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# Computer stimulated measurable structure approach: Chromatographic strategy advancement

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Abstract

**The computerization of method development and validation are useful in analysis of pharmaceuticals in pharmaceutical industry. In this article a simple and rapid high performance liquid chromatographic method has been developed for the determination of oxcarbazipine in pharmaceutical formulations. Factorial design was applied for optimization of essential factors for robustness study. A linear model was postulated and a $2^3$ full factorial design was employed to estimate the model coefficients. More specifically, experimental design helps the researcher to verify if changes in factor values produce a statistically significant variation of the observed response. The strategy is most effective if statistical design is used in most or all stages for screening and optimizing process in future method validation for method development and validation.**

**Keywords:** Column liquid chromatography, oxcarbazepine, experimental design, robustness, validation.

## INTRODUCTION

Oxcarbazepine (OXB) is a newer antiepileptic drug indicated for the treatment of partial seizures as both monotherapy and combination therapy in adults and children with epilepsy. At present, determinations of OXB have been established by the use of HPLC–UV spectrometry (Kimiskidis et al., 2007; Juenke et al., 2006; Levert et al., 2002), or LC-APCI-MS mass-spectrometry (Klys et al., 2005) However, none of these methods made the quick quantification and identification of OXB in a single run. Although methods reported for simultaneous determination of OXB and some of antiepileptic drugs has been described. (Ma et al., 2007; Yang et al.,2006; Coutin et al., 2005; Bugamelli et al., 2002; Lever, 2002; Khoschscour, 2001), that was not of use since it produced too long a chromatographic run, and had low sensitivity; it appeared that no assay existed for determination of the OXB using HPLC–MS/MS. The assay described here requires small mobile phase and sample volume, short chromatographic run and is sensitive, specific and fully validated. Computer aided statistical experimental design was used for validation to evaluate the robustness and intermediate precision.

## MATERIALS AND METHOD

### Chemicals and reagents

Oxcarbazepine, and imipramine, which was used as an internal standard (I.S.), were kindly provided by Glenmark Pharmaceuticals (Mumbai, India). HPLC-grade acetonitrile, methanol, formic acid, and diethyl ether, were purchased from Qualigens, (Mumbai, India).

### Instrumentation

A gradient HPLC system (Waters) with Waters 1525 Binary HPLC pump, Waters 2487 Dual λ Absorbance Detector and RP-$C_{18}$ column (150 × 4.6 mm i.d. partical size 5 μ) was used. The HPLC system was equipped with Waters breeze software.

### HPLC conditions

The mobile phase components, acetonitrile and $H_3PO_4$ buffer (pH adjusted to 3.0 with orthophosphoric acid) were filtered through 0.45 μm membrane filter before use and were pumped from the solvent reservoir at a ratio of 50:50 v/v into the column at a flow rate of 1 ml/min. The volume of each injection was 20 μ l. The column

was equilibrated for at least 30 min with the mobile phase flowing through the systems.

**Preparation of stock solutions**

Stock solution of Oxaliplatin and internal standard were prepared by dissolving 100 mg of oxcarbazipine 100 ml volumetric flask and then made up the solution with methanol up to the mark. Daily working standard solutions of oxcarbazipine was prepared by suitable dilutions of the stock solution with the mobile phase.

## RESULTS AND DISCUSSION

### Calibration solutions

Six sets of the oxcarbazipine solutions were prepared in mobile phase at concentrations of 0.5, 1, 2, 4, 6 and 10 μg/ml and found to linear in the range of 0.5 to 10 μg/mL with r-0.9984.

### Procedure

The standard solutions prepared as above were filtered through 0.45 μm membrane filter and the filtrate was injected six times into the column. The peak area ratio for each of the oxcarbazipine concentration was calculated. The regression of the oxcarbazipine concentration over the peak area was obtained. This regression was used to estimate the amount of oxcarbazipine in pharmaceutical dosage forms.

Oxcarbazipine solutions containing 2, 4 and 6 μg/ml were subjected to the proposed HPLC analysis for finding out the intra- and inter-day variations. The recovery studies were carried out by adding known amount of oxcarbazipine to the pre-analyzed samples and subjecting them to the proposed HPLC method. Accuracy obtained was between 95 to 104 % which is within the acceptance criteria.

### Experimental design

Experimental design techniques are powerful tools for the exploration of multivariate systems. In particular, statistical design using Response surface methodology is a way of choosing experiments efficiently and systematically to give reliable and coherent information. Response surface methodology is a collection of mathematical and statistical techniques that are useful for the modeling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response. The final step in RSM is to find a suitable approximation for the true functional relationship between response Y and the set of independent variables. Usually, a low order polynomial in some region of the independent variables is employed. If the response is well modeled by linear function of the independent variables, then the approximating function is the first order model.

$$Y = \beta_o + \beta_1 x_1 + \beta_2 x_2 + \ldots\ldots + \beta_k x_k + E$$

If there is curvature in the system, then a polynomial of higher degree must be used, such as the second order model.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum\sum_{i<j} \beta x_i x_j + E$$

All RSM problems utilize one or both of these models. RSM is a sequential procedure. The eventual objective of RSM is to determine the optimum operating conditions for the system or to determine a region of the factor space in which operating requirements are satisfied. As reported by Srinubabu et al during the validation of robustness and intermediate precision for voriconazole (Srinubabu, 2007), and pramipexole (Srinubabu, 2006), in pharmaceutical dosage forms using response surface methodology, demonstrates that the use of experimental design during optimization made the validation process easier and more cost effective.

### Factorial designs

In this design a repeated center point makes it possible to compute an estimate of the error term that does not depend on the fitted model. For this design all points except the center point appear at a distance from the origin (Yates and Mather, 1963).

### Robustness

Robustness testing is a part of method validation (ICH guidelines). Nowadays, method validation and robustness testing become increasingly important. Especially in pharmaceutical industries, extensive method validation is required in order to meet the strict regulations set by the regulatory bodies. The ICH (International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use) guidelines define robustness as: “The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage*”.* Robustness testing should be per-formed during the development of an analytical method to show its reliability when small variations occur in method parameters, that is, in operating conditions such as, for chromatographic methods, various chromatographic factors and detector conditions. When measurements are affected by these deliberate variations, precautions should be

**Table 1.** Experimental plan for robustness testing andobtained responses.

| Methanol | Flow rate | $p^H$ | Peak area ratio |
|---|---|---|---|
| 0.00000 | 1.00000 | -1.00000 | 1.94000 |
| 1.00000 | -1.00000 | 0.00000 | 1.74000 |
| -1.00000 | 0.00000 | 1.00000 | 1.86000 |
| 1.00000 | 1.00000 | 1.00000 | 1.89000 |
| -1.00000 | 1.00000 | 0.00000 | 1.87000 |
| -1.00000 | -1.00000 | -1.00000 | 1.76000 |
| 0.00000 | -1.00000 | 1.00000 | 1.81000 |
| 0.00000 | 0.00000 | 0.00000 | 1.83000 |
| 1.00000 | 0.00000 | -1.00000 | 1.85000 |

**Table 2.** ANOVA results.

| Parameter | SS | MS | F | P |
|---|---|---|---|---|
| ACN (%) | 0.0222 | 0.00101 | 2.935 | 0.254 |
| Flow rate (ml/min) | 0.0256 | 0.01281 | 37.193 | 0.026 |
| $p^H$ | 0.0029 | 0. 0014 | 4.290 | 0.189 |

to ensure that the analytical method is valid and robust when complying with these precautionary measures. For robustness study the factors selected have to reflect potential changes that may occur during validation process.

Factorial optimization design may be useful for this type of optimization study, besides it creates empirical model equations that correlate the relationship between variables and response(s). Factorial design has the following advantages: (a) to allow a complete study where all interaction effects are estimated; and (b) to give an accurate description of an experimental region around a center of interest with validity of interpolation with minimum runs (Montgomery, 2003). In factorial $k$ factors requires $3^k$ factorial runs, symmetrically spaced at ± along each variable axis, and at least one center point. In order to study the variables at no more than three levels (−1, 0, and +1). Three factors were considered: percentage v/v of methanol ($x_1$); flow rate ml $min^{-1}$ ($x_2$) and pH ($x_3$). The ranges examined were small deviations from the method settings and the corresponding responses in the peak area ratio considered ( $Y$) were observed. A factorial design with 9 experiments including two center points, the experimental plan and the corresponding responses are reported in Table 1. All experiments were performed in randomized order to minimize the effects of uncontrolled factors that may introduce a bias on the response. A classical second-degree model with a cubic experimental domain was postulated. Experimental results were computed by statistica (Stat soft, 2001) . The coefficients of the second-order polynomial model were estimated by the least squares regression. The equation model for $Y$ (found peak area ratio) was as follows:

$$Y=1.838889+0.001667x_1 0.065000x_2+0.001667x_3+0.015833x_1^2+0.015833x_2^2-0.019167x_3^2$$

The model was validated by the analysis of variance (ANOVA). The statistical analysis shows (Table 2) that the model represents the phenomenon quite well and the variation of the response was correctly related to the variation of the factors ($R^2$ = 0.9964). Optimized critical values are tabulated (Table 3).

The interpretation of the results has to start from the analysis of the whole model equation rather than from the analysis of the single coefficients. It is important for the response surface study, to consider also the factors whose coefficients are statistically non- significant. For this reason the analysis of the response surface plot is necessary. As shown in Figure 1, the analysis produces three-dimensional graphs by plotting the response model against two of the factors, while the third is held constant at a specified level, usually the proposed optimum. Figure 1 shows a graphical representation of the isoresponse surface for variation of percentage of Methanol ($x_1$) and flow rate ($x_2$), while the $p^H$ ($x_3$) is maintained constant at its optimum of 3.0. An increase in the flow rate results in a decrease of the observed peak area ratio ($Y$), while the percentage of organic modifier had no important effect on the response. Analogous interpretation may be derived by examining Figure 1 that plots the factors flow rate ($x_2$) versus $p^H$ ($x_3$)

**Intermediate precision / ruggedness**

The intermediate precision is a measure of precision between repeatability and reproducibility and it should be established according to the circumstances under which the procedure is intended to be used. The analyst should establish the effects of random events on the precision of the analytical procedure. The intermediate precision is obtained when multiple analysts, using multiple columns, on multiple days in one laboratory (Srinubabu, 2006) perform the assay. In order to study these effects simultaneously, a multivariate approach was used.

The considered variables included analysts (1 and 2), equipment (Column 1 and 2) and days (1 and 2) . The considered response was the found drug peak area ratio. A linear model ($y = b_0+b_1x_1+b_2x_2+b_3x_3$) was postulated and a $2^3$ full factorial design was employed to estimate the model coefficients. Each experiment was repeated three times in order to evaluate the experimental error variance. The analyses were carried out in a randomized order according to the experimental plan reported in Table 4. The concentration of mirtazapine was about 8µg $ml^{-1}$. No considered factor was found significant for the regression model assumed. The RSD found (5.32%, $n$ = 24) was acceptable, indicating an acceptable precision of the analytical procedure.

**Conclusion**

Rapid high performance liquid chromatographic method

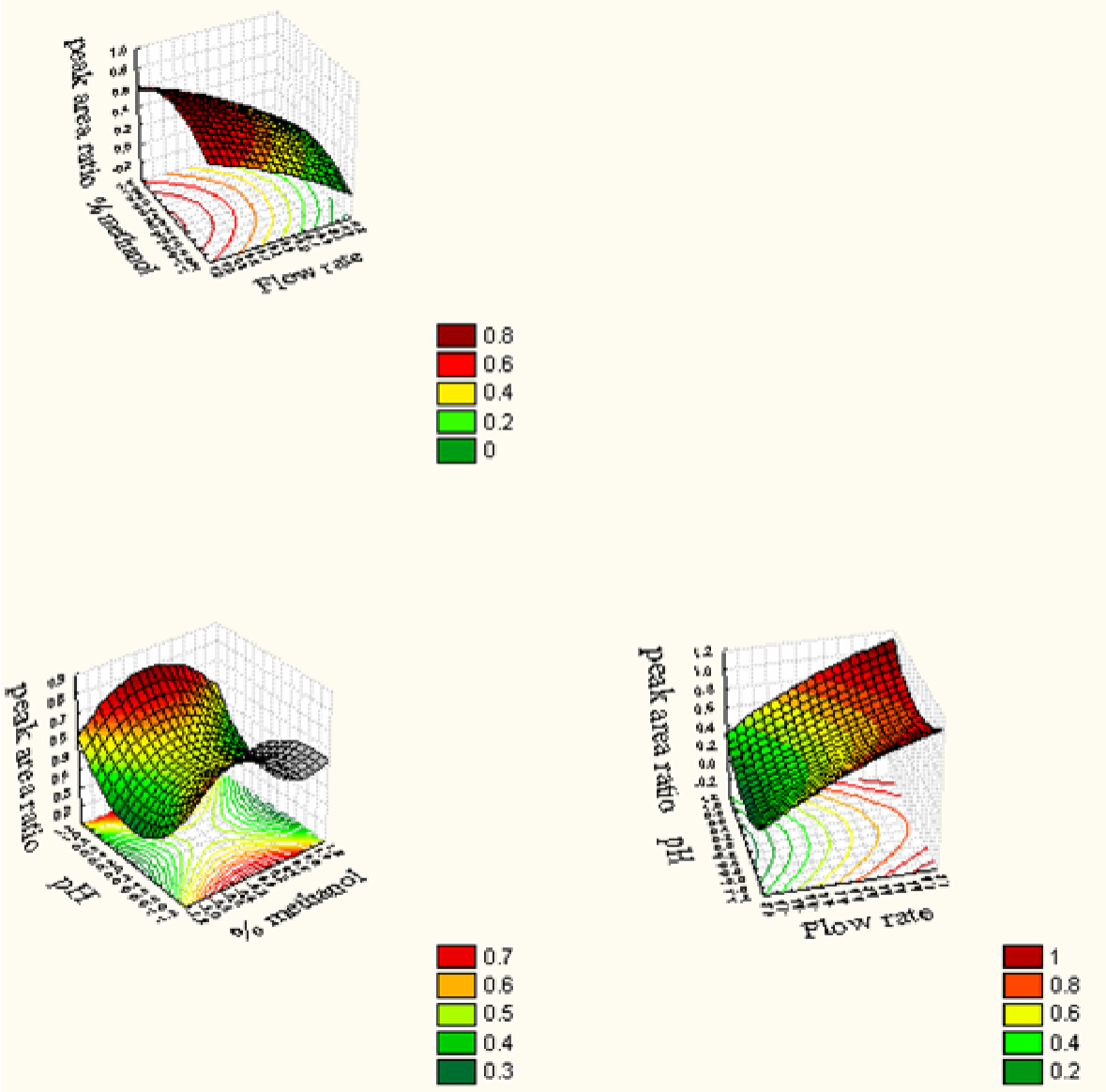


**Figure 1.** Three-dimensional plot of the response surface for *Y*(found drug peak area ratio). (a) Variation ofthe response *Y* as a function of $x_1$ (% Methanol) and $x_2$ (flow rate); fixed factor: $x_3$ ($p^H$)= 3.0 (c) Variation of the response *Y* as a function of $x_2$ (flow rate) and $x_3$ ($p^H$); fixed factor: $x_1$ (%Methanol)=50% *v/v*. (b) Variation of the response *Y* as a function of $x_1$ (% Acetonitrile) and $x_3$ ($p^H$ ) fixed factor: $x_2$ (flow rate)=1.0 ml $min^{-1}$

**Table 3.** Chromatographic conditions and range investigated during robustness testing

| Variable | optimized value | range investigated |
|---|---|---|
| Mobile phase (ACN/buffer) | 50:50 | 40 -60 |
| Flow rate (ml $min^{-1}$) | 1.0 | 0.8 - 1.2 |
| $p^H$ | 3.0 | 2.5 – 3.5 |

was developed. In particular, the use of experimental design for improvement of accuracy, precision and rapidity is a very attractive goal, and the use of experimental design during validation constitutes a basic feature of multivariate optimization, which if appropriately used can solve several problems and constitutes a powerful tool in

**Table 4.** Experimental plan for intermediate precision testing andobtained responses.

| No. exp. | Analyst | Instrument | Day | peak area ratio |
|---|---|---|---|---|
| 1 | Analyst 1 | HPLC 1 | Day 1 | 1.73 |
| 2 | Analyst 1 | HPLC 1 | Day 1 | 1.75 |
| 3 | Analyst 1 | HPLC 1 | Day 1 | 1.74 |
| 4 | Analyst 2 | HPLC 1 | Day 1 | 1.72 |
| 5 | Analyst 2 | HPLC 1 | Day 1 | 1.73 |
| 6 | Analyst 2 | HPLC 1 | Day 1 | 1.69 |
| 7 | Analyst 1 | HPLC 2 | Day 1 | 1.84 |
| 8 | Analyst 1 | HPLC 2 | Day 1 | 1.83 |
| 9 | Analyst 1 | HPLC 2 | Day 1 | 1.84 |
| 10 | Analyst 2 | HPLC 2 | Day 1 | 1.82 |
| 11 | Analyst 2 | HPLC 2 | Day 1 | 1.87 |
| 12 | Analyst 2 | HPLC 2 | Day 1 | 1.83 |
| 13 | Analyst 1 | HPLC 1 | Day 2 | 1.70 |
| 14 | Analyst 1 | HPLC 1 | Day 2 | 1.71 |
| 15 | Analyst 1 | HPLC 1 | Day 2 | 1.70 |
| 16 | Analyst 2 | HPLC 1 | Day 2 | 1.70 |
| 17 | Analyst 2 | HPLC 1 | Day 2 | 1.75 |
| 18 | Analyst 2 | HPLC 1 | Day 2 | 1.82 |
| 19 | Analyst 1 | HPLC 2 | Day 2 | 1.83 |
| 20 | Analyst 1 | HPLC 2 | Day 2 | 1.80 |
| 21 | Analyst 1 | HPLC 2 | Day 2 | 1.79 |
| 22 | Analyst 2 | HPLC 2 | Day 2 | 1.84 |
| 23 | Analyst 2 | HPLC 2 | Day 2 | 1.76 |
| 24 | Analyst 2 | HPLC 2 | Day 2 | 1.78 |

the hands of researchers.

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