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Electrochemical Behavior of Valsartan, Glibenclamide and their Interaction with Each other Using Square Wave Voltammetry

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ABSTRACT

In this work an electrochemical behavior quantification and interaction of valsartan and glibenclamide were studied using square wave voltammetric technique. The effect of temperature on the interaction was investigated and the thermodynamic parameters (ΔH, ΔS & ΔG) were calculated for the interaction and binding constant (K) also obtained. The calibration curves of each drug were linear within the range of concentration [(4.99×10^{-7}) – (6.95×10^{-6})], [(5.96×10^{-8}) – (1.15×10^{-6})] molar with R^2 value equal to 0.9819, 0.9926 for valsartan and glibenclamide respectively.

Keywords: Square wave voltammetry (SWV), Valsartan, Glibenclamide, Drug interaction.

السلوك الكهروكيميائي للفالسارتان، الغليبنكلاميد وتداخلهما مع بعضهما البعض باستخدام فولتامتري الموجة المربعة

الملخص

في هذا البحث تم دراسة السلوك الكمي الكهروكيميائي والتداخل للفالسارتان والغليبنكلاميد باستخدام تقنيـة فولتـامتري الموجة المربعة، وتم دراسة تأثير درجة الحرارة على التداخل وتم حساب القيم الثرموداينميكية (ΔG & ΔS,ΔH) وكذلك تـم الحصول ايضا على ثابت الترابط (K). كانت منحنيات المعايرة لكـل دواء خطيـة مـع مـدى تركيـز – (6.95×10^{-6})] (4.99×10^{-7})، [(5.96×10^{-8}) - (1.15×10^{-6})] مولاري وقيمة R^2 مساوية ل 0.9819, 0.9926 للفالسارتان والغليبنكلاميد على التوالي.

الكلمات الدالة: فولتامتري الموجة المربعة، الفالسارتان، الغليبنكلاميد، تداخل دوائي.

INTRODUCTION

Valsartan N-((2'-(1H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)-N-pentanoylvaline Fig. (1), is a new orally active of antihypertensive drug belonging to the family of angiotensin II receptor antagonists acting at the ATI receptor, which mediates all known effects of angiotensin II on the cardiovascular system (Nie *et al.*, 2005). Valsartan is widely used in the treatment of hypertension (Iriarte *et al.*, 2007). Therefore, an analytical method for the determination of unchanged valsartan with high accuracy is of great importance.

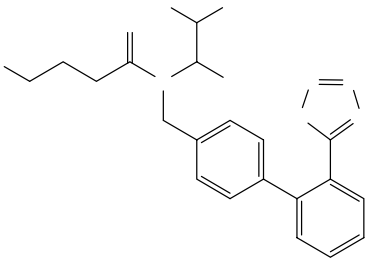

Fig. 1: Valsartan

Several methods for the determination of valsartan in pharmaceutical dosage forms have been reported in literature including high performance liquid chromatography (HPLC) with a fluorescence detector (FP) (Iriarte *et al.*, 2007; Macek *et al.*, 2006), liquid chromatography–tandem mass spectrometry (Koseki *et al.*, 2007), and spectrophotometry (Tatar and Saglik, 2002). So far, there are few electrochemical methods for the determination of valsartan, either in pharmaceutical dosage forms or in bulk form (Yan *et al.*, 2008 ; Ramadan *et al.*, 2012 ; Habib *et al.*, 2007).

Glibenclamide Fig. (2), (1-p-[2-(5-chloro-o-anisamido)ethyl[phenyl]-sulfonyl]-3-cyclohexyl urea ; glyburide) is a potent, second generation oral sulfonylurea antidiabetic agent widely-used to lower blood glucose levels in patients with type II non-insulin-dependent diabetes mellitus. It acts mainly by stimulating endogenous insulin release from beta cells of the pancreas (Radi, 2004). Glibenclamide is rapidly and completely absorbed from the gastrointestinal tract. Different methods have been used for the determination of glibenclamide among these methods, different HPLC methods coupled with UV detection (Porwal and Talele, 2017) fluorescence detection (Khatri *et al.*, 2001), or mass spectrometry (Ramos *et al.*, 2000).

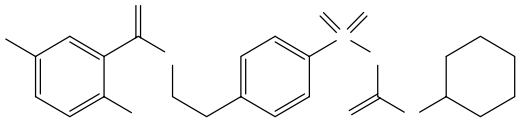

Fig. 2: Glibenclamide

A drug interaction is a situation in certain medicines can interact pharmacologically and affect the activity of other medicines. This action can be synergistic (when the drug's effect is increased) or antagonistic (when the drug's effect is decreased). These interactions may occur out of accidental misuse or due to lack of knowledge about the active ingredients involved in the relevant substances (Mohapatra *et al.*, 2018). It is therefore easy to see the importance of these pharmacological interactions in the practice of medicine, if a patient is taking two drugs and one of them increases the effect of the other it is possible that an overdose may occur. The interaction of the two drugs may also increase the risk that side effects will occur. On the other hand, if the action of a drug is reduced, it may cease to have any therapeutic use because of under dosage (Alfonso and Gayo, 2005). The SWV also used for study the interaction of different biological compounds and drugs with albumin (Sulaiman and Bader, 2009 ; Sulaiman and Al-Imam, 2012).

In this work, an electrochemical behaviour of valsartan and glibenclamide were investigated, also their interaction with each other was examined for this purpose a simple, rapid and sensitive square wave voltammetric (SWV) technique was developed. The developed method was applied to determine the valsartan and glibenclamide in pharmaceutical formulations.

EXPERIMENTAL

Apparatus

SWVs were performed using a 797 VA Computrace supplied by Metrohm, Switzerland, coupled with a three-electrode detection system and consists of hanging mercury drop electrode (HMDE), an Ag/AgCl/sat. KCl as reference electrode and 1mm platinum wire was used as an auxiliary electrode.

pH measurements were performed using a digital pH meter supplied by HANNA company, Portugal, model pH211, microprocessor pH meter with accurate to ±0.05.

HAAKE G supplied by HAAKE company, Germany, water bath was used for controlling temperature during the measurements.

Reagents and Procedure

All chemicals used were all analytical grade (Fluka, BDH). The valsartan and glibenclamide were kindly supplied by Sammira drugs industry. Stock solutions of each drug were prepared by dissolving an appropriate amount of valsartan and glibenclamide in absolute ethanol and dimethyl formamide (DMF), respectively. The supporting electrolyte was phosphate buffer (K_2HPO_4 and KH_2PO_4).

The buffer solution was placed in polarographic cell and deoxygenated via purging with N_2 gas for 5min prior the measurements. After recording the buffer voltammogram, the test solution added to the polarographic cell and the square wave voltammograms were recorded under the optimum conditions for a sequence additions of standard stock solutions of each drug, then the calibration curve was constructed for each drug.

RESULTS AND DISCUTION

Electrochemical Behavior of Valsartan

SWV of valsartan shows a well-defined reduction peak at (-1.07)V versus Ag/AgCl/sat.KCl under the default conditions of instrument in phosphate buffer (pH=7).

Optimum Condition for Valsartan

In order to optimize the conditions for measurements, various instrumental and experimental variables such as frequency, scan increment, pulse amplitude, supporting electrolyte and pH were examined and optimized, using 9.9×10^{-5}M valsartan in phosphate buffer as supporting electrolyte, the results obtained are shown in (Table 1).

Table 1: The optimum condition for 9.9×10^{-5}M valsartan in phosphate buffer (pH=6)

Start Potential (V)	-1.5
End Potential (V)	-0.5
Deposition potential (V)	-0.6
Deposition time (s)	30
Equilibration time (s)	1
Voltage step (V)	0.006
Amplitude (V)	0.06
Frequency (Hz)	50
Drop size	4
Sweep rate (V/s)	0.3

The square wave voltammograms were recorded for 4.97×10^{-5}M valsartan in phosphate buffer at different pHs, by adding appropriate amount of K_2HPO_4 and KH_2PO_4. It can be seen from Fig.(3), the reduction peak current, peak shape and peak potential depended strongly on pH. The optimum pH was found to be pH=6, which is used for determination, where as pH=7 (human blood pH) was used for interaction studies.

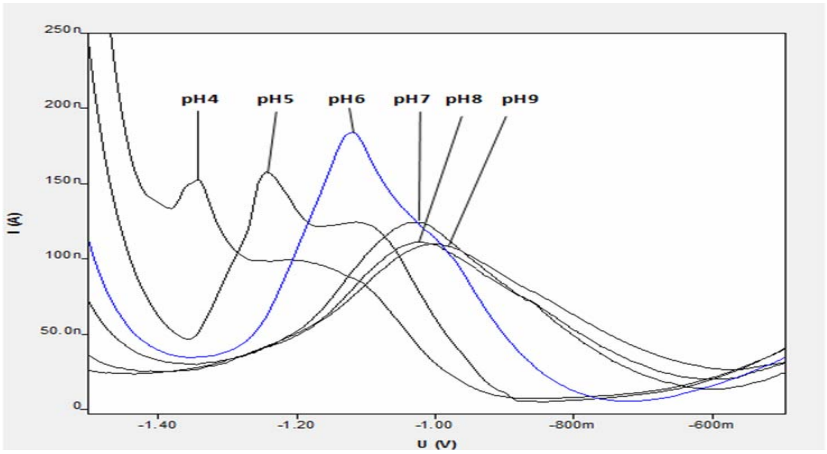

Fig. 3: Effect of pH on the reduction peak current of valsartan (4.97×10^{-5}M)

Stability of Reduction Peak

To study the stability of reduction peak of valsartan a voltammogram of 9×10^{-5}M valsartan was recorded under the mentioned optimum conditions (Table 1) versus time. The results obtained are shown in (Table 2), it is clear that the reduction peak current is stable within the time studied.

Table 2: Stability of reduction peak current of 9×10^{-5}M valsartan in pH6

Time (min)	Ip of VAL (nA)	Ep of VAL (V)
0	284	-1.08
5	287	-1.08
10	278	-1.08
15	278	-1.08
20	291	-1.08
25	291	-1.08
30	296	-1.08
35	276	-1.08
40	275	-1.08
45	278	-1.08
50	277	-1.08
55	279	-1.08
60	280	-1.08

Calibration Curve of Valsartan

The calibration curve was constructed by adding a sequence addition of standard valsartan solution (10^{-3}M) and the voltammogram was recorded for each addition Fig. (4) under the previous optimum conditions (Table 1). The plot of peak current versus concentration Fig. (5) gives a straight line with R^2= 0.9819 and concentration range [$(4.99\times10^{-7}$M) – $(6.95\times10^{-6}$M)].

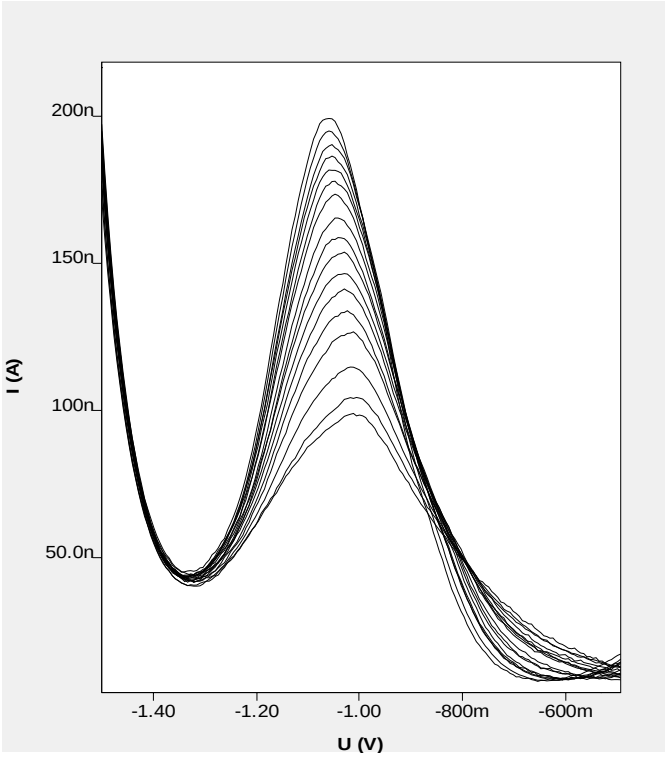

Fig. 4: Voltammograms of sequence addition of standard valsartan solution (10^{-3}M) in phosphate buffer (pH=6)

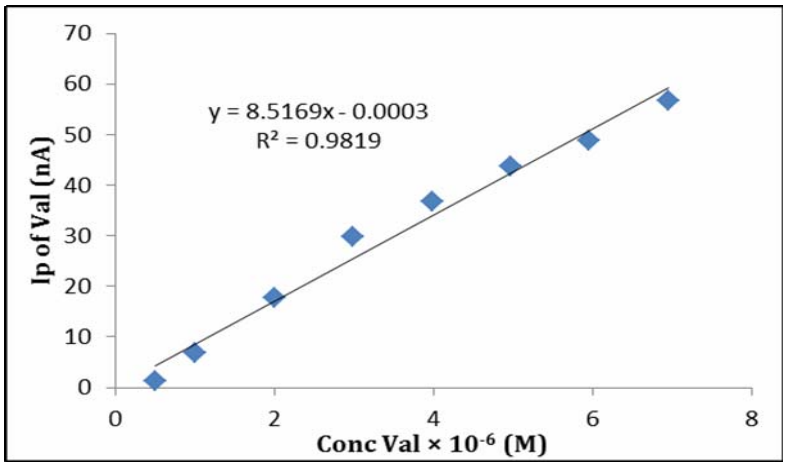

Fig. 5: The calibration curve of valsartan using phosphate buffer (pH=6)

Electrochemical Behavior of Glibenclamide

SWV of glibenclamide shows a well-defined reduction peak at (-1.37)V versus Ag/AgCl/sat. KCl under the default conditions of instrument in phosphate buffer (pH=7).

Optimum Condition for Glibenclamide

In order to optimize the conditions for measurements, various instrumental and experimental variables such as frequency, scan increment, pulse amplitude, supporting electrolyte and pH were examined and optimized, using 1.96×10^{-5}M glibenclamide in phosphate buffer as supporting electrolyte. The results obtained are shown in (Table 3).

Table 3: The optimum condition for 1.96×10^{-5}M glibenclamide in phosphate buffer (pH=8)

Start Potential (V)	-1.6
End Potential (V)	-0.8
Deposition potential (V)	-0.6
Deposition time (s)	30
Equilibration time (s)	1
Voltage step (V)	0.016
Amplitude (V)	0.06
Frequency (Hz)	50
Drop size	4
Sweep rate (V/s)	0.3

The square wave voltammograms were recorded for 1.2×10^{-4}M glibenclamide in phosphate buffer at different pHs. It can be seen from Fig. (6), the reduction peak current, peak shape and peak potential depend strongly on pH. the optimum pH was found to be pH=8.

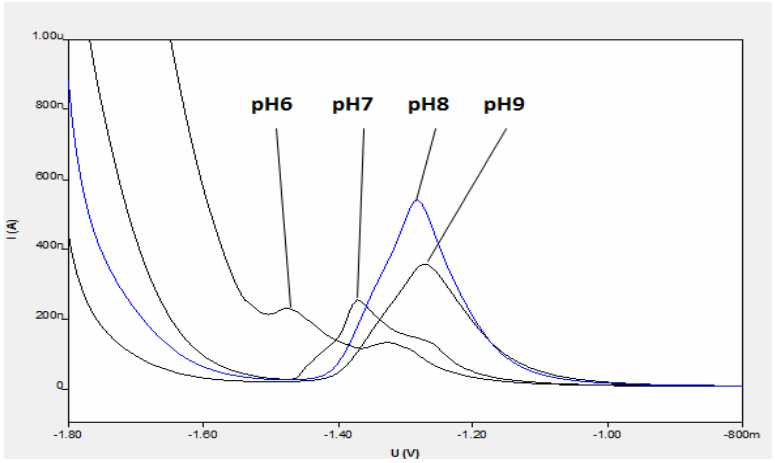

Fig. 6: Effect of pH on the reduction peak current of glibenclamide (1.2×10^{-4}M)

Stability of reduction peak

To study the stability of reduction peak of glibenclamide, a voltammogram of 1.96×10^{-5}M glibenclamide was recorded under the mentioned optimum conditions (Table 3) versus time. The results obtained are shown in (Table 4). It is clear that the reduction peak current is stable within the time studied.

Table 4: Stability of reduction peak of 1.96×10^{-5}M glibenclamide in pH8

Time (min)	Ip of GLB (nA)	Ep of GLB (V)
0	226	-1.25
5	217	-1.26
10	233	-1.25
15	213	-1.26
20	214	-1.26
25	217	-1.26
30	209	-1.26
35	227	-1.26
40	220	-1.26
45	211	-1.26
50	214	-1.26
55	215	-1.26
60	210	-1.26

The Calibration Curve of Glibenclamide

The calibration curve was constructed by adding a sequence addition of standard glibenclamide solution (10^{-5}M) and the voltammogram was recorded for each addition Fig. (7) under the previous optimum conditions (Table 3). The plot of peak current versus concentration Fig. (8) gives a straight line with R^2= 0.9926 and concentration range [(5.96×10^{-8}M) – (1.15×10^{-6}M)].

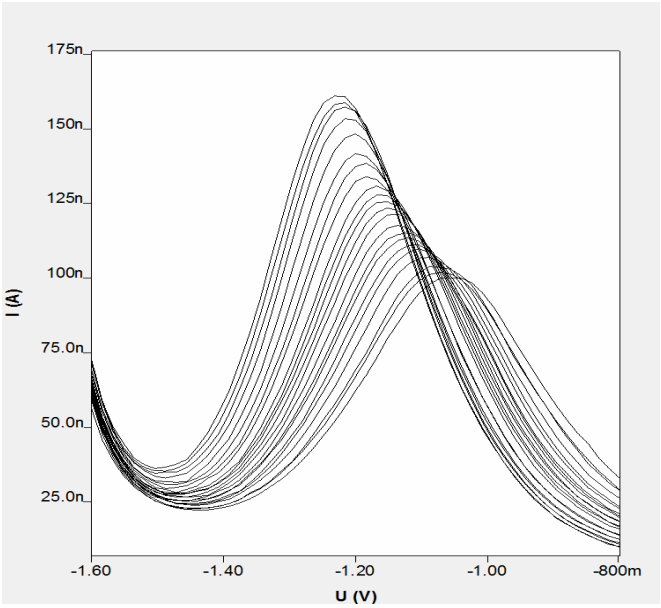

Fig. 7: Voltammograms of sequence addition of standard glibenclamide solution (10^{-5}M) in phosphate buffer (pH=8)

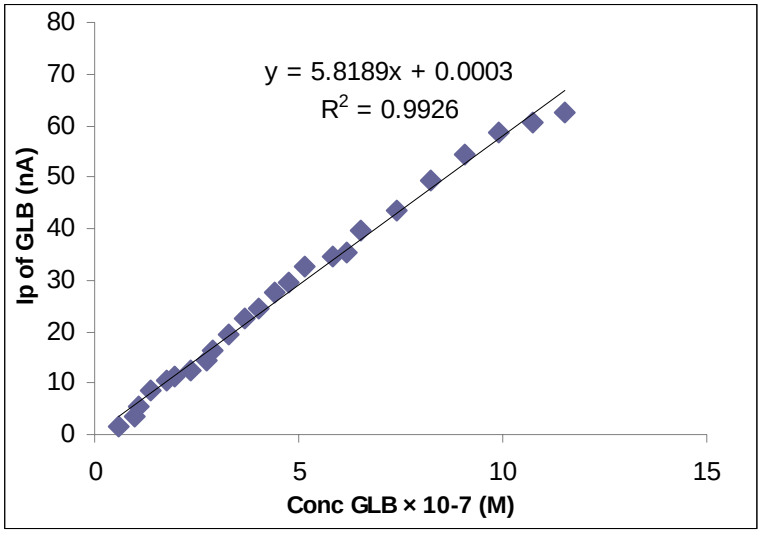

Fig. 8: The calibration curve of glibenclamide using phosphate buffer (pH=8)

Interactions of Valsartan with Glibenclamide

Square wave voltammograms of 1.4×10^{-5}M valsartan were recorded under the optimum conditions (Table 1) for a sequence additions of glibenclamide solution at different temperatures using phosphate buffer pH=7 (human blood pH).

Stability of Interaction

To study the stability of interaction peak, a voltammogram of 1.9×10^{-4}M valsartan with 1.9×10^{-7}M glibenclamide was recorded under the mentioned optimum conditions of valsartan (Table 1) in phosphate buffer pH=7 versus time. The results obtained are shown in (Table 5). It is clear that the interaction reduction peak current is stable within the time studied.

Table 5: Stability of interaction reduction peak current (1.9×10^{-4}M valsartan with 1.9×10^{-7}M glibenclamide) using phosphate buffer pH=7

Time (min)	Ip of Interaction (nA)	Ep of Interaction (V)
0	236	-1.03
5	232	-1.03
10	227	-1.03
15	227	-1.03
20	226	-1.04
25	225	-1.03
30	225	-1.03
35	228	-1.03
40	231	-1.03
45	223	-1.03
50	221	-1.03
55	232	-1.04
60	222	-1.04

Binding Constant

The decrease in peak current of valsartan with a sequence additions of glibenclamide Fig. (9) at all studied temperatures were noticed. The relations between reduction peak current and glibenclamide concentrations added were linear at all studied temperatures with R^2 equal to 0.9936, 0.9867, 0.9953, 0.9795 and 0.9922 for 288˚, 293˚, 298˚, 303˚ and 310˚K respectively. Thermodynamic parameters and binding constants were calculated (Table 6) according to equation (1) (Jalali and Dorraji, 2012) as shown in Figs. (10-14).

$$ln\left(\frac{Ip}{Ip^{\circ}-Ip}\right)=ln\left(\frac{1}{[GLB]}\right)-lnK \quad \ldots.(1)$$

where K is the binding constant, Ip° and Ip are the reduction peak currents of the free valsartan and VAL- Glibenclamide complex, respectively. The plot of ln(Ip/(Ip°-Ip)) versus ln(1/[GLB]) is linear and the binding constant was obtained from its intercept.

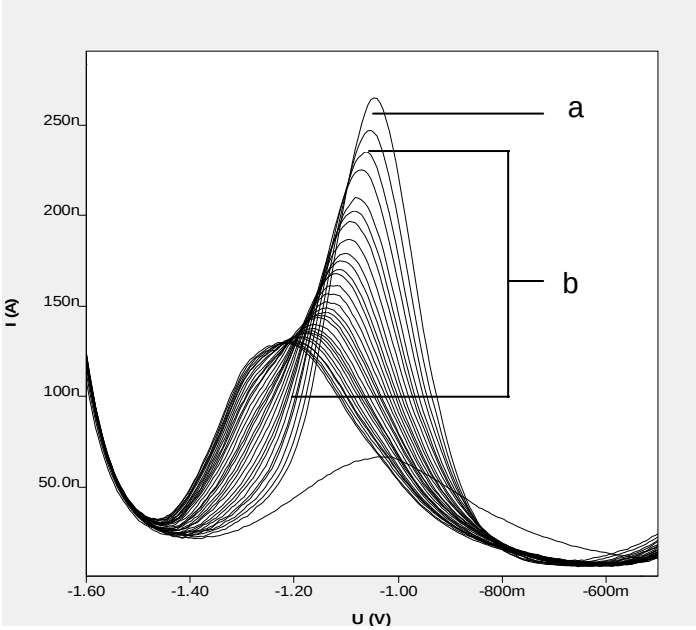

Fig. 9: The reduction peak of valsartan (a) with the sequence additions of glibenclamide (b) at (298˚K)

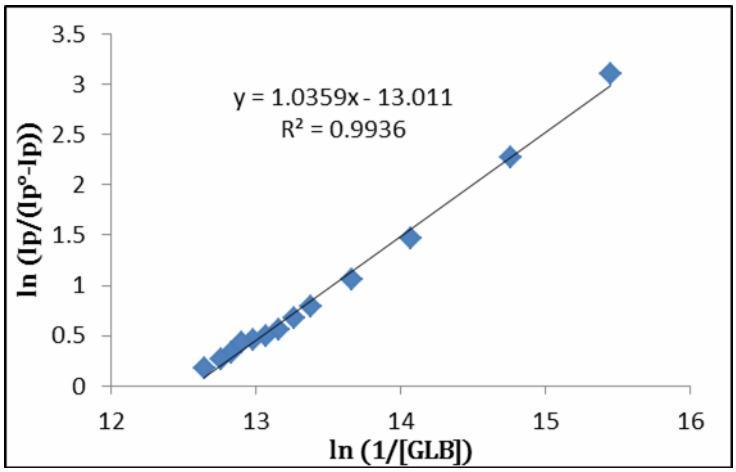

Fig. 10: Plot of ln (Ip/(Ipº –Ip)) vs ln (1/[GLB]) at 288˚K

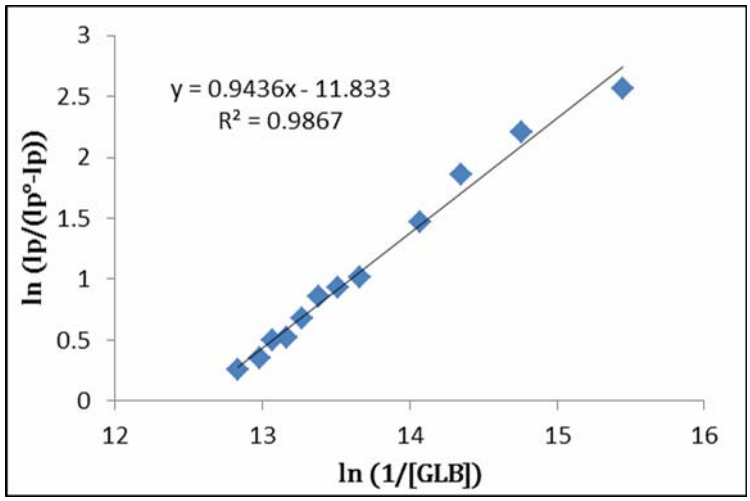

Fig. 11: Plot of ln (Ip/(Ipº –Ip)) vs ln (1/[GLB]) at 293˚K

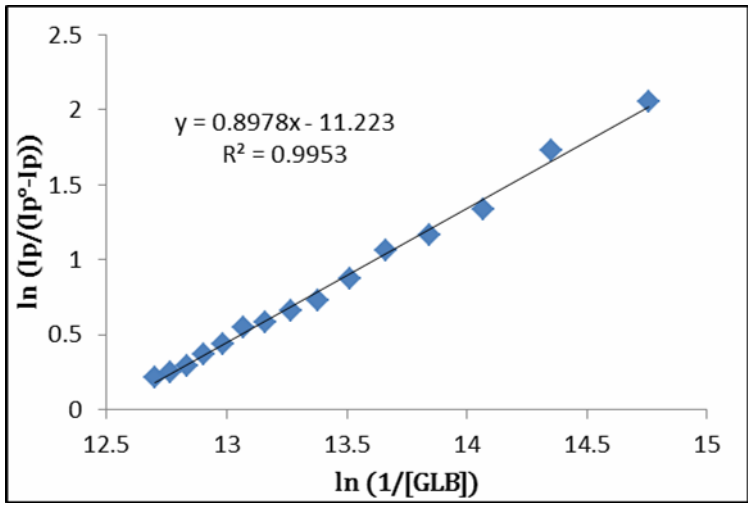

Fig. 12: Plot of ln (Ip/(Ipº –Ip)) vs ln (1/[GLB]) at 298˚K

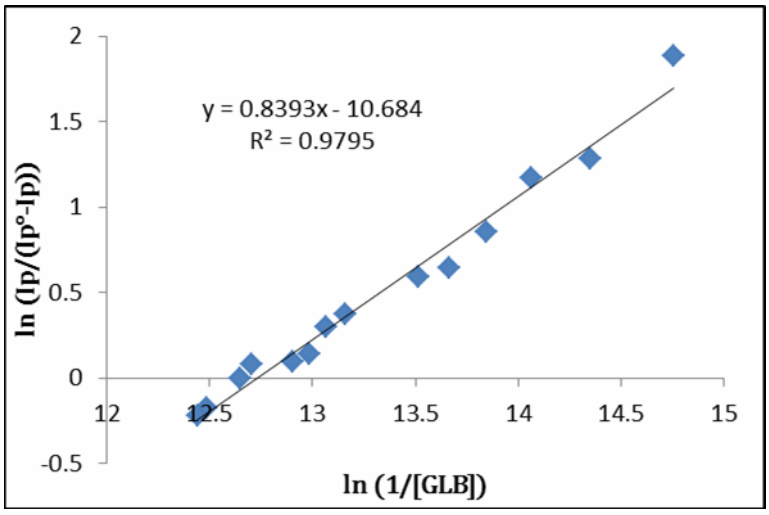

Fig. 13: Plot of ln (Ip/(Ip° –Ip)) vs ln (1/[GLB]) at 303˚K

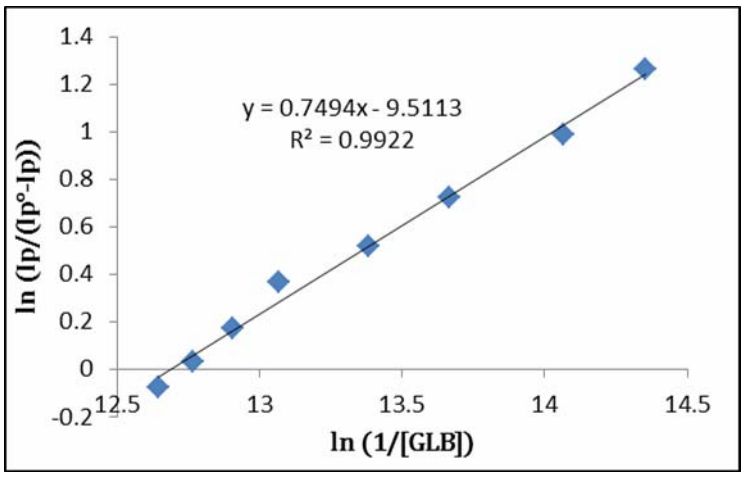

Fig. 14: Plot of ln (Ip/(Ip° –Ip)) vs ln (1/[GLB]) at 310˚K

Thermodynamic parameters

The plotting of ln K against 1/T using Van't Hoff equation (2), gives linear relationship Fig. (15). The change enthalpy (ΔH) was obtained from the slope, and other thermodynamics parameters (ΔG and ΔS) were calculated (Table 6) as follow:

$$lnK = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \ldots\ldots (2)$$

Enthalpy (ΔH) was calculated from the slope (Equation 3)

$\Delta H = -$ Slope $\times R$ …..(3) ($R = 8.314$ J. mole^{-1}. K^{-1})

The free energy (ΔG) was calculated from the equation (4) of Van't Hoff described below:

$\Delta G = -R \times T \times \ln K$ …..(4)

Entropy (ΔS) was calculated from the intercept (Equation 5)

$\Delta S =$ Intercept $\times R$ …..(5)

The plot of ln K versus 1/T gives straight line with R^2 equal to 0.9853 Fig. (18).

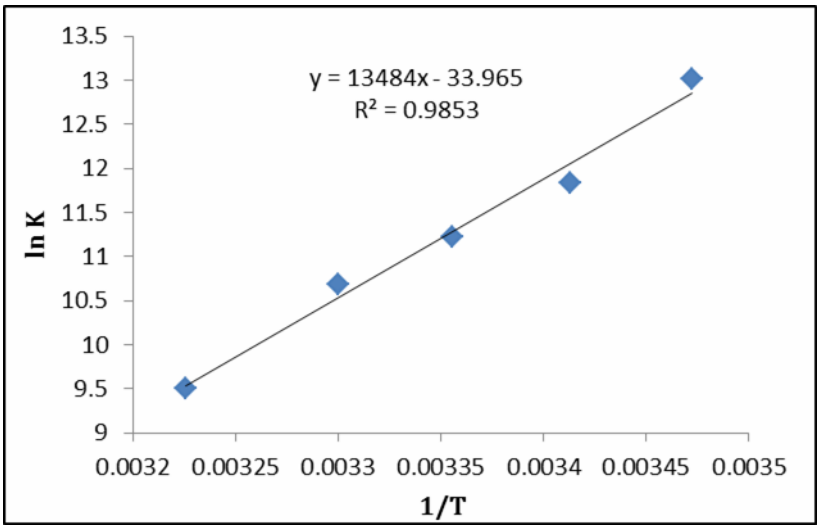

Fig. 15: Plot of ln K versus 1/T of interaction between valsartan and glibenclamide

Table 6: Thermodynamics parameters and binding constants for valsartan and glibenclamide interaction

Temp (K)	Binding constant (K_b) × 10^4 M^{-1}	ΔH ($KJ.mol^{-1}$)	ΔG ($KJ.mol^{-1}$)	ΔS ($J.mol^{-1}.K^{-1}$)
288	44.7306	-112.1059	-31.1539	-282.3850
293	13.7723		-28.8252	
298	7.4831		-27.8057	
303	4.3651		-26.9145	
310	1.3511		-24.5138	

The negative value of ΔH indicates that the binding interaction is exothermic and binding constant decrease with increasing temperature. Also ΔG becomes more positive with increasing temperature means the spontaneously of binding decreased, where as the negative value of ΔS indicates that which the system that became more ordered. The negative ΔH and ΔS values for the interaction of valsartan and glibenclamide indicate that the binding is mainly enthalpy and entropy driven, and the interaction may involve hydrogen bonding and van der Waals forces played a major role in the interaction (Ross and Subramanian, 1981).

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