gonza'lez S, Alonso RM, Jime'nez RM, Ortiz Lastra EO. Experimental Design Approach for the optimization of HPLC-fluorimetric method for the quantitation of the angiotensin II receptor antagonist in urine. J Pharm Biomed Anal. 2003;32: 847-857.
10. Krull I, Swartz M, Turpin J, Lukulay PH, Verseput R. Quality by Design methodology for rapid LC method development-part2, 2009. Last accessed on 12.10.2012.http://www.chromatographyonline.com/1cgc/A-Quality by Design methodology-for-rapid-LCmetho/Articlestandard/article/detail/579016
11. www.drugbank.com