ISSN: 0067-2904

Selective Extraction of Metformin in Pharmaceutical Preparation *via* Synthesized MIP-SPE Technique

Rana Adnan Kamal Aldeen*, Yehya Kamal Al-Bayati
Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq

Received: 12/6/2022 Accepted: 2/11/2022 Published: 30/8/2023

Abstract

This work demonstrates the synthesis and storage of molecular-imprinted polymers (MIP) at room temperature using bulk polymerisation of Metformin (Met) characterized by high sensitivity, low cost, and high stability. To ensure an acceptable adsorption capacity, the research employed 0.8:4:20 mmol ratios of template, monomer, and cross-linking agents for the polymerization. A functional monomer, 2-acrylamido-2-methyl-1-propane sulphonic acid $C_7H_{13}NO_4S$, was cross-linked with *N*,*N*-methylene bisacrylamide $C_7H_{10}N_2O_2$ to form Met-MIP, which could be characterized using a UV-VIS spectrophotometer at 236 nm, FT-IR spectroscopy, and scanning electron microscopy. The elution process that was applied to the template Metformin from the Met-MIP created cavities that were caused by the porogenic mixture solution of methanol, chloroform, and acetic acid (70:20:10, respectively). In accordance with the Freundlich isotherm model, Met-MIP had a maximum adsorption capacity of 5.2998 µmol/g and a template to monomer ratio of 1:2. A solid-phase extraction syringe packed with molecular imprinted polymers was used for the selective separation and pre-concentration of Metformin from aqueous solutions and estimation of Metformin by MIP and HPLC instruments in multiple pharmaceutical drugs of Metformin from several sources. The comparison with standard analytical techniques by MIP and RP-HPLC showed no significant difference between the two methods.

Keywords: Molecular imprinted polymer (MIP), Metformin, Isotherm process, SPE, pharmaceutical preparation.

الاستخلاص الانتقائي للميتفورمين في المستحضرات الصيدلانية عن طريق تقنية MIP-SPE المركبة

رناعدنان كمال الدين*, يحيى كمال البياتي
قسم الكيمياء ، كلية العلوم، جامعة بغداد ، بغداد، العراق

الخلاصة

يوضح هذا العمل تحضير وتخزين البوليمرات الجزيئية المطبوعة (MIP) في درجة حرارة الغرفة باستخدام البلمرة الصلدة لـ Metformin (Met) والتي تتميز بالحساسية العالية والتكلفة المنخفضة والاستقرار العالي. لضمان قدرة امتصاص مقبولة ، استخدم البحث النسب 0.8: 4: 20 ملي مول للقالب ، وعوامل المونومر وعوامل الربط التبادلية للبلمرة من أجل ضمان قدرة امتزاز مناسبة. المونومر الوظيفي 2-أكريل اميدو-2-ميثيل-1- بروبان حمض سلفونيك ($C_7H_{13}NO_4S$) مع *N،N* -ميثيلين أكريل أميد

$C_7H_{10}N_2O_2$ كرابط التشابك وبالتالي إنشاء MIP لـ Metformin كـ Met–MIP تم تمييزه باستخدام مقياس الطيف الضوئي UV–VIS عند 236 نانومتر ، والتحليل الطيفي بالأشعة تحت الحمراء والمسح المجهري الإلكتروني. أنشأت عملية الشطف التي تم تطبيقها على القالب اي انتزاع القالب المتفورمين من Met–MIP تجاويف ناتجة عن استخدام خليط مسامي من الميثانول والكلوروفورم وحمض الخليك (10:20:70 على التوالي). وفقًا لنموذج Freundlich isotherm ، كان لدى Met–MIP قدرة امتزاز قصوى هي 5.2998 ميكرو مول / غم ونسبة القالب إلى المونومر هي 1: 2. تم استخدام سرنجة للاستخلاص بالطور الصلب معبأة بالبوليمر المطبوع جزيئيًا للفصل الانتقائي والتركيز والتقدير للمتفورمين في المحاليل المائية بجهاز ال MIP, HPLC في العديد من الادوية الصيدلانية للميتفورمين من مختلف المصادر. لم تظهر المقارنة مع التقنية التحليلية القياسية بواسطة MIP و RP–HPLC أي فرق جوهري بين الطريقتين.1*

1. Introduction

Metformin (Met) or Glucofage is an oral diabetes medication that helps control blood sugar levels and is used together with diet and exercise to improve blood sugar control in adults with type 2 diabetes mellitus. It is a component of drugs like metformin-alogliptin (Kazano) and Metformin-canagliflozin (Invokamet). In the last stages of type 2 diabetes, metformin can also be used with insulin. Metformin decreases hepatic glucose production, increases insulin-mediated peripheral glucose uptake, and decreases intestinal glucose absorption. It is also used off-label to treat polycystic ovary syndrome (PCOS) [1,2]. Common adverse effects include diarrhea, nausea, and abdominal pain. It has a low risk of causing low blood sugar. High blood lactic acid levels are a concern if the medication is used in overly large doses or prescribed to patients with severe kidney problems. It is not recommended for those with significant liver disease [3]. Metformin is a biguanide antihyperglycemic agent that works by decreasing glucose production by the liver, increasing the insulin sensitivity of body tissues, and increasing GDF15 (growth differentiation factor, a protein coding gene) secretion, which reduces appetite and caloric intake [4]. Figure 1 shows the structure of metformin.

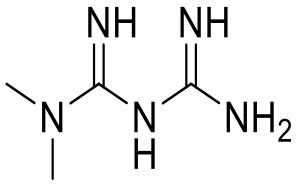


Figure 1 : Structure of Metformin [1]

In the beginning, the imprint molecule with the present monomers forms a complex in molecular imprinted polymers (MIP). The functional groups are maintained in situ following the polymerization cycle, as depicted in Figure 2, by a strongly cross-linking polymer structure [5]. In addition, the steric configuration of all these connections around a given substratum and the template is really an important characteristic for the formation of binding sites, providing additional shape, size, and flexibility to promote the selective identification followed by a high target affinity. As a result, the process of recognition in MIPs can be characterized in resemblance to enzyme-proven mechanisms-substratum-complex is formed in the (lock and key) model [6-10].

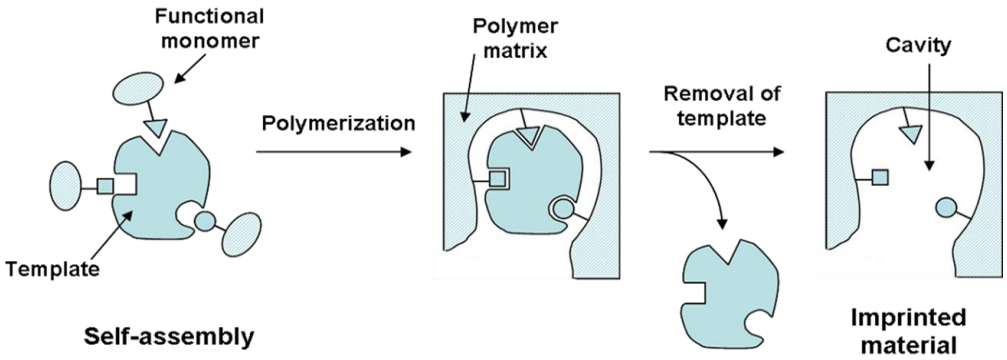


Figure 2: Molecular imprinted polymer cycle [11].

SPE and HPLC are used to prepare certain MIP applications [12-16]. The adsorption isotherm is provided by the solute concentration in the fluid phase at constant temperature. An isotherm is the relation between the concentrations of a solid and fluid, used to describe the states of a sorption process [17]. Solid phase extraction (SPE) is a technique created for quick, selective sample preparation and purification prior to chromatographic analysis (e.g. HPLC, GC, and TLC). In SPE, one or more analytes from a liquid sample are isolated by extracting, partitioning, and/or adsorbing onto a solid stationary phase, as shown in Figure 3 [18].

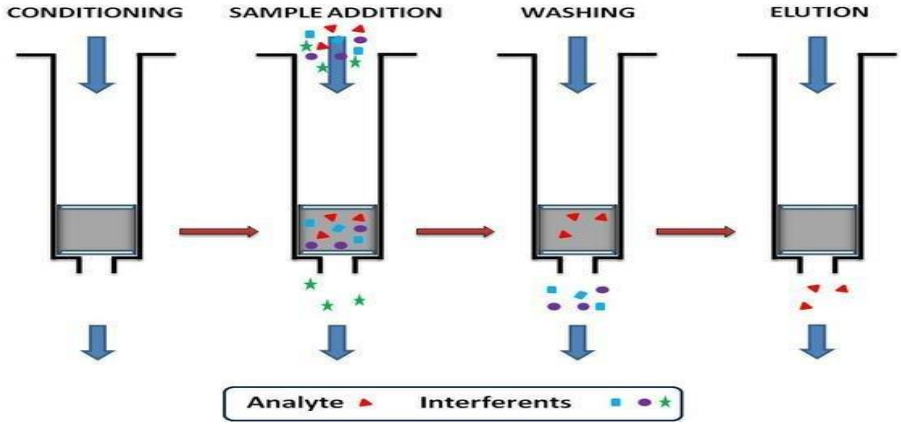


Figure 3: Illustrate the process of SPE

In this study, RP-HPLC was used because of its wide applicability and reproducibility. The preparation of MIP with recognition sites 2-acrylamido-2-methyl-1-propanesulphonic acid, $C_7H_{13}NO_4S$ as a monomer with cross-linker *N*,*N*-methylene bisacrylamide ($C_7H_{10}N_2O_2$) and benzoyl peroxide (BPO) as an initiator for the target molecule (Met). The effects of monomer dosages on the adsorption performance were studied. The adsorption behaviors of various functional monomers, cross-linking agents, and solvents were also investigated. SEM and FT-IR were used to characterize the prepared MIP. In addition, the effects of RP-HPLC, solid phase extraction, and initial Metformin concentration on adsorption capacity were studied.

2. Experimental part

2.1. Materials and method:

Metformin was obtained from Samarra/Iraq, 2-acrylamido-2-methyl-1-propanesulphonic acid (AMPS) $C_7H_{13}NO_4S$ by cross-linking *N*,*N*-methylene bisacrylamide $C_7H_{10}N_2O_2$. Benzoyl peroxide was purchased from Sigma-Aldrich (USA). Methanol and nitrogen gas (99.99%) were obtained from Al-Watan factory (Al-Nahda Street/ Baghdad/Iraq), Chloroform and

acetic acid were purchased from Merck (Germany); sulphuric acid (98%) was purchased from CDH (Central Drug House), acetonitrile for HPLC (99.9%) from Thomas Baker, phosphate buffer ($KHPO_4$ and K_2HPO_4) were purchased from AnalaR (England).

2.2. Preparation and processing

High purity grade chemicals were used for the preparation process: Met-MIP was prepared by dissolving Metformin.HCl (129.2 mg, 0.8 mmol) in methanol (4 mL), 2-acrylamido- 2 methyl-1-propanesulphonic acid (829 mg, 4 mmol) dissolved in methanol (2 mL) with H_2SO_4 (4 drops, 1 mol/L) and added to the Metformin solution, then left for few seconds at room temperature, then cross-linker *N*,*N*-methylene bisacrylamide (3.0834 g, 20 mmol) dissolved in H_2SO_4 (12 drops, 1 mol/L), methanol (6 mL), and chloroform (2 mL),), before adding a solution of benzoyl peroxide (300 mg) in chloroform, which is used as an initiator. The solution was then shacked, bubbled for 20 minutes with pure nitrogen gas to remove the dissolved oxygen from the monomer solution immediately. Following this, the tube was sealed with a rubber stopper, and the solution was left in a water bath at 60 ºC overnight. In this step, the polymerization process of Met-MIP (0.8:4:20) was completed. A white-colored polymer with a rigid structure and fine particles was formed. This was observed with the naked eye, and left to dry at room temperature overnight. The self-assembly (non-covalent) technique of bulk polymerization was used to synthesize Met-MIP. Soxhlet solid liquid phase extraction of the template has been done to remove it from the MIP using porogenic solvent v/v (acetic acid, chloroform, and methanol; 1:2:7 respectively), and has been successfully removed by repeated washing for 16-18 hours. The polymer was dried at room temperature, crushed with a mortar and sieved to a particle size of 125 µm. A plastic syringe (3 mL) of solid phase extraction vacuum (column) was used, and each syringe was packed with (100 mg) of Met-MIP and the flow rate (70 mL/min) of the Metformin standard solution. A series of Metformin.HCl standard solutions (0.02, 0.04, 0.06, 0.08, 0.1, 0.12, 0.14, 0.16, 0.18, and 0.2 µmol/mL) were prepared by dissolving Metformin (11.6 mg) in methanol (100 mL) as a stock solution. The calibration curve between Metformin concentration and its absorption (A) was achieved at 236 nm using the UV-VIS spectrophotometer. The samples of pharmaceuticals were prepared by taking the average weight of powder of Metformin tablets, as shown in Table 1. This was done by dissolving in methanol (100 mL) before filtration through a cellulose filter paper of 0.07 µm in order to obtain the concentrations from the calibration curve 1.0×10^{-4}, 1.4×10^{-4}, and 1.6×10^{-4} mmol/mL (0.1, 0.14 and 0.16 µmol/mL) of Metformin drugs (Metforal/Germany, Glucophage/Italy and Piophage/Iraqi, respectively), which have the lowest standard addition (SD) value. These were used with MIP in a solid phase extraction (SPE) column, MIP-SPE, which was prepared.

Table 1: Pharmaceutical drugs prepared for treating with Met-MIP polymer

No. of samples	Commercial name, Country, Content (500mg)	Average weight for 10 of tablets (g)	Weight of sample equivalent to 0.00166g (1.0×10^{-4}) mmol/mL of the active ingredient	Weight of sample equivalent to 0.00232g (1.4×10^{-4}) mmol/mL of the active ingredient	Weight of sample equivalent to 0.00265g (1.6×10^{-4}) mmol/mL of the active ingredient
1	Glucophage/Germany	0.5264	0.00174	0.00241	0.00279
2	Metforal / Italy	0.5598	0.00185	0.00259	0.00296
3	Piophage / Iraqi	0.5567	0.00184	0.00258	0.00295

3. Results and discussion

For a good expressive example of the advantages of the use of impressed polymers in SPE in the quantification of Metformin, Figures 4, 5, and 6 were measured by UV-VIS Spectrophotometer. The residue that has less absorption was measured using a UV-VIS Spectrophotometer, which indicates a lower concentration in the final process.

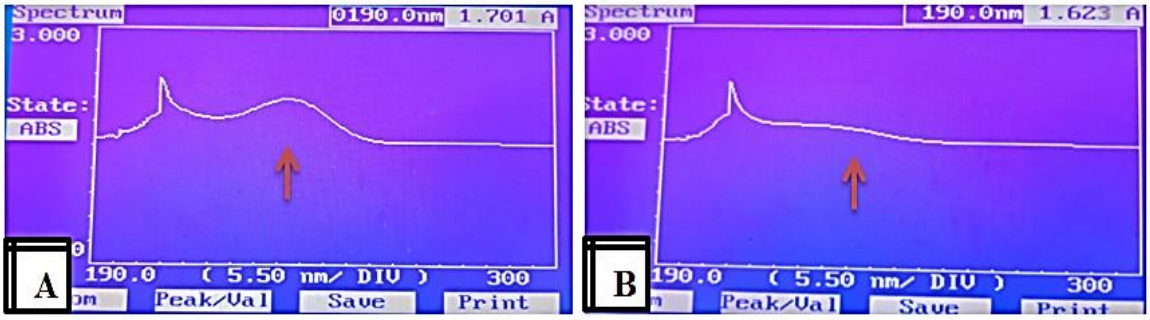


Figure 4: A,B the absorption at 236 nm of the concentration of Metformin drug (Metforal / Germany)at 1.4×10^{-4} mmol/mL (0.14 µmol/mL) before & after passing through MIP column.

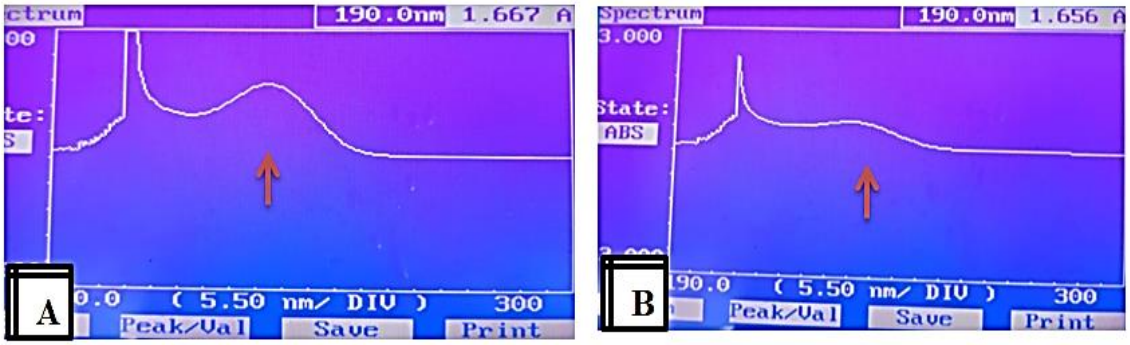


Figure 5: A,B the absorption at 236 nm of the concentration of Metformin drug (Glucophage/ Italy)at 1.6×10^{-4} mmol/mL (0.16µmol/mL)before& after passing through MIP column.

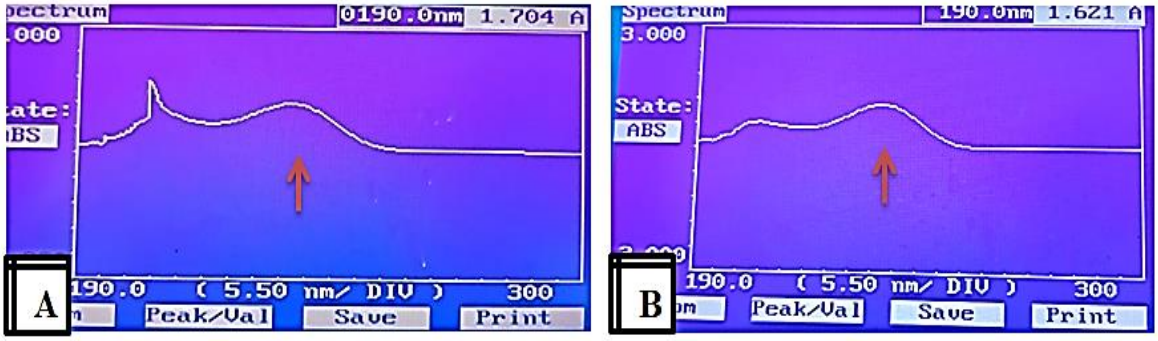


Figure 6: A,B the absorption at 236 nm of the concentration of Metformin drug (Piophage / Iraq) at 1.4×10^{-4} mmol/mL (0.14 µmol/mL) before and after passing through MIP column.

- FTIR of molecularly imprinted polymers for (Met.):

The FT-IR of molecularly imprinted polymers for Metformin: The functional groups present in a compound can be detected using a FT-IR spectrometer, which comprises a significant chemical characterization process. The FT-IR spectrum of Metformin presents multiple functional groups in addition to the Met-MIP, both before and following the Metformin template removal (see Figures 7 and 8A and 8B for Met-MIP).

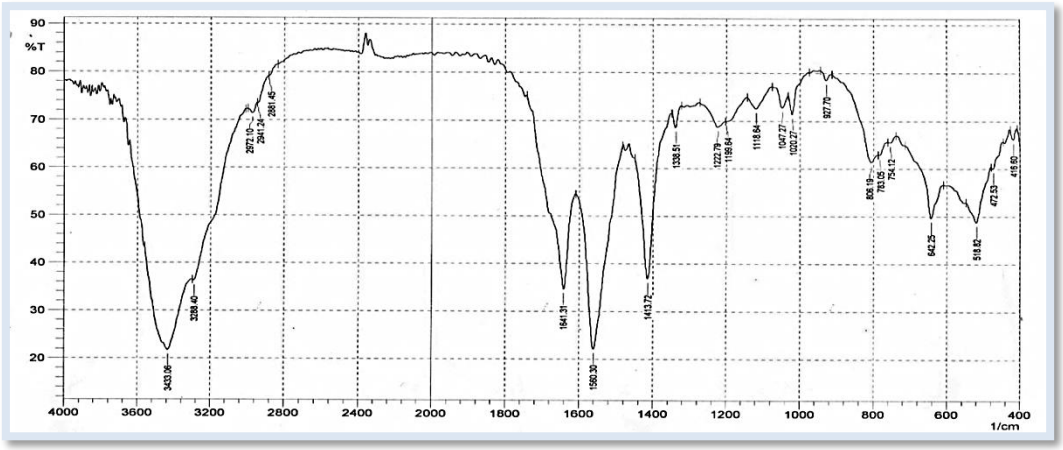


Figure 7: FT-IR spectrum of Metformin standard

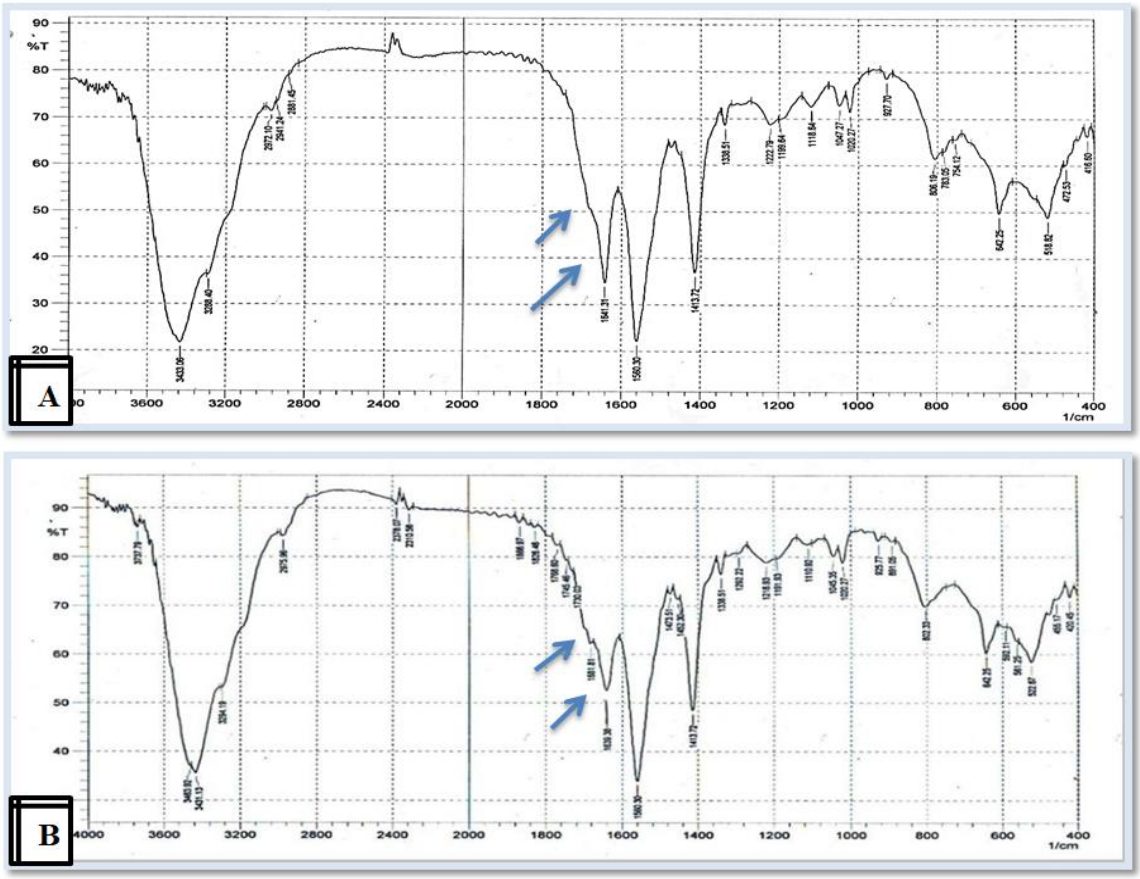
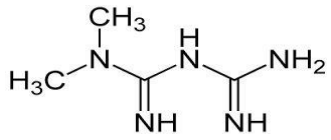
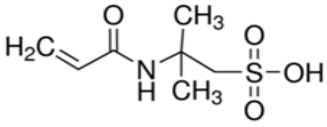
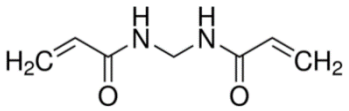


Figure 8: A and B represent the FT-IR spectra of Met-MIP before and after the extraction (after the removal of the Metformin template.

It can be seen that the spectra for Metformin and MIP before and after Metformin removal have similar bands, which means that the elution process has almost no influence on the primary polymer network structure. The spectrum of the Met shows strong bands at 3392 and 3371 cm^{-1} for NH_2 stretching. The absorptions at 3463 and 3431 cm^{-1} belong to the Met-MIP before elution, and 3433 cm^{-1} after elution. The absorptions at 3294, 3294, and 3288 cm^{-1} for N-H bands are explained in Table 2. The bands at 1625, 1639, and 1641 cm^{-1} are attributed to the C=N stretching. The C-H aliphatic bands in Metformin appeared at 2972 and 2939 cm^{-1}. The Met-MIP before elution was at 2975 cm^{-1} and after elution at 2972 and 2941 cm^{-1}. To improve that, Metformin was removed successfully, and the C=O group disappeared before eluting, which means there was interaction between C=O of the monomer and N-H of the template, and it appeared after removing Metformin, and the smallest peak of C=N indicates that the template was removed [19].

Table 2: The structures of the three main compositions and the bands of Met-MIP before &after removal of the template

 **Template (Metformin)**	 **Monomer (2-acrylamido- 2-methyl-1-propane sulphonic acid)**	 **Cross linker (*N,N*- methylene bisacrylamide)**

Band	**Drug (Template)**	**MIP before extraction**	**MIP after extraction**
N-H_2 N-H stretching	3392, 3371, 3294	3463,3431, 3294	3433 3288
C=N stretching	1625	1639	1641
C-H aliphatic	2972, 2939	2975	2972, 2941
C=O stretching	-	-	1681

The FT-IR spectroscopy is used to indicate the composition of the molecular imprinted polymer of Met drug, as is observed by the above diagram and table showing the beam at 1681 cm^{-1} for the C=O compared with the FT-IR spectrum before and after drug removal. Metformin shows the appearance of a C=O stretching vibration band, which indicates that the Metformin drug was removed and the C=N band became the smallest.

The RP-HPLC method (Liquid-Solid) has been used for quantitative estimation of Metformin.HCl in pharmaceutical dosage form. This was by a reverse phase chromatographic method utilizing HPLC column C8 (5 µm, 25 cm × 4.6 mm). Column chromatographic runs were performed at a flow rate of 1.2 mL/min with acetonitrile: phosphate buffer (65:35) as the mobile phase. The pH was adjusted to 5.5 with phosphoric acid and then filtered through a micro-membrane filter. The flow rate was 1.5 mL/min. The detection of eluent using the RP-HPLC-UV detector was at 235 nm. Figure 9 illustrates the chromatogram of the Metformin standard with a concentration of 0.1µmol/mL at two volumes; 25 and 10 µL.

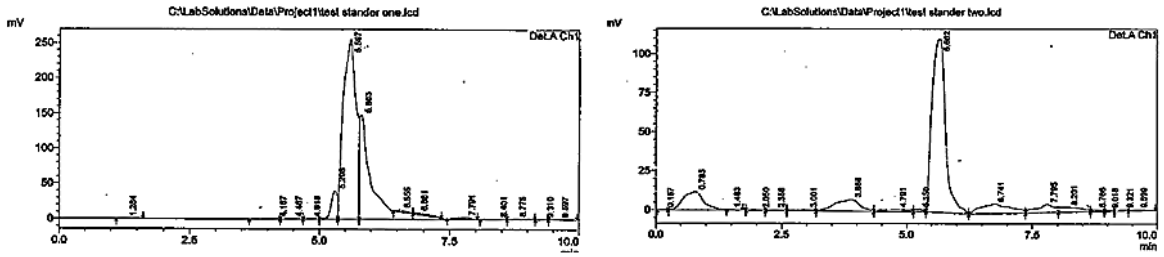


Figure 9: Chromatogram of Metformin standard at 25µL and 10µL.

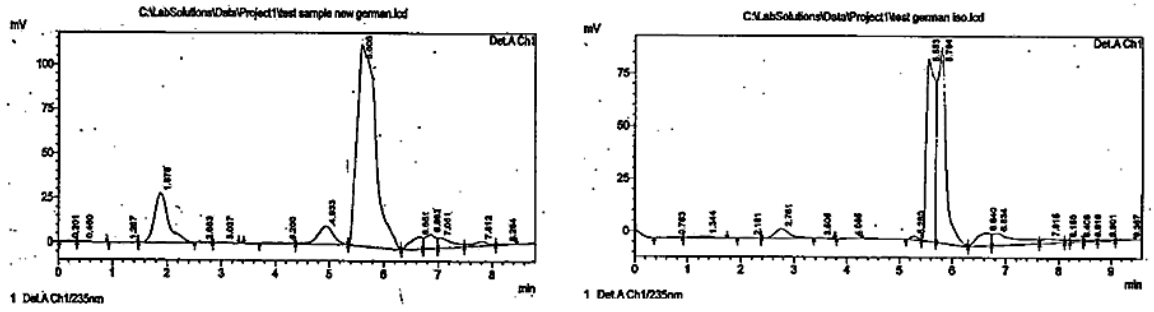


Figure 10: Chromatogram of Metformin -Germeny at 25µL before and after isotherm process.

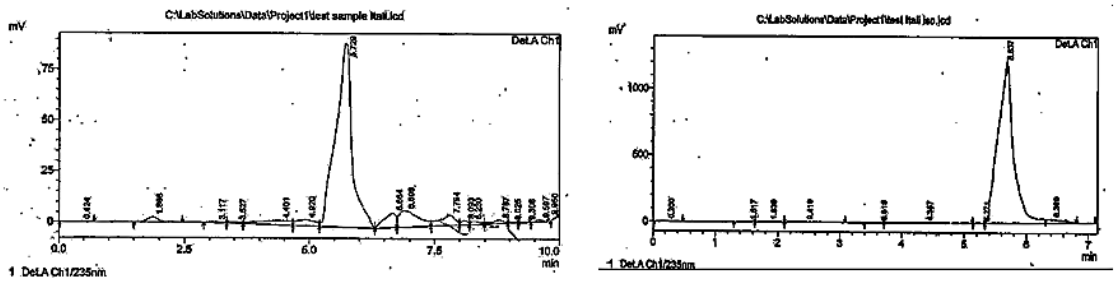


Figure11: Chromatogram of Metformin- Italy at 25µL before and after isotherm process.

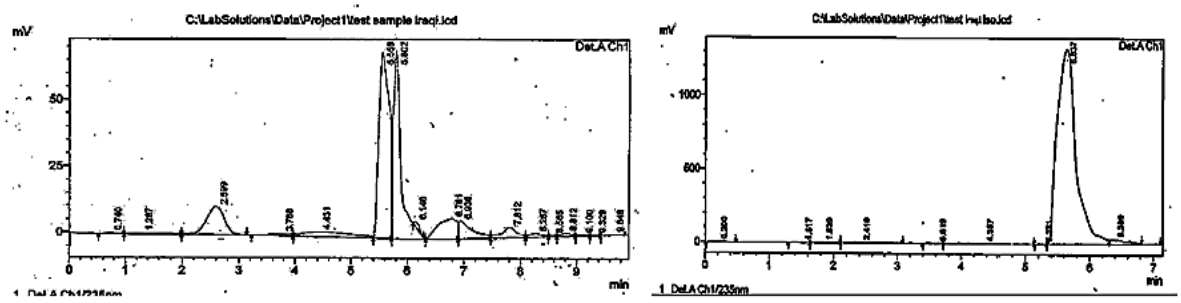


Figure12: Chromatogram of Metformin -Iraqi at 25µL before and after isotherm process.

In Figures 10, 11 and 12, the solutions at a concentration of 0.1µmol/mL for pharmaceutical drugs [Metformin-Germany (Glucophage), Metformin-Italy (Metforal), Metformin-Iraqi (Piophage)] were prepared and 25 µL was injected three times and a chromatogram was recorded, respectively. The mean retention time for Metformin was 6 minutes. A chromatogram was recorded for each solution after filtration through a 0.2 µm membrane filter. When we measure the area of the peak according to the Metformin standard peak and calculate the concentration of these drugs after the isotherm process, the effect of the

isotherm process before and after passing through the MIP-SPE column appears clear, as shown in Table 6.

Scanning electron microscopy (SEM: Metformin):

The morphological evaluation is critical to the appreciation of the morphological traits, cavity sizes, and surface configurations of MIPs both before and following the Metformin template removal. The morphology of the Met-MIPs was examined using SEM images. Figure 13 (A and B) displays the particle surface morphologies for Metformin-MIP before and after elution as well as the relative cavity calculation. Table 3 shows the surface morphologies of the particles before and after elution for Metformin-MIP and the relative cavity calculation.

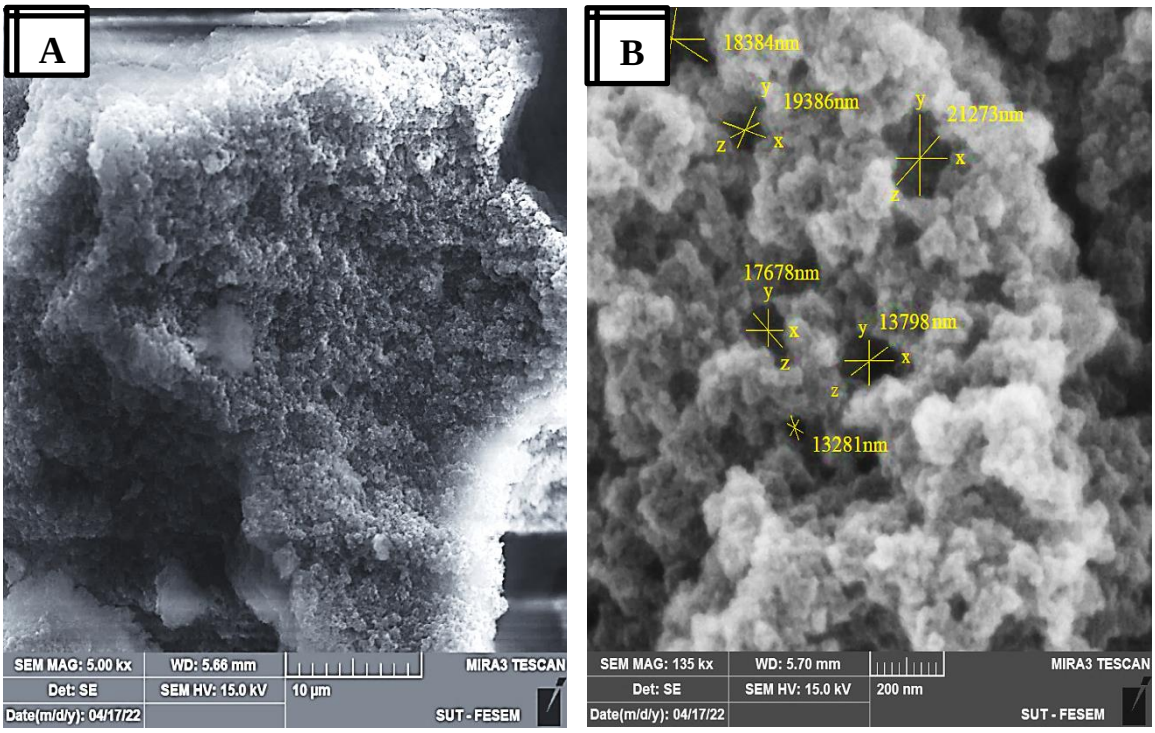


Figure 13: A and B, the surface morphologies of the particles before and after elution for Met-MIP espectively, and three dimensions of cavities with their mean.

Table 3: Calculated mean, angle, lengths of some cavities (selected six of them) and their areas using image j program

Cavities	Area	Mean (nm)	Min-Max	Angle	Length (nm)
1	188.198	13798.69	11033.45 - 17988.02	180	92.075
2	266.614	21273.02	17422.24 -28433.00	1.71	132.726
3	152.911	17678.64	16564.8 - 19310.22	0.503	75.247
4	184.278	19386.1	16620.84 - 22456.25	-6.661	91.039
5	82.337	13281.36	12859.88 - 13963.72	179.045	39.608
6	380.318	18384.56	14021.88 - 20615.71	177.614	190.255
Total Mean	209.109	17300.4	14753.86 - 20461.15	88.702	103.491
SD	102.862	3158.004	2523.887- 4841.741	98.837	52.052
Total Min - Max	82.337 - 380.318	13281.36 - 21273.02	11033.45 - 28433	-6.661-180	39.608 - 190.255

From Figure 13 and Table 3, the 3D of cavities between the minimum mean area (13281.36 nm) and maximum mean area (21273.02 nm), it was noticed that the holes vary in diameter range between 13281.36 and 21273.02 nm, and most of the holes are large, which leads to the

retention of large quantities of the drug, which is consistent with the high value of the capacity in isotherm.

Concentration of Metformin µmol /mL	Absorption
0.02	0.3836
0.04	0.5749
0.06	0.7889
0.08	0.9656
0.10	1.1281
0.12	1.2688
0.14	1.4575
0.16	1.6705
0.18	1.8855
0.20	1.9987

Relation between initial concentration and capacity

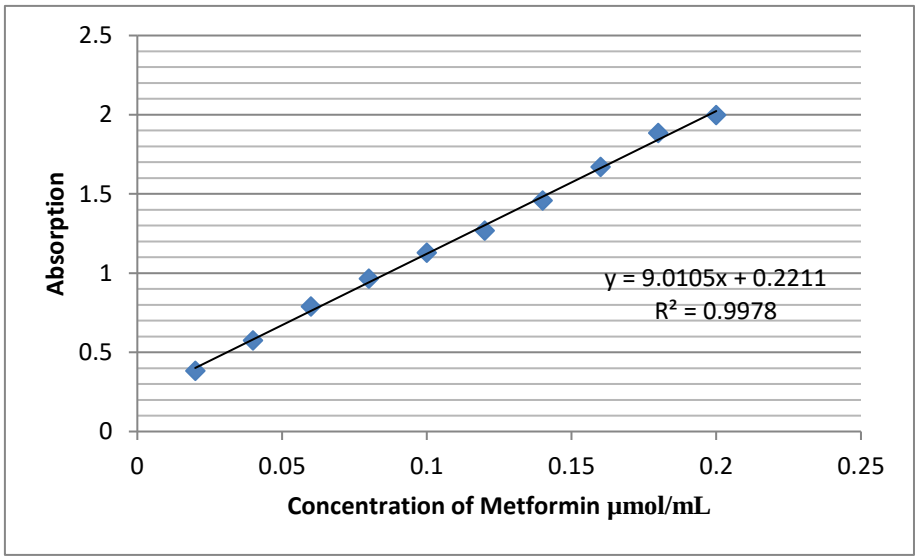


Figure 14: Calibration curve between concentrations of Metformin standard µmol/mL and its absorptions.

Adsorption capacity and pre-concentration:A series of absorption achievements for different initial concentrations of Met-MIP ranging from 0.02 to 0.2 µmol/mL on adsorption capacity µmol/g was studied using the following equation [20]:

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

The process of attaining a high local concentration at the sensor surface is referred to as pre- concentration.*1 The concentrations from 0.02 to 0.08 µmol/mL consume volumes of 27-15 mL, while the concentrations between 0.1 and 0.2 µmol/mL consume 4-3 mL only when using Met-MIP (100 mg), as shown in Table 4.

Table 4: The optimal synthesis conditions for the molecularly imprinted polymer for Metformin developed in this study

W/ MI (g)	Ci (ppm)	Ci (µmol/mL)	Cf (µmol/mL)	Vol (mL)
0.1	3.3124	0.02	0.0148	27
	6.6248	0.04	0.0319	26
	9.9372	0.06	0.0476	22
	13.2496	0.08	0.0583	15
	16.5620	0.10	0.0741	4
	19.8744	0.12	0.0820	3
	23.1868	0.14	0.1001	3
	26.4992	0.16	0.1160	3
	29.8116	0.18	0.1329	3
	33.1240	0.20	0.1510	3

The relation between initial concentration Ci (µmol/mL) and capacity Q (µmol/g)

Ci initial concentration (µmol/mL)	**Q capacity (µmol/g)**
0.02	1.404
0.04	2.106
0.06	2.728
0.08	3.255
0.1	1.036
0.12	1.140
0.14	1.197
0.16	1.320
0.18	1.413
0.2	1.47

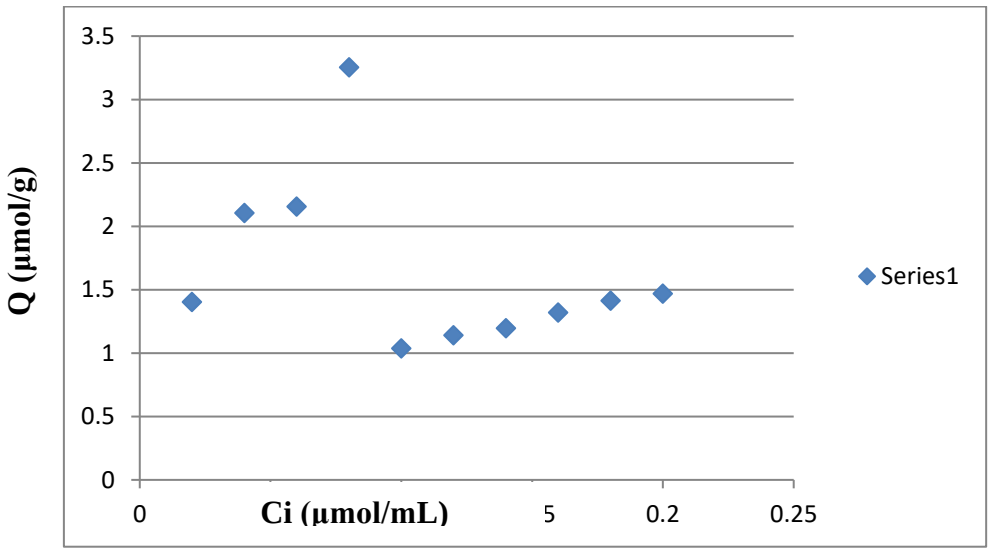


Figure 15: Illustrate Freundlich isotherm model of adsorption equilibrium

The relation between capacity Q (µmol/g) and Q/Cf (mL/g):

Q µmol/g	**Q/Cf mL/g**
1.404	94.865
2.106	66.018
2.156	57.311
3.255	55.832
1.036	13.981
1.140	13.902
1.197	11.958
1.320	11.379
1.413	10.632
1.470	9.735

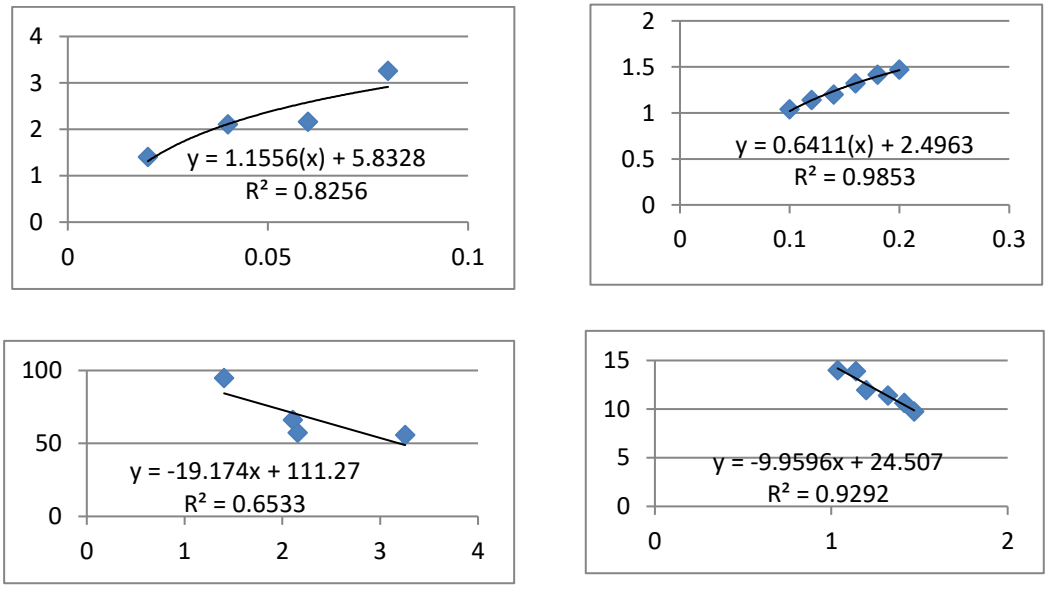


Figure 16: Shows the two slopes due to Freundlich isotherm model

Slope = -1/kd
-19.174= -1/ kd
Kd= 0.0522
Intersept = 111.27
Intersept = Qmax/ kd
Qmax =111.27 * 0.0522
= 5.808 µmol/g

Slope = -1/kd
-9.9596= -1/ kd
Kd= 0.100
Intersept= 24.507
Intersept= Qmax/ kd
Qmax=24.507*0.1
= 2.461 µmol/g

As a result, Met-Mip has two capacities, with a range of 5.808 to 2.461 mol/g. It follows the Freundlich isotherm model, which has scattered values and two slopes.

Table 5: Precision and accuracy of the analysis of pharmaceutical drugs in the UV-VIS spectrophotometer before and after the isotherm process

Drug name 500mg	MIP	Concentration Ci (µmol/mL)	Absorption before isotherm process	Absorption after isotherm process	Concentration Cf (µmol/mL)	Vol (mL)	Q (µmol/g)	RSD% = (δn-1/Mean) *100 Precision	Rec. % = (practical value/True value)*100 Accuracy	Re% = 100-Rec
Metforal/Germany	MIP 0.1 g	0.10	1.1352	0.5936	0.0523	6	2.862	0.1620	100.62	0.62
		0.12	1.2693	0.6940	0.0656	6	3.264	0.1570	100.04	0.04
Glucophage/Italy		0.10	1.1381	0.6794	0.0597	8	3.224	0.1820	100.01	0.01
		0.12	1.2886	0.7547	0.0703	7	3.479	0.1650	101.56	1.56
Piophage/Iraq		0.10	1.0803	0.6352	0.0588	7	2.884	0.1610	95.76	-4.24
		0.12	1.2079	0.6770	0.0672	7	3.696	0.1450	95.20	-4.80

* For n = 5 absorptions of drugs before isotherm process (passing through MIP column), *

The true value is the absorption at 0.1,0.12 µmol/mL in calibration curve of Metformin. In Tables 5 and 6, the volumes that pass through the MIP column for pharmaceutical drugs consume milliliters more than standard due to interferences, additions, and active materials used in the manufacture of drugs.

Table 6: Quantification of Metformin in pharmaceutical drugs by the RP-HPLC method before and after the isotherm process

Drug name 500mg	**Concentration Ci (µmol/mL) from peak area of RP- HPLC before passing through MIP column**	**Concentration Cf (µmol/mL) from peak area of RP- HPLC after passing through MIP column**	**Vol (mL) consuming in MIP**	**Capacity Q (µmol/g) Q = (Ci-Cf)(µmol/mL)*(vol (mL))/(Wof MIP(g))**
Metforal/ Germany	0.0734	0.0270	6	2.7816
Glucophage/ Italy	0.0754	0.0366	8	3.1040
Piophage/ Iraq	0.0682	0.0245	7	3.0590

*****The concentration of Metformin standard 0.1 µmol/mL

To compare the capacity of Metformin selectivity between two analytical techniques by RP HPLC and our method of MIP determination of Metformin in pharmaceutical drugs (Table 7).

Table 7: Compare the capacity between two analytical techniques for MIP by RP-HPLC and our method MIP by UV determination of Metformin drug

Drug name (500mg)	**Capacity Q (µmol/g) for MIP-solid phase by RP-HPLC technique**	**Capacity Q (µmol/g) for MIP-solid phase by UV technique**
Metforal/ Germany	2.7816	2.8620
Glucophage/ Italy	3.1040	3.2240
Piophage/ Iraq	3.0590	2.8840

*At concentration 0.1 µmol/mL.

3. Conclusion

The monomer 2-acrylamido-2-methyl-1-propane sulphonic acid (AMPS) $C_7H_{13}NO_4S$ and the crosslinker *N*,*N*-methylene bis-acrylamide $C_7H_{10}N_2O_2$ as Met-MIP were used to generate a novel bulk polymer. Numerous studies and experiments were conducted to achieve the selective molecular imprinted polymer. This was achieved by preparing and optimizing the required monomers and cross-linkers using suitable solvents, porogen solvents for template removal, and the optimal molar ratios of template (Metformin) to monomer to cross-linker. SEM reveals an irregular three-dimensional network structure of polymer before and after template removal; FT-IR, HPLC, and isotherm processing all improve the healthy work. Two slops are gained when studying the capacity of adsorption of Met-MIP, which follows the Freundlich isotherm model with scatter values (heterogeneous structure) and the ratio of template to monomer is 1:2. The maximum adsorption capacity of Met-MIP was 5.2998 µmol/g and when comparing the capacity between two analytical techniques by RP-HPLC and our method MIP by UV for Metformin drugs, there was no significant difference between the two methods.

References

[1] M. S. Sinha, "Metformin is a biguanide antihyperglycemic agent Metformin Hydrochloride", *The American Society of Health-System Pharmacists*. Last updated 2021.

[2] D. G. Rena, H. Grahame, and R. Ewan,"The mechanisms of action of metformin", *Springer Diabetologia*, vol. 60, no.9, pp.1577-1585, 2017.

[3] S. A. Ismaeel and Y. K. Al-Bayati,"Determination of trace metformin in pharmaceutical preparation using molecularly imprinted polymer based pvc-membrane", *Eurasian Chemical communications*, vol. 3, no.11, pp. 812-830, 2022.

[4] C. Sirtori, G. Franceschini, M. Galli-Kienle, G. Cighetti, G. Galli, A. Bondioli, and F. Conti,"Disposition of metformin (*N*,*N*-dimethylbiguanide) in man", *Clinical Pharmacology and Therapeutics*, vol. 24, no.6, pp. 683-693, 1978.

[5] C. Alvarez-Lorenzo and A. Concheiro, "Handbook of Molecularly Imprinted Polymer", *Smithers Rapra Technology*, Ltd. UK, vol. 211, pp. 1-41, 2013.

[6] Y. K. Al-Bayati and M. Farhan Abd, "Determination of methamphetamine drug by GC-MS based on molecularly imprinted solid-phase used meth acrylic acid and acryl amide as functional monomers", *Iraqi Journal of Science*, vol.58, no.4B, pp. 2022-2034, 2017.

[7] Y. K. Al-Bayati "Preparation of Selective Sensors for Cyproheptadine Hydrochloride based on Molecularly Imprinted Polymer used N, N-Diethylaminoethyl Methacrylate asFunctional Monomer," Eurasian Journal of Analytical Chemistry, vol. 13, no. 5, pp. 1-10, 2018.

[8] S. A. Jaber and Y. K. Al-Bayati, "Synthesis of the new Naproxen selective electrode based on impinted polymer using different monomers and its determination at pharmaceutical preparation", *Iraqi journal of market research and consumer protection*, vol.12, no. 2, pp. 1-11, 2020.

[9] Y. K. Al-Bayati and I. H. Al Khafaji, "Potentiometric Determination of Mebeverine Hydrochloride Using Imprinted Molecular Polymer in PVC Matrix Membrane", *Iraqi Journal of Science*, vol. 57, no.4c, pp. 2790-2799, 2016.

[10] L. I. Andersson, "Application of molecular imprinting to the development of aqueous buffer and organic solvent based radioligand binding assays for (*S*)-propranolol". *Anaytical Chemistry*, vol. 68, no. 1, pp.111, 1996.

[11] Tauha, "Molecularly Imprinted Polymer Molecule Molecular Imprinting Molecular Configuration PNG (Portable Graphics Format) PNG image on June 11, 2018.

[12] M. Sha'at, A. Florin Spac, I. Stoleriu, A. Bujor, M. Stamate Cretan, M. Hartan, and L. Ochiuz, "Implementation of QbD Approach to the Analytical Method Development and Validation for the Estimation of Metformin-Hydrochloride in Tablet Dosage Forms by HPLC". *Pharmaceutics*, vol.14, no.6, pp. 2-22, 2022.

[13] M. A. Abdelgawad, M. Elmowafy, A. Musa, M. M. Al-Sanea, A. A. Nayl, M. M. Ghoneim ,Y. M. Ahmed, H. M. Hassan, A. M. AboulMagd and H. F. Salem, "Development and Greenness Assessment of HPLC Method for Studying the Pharmacokinetics of Co-Administered Metformin and Papaya Extract". *Molecules*, vol. 27, no.375, pp. 1-14, 2022.

[14] H. H. Lim, Y. S. Oh, and H. S. Shin, "Determination of *N*-nitrosodimethylamine and N-nitrosomethylethylamine in drug substances and products of sartans, metformin and ranitidine by precipitation and solid phase extraction and gas chromatography–tandem mass spectrometry", *Journal of Pharmaceutical and Biomedical Analysis*, vol.189, no.10, pp. 113460, 2020.

[15] S. Knoll, S. Jacob, Susanna Mieck and R. Triebskorn, "Thomas Braunbeck and Carolin Huhn, Development of a capillary electrophoresis–mass spectrometry method for the analysis of metformin and its transformation product guanylurea in biota" *Analytical and Bioanalytical Chemistry*, vol. 412, pp. 4985-4996, 2020.

[16] A. J. Al-Safi and Y. K. Al-Bayati, "Synthesis and Characterization of molecularly imprinted Polymer for Tramadol HCL Using Acryl Amide And 2-Hydroxyethyl Meth Acrylate AS Monomers," Current Issues in Pharmacy and Medical Sciences, vol. 31, no. 2, pp. 81-88, 2018.

[17] H. Yu Z. Chen, Y. Fu, L. Kang, M. Wang, and X. Du, "Synthesis and optimization of molecularly imprinted polymers for quercetin", *Polymer International*, vol. 61, no. 6, pp. 1002-1009, 2012.

[18] M. Nakamura, M. Matsubara, T. Taguchi, and K. Takayama ,"Development of a rapid method using solid-phase extraction in the acid hydrolysis method of lipid analysis" *Nippon Shokuhin Kagaku Kogaku Kaishi* , vol. 69, no. 2, pp. 55-60, 2022.

[19] Morrison and Boyd, 2010, *Organic chemistry*, 7th Ed., Pearson Education India, p. 1508.

[20] Y. J. Huang, R. Chang, and Q. J. Zhu, "Synthesis and Characterization of a Molecularly Imprinted Polymer of Spermidine and the Exploration of its molecular recognition properties", *Polymers*, vol. 10, no. 12, pp.1389-1399, 2018.