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Research Article

EFFECT OF MONOMER ON THE SYNTHESIS OF MOLECULARLY IMPRINTED POLYMERS FOR QUERCETIN

Nitu Trehan, Supreet Kaur,Bhavkiranbir Kaur, Gaganpreet Kaur
Biotechnology Department, Mata Gujri College, Fatehgarh Sahib-140406, India

Corresponding Author: Nitu Trehan,, **Email:** nitutrehan@yahoo.com

Abstract: In this paper, the effect of change of monomer on the synthesis of molecularly imprinted polymers prepared by bulk polymerization is described. Polymers were prepared under optimized polymerization conditions by changing the monomer using quercetin as a template molecule and EGDMA as a cross linker. The synthesized polymers were characterized by HPLC analysis for their binding capacity for the template and template analogue molecule rutin. The imprinting factor was calculated by calculating the binding capacity of MIPs and NMIPs for the template molecule. It was concluded that the MIPs prepared by using Itaconic acid as a functional monomer are better than acrylamide MIPs

Key**words:** molecularly imprinted polymers, bulk polymerization, HPLC, quercetin, rutin

1.INTRODUCTION

Molecularly imprinted polymers (MIPs) are the polymeric matrices that act as a molecular recognition element that can mimic like the natural recognition entities such as antibodies, biological receptor and also able to identify and separate the various types of samples. They are able to recognize both biological and chemical molecules such as amino acid, protein, drugs and pollutant component etc. The design of synthetic material, which are capable of acting like the other recognition system such as sensors has become an important area of research in recent years because of its application in separation science, purification and chemical sensors. Molecular Imprinting Technology (MIT) is used for the synthesis of molecularly imprinted polymer. This technology is based on the formation of complex between the template and the monomer. In the presence of cross-linking agent a three dimensional polymer network is formed. After the polymerization process, from the polymer the template is removed which then leaves the specific recognition site which is complementary in shape, size etc. to the template removed. Intermolecular interactions like hydrogen bonds and ionic interactions between the template molecule and functional groups present in the polymer matrix drive the molecular recognition phenomenon. Thus, the resultant polymer recognizes and binds selectively only the template molecules [1]. Molecularly imprinted polymers (MIPs) are the polymer that performs the function similar to that of sensor recognition. Over the last decades, molecularly imprinted polymer (MIPs) gained more importance due to their application in analytical chemistry and separation science. These imprinted polymers are the solid material that can be synthesized through a molecule imprinting process in which the target compound known as template is present during the process of polymerization. The template molecule is imprinted into the polymer through the process of polymerization. After the removal of template, the polymer so formed is known as molecular imprinted polymer (MIPs) which contains the specific binding site which is similar in shape and functional group to that of the template molecule. Due the presence of specific binding site in MIPs they show specific selectivity for the template or the other molecule which are similar to the template molecule [2]. The molecularly imprinted polymers are the artificial receptor for recognition of molecule which is similar to template molecule. The relation between template and imprinted cavity is similar to that of LOCK AND KEY mechanism proposed by EMIL FISCHER which explains the enzyme – substrate relationship [3].

The process of synthesis require good understanding of chemical equilibrium, molecular recognition theory, polymer chemistry for achieving high level of molecular recognition. The rigid structure of polymer is responsible for maintaining the structure of cavity after removing the template. The functional monomer establish complementary interaction with the template. The template-monomer ratio effects the imprinting process. The functional monomer are responsible for binding interaction in molecular recognition technology. The cross-linker provides mechanical strength to polymer and it maintains the stability of recognition sites. In formation of porous structure of MIP, porogenic solvent plays an important role. It also solubilises all the component before polymerization [4].

The main advantages of molecularly imprinted polymers (MIPs) are their high selectivity and affinity for the target molecule used in the imprinting procedure. Imprinted polymers compared to biological systems such as proteins and nucleic acids, have higher physical robustness, strength, resistance to elevated temperature and pressure and inertness towards acids, bases, metal ions and organic solvents. In addition, they are also less expensive to be synthesized and the storage life of the polymers can be very high, keeping their recognition capacity also for several years at room temperature. A bulk of research has focused

on different method for development of imprinted polymer, this research allows the expand of imprinting technology into various scientific area such as Nanotechnology, Biotechnology etc. The concept of molecular imprinting technology has been widely used as the promising methodology for preparation of different type of polymer having different selective binding cavities. Major advantages of MIPs other than possessing antibody-like molecular selectivity, include resistance to temperature, pressure as well as low production and ease of preparation [5]. The synthesis of polymer require a good understanding of chemical equilibrium, molecular recognition theory, thermodynamics and polymer chemistry in order to determine the high level of molecular recognition. MIPs can be prepared by many methods but the conventional method is to prepare the MIPs in bulk polymerization. After grinding the so formed polymer are passed through the sieve of desired size. Although this method is simple and easy to perform but grinding often produce the particle of irregular in shape. MIPs show very good thermal and chemical stability over the repeated operation without the loss of activity, high mechanical strength. Because of these advantages MIPs has been widely used as a stationary phase in affinity and column chromatography [6].

2. EXPERIMENTAL SECTION

Materials Required

Acrylamide, Itaconic Acid, Ethylene Glycol Dimethacrylate (EGDMA), Azobisisobutyronitrile (AIBN), quercetin, rutin from Sigma and methanol (HPLC grade) and syringe filters (0.2 µm).

2.1Preparation of polymers by changing the functional monomer

The MIP were prepared by bulk polymerization method template quercetin (0.13g), monomer acrylamide (0.2g) during the synthesis of MIP1 and monomer itaconic acid (0.364g) during the synthesis of MIP2 were dissolved in methanol (5ml) in a thick – walled glass tube as described in table no.1. The solution was sonicated, cross-linker EGDMA (3.5 ml) and initator AIBN(10 mg) were dissolved in the previous mixture. The obtained solution was again sonicated for 10 min. The mixture was then incubated in preheated water bath at 60^{o} C for 24 hours. The resultant bulk rigid polymer were crushed , ground into powder and sieved through a 63 µm pore sized stainless steel sieve. These imprinted polymer were than washed with methanol NMIPs were prepared with similar procedure in the absence of template quercetin [7].

Table No. 1: MIPs preparation by changing the monomer

S.No.	Polymer	(Quercetin)	Monomer gm	Crosslinker (EGDMA) ml	Initiator (AIN) gm	Solvent (Methanol) ml
1.	MIP1	0.13	0.2	3.5	0.01	5
2.	MIP 2	0.13	0.364	3.5	0.01	5

2.2 Binding Studies

2.2.1 Template molecule binding analysis:

The binding analysis of the prepared and washed MIPs was studied by HPLC analysis. In this process 100 mg of each washed MIP sample is taken in packed column separately. 100 ppm of quercetin was prepared in methanol and 1ml of this prepared solvent is allowed to pass through the packed column .Elution was collected in test tube. Now this elution sample was load for HPLC analysis for to check the absorption concentration.

2.2.2 Template analogue molecule (rutin) binding analysis:

To predict the binding capacity of MIPs and NMIPs an experiment was carried out with the help of HPLC. Column was prepared by tightly packing of syringe with glass wool. 100mg MIP was taken in a column and then 100 ppm of Rutin was loaded and then it was tested for binding along with the control polymer NMIP. Various polymers were then screened for their binding capacity using HPLC.

2.2.3Calculation of binding Capacity (Q):

Binding Capacity (Q) was defined as mg of substrate bound per gram of microspheres and it is calculated by the change of quercetin concentration after and before adsorption by the polymers.

The binding capacity (Q) can be calculated by the equation:

$$\mathbf{Q= (C_o – C) * V / W}$$

In the above equation, C_o is the initial concentration and C is the free concentration of quercetin in supernatant respectively. Here V ml is the volume of adsorption and W represents the mass of microspheres in grams.

Imprinting Factor (IF):

After calculating the binding capacity (Q), the imprinting factor can be calculated by dividing the binding capacity of MIPs by the binding capacity of NMIPs as:

IF = QMIPs / QNMIPs

3. RESULTS AND DISCUSSION:

3.1Standardization of Template:

A stock solution of quercetin (1000ppm) was prepared by dissolving 10 mg quercetin in 10ml of methanol and then from the stock solution 100ppm of standard solution was prepared. About 20 µL of standard was loaded in the HPLC column. The wavelength for maximum absorption of quercetin is 365 nm and the flow rate was maintained at 0.5ml / min. At this wavelength, the retention time and peak area was observed as given in table no.2 and shown in figure 1 that is the HPLC chromatogram of quercetin.

Table No. 2: HPLC of Quercetin Standard

S. No.	Template	Retention time	Peak area	Conc. of Quercetin Insupernatant
1.	Quercetin	16.730	14427658	100ppm

3.2 Binding experiment:

3.2.1Quercetin binding analysis of MIPs and NMIPs having different monomer:

Table No. 3: Quercetin binding analysis of MIPs having different monomer

S.No.	Monomer	Retention Time	Peak area	Conc. of quercetin in supernatant	Binding capacity (Q)
1.	Acrylamide	19.189	11862048	820 µg/g	1.8
2.	Itaconic Acid	16.566	8370266	580 µg/g	4.2

From the table no. 3, it has been observed that Itaconic acid (functional monomer) MIPs are the best MIPs as they have high binding capacity for the quercetin molecule as less quercetin is eluted out in supernatant. The binding capacity of Itaconic acid and Acrylamide MIPs is 4.2 and 1.8 respectively.

Table No. 4 : Quercetin binding analysis of NMIPs having different monomer

S.No.	Monomer	Retention Time	Peak area	Conc. of quercetin in supernatant	Binding capacity (Q)
1.	Acrylamide	17.199	6356254	440µg/g	5.6
2.	Itaconic Acid	15.895	11455028	790µg/g	2.1

3.2.2Calculation of Imprinting Factor:

Table No. 5: Imprinting Factor of Polymer having different Monomer (IF)

S.No.	Polymer	QMIPs	QNMIPs	IF
1.	Itaconic Acid	4.2	2.1	2
2.	Acrylamide	1.8	5.6	0.32

From table no.5, it is concluded that the imprinting factor of itaconic acid MIPs is 2,which is higher than that of Acrylamide 0.32. The MIPs prepared using itaconic acid as fuctional monomer shows excellent binding ability because the hydrogen binding interaction between the functional groups of quercetin and itaconic acid and its imprinting factor is high thus due to this reason these are selected as the best MIPs on the basis of high imprinting factor [8] .

Standardization of structural analogue:

A stock solution of rutin (1000ppm) was prepared by dissolving 10mg rutin in 10ml of methanol and then from the stock solution 100ppm of standard solution was prepared. About 20 µL of standard was loaded in the HPLC column. The wavelength for maximum absorption of quercetin is 365 nm and the flow rate was maintained at 0.5ml / min. At this wavelength, the retention time and peak area was observed as given in table no.6 and shown in figure 4 that is the HPLC chromatogram of quercetin.

Table No. 6: HPLC of rutin standard

S.No.	Retention time	Peak area	Conc. of rutin in supernatant
1.	6.982	16606285	100ppm

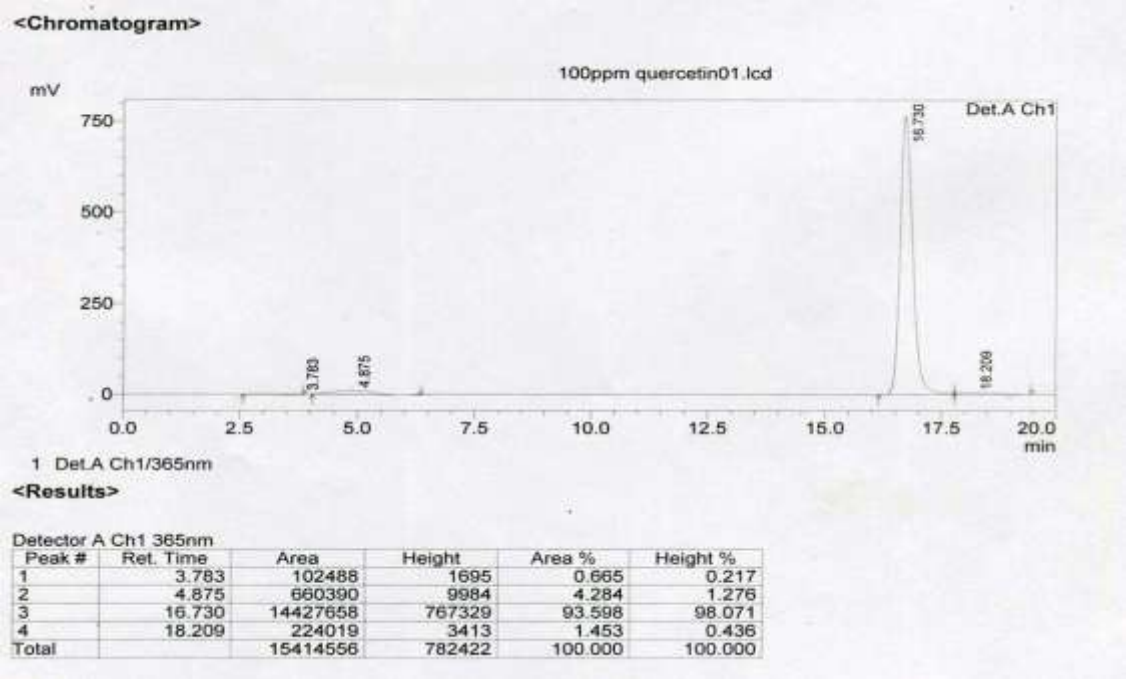
Rutin binding analysis of MIPs:

Table No. 7: Rutin binding analysis of MIPs having different monomer

S.No.	Monomer	Retention Time	Peak Area	Free Conc. of rutin in supernatant solution	Binding capacity(Q)
1.	Itaconic Acid	6.798	11762359	700 µg/g	3
2.	Acrylamide	6.352	2493449	150 µg/g	8.5

From the table no.7, it is concluded that Itaconic acid MIPs are the best MIPs as they have less binding capacity for template analogue molecule than the acrylamide MIPs for same. Thus the Itaconic acid MIPs are specific for template molecule binding.

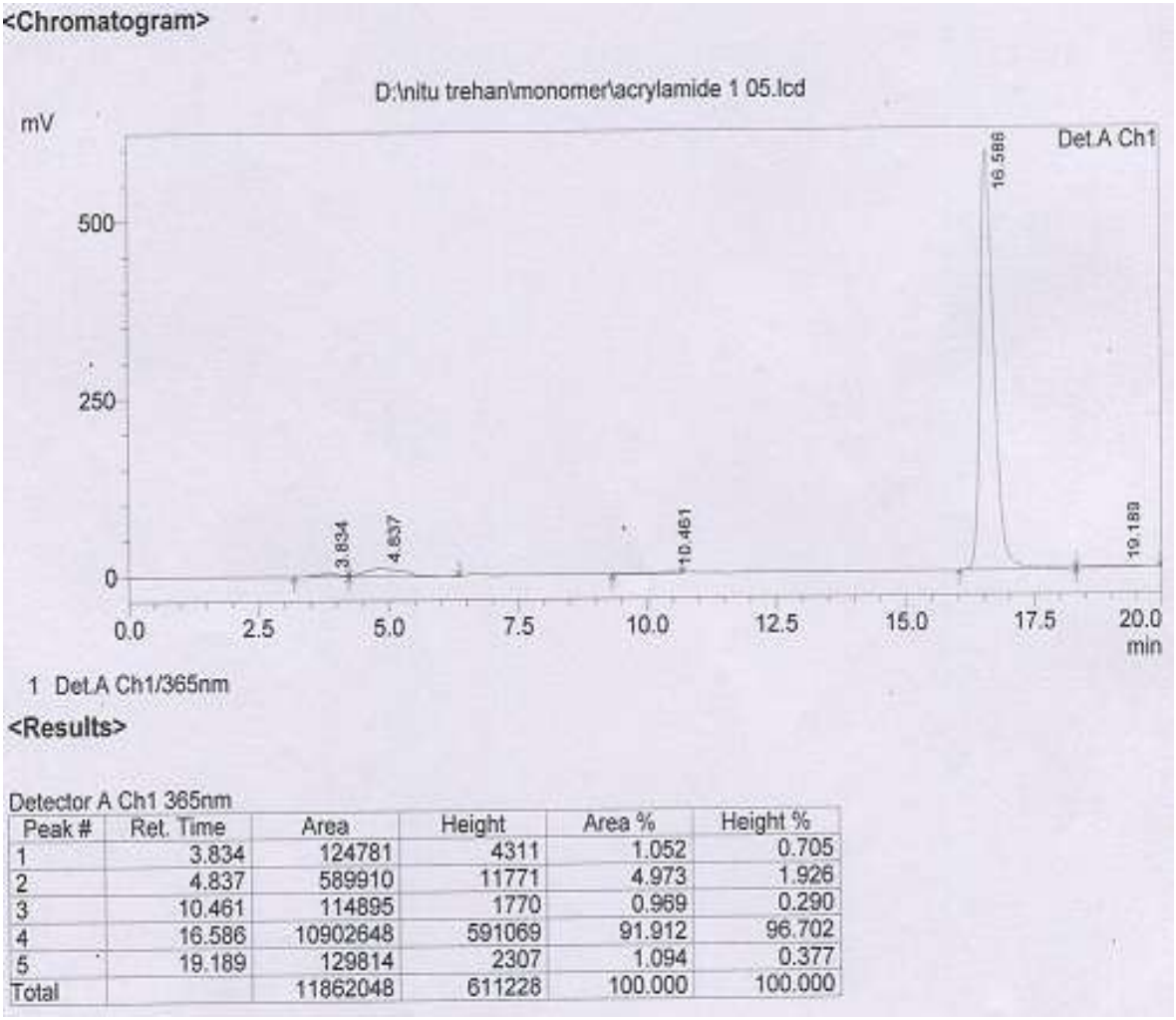
Chromatograms of rutin binding analysis of MIPs having different monomer:



Detector A Ch1 365nm

Peak #	Ret. Time	Area	Height	Area %	Height %
1	3.783	102488	1695	0.665	0.217
2	4.875	660390	9984	4.284	1.276
3	16.730	14427658	767329	93.598	98.071
4	18.209	224019	3413	1.453	0.436
Total		15414556	782422	100.000	100.000

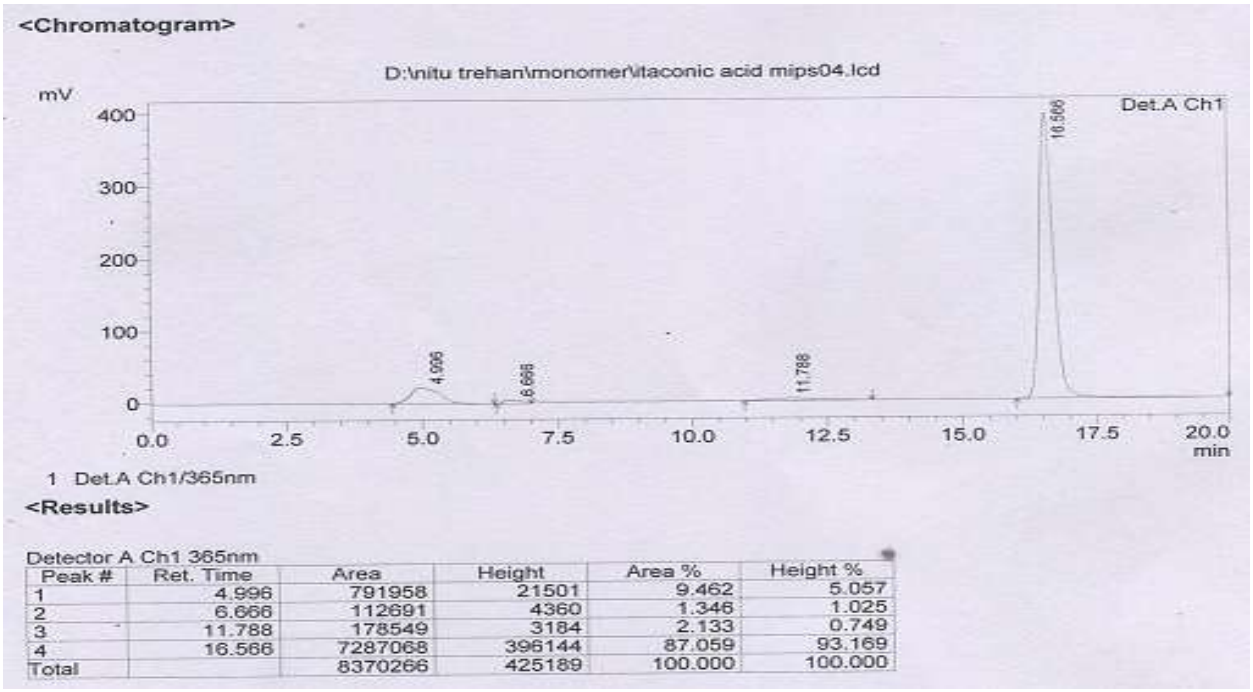
Fig.1: HPLC chromatogram of quercetin 100ppm



Detector A Ch1 365nm

Peak #	Ret. Time	Area	Height	Area %	Height %
1	3.834	124781	4311	1.052	0.705
2	4.837	589910	11771	4.973	1.926
3	10.461	114895	1770	0.969	0.290
4	16.586	10902648	591069	91.912	96.702
5	19.189	129814	2307	1.094	0.377
Total		11862048	611228	100.000	100.000

Fig. 2: HPLC Chromatogram of Acrylamide MIPs

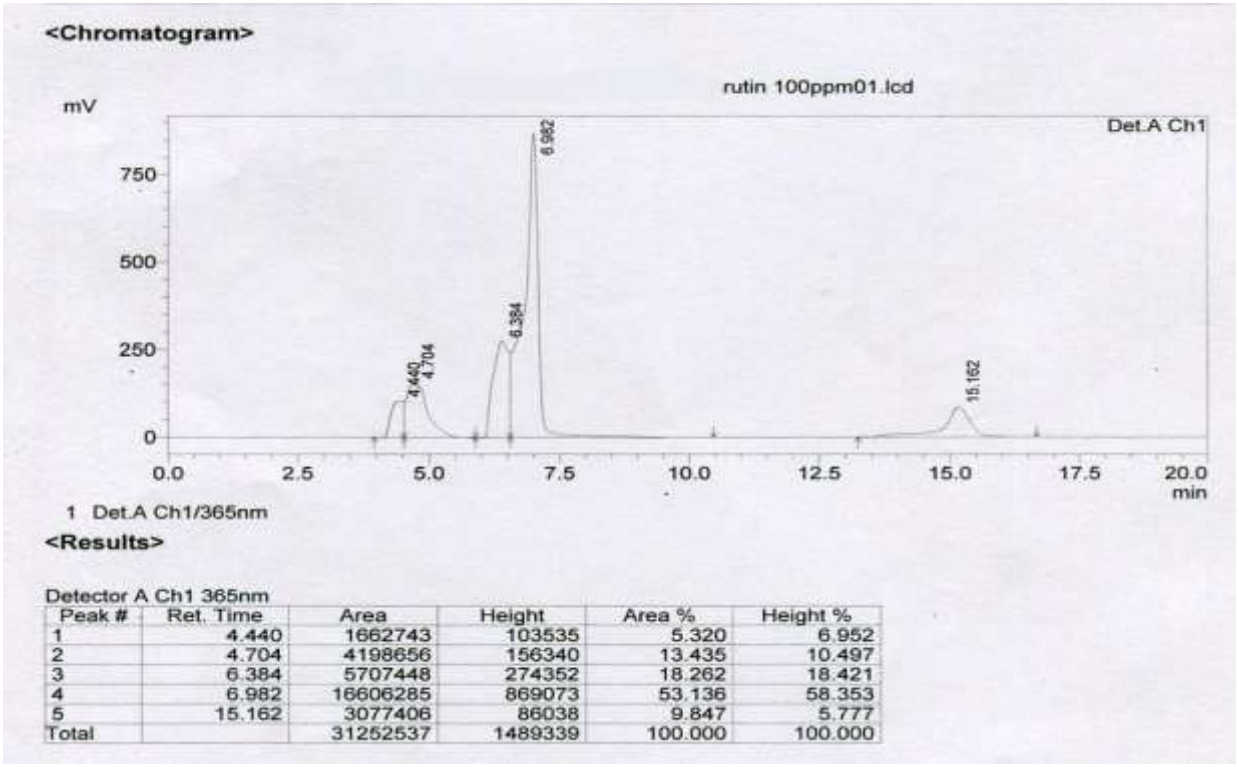


<Results>

Detector A Ch1 365nm

Peak #	Ret. Time	Area	Height	Area %	Height %
1	4.996	791958	21501	9.462	5.057
2	6.666	112691	4360	1.346	1.025
3	11.788	178549	3184	2.133	0.749
4	16.566	7287068	396144	87.059	93.169
Total		8370266	425189	100.000	100.000

Fig 3: HPLC chromatogram of itaconic acid MIPs



<Results>

Detector A Ch1 365nm

Peak #	Ret. Time	Area	Height	Area %	Height %
1	4.440	1662743	103535	5.320	6.952
2	4.704	4198656	156340	13.435	10.497
3	6.384	5707448	274352	18.262	18.421
4	6.982	16606285	869073	53.136	58.353
5	15.162	3077406	86038	9.847	5.777
Total		31252537	1489339	100.000	100.000

Fig 4: HPLC chromatogram of rutin

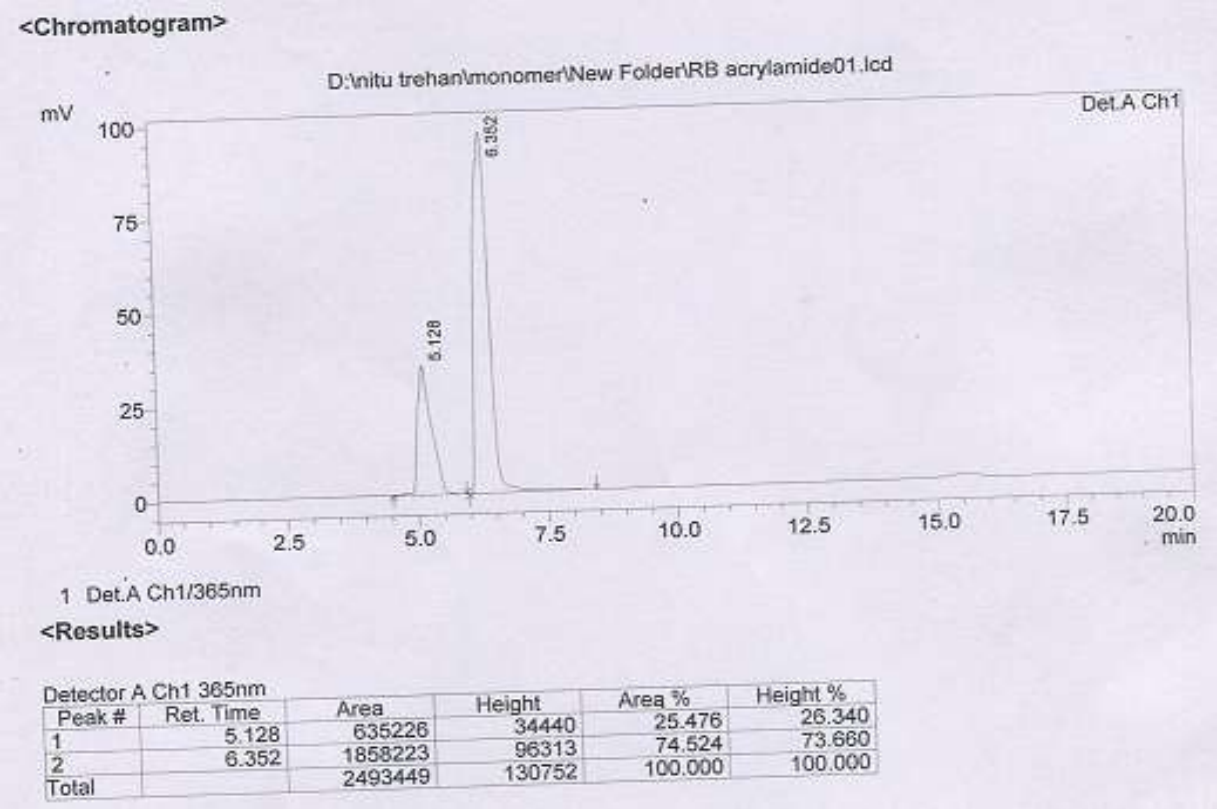


Fig 5 : HPLC chromatogram of rutin binding Acrylamide MIPs

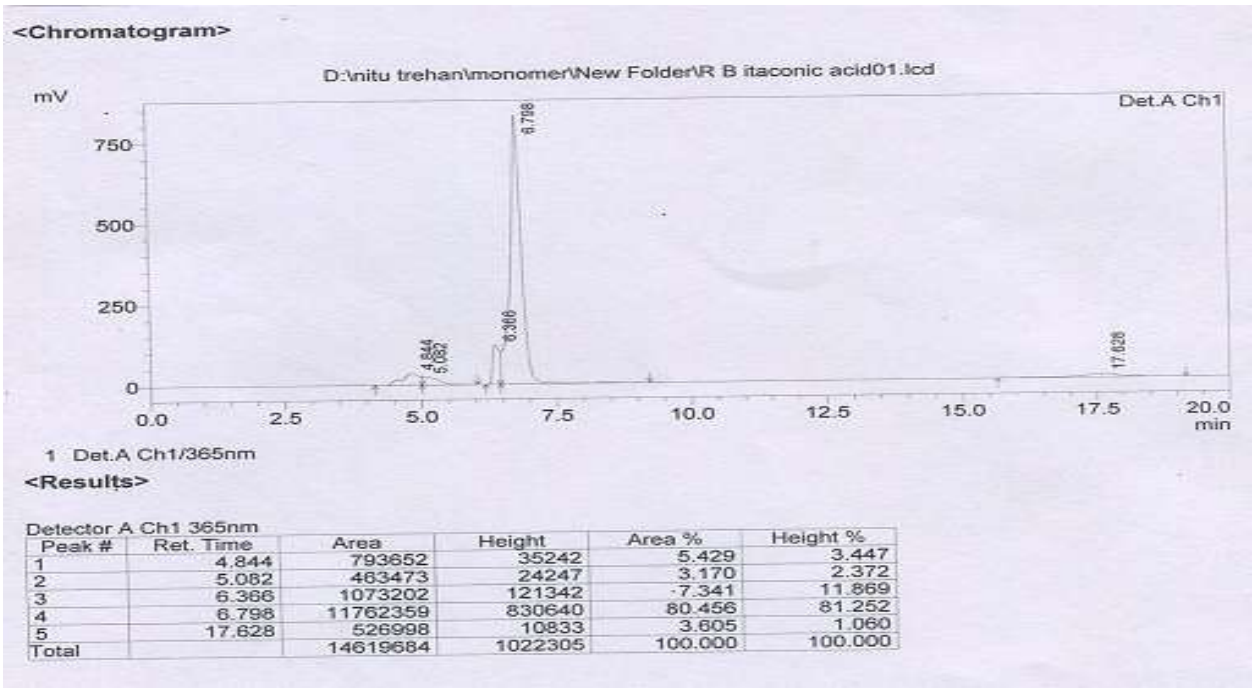


Fig 6: HPLC chromatogram of rutin binding itaconic acid

Conclusion

Molecular imprinting as a technique for the creation of artificial receptor - binding sites with a "memory" for the shape and functional group position of the template molecule and has become increasingly attractive in many fields of analytical chemistry in recent years. Here in this project work the molecularly imprinted polymer by changing the monomer were prepared and by their HPLC it is concluded that the binding capacity of MIPs is greater than the control ones (NMIPs) for the template molecule (Quercetin). Out of all, the MIPs which were synthesized using Itaconic Acid as a monomer and EGDMA as a cross-linker showed the best binding capacity and also they have high imprinting factor. But the MIPs synthesized using Itaconic Acid as a monomer and EGDMA as a cross-linker showed less binding capacity for the template analogue molecule rutin. Therefore, the resulted imprinted microspheres for quercetin have good selectivity and high binding capacity, and they are used as adsorption media, even also used as chromatographic stationary phase.

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