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وحماية المستهلك

SELECTIVE EXTRACTION OF GLIMEPIRIDE IN PHARMACEUTICAL PREPARATION AND IN HUMAN SERUM VIA SYNTHESIZED MIP-SPE TECHNIQUE

Rana A. Kamal Aldeen[1]* , Yehya K. Al-Bayati[2]
[1]Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq, ranaadnankamalaldeen@gmail.com
[2] Professor PhD, Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq, yahyaalbayti@yahoo.com

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ABSTRACT

This paper demonstrates that the synthesizing and storage of molecular-imprinted polymers (MIP) at room temperature using bulk polymerisation of Glimepiride (Glim.) is characterized by high sensitivity, reduced costs, increased stability, and extended life. The research used 1:15:20 mmol ratios of template, monomer and cross-linking agents for the polymerisation in order to ensure an appropriate adsorption capacity. Benzoyl peroxide BPO was employed as the initiator for the functional monomer Allyl chloride C_3H_5Cl , cross-linked with Ethylene glycol dimethacrylate EGDMA $C_{10}H_{14}O_4$, thereby creating MIP for Glimepiride (Glim-MIP) that could be characterised with UV-Visible Spectrophotometry at 274.5nm, for pharmaceutical drugs. Fourier-transform infrared spectroscopy (FTIR) and Scanning electron microscopy (SEM) was used for the human serum. The elution process that was applied to the template (Glim.) from the Glim-MIP created cavities that were caused by the porogenic mixture solvents that were created from (acetic acid, methanol) 1:9 respectively, successfully removed by repeated washing for 20 hours, the polymer was dried at room temperature. The maximum adsorption capacity was 11.7797 µmol/g using (0.1) g weight of Glim-MIP. which adhered to the Langmuir isotherm model. A solid-phase extraction (SPE) syringe packed with molecular imprinted polymers (MIPs) was employed to selectively separate and pre-concentrate the Glimepiride in multiple pharmaceutical drugs from several sources. The human serum was based on the use of deionized water to dilute the serum, followed by heating of the serum with methanol. Subsequently, few drops of 1N hydrochloric acid were applied to detect Glimepiride at UV region 274.5 nm by applying the standard addition method.

Keywords: Isotherm process, Glimepiride, (Molecular Imprinted Polymers) MIP, Serum, (Solid-Phase Extraction) SPE.

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الاستخلاص الانتقائي للكليميبيرايد في المستحضرات الصيدلانية وفي مصل الانسان عن طريق تقنية MIP-SPE المركبة

رنا عدنان كمال الدين[1], يحيى كمال البياتي[2]

[1]قسم الكيمياء، كلية العلوم، جامعة بغداد، بغداد، العراق، ranaadnankamalaldeen@gmail.com

[2]الاستاذ الدكتور، قسم الكيمياء، كلية العلوم، جامعة بغداد، بغداد، العراق، yahyaalbayti@yahoo.com

الخلاصة

يوضح هذا البحث تحضير وتخزين البوليمرات الجزيئية المطبوعة (MIP) في درجة حرارة الغرفة عن طريق البلمرة الصلدة لـ Glimepiride (Glim) والتي تتميز بالحساسية العالية والتكلفة المنخفضة والاستقرار العالي. اذ تم أخذ نسب 1: 15: ٢٠ ملي مول للقالب، و للمونومر ولعوامل الربط المتصالب للبلمرة من أجل ضمان قدرة امتزاز مناسبة. المونومر الوظيفي اليل كلورايد C_3H_5Cl تم ربطه مع إيثيلين جليكول ثنائي ميثاكريلات EGDMA $C_{10}H_{14}O_4$ رابط التشابك وبالتالي إنشاء MIP لـ Glimepiride كـ Glim-MIP تم تمييزه باستخدام مقياس الطيف الضوئي UV-VIS عند 274.5 نانومتر، والتحليل الطيفي بالأشعة تحت الحمراء والمسح المجهري الإلكتروني. أنشأت عملية الشطف التي تم تطبيقها على القالب اي انتزاع القالب ال Glim من Glim-MIP تجاويف ناتجة عن استخدام خليط مسامي من الميثانول وحمض الخليك (10:90) على التوالي. اذ اجريت عملية الشطف بنجاح لمدة ٢٠ ساعة ويجفف البوليمر بدرجة حرارة الغرفة ،كانت السعة القصوى للامتزاز Glim-MIP هي 11.7797 ميكرو مول / غم عند استخدام وزن ٠,١ غم من Glim-MIP. يتبع الامتزاز بواسطة Glim-MIP نموذج Langmuir isotherm. تم استخدام حقنة استخلاص ذات المرحلة الصلبة (SPE) معبأة ببوليمرات مطبوعة جزيئيًا (MIPs) للفصل الانتقائي والتركيز المسبق للكليميبيرايد في العديد من الأدوية الصيدلانية من عدة مصادر. اعتمد المصل البشري على استخدام الماء منزوع الأيونات لتخفيف المصل، يليه تسخين المصل بالميثانول. بعد ذلك، تم تطبيق بضع قطرات من حمض الهيدروكلوريك N١ للكشف عن الكليميبيرايد في منطقة الأشعة فوق البنفسجية 274.5 نانومتر من خلال تطبيق طريقة الإضافة القياسية.

الكلمات المفتاحية: عملية الايزوثرم ، كليميبيرايد ، بوليمرات الطبعة الجزيئية ، المصل، استخلاص الطور الصلب.

INTRODUCTION

Glimepiride; Amaryl a second-generation of antidiabetics sulfonylurea, it was patented in 1979 and approved for medical use in 1995**(Basit *et al*., 2012)**
Glimepiride stimulates pancreatic beta cells to secrete insulin and improves the sensitivity of peripheral tissues to insulin thereby increasing peripheral glucose uptake, and reducing plasma blood glucose levels and glycated hemoglobin (HbA1C) levels **(Zekry, *et al*. 2023; Sola *et al*., 2015)**
Molecular Formula: $C_{24}H_{34}N_4O_5S$, Molecular Weight : 490.62 , figure (1) illustrates the structure of Glimepiride

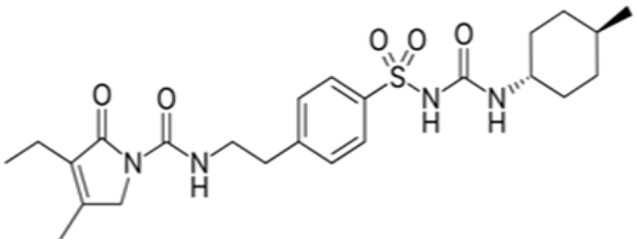

Figure (1): Structure of Glimepiride

1-[[p-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1carboxamido) ethyl] phenyl] sulfonyl]-3-(trans-4-methylcyclohexyl)urea) **(Basit *et al*., 2012).**

It was found to be practically insoluble in water, slightly soluble in dichloromethane and very slightly soluble in methanol. It was soluble in DMSO (>10 mg/ml) and ethanol (<1 mg/ml). In acidic and neutral aqueous solutions glimepiride exhibits very poor solubility at 37 ^oC (<0.004 mg/ml) **(Kari *et al*., 2023)**.

A molecular imprinting polymer (MIP) creates a multifaceted monomer (**Samarth *et al*, 2015).** A highly cross-linked polymer structure was used to secure functional groups in situ. Moreover, the steric patterns of these connections and the template are significant for the formation of binding sites that supply the shape, size, and flexibility required to encourage selective identification, in addition to elevated target correspondence. Consequently, the process can be deemed to be comparable to enzyme-proven mechanisms or substrata. Hence, the complex was created in a manner akin to a lock/key model **(Al-Bayati & Hadi, 2022; Aljabari & Al-Bayati, 2023; Mohsen & Al-Bayati, 2022).** Figure (2) presents the polymerization cycle.

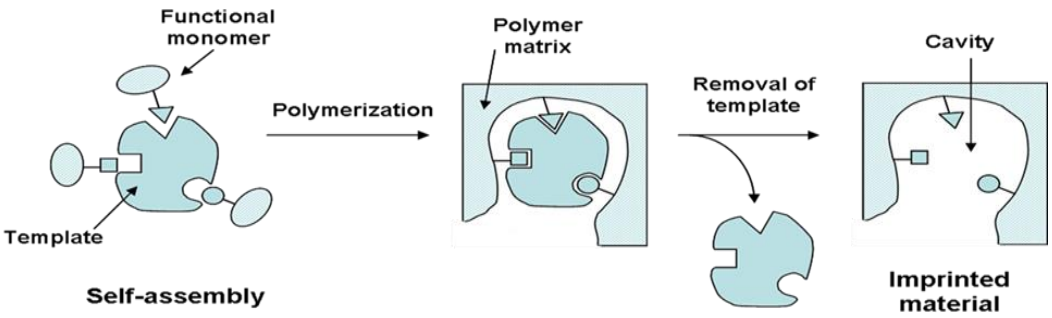

Figure (2): Molecular imprinted polymer cycle **(Yan & Row, 2006)**

After this cycle, certain MIP have been prepared using SPE **(Knoll *et al.*, 2020; Lim, Oh *et al.* 2020)**

The solute concentration in the fluid phase at a constant temperature provides an adsorption isotherm. An isotherm is the relation between the concentrations of a solid and fluid, used to describe states of the sorption process **(Yu *et al.*, 2012).**

- Solid phase extraction (SPE) is a technique designed for rapid, selective sample preparation and purification **(Mahdi Z & Al-Bayati ٢٠٢٠; Abd Jaber & Al-Bayati, 2020)** prior to the chromatographic analysis (e.g. HPLC, GC, TLC) **(Nakamura *et al.*, 2022; Qasim *et al.* 2020)**. In SPE, one or more analytes from a liquid sample are isolated by extraction, partitioning, and/or adsorption onto a solid stationary phase, washing and elution to cover the analyte under investigation as been by Figure (3).

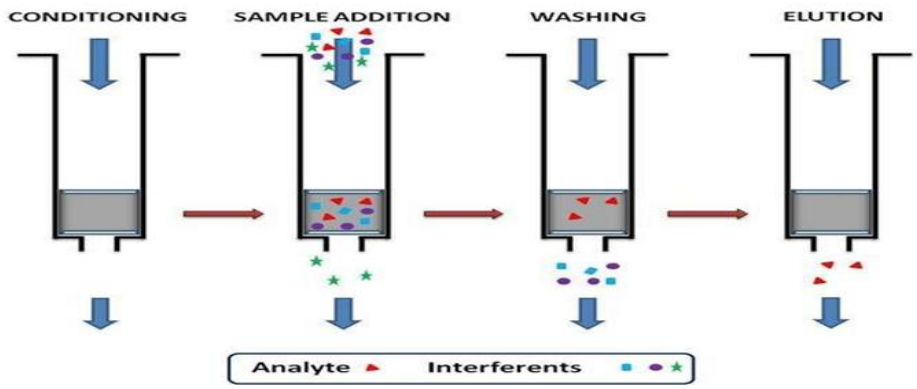

Figure (3): Illustrate the process of SPE.

In this work identify the MIP preparation was performed in conjunction with the recognition site Allyl chloride C_3H_5Cl with crosslinking Ethylene glycol dimethacrylate EGDMA $C_{10}H_{14}O_4$, whereby benzoyl peroxide BPO functioned as the target molecule (Glimepiride) initiator. Subsequently, the impact of monomer dosage on adsorption performance was observed. This study also examined the adsorption behavior of diverse functional monomers, cross-linking agents, and solvents. SEM, FTIR was employed to characterise the primed MIPs. Furthermore, this study investigated the impact of solid phase extraction and initial Glimepiride concentration on the adsorption capacity.

MATERIALS AND METHODS

MATERIALS

Glimepiride standard as template from Samarra/Iraq was provided , Allyl chloride as monomer, EGDMA as cross-linker and Benzoyl peroxide as initiator were purchased from Sigma Aldrich (St. Louis, MO, USA, www.sigma-aldrich.com) , Methanol, Nitrogen gas (99.99) supplied by Al-Watan factory (Al-Nahda street/ Baghdad/Iraq), Chloroform and Acetic acid were purchased from Merck (Darmstadt, Germany), Glimepiride/UK and Glypride/ julphar Emirate as pharmaceutical drugs of glimepiride purchased from pharmacy.

METHODOLOGY

With the recognition sites of monomer allylchloride C_3H_5Cl, crosslinking Ethylene glycol dimethacrylate EGDMA $C_{10}H_{14}O_4$ with benzoyl peroxide BPO as initiator was synthesized for the target molecule Glimepiride:

1. Glim-MIP was prepared by dissolving 1mmol of Glimepiride 0.4906g in 5-6 drops of 1N HCl after that methanol was added. The resultant solution was stirred and warmed for 10-20 seconds to obtain a transparent solution.
2. A 15 mmol of allylchloride 1.1480g with 2 ml methanol was added.
3. The mixture (step1 and 2) was allowed to stand for a few seconds at room temperature.
4. A cross-linker 20 mmol Ethylene glycol dimethacrylate EGDMA 3.9644g with 2ml methanol and 0.3 g benzoyl peroxide dissolved in chloroform as an initiator were added to the above solution.
5. The ratio 1:15:20 Glim-MIP was completed, the solution was shaken and bubbled for 20 min with pure nitrogen gas to remove the dissolved oxygen from the monomer solution immediately.
6. The tube was sealed with a rubber stopper. The solution was left overnight in a water bath at 60 ºC for 72 hours.

A white color polymer with a fluff structure was formed, Figure 4.

7. Soxhlet solid liquid phase extraction for the template was performed to remove the template glimepiride from MIP using a porogenic solvent (acetic acid, methanol) 1:9 respectively, successfully removed by repeated washing for 20 hours,
8. The MIP was dried at room temperature, after that it was crushed with mortar, sieved to particle size 125µm.
9. A plastic syringe (10 ml) of solid phase extraction vacuum (column) was used, and each syringe packed with 0.1 g of Glim-MIP.

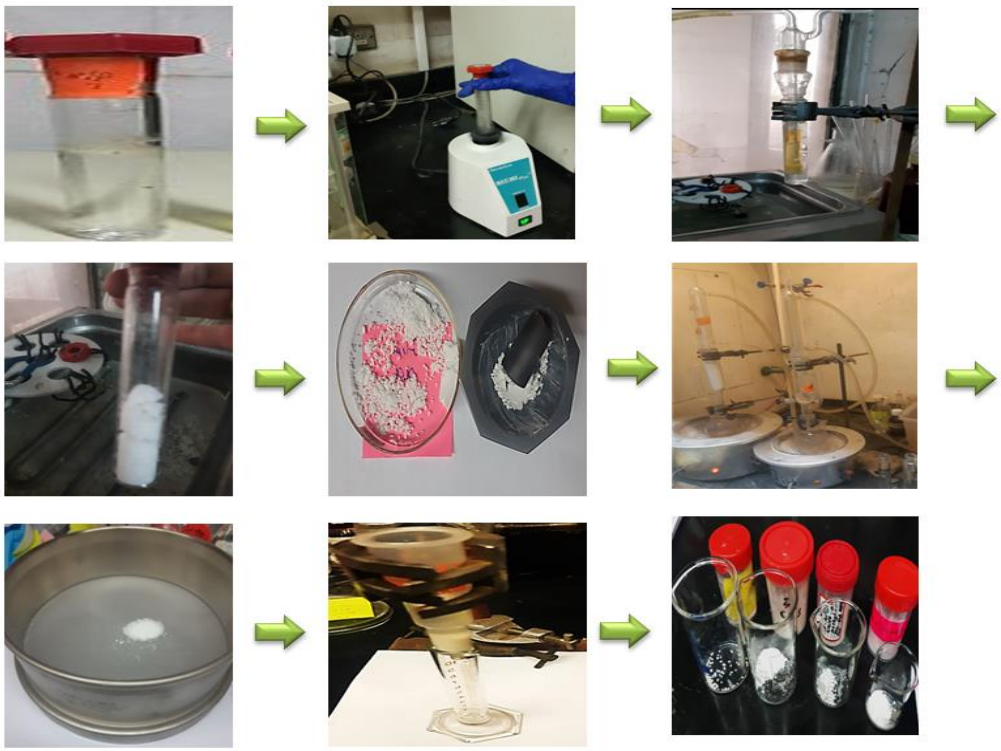

Figure (4): The preparation of imprinting polymer in the laboratory: firstly combined template, monomer, cross linker and initiator, mix well in shaker, bubbled with nitrogen gas, the polymer become solid MIP after placed in a water bath, drying and grinding, Soxhlet solid liquid extraction to separate the template, crushed and sieved the MIP to required a suitable particle size(using 125µm), packed in a cartridge to prepare a column for isotherm process, finely store MIPs in suitable containers**.**

A solutions (standard solution, pharmaceutical drugs of glimepiride and serum) was poured from the top of the column and the movement of the solution was by electric vacuum at 70 rpm. A series of standard solutions of Glimepiride (0.04, 0.06, 0.08, 0.1, 0.2, 0.4, 0.6, 0.8, 1, 1.2) µmol/ml was prepared by dissolving 0.0589g Glim. in 1-2 drops of 1N HCl to create a buffer solution, after which methanol was added. The resultant solution was stirred and heated for approximately 15-20 seconds methanol volumetric flask 100 ml as a stock solution. A calibration curve was constructed between concentration of Glim. and its absorbance A, This was achieved using at (274.5 nm by UV-VIS instrument).

Sample preparation of Glimepiride (pharmaceutical samples)

Ten tablets were weighted then crushed and grinded. Tablets containing 4 mg of Glimepiride were weigh 0.1422g, 0.1418g, (equivalent to 0.0196g& 0.0343g of active ingredient, 4×10^{-4}, 7×10^{-4}mMol/L) for Glimepiride (Glimepiride/UK, Glypride/ julphar

Emirate) respectively (Table 1) and dissolved in several drops of 1M HCl in a 100 mL volumetric flask. Methanol was added agitated and warmed using a magnetic stirrer for at least 30 minutes, the solution was filtered to get rid of undissolved materials, the residue was washed with methanol and completed the volume to 100ml with methanol.

Table (1): Pharmaceutical drugs prepared for treating with Glim-MIP polymer

No. of samples	Commercial name, Country Content 500mg	Average weight for 10 of tablets	Weight of sample equivalent to 0.0196g (4×10^{-4}) mmol/mL (0.4µmol/mL)of the active ingredient	Weight of sample equivalent to 0.0343g (7×10^{-4}) mmol/mL (0.7 µmol/mL) of the active ingredient
1	Glimepiride/UK	0.1422	0.6968	1.2194
2	Glypride/ julphar Emirate	0.1418	0.6950	1.2159

RESULTS AND DISCUSSION

RESULTS

Accuracy of the work for extraction and determination of Glimepiride

UV-VIS Spectrophotometry

A calibration curve between concentrations of standard Glimepiride (0.04-1.2) µmol /ml and their absorbance was plotted figure 5.

Concentration of Glimepirideµmol /ml	Absorbance
0.04	0.0688
0.06	0.1191
0.08	0.141
0.10	0.1712
0.20	0.3314
0.40	0.5978
0.60	0.9688
0.80	1.2856
1.00	1.5473
1.20	1.8367

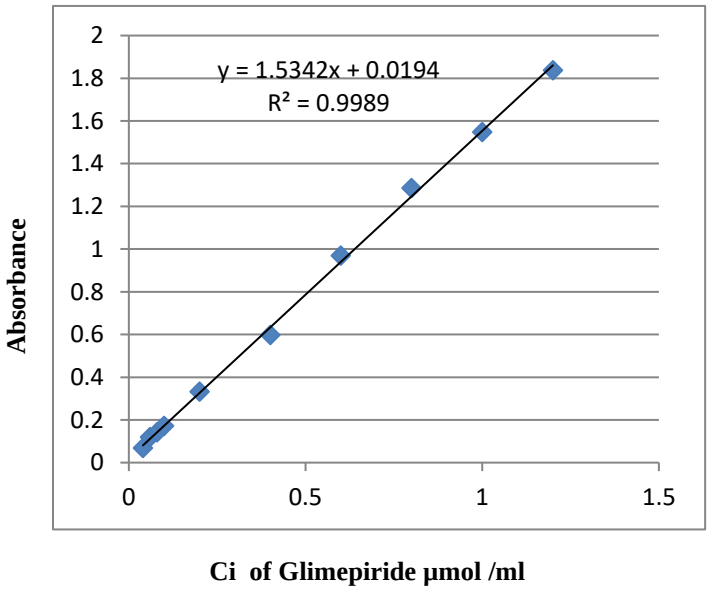

Figure (5): Calibration curve of standard concentrations of Glimepiride and its absorptions.

After passing the solution of Glimepiride in syringe packed with Glim-MIP the residue which has less absorption was measured by UV-VIS that indicate to lower concentration at final process, for good expressive example of the advantages of the use of impressed polymers in SPE in the quantification of the Glimepiride, figure 6, 7 and 8.

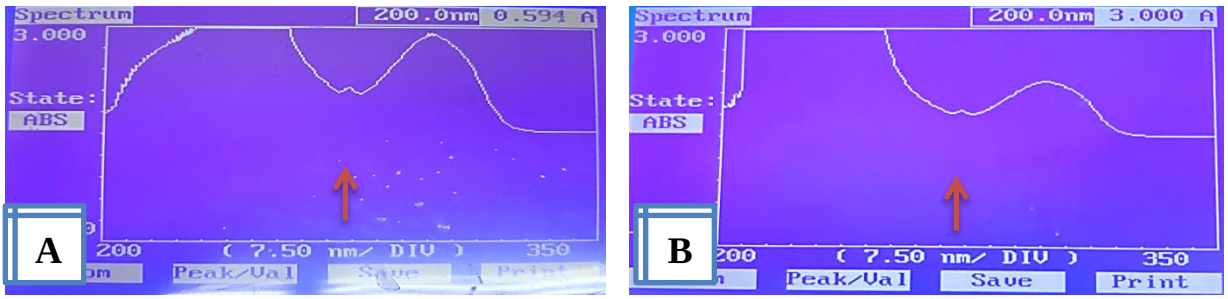

Figure (6): A, B the absorption at 274.5 nm of Glimepiride standard at 4.0×10^{-4} , 7.0×10^{-4} mmol/mL (0.4, 0.7 µmol/ml)) respectively

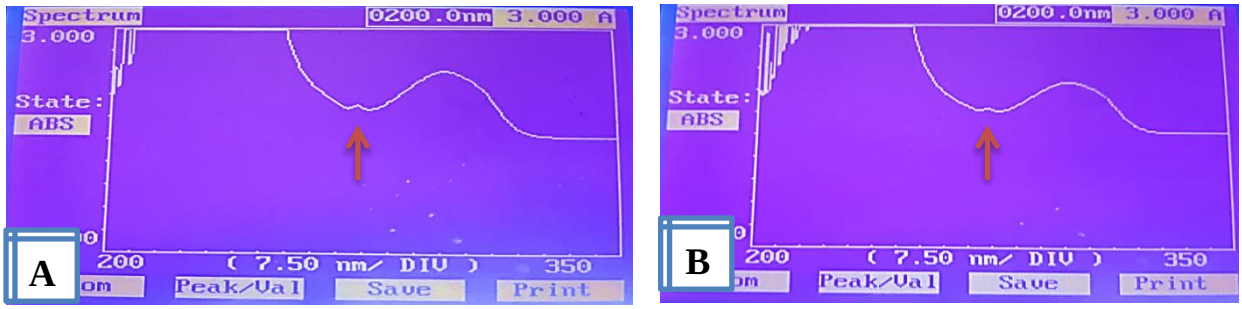

Figure (7): A,B the absorption of the concentration of Glimepiride drug (Glypride/ julphar Emirate) at 7×10^{-4} mmol/mL (0.7 µmol/ml) before& after passing through Glib-MIP column at wave length 274.5 nm

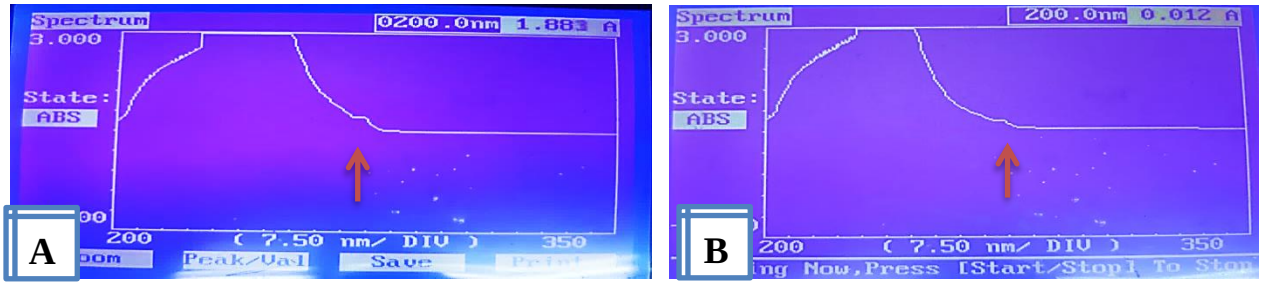

Figure (8): A,B the absorption of the concentration of Glimepiride drug (Glimepiride/UK) at 4×10^{-4} mmol/mL (0.4 µmol/ml) before& after passing through Glib-MIP column at wave length 274.5 nm.

FT-IR spectrum of standard and molecularly imprinted polymers for (Glim.)

Fourier transmission infrared spectrometer is an important chemical characterization process to detect the functional groups which have been presented in a compound. The standard of Glimepiride FT-IR spectra shows several functional groups and Glim-MIP before and after removal of template in the following Figures 9& 10A,B for Glim-MIP

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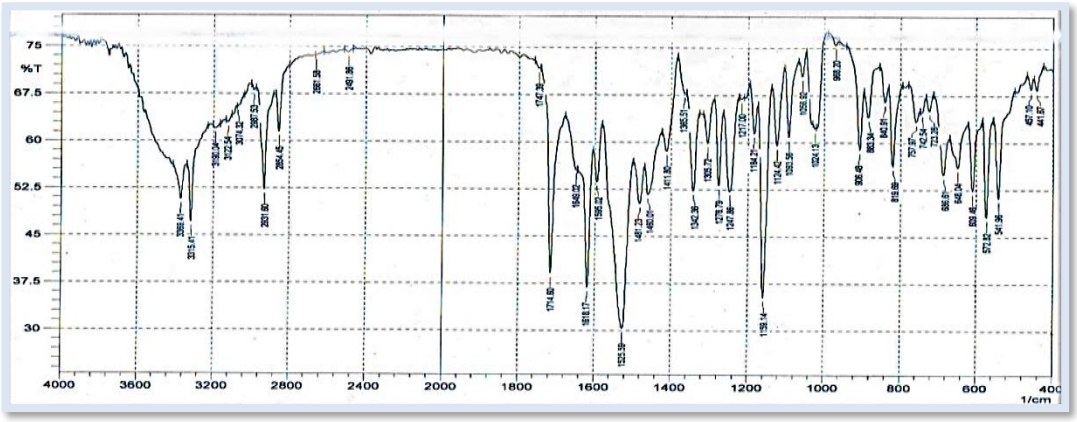


Figure (9): FTIR spectra of standard Glimepiride

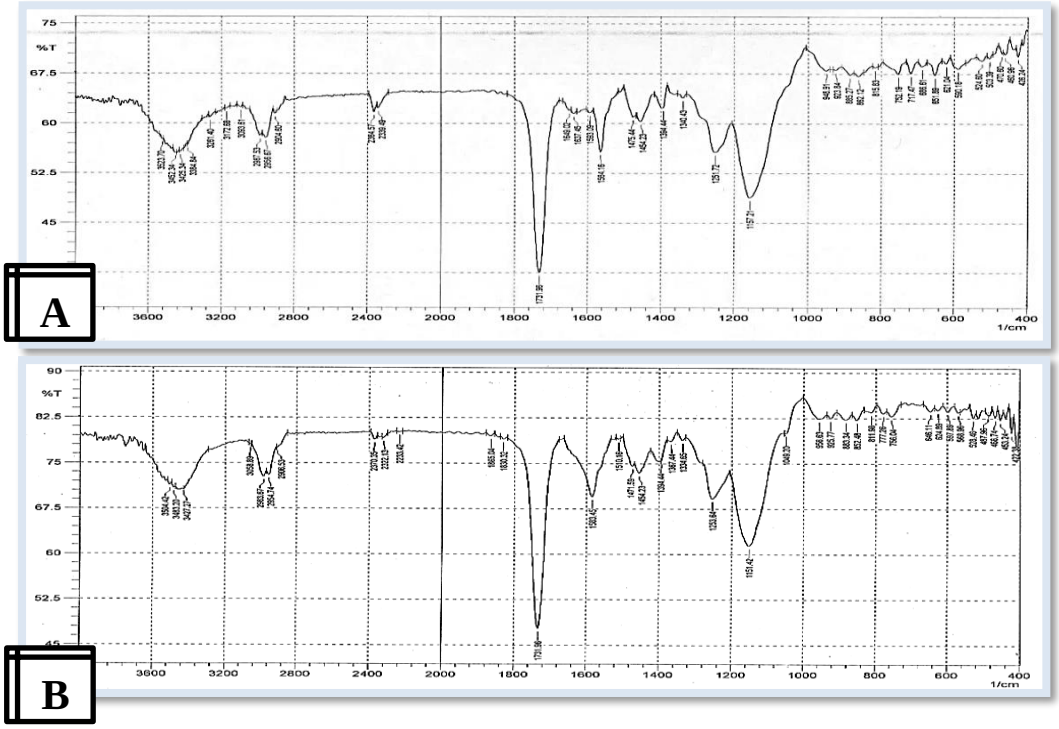
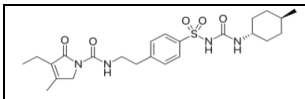
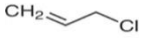
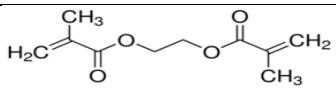


Figure (10): A,B FTIR spectrum of Glim-MIP before and after extraction (after removal the template Glimepiride.

It can be seen that the spectra for Glim- MIP before and after removal Glimepiride have approximately similar bands, that mean elution process has a slight impact on the architecture structure Table 2.

Table (2): The structures of the main three compositions of Glim-MIP and the bands indicate MIP before &after removal template

 Template Glimepiride	 Monomer Allyl chloride	 Cross linker EGDMA	
Band	**Drug(Template)**	**MIP before extraction**	**MIP after extraction**
N-H str.	**3369** **3315**	**3384** **3452**	/
Ar-H aromatic	**3074**	**3093**	/
C-H aliph.	**2931** **2854**	**2987** **2956**	**2954** **2906**
C-O est.	**1247**	**1251**	**1253**
C=O	**1714**	**1731**	**1731**
C=C ole.	**1595**	**1593**	**1583**
C=C arom.	**1618**	**1637**	/

SEM of molecularly imprinted polymers for (Glim.):

Figure 10 (A,B) shows the surface morphologies of the particles before and after elution for Glim-MIP and table 3 shows the measurements of 5 selected cavities

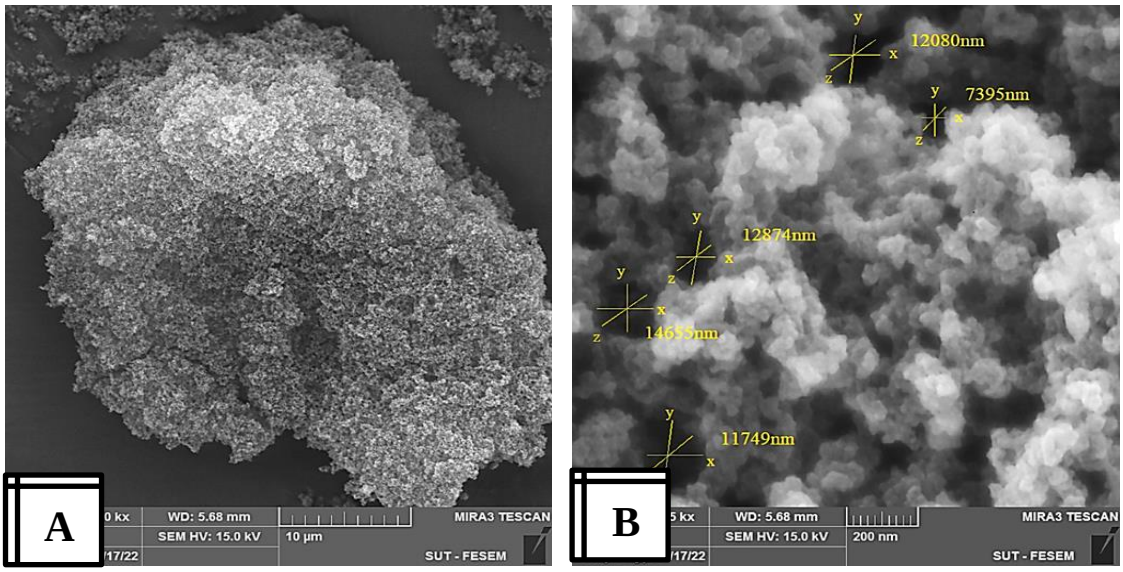


Figure (11): A, B surface morphologies of the particles before and after elution for Glim- MIP respectively, and three dimensions of cavities with their areas.

Table (3): Calculated mean, angle , lengths of some cavities (selected six of them) and their areas.

Cavities	Area	Mean	Min-Max	Angle	Length
1	305.822	11749.35	10172.19 - 15806	-0.744	152.481
2	262.694	14655.9	11989 - 20501	0	130.687
3	164.674	12874.92	11196.27 - 16132.14	0	80.524
4	176.436	7395.47	5613.045- 13208	-178.698	87.147
5	101.941	12080.53	9467.92- 18686	-177.709	49.542
Total Mean	202.313	11751.23	9687.684- 16866.63	-71.43	100.076
SD	81.426	2682.389	2472.731-2809.303	97.471	41.19
Min-Max	101.941-305.822	7395.47-14655.9	5613.045-13208 11989-20501	-178.698-0	49.542-152.481

From Figure 10 and Table 3 the 3D of Cavities between min = 7395.47nm (7.3954µm) to max = 14655.9nm (14.6559µm) we notice that the holes vary in diameter range between (7395.47-14655.9)nm and most of the holes are deep, which leads to the retention of large quantities of the drug and this is consistent with the high value of the capacity in isotherm.

Adsorption capacity and pre-concentration for Glim-MIP: A series of absorption achievement for different initial concentrations of Glim-MIP ranging from 0.04 to 1.2 µmol/ml on adsorption capacity µmol/g was studied using the following equation **(Al-Janabi, 2017; Huang *et al*, 2018)**.

$$\mathbf{Q} = (\boldsymbol{Ci} - \boldsymbol{Cf})(\boldsymbol{\mu mol/ml}) * \frac{\boldsymbol{vol\ (ml)}}{\boldsymbol{Wof\ Mip(g)}}$$

Ci- initial concentration , Cf - final concentration (after passing through column packed with Glim-MIP)

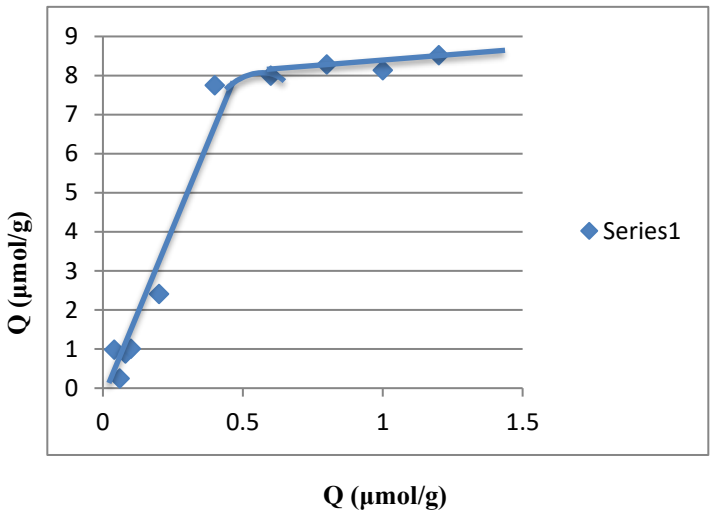
Pre-concentration refers to the process of obtaining a high local concentration at the sensor surface, the concentrations from (0.06-1.2)µmol/ml consume (3)ml range of volumes while at concentration 0.04µmol/ml consume (4)ml, when using 0.1g weight of Glim-MIP, Table 4.

Table (4): The optimal synthesis conditions for the molecularly imprinted polymer for Glimepiride developed in this study in 0.1 g of MIP

W/ MIP (g)	Ci Conc.in (ppm)	Ci Conc.in (µmol/ml)	Cf (µmol/ml)	Vol (ml)
0.1	19.6247	0.04	0.0153	4
	29.4370	0.06	0.0517	3
	39.2494	0.08	0.0503	3
	49.0617	0.10	0.0662	3
	98.1234	0.20	0.1196	3
	196.2468	0.40	0.1417	3
	294.3702	0.60	0.3334	3
	392.4936	0.80	0.5237	3
	490.6170	1.00	0.7288	3
	588.7404	1.20	0. 9218	3

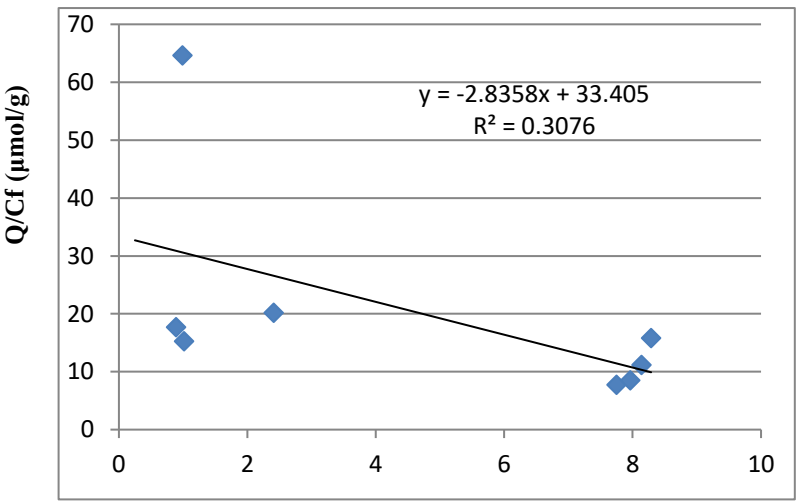
Ci (µmol/ml)

Relation between initial concentration Ci (µmol/ml) and capacityQ (µmol/g):



Ci (µmol/ml)	Q (µmol/g)
0.04	0.9884
0.06	0.2493
0.08	0.891
0.1	1.0128
0.2	2.412
0.4	7.7484
0.6	7.998
0.8	8.288
1	8.1355
1.2	7.9639

Figure (12): Illustrate Langmuir isotherm model The relation between capacity Q (µmol/g) and Q/Cf (µmol/g):



Q µmol/g	Q/Cf (ml/g)
0.9884	64.6436
0.2493	4.82293
0.891	17.7137
1.0128	15.2899
2.412	20.1672
7.7484	7.7484
7.998	23.9461
8.288	15.8278
8.1355	11.1629
7.9631	8.5192

Figure (13): The slope of Langmuir isotherm model

Table (5): Results of maximum capacity in µmol/g for Glim-MIP using 0.1g weight of MIP

Slope	Kd = -1/ slope	Intersept	Qmax= Intersept × Kd µmol/g
-2.8358	0.3526	33.405	11.7797

Qmax = 11.7797 µmol/g for Glim-MIP 0.1g weight

In human serum

1- Sample collection

In total, 5 ml of blood was gathered and placed in serum separator tubes (SST). The clot activator SST contained a gel in the form of an inert thixotropic polymer**(Schrapp *et al.*, 2019; Yasar & Konukoglu, 2020)**, which was located at the bottom, its purpose being to separate blood cells from serum through centrifugation. This was performed for each patient and healthy individual. Blood samples were allowed to stand for 5 minutes following centrifugation at ~ 2000 rpm. the serum was kept at 20ºC so that it could later be employed for the estimation of Glimepiride.

2- Procedure

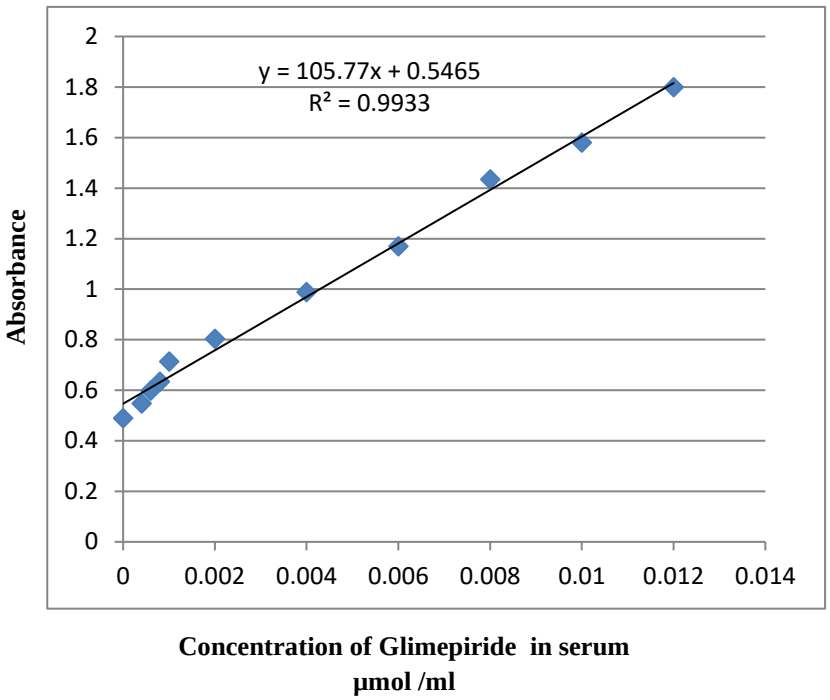
This method uses one ml of each human serum. In other words, it requires serum from the control group (healthy individuals who do not take Glimepiride) and the patient group (who take Glimepiride drug), both of which were diluted in 10 ml of deionized water. Subsequently, 1 ml of diluted serum was placed in a 10 ml volumetric flask, to which was added 2-3 drops of 1 N HCl solution, the purpose being to eliminate the viscosity of the serum **(Constable *et al.*, 2019)**. Methanol was used to make the volume up to 10 ml. The solutions were then warmed in a water bath for 10-15 minutes at a temperature not exceeding 60 ºc in order to create a transparent solution.

Several series of solutions were created for each control and patient group. This was realized through the transferal of 1 ml to each eleven volumetric flask (10 ml) (We doubled the amount of serum to get the quantity needed for 11 volumetric flask) followed by the addition of constant volumes of standard Glimepiride (0.1 ml) from different concentrations (0, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4, 0.6, 0.8, 1, 1.2) µmol/ml to obtain (0, 0.0004, 0.0006, 0.0008, 0.001, 0.002, 0.004, 0.006, 0.008, 0.01, 0.012) µmol/ml. Flask No.1 is the sample (serum). The findings were subjected to mathematical evaluation ($M_1V_1=M_2V_2$ for the standard addition method) (see Table 6). Furthermore, the absorption recorded for each volumetric flask was gauged with the assistance of UV-Visible spectrophotometry, which focused on the control serum and then measured the patient serum at the maximum 274.5 nm absorption, the objective being to eradicate the majority of interferences. Subsequently, the resultant solution was scanned in the 200-350 nm range. Fig. 13 presents the calibration curve that was plotted between the concentrations and absorptions.

Table (6): Results of standard addition for the estimation of Glim in human serum.

Human	**Diluted Serum**	**1N HCl**	**Glimepiride µmol/ml**										
Control	**1 ml**	**2-3 drops**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Patient	**1 ml**	**2-3 drops**	**0**	**$4x10^{-4}$**	**$6x10^{-4}$**	**$8x10^{-4}$**	**$1x10^{-3}$**	**$2x10^{-3}$**	**$4x10^{-3}$**	**$6x10^{-3}$**	**$8x10^{-3}$**	**$1x10^{-2}$**	**$1.2x10^{-2}$**

Glimepiride in serum was statistically evaluated by considering the length of time the drug was in the body of the patient, the rate at which it was metabolized, and the medication dose. These variables differ between patients. In addition, Glimepiride is reported to undergo hepatic metabolism, the elimination half-life of glimepiride is approximately 5 -8 hours **(Li *et al.*, 2016)** Calibration curve between concentrations and absorptions.



Concentration of Glimepiride in serum µmol /ml by standard addition method	Absorbance of Glimepiride in serum by standard addition method
0	0.4887
0.0004	0.5467
0.0006	0.5982
0.0008	0.6331
0.001	0.7125
0.002	0.8024
0.004	0.9877
0.006	1.1695
0.008	1.4337
0.010	1.5788
0.012	1.7989

Figure (14): Calibration curve between concentrations of Glimepiride in serum using standard addition method µmol/ml and its absorbance.

When y= 0.4887 that mean the absorbance of Glimepiride in this sample of serum is 0.4887 It found that the absorption 0.4887 are nearest to the absorption 0.3314 which has concentration 0.20 µmol /ml in calibration curve (Figure 12) and substituting for y= 0.4887 the concentration is 0. 209µmol /ml. That mean the concentration of Metformin in this sample of serum is 0.2949 µmol /ml by ratio and proportion. so, a comparison for absorption of this concentration after passing through Glim-MIP column has been studied in pharmaceutical drugs solution and human serum.
*To know the concentration of drug in human serum we must multiply this concentration 0.2949µmol /ml x 10(Dilution coefficient).

DISCUSSION

This paper presents a comparison between two approaches to the drug Glimepiride The T-Test statistical evaluation**(Deckard, 2016; Haaland & Thomas, 1988)**, was designed to facilitate a comparison between the identification of Glimepiride once it had passed through the Glim-MIP syringe solid phase extraction process and the human serum at 274.5 nm:

$$/t/= \bar{X}i1 - \bar{X}i2/(S(\sqrt{1/n1 + 1/n2}))$$

If $\bar{X}i1 = \bar{X}i2$ $\longrightarrow$ **Null hypothesis when** $t_{calculated} < t_{tab}$

That mean $\bar{X}i1 - \bar{X}i2$ **= zero**

$Xi1 \neq Xi2$ ⟶ **Alternative hypothesis when** $t_{calculated} > t_{tab}$

That mean $\bar{Xi}1 - \bar{Xi}2 > < zero$

* $\bar{Xi}1 = 0.1248$ Mean for n1=3 absorption value after passing through Glim-MIP column in pharmaceutical drugs solution with S1 variance= 0.086

$\bar{Xi}2 = 0.1625$ Mean for n2=3 absorption value after passing through Glim-MIP column in human serum with S2 variance=0.075

$$S^2 = (n1 - 1)*S^2_1 + n2 - 1)*S^2_2 / n1 + n2 - 2$$

$$t\ calculated = 0.574,\ t\ tab = t_{0.05/2,\ (n1+n2)-2} = 2.776$$

It found $t_{calculated} < t_{tab}$ at confidence level 95% therefor there is no significant difference between two approaches, So Null hypothesis will be accepted.

CONCLUSION

New and novel bulk polymers were created by using Allyl chloride C_3H_5Cl & crosslinking Ethylene glycol dimethacrylate EGDMA $C_{10}H_{14}O_4$ as Glim-MIP, different studies and experiments were used to reach for selective molecular imprinted polymer by prepare and optimize required monomers, cross-linker using suitable solvents, porogen solvent for template removal and the optimal molar ratios of Template (Glimepiride)to monomer to cross-linker. Irregular shapes three-dimension network structure of polymers can be seen by SEM before and after removal template, FTIR, isotherm processing all improves the healthy work.

one slope gain when studied the capacity of adsorption of Glim-MIP which follow Langmuir isotherm model with uniform values (homogeneous structure), The maximum adsorption capacity was 11.7797 µmol/g for 0.1g of Glim-MIP. A standard addition method using to eliminate the interferences when detect the concentration of Glimepiride in human serum. T-Test statistical evaluation was designed to facilitate a comparison between the identification of Glimepiride once it had passed through the Glim-MIP syringe solid phase extraction process and the human serum at 274.5 nm and when it found that $t_{calculated} < t_{tab}$ at confidence level 95% by UV for Glimepiride drug therefor there is no significant difference between two methods, So Null hypothesis will be accepted.

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