Journal of Medicinal Chemistry and Drug Discovery
ISSN: 2347-9027
Special Issue
Analytical Chemistry Teachers And Researchers Association
National Convention/Seminar
18 January 2015

USE OF QUALITY BY DESIGN (QBD) APPROACH IN DEVELOPMENT OF HPLC METHOD FOR EPROSARTAN

Zahid Zaheer *, Jaiprakash N. Sangshetti, Rana Z. Ahmed, Hanumant Kavhale, Nikhil Kadam,
Y. B. Chavan College of Pharmacy, Dr. Rafiq Zakaria Campus, Rauza Baugh, Aurangabad-431001, India.
Corresponding Author E-mail: zaheerzahid@gmail.com

Abstract: A simple and robust high-performance liquid chromatographic (HPLC) method was developed and validated for the quantitative estimation of eprosartan in bulk and formulation. 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-4.5): Acetonitrile (60:40 v/v) degassed in a sonicator for 10 min and filtered through 0.2µ membrane filter before use. The optimized chromatographic method was validated according to the International Conference on Harmonization (ICH) Q2 (R1) guidelines for linearity, range, accuracy, precision and robustness. For system suitability, standard solution of 100µg/ml of Eprosartan was prepared by diluting and mixing drug with methanol.

Introduction

Quality by Design (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 as, “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.”As such, both industry and regulators Recognize the benefits of adopting a QbD approach to drug-product development and Manufacture , with key concepts described in International Conference on Harmonization (ICH)

guidelines [3]. QbD can be applied for various analytical methods which Includes, Chromatographic techniques like HPLC (For stability studies, method development and determination of impurities in pharmaceuticals). There are many Qbd Techniques, but we have used the systematic approach for The HPLC method development for the determination of Eprosartan [25,49] the Chemical name is 4-({2-butyl-5-[2-carboxy-2-(thiophen-2-yl methyl) eth-1-en-1-yl]-1H-imidazol-1-yl} methyl) benzoic acid.

Therapeutically Eprosartan is used as an Antihypertensive agent. The mechanisam of action Of eprosartan is It acts on rennin angiotensin system in two ways to decrease peripheral resistance. Firstly it blocks the binding of angiotensin II to AT_1 receptors in vascular smooth Muscles, causing vascular dilatation. This is followed by second step of inhibition of sympathetic nor-epinephrine production, which further reduces Blood Pressure.

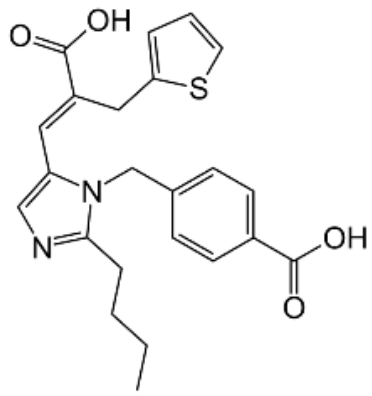


Fig-1 **chemical structure of Eprosartan**

Reference standard of Eprosartan 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 phase C-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 400 nm.

Eprosartan sample preparation

Eprosartan stock solution for optimization of experiments was prepared by accurately weighing 10mg of Eprosartan and dissolving in 100ml Methanol to yield a final concentration of 100µg/ml Eprosartan. From above stock solution 5µg/ml sample was prepared for analysis.

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 4.5 with glacial acetic acid. Buffer solution was degassed with sonicator and filtered prior to use for HPLC analysis.

Wavelength selection for analysis

Appropriate dilutions of Eprosartan were prepared and samples were scanned using UV spectrometer in the range of 200nm to 400nm. An absorbance maximum was obtained at 217nm.

Analytical target profile

"QbD is systematic approach to product, process design and development."[6]. Hence it begans with determination of goal or method intent. In emphasis given on the product and process understanding [4]. Here method intent was to develop HPLC method of Eprosartan 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.

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.

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-4.5): Acetonitrile (60:40 v/v) degassed in a sonicator for 10 min and filtered through 0.2µ membrane filter before use. Peak was obtained at retention time of 6.06 min flow rate of 0.8 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 217nm. Further changes were done according to optimization model. pH was change by using acetic acid.

Method design

Optimization was done by response surface methodology, applying a three level Box Behnken design with three centre points **(Table 1)**. Three factor selected were flow rate, wavelength and acetonitrile 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 30 ^{0}C were kept constant as their effect on tailing was less significant. Experiments were conducted by making triplicate injections (total 51 runs) of standard Eprosartan 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

$Y= \beta_0+\beta_1X_1+\beta_2X_2+ \beta_3X_3+ \beta_{11}X_1^2+ \beta_{22}X_2^2+ \beta_{33} X_3^2 + \beta_{12} X_1 X_2+ \beta_{13} X_1X_3+ \beta_{23}X_2X_3$

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}, $\beta_{13}$and β_{23} are interaction coefficients [27,37].

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

Chromatographic Condition	Level used		
	Low	Centre	High
Flow rate (X_1)	0.7	0.8	0.9
ACN Conc.(X_2)	35	40	45
Wavelength (X_3)	215	217	219

Table 2: Box Behnken method used for Eprosartan 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+	0.9	40	219
2	-0+	0.7	40	219
3	000	0.8	40	217
4	000	0.8	40	217
5	++0	0.9	45	217
6	0++	0.8	45	219
7	-0-	0.7	40	215
8	+0-	0.9	40	215
9	0+-	0.8	45	215
10	+-0	0.9	35	217

11	000	0.8	40	217
12	-+0	0.7	45	217
13	0-+	0.8	35	219
14	000	0.8	40	217
15	--0	0.7	35	217
16	0--	0.8	35	215
17	000	0.8	40	217

Critical Quality Attribute (CQA)

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

Method validation

The optimized chromatographic method was validated according to the International Conference on Harmonization (ICH) Q2 (R1) [3] guidelines for linearity, range, accuracy, precision and robustness. For system suitability, standard solution of 100µg/ml of Eprosartan 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 Eprosartan 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.

Table 3: Validation of method in term of linearity, precision and accuracy of Eprosartan

Validation parameter	**Result**	**Acceptance criteria**
Linearity (20-100 ug/ml)	Coefficient of Correlation-0.999	Coefficient of Correlation0>0.999

Accuracy	Recovery-99.5%	Recovery 98-102%
Precision		
Repeatability	RSD: 0.19%	RSD less than 2%

Linearity

A set of six solution of Eprosartan at concentration ranging from 20-100 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 0.998 **(Table 4) (Fig.2)**

Table 4: Linearity of Eprosartan

Standard Concentration (µg/ml)	**Peak area of EPR**
20	4523
40	8456
60	11568
80	15897
100	19452
Regression equation	y= 186.5x+789.5
Regression coefficient	0.998

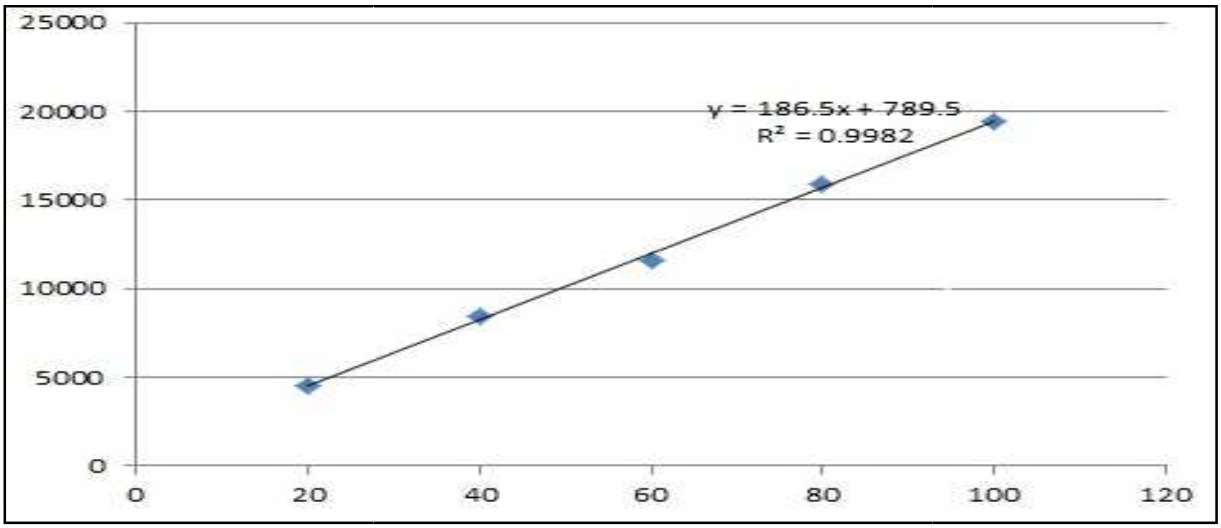


Figure 2: Linearity plot of Eprosartan

Repeatability

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

Table 5: Repeatability of Eprosartan

Sr.no	Concentration (ug/ml)	Peak Area
1	20	4529
2	20	4533
3	20	4540
4	20	4523
5	20	4542
6	20	4547
Average		4535.6
%RSD		0.19

Results and discussion for Eprosartan

Eprosartan is an angiotensin II receptor antagonist chemicaliy, eprosartan is 4-({2-butyl-5-[2-carboxy-2-(thiophen-2-ylmethyl)eth-1-en-1-yl]-1*H*-imidazol-1-yl}methyl) benzoic acid. It contains amino group in its structure hence it may be more retained on C-18 column hence flow rate has to be increased in order to carry drug substance with mobile phase also retention time has to be consider while optimization. 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-4.5): Acetonitrile (60:40 v/v) Peak was obtained at retention time of 6.06 min, flow rate of 0.8 ml/min, at 217nm wavelength. Further optimization was done by carrying runs as by Box-Behnken 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 coefficient and p-values obtained from software generated report are given in **(Table 6**)

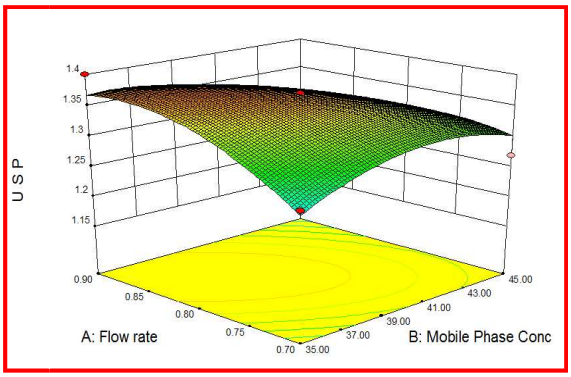
Table 6: Regression coefficients and associated probability values (p-values) for USP tailing of Eprosartan

Term	Coefficient	p-value
Intercept	1.37	0.0012
Flow rate	0.012	0.2036
ACN conc.	-0.020	0.0598
Wavelength	0.015	0.1363
Flow rate x ACN conc.	-0.040	0.0156
Flow rate x Wavelength	0.000	1.0000
ACN conc. x Wavelength	0.000	1.0000
Flow rate x Flow rate	-0.038	0.0185

ACN conc. x ACN conc.	-0.038	0.0185
Wavelength x Wavelength	-0.11	<0.0001

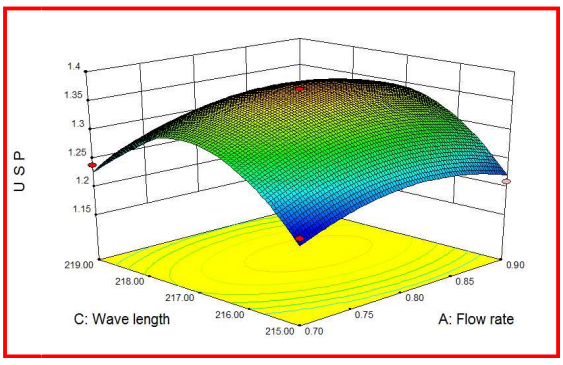
Response surface and contour plot were studied to visualize effect of factor and their interaction so as to develop design space for robust method 3D graph are given below in **Fig.3** (A.B.C)

From **(Fig.3A)** showing effect of flow rate and ACN conc.(where wavelength is constant at 217nm), it can be observed that tailing was in limit at flow rate of 0.7-0.9 ml and ACN conc. should be between 35-43%. If flow rate and ACN conc. gets increased then the tailing gets affected.

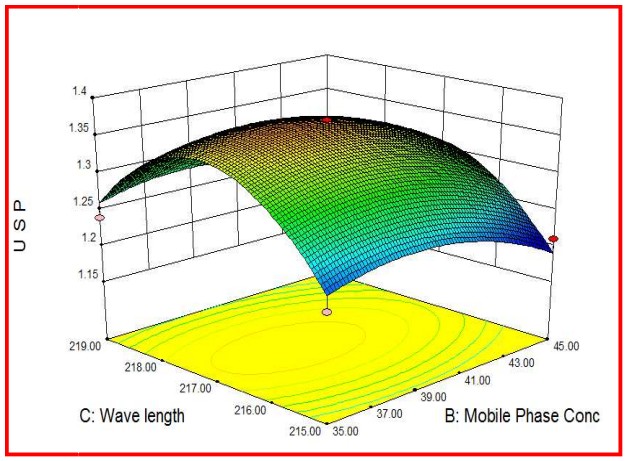


[A]

When ACN conc. Was kept constant at 40%. Flow rate and wavelength was studied. **(Fig. 3B)** it was found that at flow rate between 0.75-0.9 ml and wavelength should be below 218 nm.



When flow rate was kept constant at 0.8 ml and wavelength and ACN concentration was studied. Wavelength should be within 218nm. **(Fig.3 C**).



[C]

Figure 3 (A B C) : Response surface (3D) and contour plots showing the effects of flow rate, ACN conc. and wavelength on USP tailing factor of Eprosartan

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

C) Effect of ACN conc. and wavelength

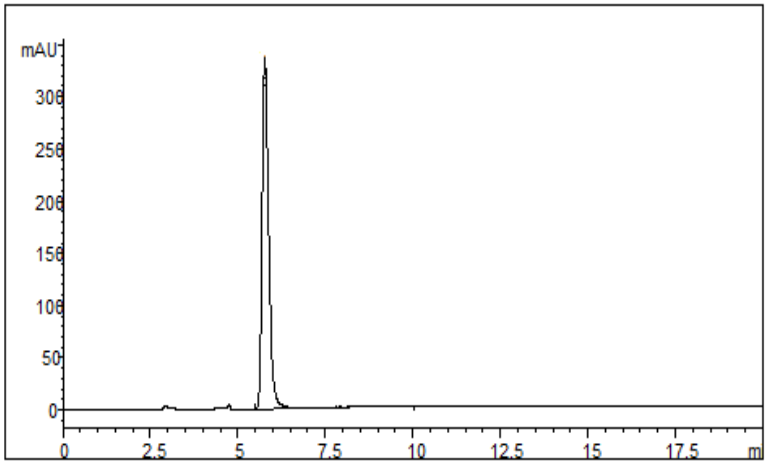


Figure 4: Chromatogram of Eprosartan

From the three of diagrams (i.e A B C) it can be concluded flow rate should be between 0.75-0.9ml ACN concentration has lesser effect on tailing but at lower 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 1.37.

Optimum condition having desirability was chosen from obtained runs i.e. flow rate of 0.8 ml, ACN concentration of 40% and wavelength at 217 nm **(Fig.10**).Set of conditions were analysed to compare predicted response with actual response. six replicates of 20µg/ml of solution at above specified conditions were taken. Difference in the response was not more than 3%.

Conclusion

The Quality by Design approach has been successfully used to develop HPLC method for eprosartan and trospium chloride API. All key aspect of QbD were tried to be implemented in said study. Systematic approach was utilized to develop an efficient and robust method which includes beginning with determination of target profile characteristics, 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.

Acknowledgement

The authors are thankful to Mrs. Fatima Rafiq Zakaria, Chairman, Maulana Azad Educational Trust and Principal, Y.B. Chavan College of Pharmacy, Dr. Rafiq Zakaria Campus, Aurangabad 431 001 (M.S.), India for providing the facility.

References

1. Borman P, Schweitzer M, Pohl M, Hanna-Brown M, Nethercote P, Hansen G, Smith K and Larew J. Implication and opportunities of applying QbD principles to analytical measurements. Pharm Technology, 2010, 34, 52-59.

2. Chatwal GR and Anand SK. Instrumental methods of chemical analysis, 5th edition, Mumbai: Himalaya Publishing House; 2007, 2.149-2.150.

3. www.drugbank.com

4. ICH Harmonised tripartite guideline pharmaceutical development Q8 (R2). www.ich.org. last accessed on .2013.

5. 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.

6. Awotwe-Otoo D, Agarabi C, Faustino PJ, Habib MJ, Lee S, Khan MA, Shah RB. Application of quality by design elements for the development and optimization of an analytical method for protamine sulphate. J pharm biomed anal. 2012, 25, 61-67.

7. 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.

8. ICH Harmonised tripartite guideline validation of analytical procedure: text and methodology Q8 (R2). . Www. Ich.org. last accessed on 2013.