

# Formulation, *in-vitro* evaluation and optimization of valsartan nano-lipid complex by Box-Behnken design

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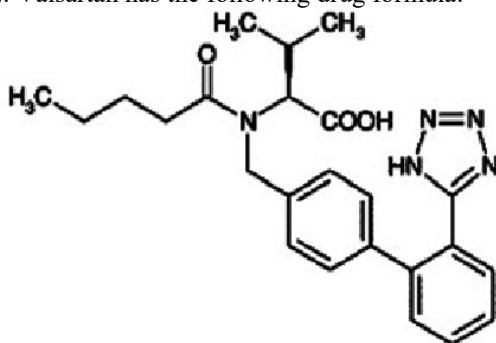
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**Abstract:** The antihypertensive drug valsartan, has an imperfect bioavailability due to its low solubility, permeability and excessive first pass hepatic metabolism. So, the goal of this article is to improve the physicochemical properties of valsartan to increase its bioavailability. In order to achieve this goal, valsartan- phospholipid complexesomes (VAL-PLC) were developed by the technique of solvent evaporation using Box-Behnken experimental design to optimize variables in the production process. The box- Behnken design revealed that the formula F3 prepared using 60% lipid percentage at 60 °C reaction temperature for 2h reaction time offered the optimum conditions for VAL-PLC preparation where the percent drug content reached 92.24%, average particle diameter was 189.17nm and polydispersity index was 0.289. Drug release experiment indicated that the dissolution of both raw valsartan and its commercial dosage form was dependent upon pH where it was extremely low at pH 1.2 and low in distilled water and it had a high dissolution at pH 6.8. On the contrary, the optimized VAL-PLC formula showed a high and pH-independent dissolution rate whatever the type of dissolution medium was. Therefore, it was concluded that valsartan-complexsomes may be considered as an encouraging approach for improving physicochemical properties and increasing bioavailability of valsartan.

**Keywords:** Valsartan, complexesomes, phospholipid, box-behnken design.

## INTRODUCTION

Valsartan is an angiotensin II receptor antagonist which is used for the treatment of hypertension (Leidig *et al.*, 2008). It relieves symptoms and improves the life quality in heart failure patients (Baumhäkel *et al.*, 2008). Valsartan is used without risk as an antihypertensive drug in children smaller than 6 years old (Flynn *et al.*, 2008). Valsartan is commercially available as oral tablets with strengths of 40, 80 and 160mg (Ibrahim and El-Setouhy, 2010). Valsartan has the following drug formula:



### Chemical structure of valsartan

In 2018, the FDA declared the presence of carcinogenic nitrosamine impurities such as nitrosodiethylamine (NDEA) in some of the products, including valsartan and losartan and drugs' recall procedures were started.

Consequently, they should be controlled to be below the acceptable cancer risk level to assure safety of the pharmaceutical products. Abd El-Hay and co-workers have proposed a green HPLC method for the detection of valsartan, losartan and their NDEA impurity in their pharmaceutical dosage forms (Abd El-Hay *et al.*, 2022). Recently, green analytical chemistry has been used in chromatography to minimize the amount of organic solvents consumed without loss in chromatographic performance (Hegazy *et al.*, 2022; El-Awady *et al.*, 2022; Hemdan *et al.*, 2022).

As valsartan had low bioavailability, pH-dependent solubility and low permeability (Wu and Benet, 2005), changing the drug physicochemical properties may increase its bioavailability. In this study, changing valsartan physicochemical properties was achieved by preparation of complexesomes (Dora *et al.*, 2017). Complexsomes are intramolecular complexes of polyphenolic compounds linked covalently to phospholipids, mainly phosphatidylcholine (PC) (Gulati *et al.*, 1998; Semalty *et al.*, 2009). For the drug to be esterified to the lipid, it must possess an active hydrogen atom (-OH, -NH<sub>2</sub>, -COOH etc.) (Semalty *et al.*, 2009). In aqueous colloidal dispersions, complexesomes consist of vesicular or micellar formations (Vaizoglu and Speiser, 1986). As the drug molecules are covalently bound to lipids, complexesomes circumvent the common problems

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related to liposomes like involvement of polar drugs, low drug inclusion, leakage and stability problems (Gulati *et al.*, 1998).

Krishnaiah *et al.* (2010) and Alodaini *et al.* (2019) showed that valsartan stability was affected by acidic pH and an accurate stability-indicating UPLC method was developed for the quantitative determination of purity of valsartan in pharmaceutical dosage forms in the presence of its impurities and degradation products. However, formulation of valsartan in the form of complexosomes will not only improve its solubility but also it can provide a means for its protection in a low pH environment. Peppas and co-workers (Peppas *et al.*, 2004) proved that formulation of insulin as complexation hydrogels, exhibited a pH-sensitive swelling behavior that protected insulin from acidic environment.

The intent of this research is to enhance the physicochemical characters of valsartan with the ultimate goal of increasing its bioavailability. This was done through the preparation of valsartan phospholipid complexosomes (VAL-PLC). During the study, the best conditions for preparation of valsartan complexosomes were estimated using a Box-Behnken design, followed by physicochemical characterization of the formed valsartan complexosomes.

## MATERIALS AND METHODS

### Materials

Valsartan was helpfully donated by the Egyptian International Pharmaceutical Industries Company (EIPICO), Egypt, while lecithin phospholipid and tetrahydrofuran were purchased from Pharco Company, Egypt. Methanol and glacial acetic acid were bought from El-Nasr Pharmaceutical Chemicals Co., Cairo, Egypt. All other chemicals were of pure analytical grade.

### Experimental design

Box-Behnken design was used for designing polynomial model for optimization of valsartan- phospholipid complexosomes (VAL-PLC) keeping 3 independent and 3 dependent variables. The independent variables were reaction time, reaction temperature and lipid %, whereas the dependent variables were % drug content, particle size (PS) and polydispersity index (PDI) (Varshosaz *et al.*, 2010).

### Box-behnken design (BBD)

A 3-variable 3-level BBD with Design Expert® software V.13 (Stat-Ease Inc., Minneapolis, MN) was employed for the purposes of optimizing the complex of valsartan and phospholipid and investigating the interdependence between the experimental factors and responses (Badawi *et al.*, 2020; Goo *et al.*, 2020). The same concept was followed by Usta *et al.* (2022) and Mohit *et al.* (2022)

who employed the Box-Behnken design to develop and optimize the formulation of bosentan and Gefitinib respectively, to solve their low bioavailability problem.

### Preparation of valsartan complexosomes

Valsartan complexosomes were prepared by refluxing a weighted amount of valsartan and phospholipids in a vacuum flask (100ml) containing a measurable amount of tetrahydrofuran as a reaction solvent at the specified temperature for the specified time (Morsi *et al.*, 2014). The reaction temperature for valsartan complexosomes preparation was controlled at 40, 50 and 60°C using valsartan: phospholipid ratios of 1:1, 1:1.5 and 1:2 which correspond to 50%, 60% and 70% w/w of lipid respectively. Reaction times of 1h, 1.5h and 2h were used to determine the impact of time of the reaction on the preparation of valsartan phospholipid complexosomes (Guo *et al.*, 2014). Based on Box Behnken design, the experimental design suggested preparation of 14 formulae. The suggested formulae were prepared and all formulae were subjected to measurement of % drug content, particle size (PS) and polydispersity index (PDI) as shown in table 2.

### Evaluation parameters

#### % Drug content of valsartan phospholipid complexosomes

The valsartan complexosomes prepared as stated above were dissolved in methanol to determine the total valsartan content in valsartan complexosomes spectrophotometrically at  $\lambda_{\max}$  250nm. To determine the un-reacted valsartan, the prepared valsartan complexosomes were washed by dispersion in 1% glacial acetic acid to dissolve un-reacted valsartan. The dispersion was then filtered and the un-reacted valsartan in glacial acetic acid was assayed by UV spectrophotometry at  $\lambda_{\max}$  250nm. The % drug content was calculated by applying the following equation:

$$\% \text{ Drug content} = (a-b/a) \times 100$$

where a is the total valsartan and b is the free un-reacted valsartan (Morsi *et al.*, 2017).

#### Estimation of particle size (PS) and polydispersity index (PDI)

The average PS and PDI of the developed Val-PLC were estimated using Malvern Zetasizer (Malvern Instrument Ltd., Worcestershire, UK) at 25°C. The PDI was estimated to give an implication of the uniformity of particle size and size distribution of the formulated valsartan complexosomes. Small values of PDI (<0.3) implies homogenous particle size distribution (Malviya, 2021).

#### Characterization of optimized valsartan phospholipid complexosomes

##### 1- FT-IR, DSC, XRD characterization of valsartan phospholipid complexosomes

An FT-IR spectrophotometer (FT-IR Spectrometer, JASCO 6100, Japan) was employed to detect any

interaction between valsartan and the phospholipid (PL). The IR spectra of valsartan, lecithin, their physical mixture and the optimized VAL-PLC formula were obtained by the potassium bromide method where an appropriate amount of the powder was admixed with potassium bromide and then compressed into discs. The spectra were then obtained at the functional group region of  $4000\text{ cm}^{-1}$  to  $400\text{ cm}^{-1}$ .

DSC is a rapid and dependable method that provides some knowledge about the probable interaction of VAL with the phospholipid (Tan *et al.*, 2012). DSC thermograms of valsartan, lecithin, their physical mixture and the optimized VAL-PLC formula were obtained by a differential scanning calorimeter (LABSYS evo SETARAM, France).

XRD technique was used for characterization of crystallinity and polymorphism of VAL-PLC. XRD patterns of pure valsartan powder and the optimized VAL-PLC formula were obtained by an XRD equipment at room temperature (Bruker D8 Discover Diffractometer, Germany). The relative intensities (I/I<sub>0</sub>) and reflection peaks over a  $2\theta$  range of  $5^\circ$  -  $100^\circ$  were obtained.

### 2- Transmission electron microscopy (Tem)

The optimized VAL-PLC formula was observed under a transmission electron microscope operated at 80kv (Model JEM-2000, Jeol, Tokyo, Japan).

### 3- In vitro release studies

A dialysis bag (Dialysis cellulose membrane, Sigma Co., USA; Molecular weight cut-off 12,000–14,000) was employed to examine the release of valsartan from its optimized VAL-PLC formula, commercial tablet (Tareg® 40mg) and pure valsartan powder (Munyendo *et al.*, 2013). Drug release was inspected in three vehicles: distilled water, in addition to 0.1 N HCl of pH 1.2 and phosphate buffer of pH 6.8 to mimic the pH gradient of the stomach and intestine respectively (Sharma *et al.*, 2015, Kassem *et al.*, 2017). Two milliliters from each formula were infused in the dialysis bag which was then inserted in 250ml glass flask containing 100ml of the release medium and stirred at  $37\pm0.5^\circ\text{C}$  by a magnetic stirrer. Two milliliters were taken at appropriate time intervals (10, 20, 30, 60, 90, 120, 150, 180 mins) and substituted by equal amount of the fresh medium. Then samples were analyzed spectrophotometrically for valsartan concentration at  $\lambda_{\text{max}}$  250nm (Gupta *et al.*, 2010). The cumulative drug release percentages were calculated and plotted versus time on a graph. All measurements were carried out in triplicates.

## STATISTICAL ANALYSIS

Data were expressed as the mean $\pm$ SD (n=3). One way ANOVA was used to decide the statistical significance of

the data using SPSS® software V. 22 (Statistical Package for the Social Sciences; SPSS Inc., Chicago, IL, USA). The parameters were significant for the p values $\leq$ 0.05.

## RESULTS

In this study, 14 runs were done using Box-Behnken as an experimental design for optimization of valsartan complexosomes, using 3 independent and 3 dependent variables. The formed complexosomes were subjected to characterization, including the percent drug content, particle size and PDI. The impact of independent or formulation variables on dependent or response variables was examined and used to develop contour plots. Table 2 exhibits the mean % drug content, PS and PDI of VAL-PLC formulations. Polynomial equation of each dependent variable were generated which explained the individual and interaction effects of independent variables on dependent variables.

### % Drug content

As observed in the table 2, the % drug content in all VAL-PLC formulae ranged from 84 to 99.1%. Model F value for % drug content was 133.9 (P<0.05), reflecting the significance of the model as shown in table 3. F-value of 1.03 (P>0.05), which is non-significant, reflects a good model fit. As observed in table 4, the correlation coefficient for the polynomial equation ( $R^2 = 0.9967$ ) indicates significant data fitting in the model. The predicted  $R^2$  of 0.9568 agreed with the adjusted  $R^2$  of 0.9892 which indicates the suitability of the model to predict the response of % drug content of the drug (Malviya, 2021). Adequate precision measures the signal to noise ratio. A ratio higher than 4 is desirable. Adequate precision in case of % drug content is 31.746, indicating an adequate signal and can be used to navigate the design space. The second order polynomial equation relating the response of % drug content is mentioned below:

$$\% \text{ Drug content} = + 90.40 - 0.4250A + 0.5000B + 6.90C - 0.0000AB - 0.2500AC - 0.0500BC - 0.5750A^2 + 0.3250B^2 + 1.37C^2$$

The equation shows that increase in the reaction temperature (B) and lipid % (C) led to an increase in % drug content. On the contrary, the increase in the reaction time (A) led to a decrease in % drug content. Fig. 1A exhibits the 3-D response surface plot of % drug content.

### Particle size (PS)

The mean PS of the prepared VAL-PLC is shown in table 2. The particle diameters of all VAL-PLC formulations ranged between 159.3 and 405nm. Table 3 shows that model F value for particle size was 25.85 (P<0.05) which reflects the significance of the model. F-value of 0.32 (P>0.05), which is non-significant, indicates good model fit. As observed in table 4, the correlation coefficient for

the polynomial equation ( $R^2=0.9831$ ) indicates significant data fitting in the model. The predicted  $R^2$  of 0.8331 which is close to the adjusted  $R^2$  of 0.9451 reflects the suitability of the model to predict the response of PS (Hakeem *et al.*, 2020). Adequate precision in case of particle size was 12.4113, indicating an adequate signal and can be used to navigate the design space. The second order polynomial equation relating the response of particle size is shown below:

$$PS = + 202.10 - 1.45A + 5.66B + 13.39C - 17.4AB - 6.00AC + 5.27BC - 15.01A^2 - 4.59B^2 + 194.41C^2$$

As shown in the equation, the increase in the reaction temperature (B) and lipid % (C) led to a concomitant increase in particle diameter. Nevertheless, the increase in reaction time (A) was accompanied by a decrease in the particle diameter. The 3-D response surface plot of particle size is exhibited in fig. 1B.

#### **Polydispersity index (PDI)**

As presented in the table 2, the PDI in all VAL-PLC formulations ranged from 0.265 to 0.658. Table 3 shows that model F value for PDI was 164.40 ( $P<0.05$ ), reflecting the significance of the model. F-value of 119.13 ( $P>0.05$ ), which is non-significant, indicates a good model fit. As observed in table 4, the correlation coefficient for the polynomial equation ( $R^2=0.9973$ ) revealed significant data fitting in the model. The predicted  $R^2$  of 0.9570 was in good agreement with the adjusted  $R^2$  of 0.9912, indicating the suitability of the model to predict the response of PDI (Hakeem *et al.*, 2020). Adequate precision in case of PDI was 34.3843, indicating an adequate signal and can be used to navigate the design space. The second order polynomial equation relating the response of PDI is given below:

$$PDI = + 0.3700 - 0.0178 A - 0.0094 B - 0.0436 C - 0.0065 AB + 0.0105 AC + 0.0028 BC - 0.0314 A^2 - 0.0281 B^2 + 0.2554 C^2$$

As shown in the equation, the increase in the reaction time (A), reaction temperature (B) and lipid % (C) led to a decrease in PDI. Fig. 1C demonstrates the 3-D response surface plot of PDI.

#### **Optimization using the desirability functions and model validation**

The final optimized formulation was chosen based on specific parameters including minimum particle size and PDI with maximum % drug content. Design expert software analyzed all the probable combinations of variables within the design space to fulfill the desirability specifications. Seven combinations were found to have maximum value of desirability as observed in table 5. In the present assessment, seven combinations of variables having desirability value 0.766 were selected. Amongst these seven combinations, one combination of variables was selected depending on the criteria of minimum PS

and PDI and maximum % drug content. As shown in table 5, the selected combination had % drug content 92.245, PDI 0.289 and particle size 189.172nm, at reaction time 2h, reaction temperature 60 °C and lipid % 60. Fig. 2 depicts the bar chart and 2-D contour plots illustrating the desirability.

#### **In vitro release studies**

Valsartan release was checked for optimized valsartan-phospholipid complexes formula, commercial tablet (Tareg-40®) and pure valsartan powder using dialysis bag method. The release of valsartan was evaluated in 3 media; distilled water and two buffers of pH 1.2 and pH 6.8 to simulate GIT conditions. The dissolution of both pure valsartan powder and commercial dosage form was low and pH-dependent. Thus, the drug release was extremely low in pH 1.2 (<20% after 3h) and low in distilled water (<40% after 3h), while it was high and fast in pH 6.8 (>80% after 2h). These findings were in accordance with those obtained with other researchers during their attempts to improve the dissolution of telmisartan (Tran *et al.*, 2008; Zhang *et al.*, 2010). In contrast, optimized VAL-PLC formula showed a high dissolution rate in all dissolution media irrespective of their pH values, where it reached over 80% within 20 min. (fig. 3). All in all, there was a remarkable enhancement in the dissolution rate of valsartan from its optimized VAL-PLC formula.

#### **FT-IR, DSC and XRD characterization of valsartan Complexsomes**

FTIR spectroscopy is primarily utilized to detect any chemical reaction between the drug and the additives utilized in its formulation (Larkin, 2017). The FTIR spectra for the pure valsartan, lecithin, valsartan / lecithin physical mixture and the optimized formula of valsartan complexesomes (F3) are shown in fig. 4. Pure valsartan showed 2 carbonyl absorption bands at 1600.62 $\text{cm}^{-1}$  and 1732.08  $\text{cm}^{-1}$ , distinctive for the stretching of amide carbonyl and carboxyl carbonyl respectively. These bands offered an important tool to illustrate the drug-phospholipid complexation. The spectrum of the drug in its physical mixture with lecithin showed the main peaks of the drug without any shift or disappearance of any of these peaks. However, there was a slight shifting of amide carbonyl band in the spectrum of the optimized formula from 1600.62  $\text{cm}^{-1}$  to 1635.64  $\text{cm}^{-1}$ .

The DSC is a widely employed method to estimate thermal behavior, structural changes and any interactions between drugs and other chemicals. The DSC thermogram of valsartan exhibited in fig. 5 displays a distinctive acute endothermic peak at 80.5 °C that was in line with the drug melting temperature (Skotnicki *et al.*, 2013). This distinctive peak of valsartan was still detected in the DSC graph of the physical mixture of valsartan/lecithin. However, the DSC thermogram of the

phospholipid complex of the optimized formula showed the endothermal peak of the drug at a lower temperature of 57.6.

The purpose of the XRD analysis was to describe the physical nature of the drug. As observed in fig. 6, valsartan exhibited sharp diffraction peaks in its XRD pattern. However, these crystalline sharp peaks of the drug were missed in the XRD pattern for the drug phospholipid complex of the optimized formula.

#### **Transmission electron microscopical examination (TEM)**

TEM was performed to describe the shape of valsartan-phospholipid complexosomes diffused in the aqueous solution. As exhibited in fig. 7, the optimized formula of valsartan-phospholipid complexosomes formed a uniform spherical vesicular structure in aqueous dispersions.

### **DISCUSSION**

#### **% Drug content**

The high % drug content denotes the suitability of the technique of solvent evaporation for the preparation of valsartan-complexosomes.

The direct relation between the reaction temperature and the % drug content was in agreement with other results reported by Morsi and co-workers who proved that reaction temperature had a synergistic effect on the % drug content of mosapride citrate pharmacosomes (Morsi *et al.*, 2014). It was also reported in the literature that the complexation efficiency increased with an elevation of reaction temperature, but the increasing range was rather limited and reached a plateau when the temperature was above 60°C. Furthermore, oxidation of phospholipid was increased when the temperature exceeds 60°C. All in all, to promote the complexation reaction and minimize the oxidation of phospholipid, the optimal temperature range was determined to be 40-60°C (Chen *et al.*, 2022).

The increase in % drug content with the increase of lipid % may be as a consequence of the improved solubility of the drug in presence of greater amounts of lipid in view of the hydrophobic nature of the drug. Accordingly, more amounts of the solubilized drug will be available to interact with the phospholipid to form the complexosomes.

#### **Particle size (PS)**

All the prepared VAL-PLC formulations were in the nanometer size. These nano-sized particles can have a great entry to the human body and also show an effective tissue adherence due to the increase in touch area for mucoadhesive interactions (Lamprecht *et al.*, 2001, Gupta and Kompella, 2006).

The synergistic effect of the lipid % on the particle diameter was similarly reported by Xia, Kassem and co-workers (Xia *et al.*, 2013, Kassem *et al.*, 2017) during preparation of drug-phospholipid complexes of protopanaxadiol and repaglinide respectively. They reported that an increase in the content of phospholipid used for complex formation led to a concomitant increase in particle diameter.

#### **Polydispersity index (PDI)**

The low PDI values in all VAL-PLC formulations indicate homogeneity of the particle size. There was a direct relation between PS and PDI. Thus, a decrease in PS was accompanied by a concomitant decrease in PDI.

#### **In vitro release investigation**

*In vitro* release studies revealed that dissolution of both raw valsartan and its commercial dosage form was dependent upon pH where it was extremely low at pH 1.2 and low in distilled water and it had a high dissolution in pH 6.8. Valsartan possesses two weakly ionizable groups, a tetrazole group ( $pK_a=4.73$ ) and carboxylic acid group ( $pK_a=3.9$ ), which may explain its pH-dependent solubility attribute (Yeom *et al.*, 2017). In contrast, optimized VAL-PLC formula showed a high dissolution rate in all dissolution media irrespective of their pH values. The improvement in valsartan dissolution in its optimized VAL-PLC formula may be firstly owing to the transformation of valsartan from its crystalline to an amorphous state as verified by the DSC and XRD data (Liu *et al.*, 2010). Secondly, the reduced particle diameter of the drug to the nano-size provided a high surface area and surface free energy which eventually gave rise to a high enhancement in valsartan release from its complexosomes.

#### **FT-IR, DSC and XRD characterization of valsartan complexosomes**

Shifting of amide carbonyl band in the infra-red spectrum of the optimized formula shown in fig. 4D from  $1600.62\text{ cm}^{-1}$  to  $1635.64\text{ cm}^{-1}$  was in the range of amide group ( $1695\text{ cm}^{-1}$  to  $1600\text{ cm}^{-1}$ ), demonstrating no chemical changes occurred in the drug molecule (Shah *et al.*, 2015). The little moving of the absorption band of the carbonyl group of amide to a higher frequency might refer to disruption of the inter-molecular hydrogen bonds within the drug molecule and development of hydrogen bonds between the drug and the phospholipid. These results imply that some weak physical interactions had occurred between valsartan and the phospholipid in the formation of valsartan complexosomes (Cai *et al.*, 2012).

The sharpness of valsartan peak shown in its DSC thermogram in fig. 5 reveals the drug's crystalline nature. Appearance of the distinctive peak of valsartan at a lesser temperature of 57.6 suggests that some interactions had occurred between valsartan and the phospholipid during formation of the complexosomes which resulted in the change of the drug to an amorphous state (Skotnicki *et al.*, 2013).

**Table 1:** Dependent and independent variables for preparation of valsartan-phospholipid complexesomes

Level			Independent variables
+1	0	-1	
2h	1.5h	1h	Reaction time (X <sub>1</sub> )
60 <sup>o</sup> C	50 <sup>o</sup> C	40 <sup>o</sup> C	Reaction temp. (X <sub>2</sub> )
70%	60%	50%	Lipid % (X <sub>3</sub> )
Limitations			Dependent variables
Maximize			% Drug content (Y <sub>1</sub> )
Minimize			Particle size (Y <sub>2</sub> )
Minimize			PDI (Y <sub>3</sub> )

**Table 2:** Independent factors and investigated responses of the prepared VAL-PLC formulations

Formula	Factors			Responses		
	Reaction time (h)	Reaction temp. (°C)	Lipid (%)	Drug content (%) ± SD	Particle size (nm) ± SD	PDI ± SD
1	1.5	50	60	90±0.55	177.5±18.11	0.369±0.011
2	1.5	60	70	99.1±0.51	401.1±37.19	0.551±0.017
3	2	60	60	92.245±0.36	189.17±6.81	0.289±0.01
4	1.5	40	50	85±0.87	393.3±28.42	0.649±0.012
5	1	60	60	91.3±0.48	219.5±22.31	0.322±0.013
6	1	50	70	98.9±0.46	405±40.25	0.557±0.016
7	1.5	40	70	98.3±0.47	393.3±29.32	0.549±0.017
8	2	50	70	97.9±0.71	401±42.71	0.470±0.013
9	1.5	60	50	86±0.87	380±26.14	0.640±0.018
10	1.5	50	60	90.8±0.42	226.7±21.53	0.371±0.011
11	1	40	60	90.2±0.51	159.3±11.32	0.343±0.010
12	2	50	50	84±0.67	370±18.84	0.610±0.015
13	2	40	60	89±0.45	180.3±13.15	0.312±0.012
14	1	50	50	84±0.69	350±24.65	0.658±0.017

**Table 3:** Analysis of variance (ANOVA) of the calculated model for responses

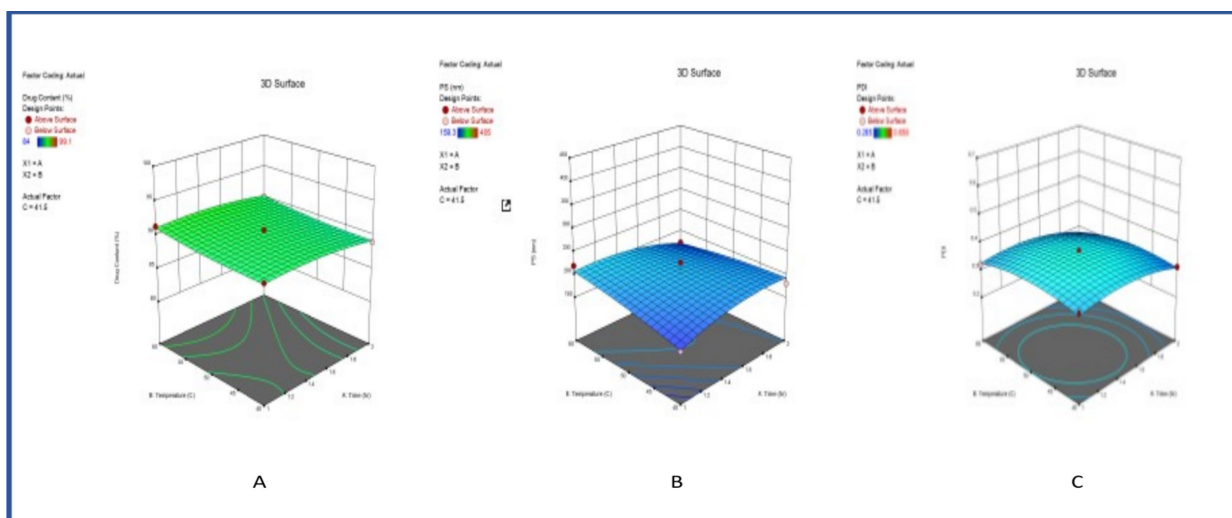
Result of the ANOVA regression	% Drug content	Particle size	PDI
Sum of Squares	393.17	1.38E+05	0.2651
Degrees of freedom (Df)	9	9	9
Mean square	43.69	15320.17	0.0295
F-value	133.9	25.85	164.4
p-value	0.0001	0.0034	0.0001
Conclusion	Significant	Significant	Significant
Lack of fit tests			
Sum of Squares	0.985	1160.33	0.0007
Degrees of freedom (Df)	3	3	3
Mean square	0.3283	386.78	0.0002
F-value	1.03	0.3196	119.13
p-value	0.6037	0.825	0.0672
Conclusion	Not significant	Not significant	Not significant
Residual			
Sum of Squares	1.31	2370.65	0.0007
Degrees of freedom (Df)	4	4	4
Mean square	0.3263	592.66	0.0002

**Table 4:** Main output data of the Box-Behnken design analysis for valsartan phospholipid complexesomes

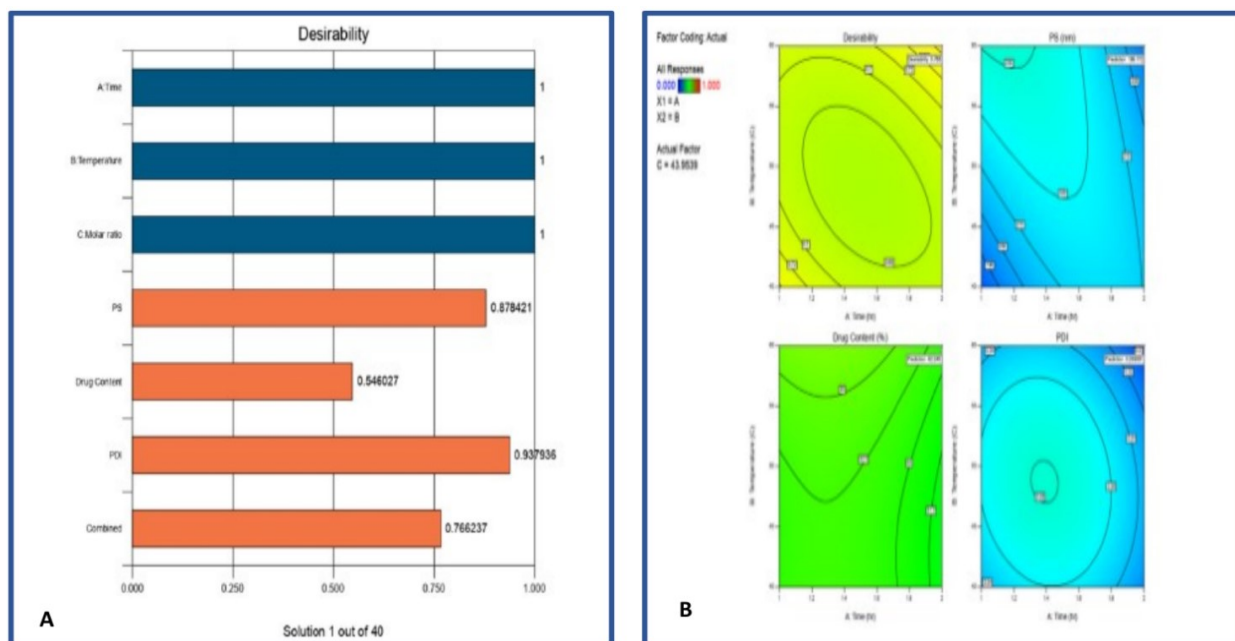
Response	R <sup>2</sup>	Adjusted R <sup>2</sup>	Predicted R <sup>2</sup>	Adeq. Precision	SD	Mean	CV %
% Drug content	0.9967	0.9892	0.9568	31.746	0.5712	91.04	0.6274
Particle size	0.9831	0.9451	0.8331	12.4113	24.34	302	8.06
PDI	0.9973	0.9912	0.9570	34.3843	0.0134	0.482	2.78

**Table 5:** Final optimized combinations of variables

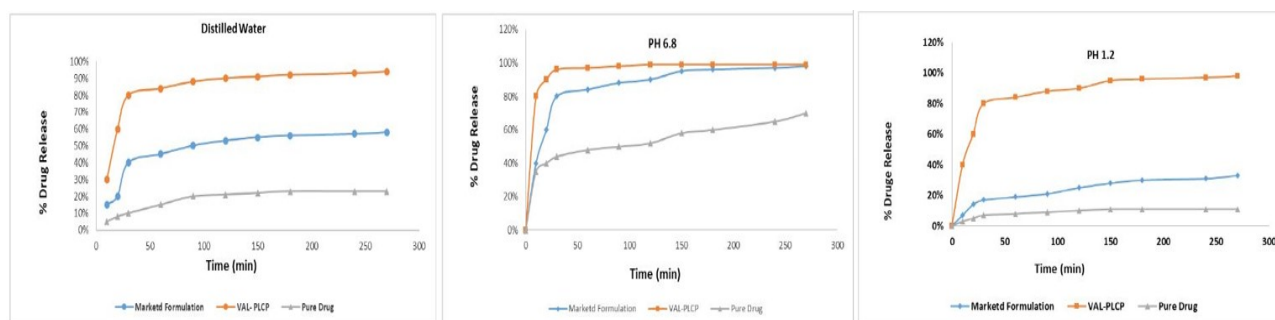
Number	Time (h)	Temp. (°C)	Lipid (%)	Drug Content (%)	PS (nm)	PDI	Desirability	
1	2	60	60	92.245	189.172	0.289	0.766	Selected
2	2	60	60	92.24	189.086	0.289	0.766	
3	2	59.99	60	92.187	188.209	0.288	0.766	
4	2	60	60	92.341	190.822	0.291	0.766	
5	2	60	60	92.095	186.706	0.287	0.766	
6	2	60	60	92.039	185.829	0.286	0.766	
7	2	59.99	60	92.533	194.302	0.294	0.765	



**Fig. 1:** 3-D response surface plots showing the effect of reaction time and reaction temperature on (A) % drug content, (B) particle size and (C) polydispersity index.

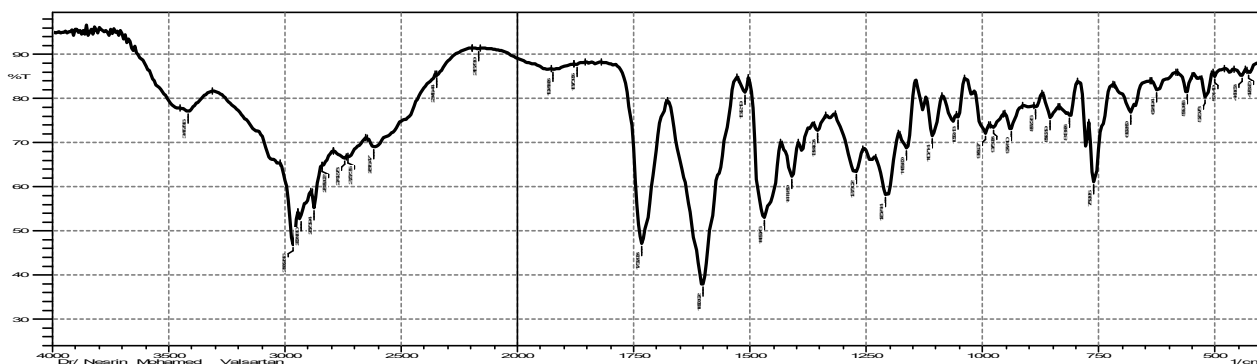


**Fig. 2:** Desirability functions: (A) bar chart, (B) 2-D contour plots.

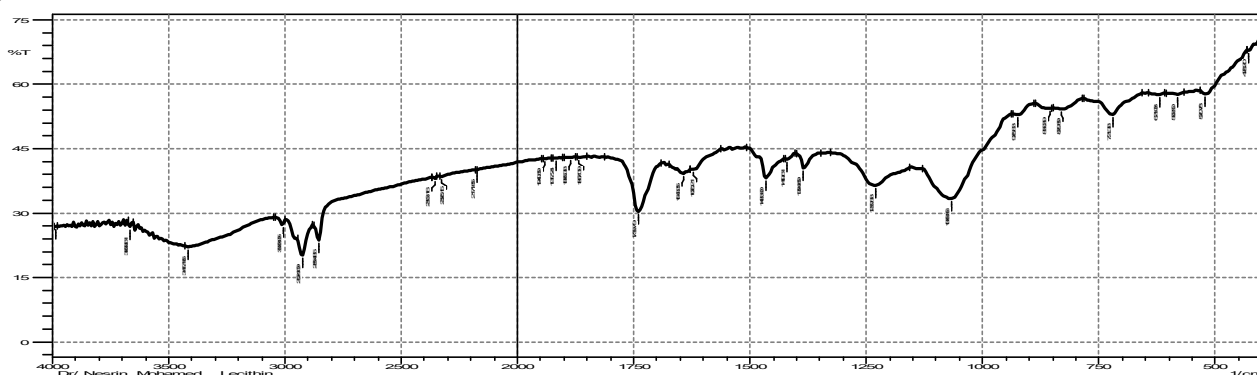


**Fig. 3:** In-vitro release outlines of valsartan from its different formulations in distilled water, pH 1.2 and pH 6.8.

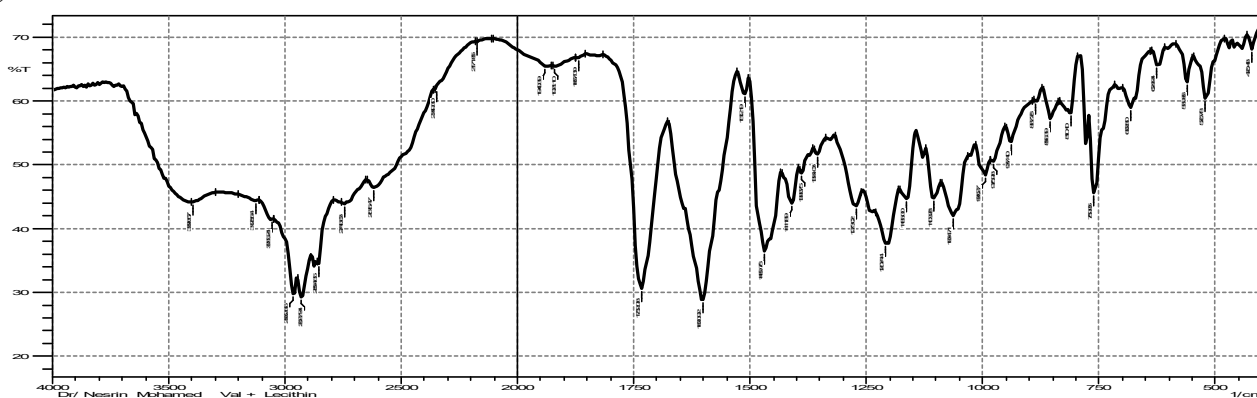
A



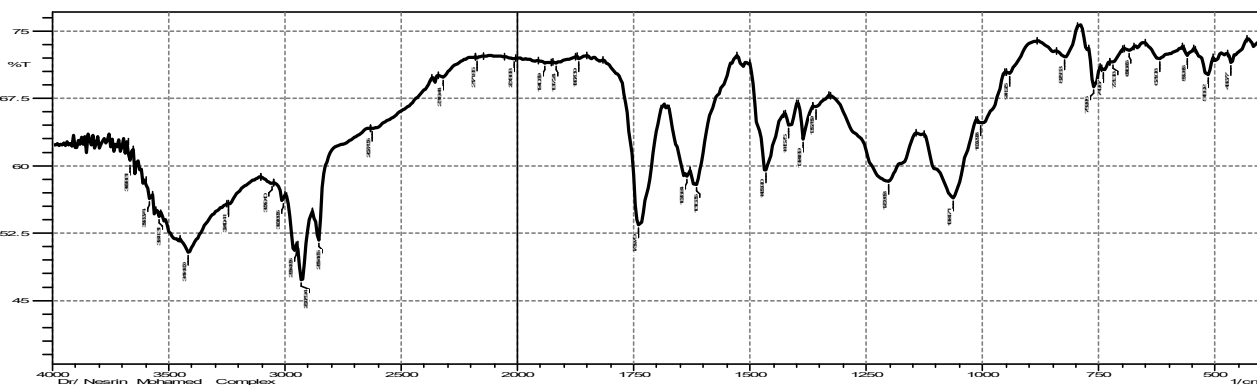
B



C

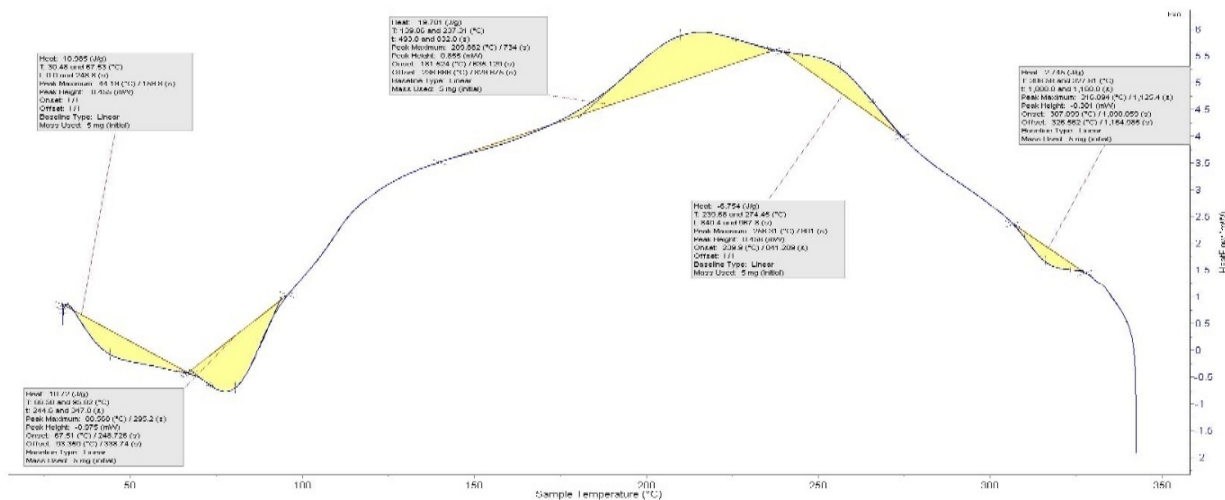


D

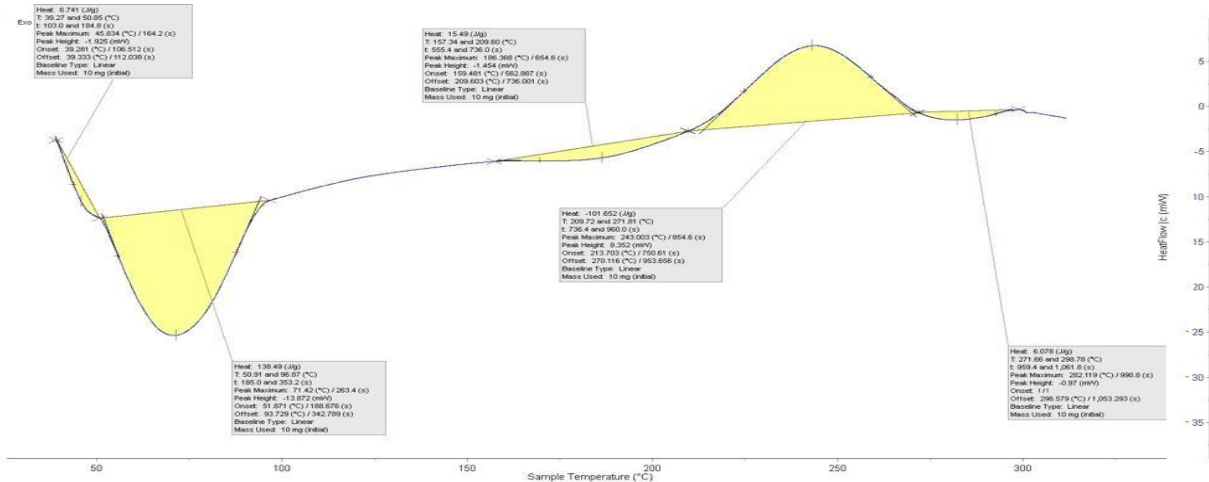


**Fig. 4:** FTIR spectra of A) pure valsartan, B) lecithin, C) valsartan/lecithin physical mixture and D) valsartan-complexesomes of the optimized formula (F3).

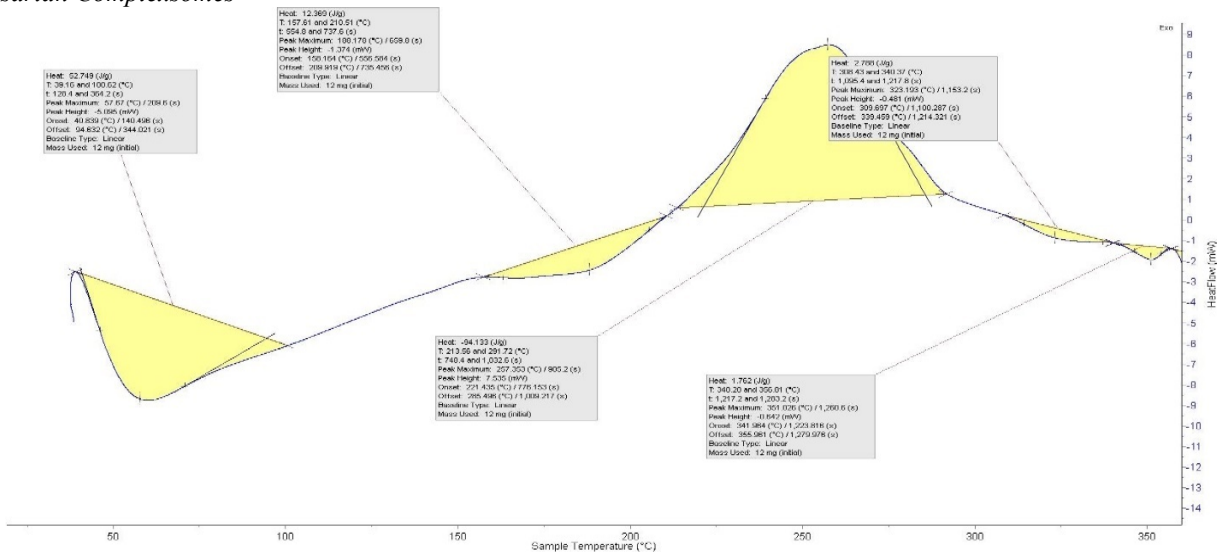
## Valsartan



## Physical Mixture

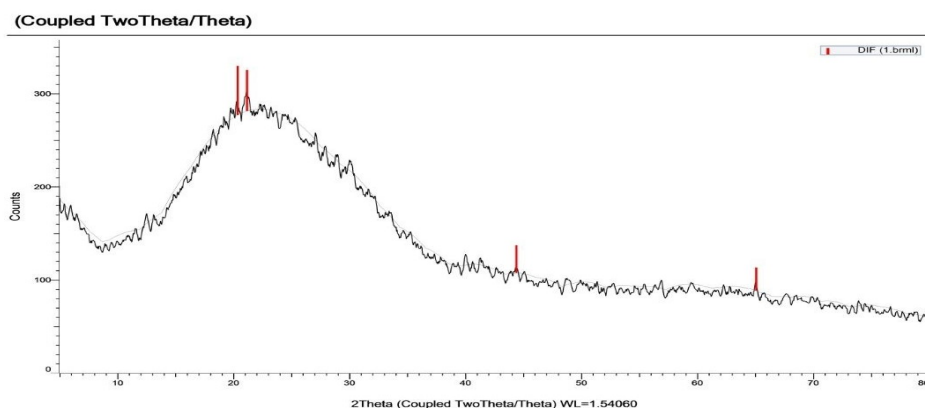


## Valsartan Complexsomes

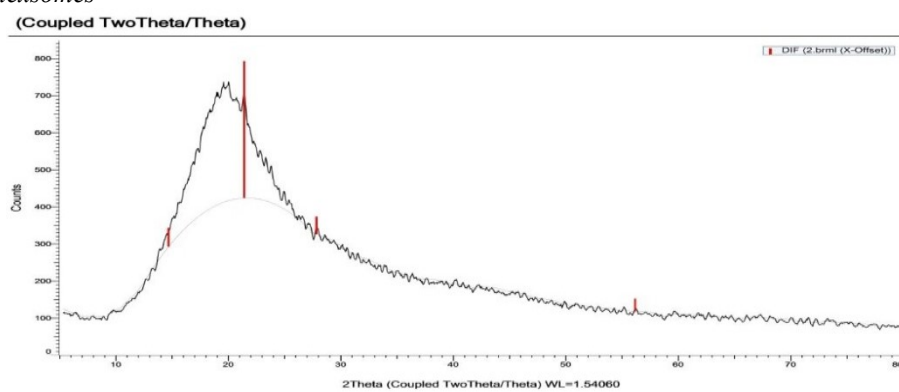


**Fig. 5:** DSC thermograms of pure valsartan, valsartan / lecithin physical mixture and valsartan-complexsomes of the optimized formula (F3).

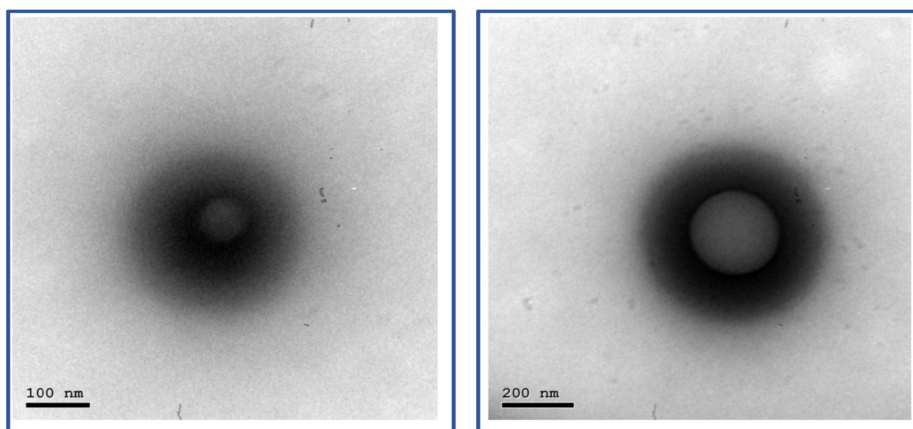
## Valsartan



## Valsartan Complexsomes



**Fig. 6:** XRD of pure valsartan and valsartan-complexsomes of the optimized formula (F3).



**Fig. 7:** Transmission electron micrograph of valsartan complexesomes optimized formula.

The sharp diffraction peaks exhibited by valsartan in its X-ray diffractogram in fig. 6, indicates crystallinity of the drug. Disappearance of such distinct peaks in the XRD pattern for the drug phospholipid complex of the optimized formula confirmed that the drug has been converted from its crystalline nature to an amorphous state upon its complexation with the phospholipid (Vuppapapati *et al.*, 2016). The lowering in the crystallinity of the drug might be behind the enhancement

in the dissolution of valsartan in its optimized VAL-PLC formula as noticed from the *in vitro* release studies.

### Transmission Electron Microscopical Examination (TEM)

The particle diameter of the drug in its optimized VAL-PLC formula shown in its TEM micrograph (fig. 7), was close to that obtained by the light scattering technique using Malvern Zetasizer.

## CONCLUSION

In this study, valsartan phospholipid complexosomes were successfully developed by the solvent-evaporation technique. A 3-factor, 3-level Box Behnken design (BBD) was employed to optimize the most desirable formula having the lowest particle size and polydispersity index with maximal % drug content. Design expert program was employed to assess the interaction and quadratic effects of selected three factors having direct effect on % drug content, particle size and polydispersity index. The factors chosen were reaction time, reaction temperature and lipid %. Fourteen formulations were developed and their responses were analyzed and ultimate optimized values of factors were determined based upon desirability value of 0.766. The complex formation was verified by FT-IR studies. The high % drug content confirmed the suitability of the solvent evaporation method for preparing valsartan-complexosomes. The small particle size indicated that valsartan complexosomes had a high drug dissolution rate. VAL-PLC exhibited pH-independent high release profiles while the free drug exhibited a high release at 6.8-pH and low release at 1.2-pH. To summarize, BBD can be used to optimize the formulation of valsartan phospholipid complexosomes as an encouraging approach for improving the antihypertensive effect of valsartan.

## REFERENCES

- Abd El-Hay SS, Elhenawee M, Maged K and Ibrahim AE (2022). Cost-effective, green HPLC determination of losartan, valsartan and their nitrosodiethylamine impurity: Application to pharmaceutical dosage forms. *R. Soc. Open Sci.*, **9**(6): 220250.
- Alodaini H, Alotaibi NT, Ahmad D, Omer M, Vasudevan S and Mansour M (2019). Stability study of valsartan in aqueous solutions: Effect of different pH, time and temperature. *IJRPS*, **8**(3): 1-15.
- Badawi N, El-Say K, Attia D, El-Nabarawi M, Elmazar M and Teaima M (2020). Development of pomegranate extract-loaded solid lipid nanoparticles: Quality by design approach to screen the variables affecting the quality attributes and characterization. *ACS Omega*, **5**(34): 21712-21721.
- Baumhäkel M, Müller U and Böhm M (2008). Valsartan improves symptoms and quality of life in patients with chronic heart failure. *MMW Fortschr. Med.*, **150**(1): 48-53.
- Cai X, Luan Y, Jiang Y, Song A, Shao W, Li Z and Zhao Z (2012). Huperzine A-phospholipid complex-loaded biodegradable thermosensitive polymer gel for controlled drug release. *Int. J. Pharm.*, **433**(1-2): 102-111.
- Chen S, Xie Q, Yang M, Shi Y, Shi J and Zeng X (2022). Scutellaria baicalensis extract-phospholipid complex: Preparation and initial pharmacodynamics research in rats. *Curr. Pharm. Biotechnol.*, **23**(6): 847-860.
- Dora CP, Kushwah V, Katiyar SS, Kumar P, Pillay V, Suresh S and Jain S (2017). Improved oral bioavailability and therapeutic efficacy of erlotinib through molecular complexation with phospholipid. *Int. J. Pharm.*, **534**(1-2): 1-13.
- El-Awady MI, Abdel-Razeq S, Barghash S and Belal F (2022). Decision-making tree for improving selectivity and sensitivity of sweeping-MEKC determination of hydrophobic analytes: Application to five cardiovascular drugs with greenness assessment. *Microchem. J.*, **181**(10): 107792.
- Flynn JT, Meyers KE, Neto JP, De Paula Meneses R, Zurowska A, Bagga A, Mattheyse L, Shi V, Gupte J and Solar-Yohay S (2008). Efficacy and safety of the angiotensin receptor blocker valsartan in children with hypertension aged 1 to 5 years. *Hypertension*, **52**(2): 222-228.
- Goo YT, Park SY, Chae BR, Yoon HY, Kim CH, Choi JY, Song SH and Choi YW (2020). Optimization of solid self-dispersing micelle for enhancing dissolution and oral bioavailability of valsartan using Box- Behnken design. *Int. J. Pharm.*, **585**(7): 119483.
- Gulati M, Grover M, Singh S and Singh M (1998). Lipophilic drug derivatives in liposomes. *Int. J. Pharm.*, **165**(2): 129-168.
- Guo B, Liu H, Li Y, Zhao J, Yang D, Wang X and Zhang T (2014). Application of phospholipid complex technique to improve the dissolution and pharmacokinetic of probucol by solvent-evaporation and co-grinding methods. *Int. J. Pharm.*, **474**(1-2): 50-56.
- Gupta K, Mahapatra A, Wadodkar A and Wadodkar S (2010). Simultaneous UV spectrophotometric determination of valsartan and amlodipine in tablet. *Int. J. Chem. Tech. Res.*, **2**(1): 551-556.
- Gupta RB and Kompella UB (2006). Nanoparticle technology for drug delivery. Taylor & Francis New York, USA. **159**(1): 1-427.
- Hakeem EA, El-Mahrouk GM, Abdelbary G and Teaima MH (2020). Freeze-dried clopidogrel loaded lyotropic liquid crystal: box-Behnken optimization, *in-vitro* and *in-vivo* evaluation. *Curr. Drug Deliv.*, **17**(3): 207-217.
- Hegazy AM, Batubara AS, Abdelgawad MA, El-Sherbiny M, Ghoneim MM, Ahmed AM and Gamal M (2022). Recommended and verified stability indicating GC-MS procedures for green separation of quaternary mixture of naphazoline, ephedrine, methylparaben and naphazoline impurity. *Microchem. J.*, **183**(1): 108058.
- Hemdan EA, Magdy R, Farouk M and Fares NV (2022). Central composite design as an analytical optimization tool for the development of eco-friendly HPLC-PDA methods for two antihypertensive mixtures containing the angiotensin receptor blocker valsartan: Greenness assessment by four evaluation tools. *Microchem. J.*, **183**(12): 108105.
- Ibrahim HK and El-Setouhy DA (2010). Valsartan orodispersible tablets: formulation, *in vitro/in vivo* characterization. *AAPS PharmSciTech*, **11**(1): 189-196.
- Kassem AA, Abd El-Alim SH, Basha M and Salama A (2017). Phospholipid complex enriched micelles: A novel drug delivery approach for promoting the

- antidiabetic effect of repaglinide. *Eur. J. Pharm. Sci.*, **99**(3): 75-84.
- Krishnaiah C, Reddy AR, Kumar R and Mukkanti K (2010). Stability-indicating UPLC method for determination of Valsartan and their degradation products in active pharmaceutical ingredient and pharmaceutical dosage forms. *J. Pharm. Biomed. Anal.*, **53**(3): 483-489.
- Lamprecht A, Schäfer U and Lehr CM (2001). Size-dependent bioadhesion of micro-and nanoparticulate carriers to the inflamed colonic mucosa. *Pharm. Res.*, **18**(6): 788-793.
- Larkin P (2017). Infrared and Raman spectroscopy: principles and spectral interpretation. Elsevier. **1**(2): 1-5.
- Leidig M, Bambauer R, Kirchertz E, Szabã T, Handrock R, Leinung D, Baier M and Schmieder R (2008). Efficacy, safety and tolerability of valsartan 80mg compared to irbesartan 150mg in hypertensive patients on long-term hemodialysis (VALID study). *Clin. Nephrol.*, **69**(6): 425-432.
- Liu Y, Sun C, Hao Y, Jiang T, Zheng L and Wang S (2010). Mechanism of dissolution enhancement and bioavailability of poorly water soluble celecoxib by preparing stable amorphous nanoparticles. *J. Pharm. Pharm. Sci.*, **13**(4): 589-606.
- Malviya V (2021). Preparation and evaluation of emulsomes as a drug delivery system for bifonazole. *IJPER*, **55**(1): 86-94.
- Mohit Kumar P, Solanki P, Mangla B and Aggarwal G (2022). Formulation development, optimization by box-behnken design and *in vitro* characterization of gefitinib phospholipid complex based nanoemulsion drug delivery system. *J. Pharm. Innov.*, **17**(11): 1-11.
- Morsi NM, Abdelbary GA and Ahmed MA (2014). Preparation and physicochemical characterization of mosapride citrate pharmacosomes. *Literatures*, **13**(1): 14.
- Morsi NM, Abdelbary GA, Elshafeey AH and Ahmed MA (2017). Engineering of a novel optimized platform for sublingual delivery with novel characterization tools: *In vitro* evaluation and *in vivo* pharmacokinetics study in human. *Drug Deliv.*, **24**(1): 918-931.
- Munyendo WL, Zhang Z, Abbad S, Waddad AY, Lv H, Baraza LD and Zhou J (2013). Micelles of TPGS modified apigenin phospholipid complex for oral administration: Preparation, *in vitro* and *in vivo* evaluation. *JBN*, **9**(12): 2034-2047.
- Peppas NA, Wood KM and Blanchette JO (2004). Hydrogels for oral delivery of therapeutic proteins. *Expert Opin. Biol. Ther.*, **4**(6): 881-887.
- Semalty A, Semalty M, Rawat BS, Singh D and Rawat M (2009). Pharmacosomes: The lipid-based new drug delivery system. *Expert Opin. Drug Deliv.*, **6**(6): 599-612.
- Shah S, Patel R, Soniwala M and Chavda J (2015). Development and optimization of press coated tablets of release engineered valsartan for pulsatile delivery. *Drug Dev. Ind. Pharm.*, **41**(11): 1835-1846.
- Sharma M, Kohli S and Dinda A (2015). *In-vitro* and *in-vivo* evaluation of repaglinide loaded floating microspheres prepared from different viscosity grades of HPMC polymer. *SPJ*, **23**(6): 675-682.
- Skotnicki M, Gawel A, Cebe P and Pyda M (2013). Thermal behavior and phase identification of Valsartan by standard and temperature-modulated differential scanning calorimetry. *Drug Dev. Ind. Pharm.*, **39**(10): 1508-1514.
- Tan Q, Liu S, Chen X, Wu M, Wang H, Yin H, He D, Xiong H and Zhang J (2012). Design and evaluation of a novel evodiamine-phospholipid complex for improved oral bioavailability. *AAPS PharmSciTech.*, **13**(2): 534-547.
- Tran PHL, Tran HTT and Lee BJ (2008). Modulation of microenvironmental pH and crystallinity of ionizable telmisartan using alkalizers in solid dispersions for controlled release. *J. Control. Release*, **129**(1): 59-65.
- Usta DY, Timur B and Teksin ZS (2022). Formulation development, optimization by box-behnken design, characterization, *in vitro*, *ex-vivo* and *in vivo* evaluation of bosentan-loaded self-nanoemulsifying drug delivery system: A novel alternative dosage form for pulmonary arterial hypertension treatment. *Eur. J. Pharm. Sci.*, **174**(19): 106159.
- Vaizoglu M and Speiser P (1986). Pharmacosomes -- a novel drug delivery system. *Acta Pharm. Suec.*, **23**(3): 163-172.
- Varshosaz J, Tabbakhian M and Mohammadi MY (2010). Formulation and optimization of solid lipid nanoparticles of buspirone HCl for enhancement of its oral bioavailability. *J. Liposome Res.*, **20**(4): 286-296.
- Vuppapapati L, Cherukuri S, Neeli V, Reddy Yeragamreddy P and Reddy Kesavan B (2016). Application of central composite design in optimization of valsartan nanosuspension to enhance its solubility and stability. *Curr. Drug Deliv.*, **13**(1): 143-157.
- Wu CY and Benet LZ (2005). Predicting drug disposition via application of BCS: Transport/absorption/elimination interplay and development of a biopharmaceutics drug disposition classification system. *Pharm. Res.*, **22**(1): 11-23.
- Xia Hj, Zhang Zh, Jin X, Hu Q, Chen Xy and Jia Xb (2013). A novel drug-phospholipid complex enriched with micelles: Preparation and evaluation *in vitro* and *in vivo*. *Int. J. Nanomed.*, **8**(1): 545-554.
- Yeom DW, Chae BR, Son HY, Kim JH, Chae JS, Song SH, Oh D and Choi YW (2017). Enhanced oral bioavailability of valsartan using a polymer-based supersaturable self-microemulsifying drug delivery system. *Int. J. Nanomedicine*, **12**(5): 3533-3545.
- Zhang Y, Zhi Z, Jiang T, Zhang J, Wang Z and Wang S (2010). Spherical mesoporous silica nanoparticles for loading and release of the poorly water-soluble drug telmisartan. *J. Control. Release*, **145**(3): 257-263.