VINYL IMIDAZOLE BASED GALLIC ACID IMPRINTED POLYMERS

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Abstract

The aim of this study was to prepare vinyl imidazole (VIM) based gallic acid (GA) imprinted polymers and to optimize their adsorption properties. GA, that is a natural bioactive compound with anti-oxidant, anti-inflammatory, anti-cancer, and anti-viral activities, imprinted by emulsion polymerization using VIM as the functional monomer and EGDMA as the cross-linker. GA-MIPs were characterized by FTIR spectroscopy, Zeta sizer and Zeta potential analysis. GA adsorption onto GA-MIPs was optimized and results demonstrated that maximum adsorption capacity was determined as 1012.4 ± 44.9 mg GA/g MIPs in the optimum conditions (75 min using 0.9 mg GA-MIPs in H_2O at 25°C). IF value for 100 ppm was calculated higher than other concentrations and thus, selectivity studies were performed using 100 ppm GA and 100 ppm its analogue. Selectivity coefficient values showed that GA-MIPs adsorbed more GA as compared to HB and CA analogues. All relative selectivity coefficients greater than 1 has showed that GA-MIPs adsorbed more GA than its analogues as regards to GA-NIPs. Relative selectivity values for GA-CA and GA-PCA pairs were calculated high as 5.6 and 5.1, respectively. Brewed tea and clove extract were selected as real samples due to extensive use in Turkey. GA was recovered selectively with high percentages recoveries from GA-MIPs.

Keywords: *gallic acid, molecular imprinting, 1-vinylimidazole, selectivity*

1. INTRODUCTION

Molecularly imprinted polymers (MIPs) are frequently used in applications for recognition of numerous molecules [1] and these synthetic materials resemble natural receptors which bind a target molecule [2,3]. MIPs are produced in the presence of template molecule using functional monomers and cross-linker by several interactions such as covalent, non-covalent and H-bonding. Template removal after polymerization provides gaps specific for the shape and size of template. These gaps bind template/target molecule selectively [4]. Therefore, MIPs with many advantages such as resistance to chemical and mechanical conditions, thermal and storage stabilities, low cost, and repeated use [5,6] could be used in many applications for sensing, chemical synthesis, and separation/isolation. [7-8]. For the purpose of separation, MIPs are widely used as solid phase extraction material (known as molecularly imprinted solid-phase extraction) in pre-concentration and purifying processes. MIPs provide advantage for the separation/isolation of target molecules from complex matices [9-11].

GA also known as 3,4,5-trihydroxybenzoic acid of which the chemical formula is $C_6H_2(OH)_3CO_2H$ [12]. It is a natural bioactive compound classified as a polyphenolic acid and used in the pharmaceutical and biomedical industries due to its anti-oxidant, anti-inflammatory, anti-cancer, and anti-viral activities [13-15]. It is present at several fruits and fruit seeds, fruit derived food and beverages, and tea leaves [16-18]. GA has an enormous request worldwide because it has pharmaceutical and industrial importance and usage [19]. GA is generously existing in natural sources and can be extracted from several herbs and fruits. Despite this, many interferences during the selective extraction of GA are eluted along with because of the complex nature of natural materials and high polarity of GA. Therefore, development of selective and useful materials for the isolation and detection of GA is essential [20, 21].

For this purpose, MIPs are used in several studies for isolation, detection and extraction of GA. Pardeshi et al. prepared micro- and nano-particles by precipitation polymerization and used for GA extraction from *Emblica offocinalis* [21]. MIPs on mesoporous magnetic silica for selective extraction and detection of GA from fruit juices as grape, apple, peace and orange in the study of Hu et al. [22]. Hao et al. synthesized magnetic carbon nanotubes-based surface imprinted polymers and used for fast

separation and detection of GA from pomegranate rind [23]. Li and Rwo prepared hydrophilic molecularly imprinted chitosan for the enrichment of GA in red ginseng tea [24]. Badea et al. prepared reduction-oxidation microsensors and carbon sensors using differential pulse voltammetry for GA detection. Real samples such as wine, green tea, apple juice and serum fortified with GA were used for the validation of methods [25]. Inanan prepared GA imprinted polymers using acrylamide as the functional monomer and used for selective GA separation from plant samples [26].

In this study, VIM based GA imprinted polymers were synthesized by emulsion polymerization. After template removal, GA-MIPs and GA-NIPs were characterized by FTIR spectroscopy and Zeta sizer and Zeta potential analysis. GA adsorption onto GA-MIPs and GA-NIPs were optimized by testing many parameters. Selectivity experiments were performed using GA and 4-hydroxybenzoic acid (HB), caffeic acid (CA) and p-cumaric acid (4-hydroxycinnamic acid, PCA) as analogues, and analyzed by HPLC. Finally, GA-MIPs were used for GA isolation from brewed tea and clove extract.

2. MATERIALS AND METHODS

2.1. Materials

GA, polyvinyl alcohol (PVA), ammonium persulphate (APS), and methanol (MeOH) were purchased from Merck. 1-vinylimidazole (VIM) was purchased from Alfa Aesar. HBA, PCA, and ethylene glycol dimethacrylate (EGDMA) were achieved from sigma and CA were from Isolab. All other chemicals were analytical grade and distilled water were procured by Millipore S.A.S 67120 system. Zorbax SB-phenyl column used in HPLC analysis were obtained from Agilent.

2.2. Methods

2.2.1. Synthesis of GA-imprinted VIM-based polymers and template removal

Pre-polymerization complex between GA (3 mmol solved in methanol) and VIM (9 mmol) was prepared overnight at dark and then polymerized by emulsion polymerization similar in the study of Inanan et al., 2016 [27]. 0.2275 g PVA was dissolved in hot water as a stabilization solution and pre-polymerization complex was added in this solution. EGDMA (3 mmol) as cross-linker and APS (25 mg) as initiator were added into polymerization solution and sonicated in ultrasonic bath for 15 min. After that, N_2 gas was passed through the polymerization solution to remove dissolved oxygen. Polymerization reaction was carried out at 70°C and 65 rpm for 6 h. Non-imprinted polymers were prepared in a similar manner without adding GA.

Obtained GA-MIPs and GA-NIPs were sonicated in ultrasonic bath for 30 min and centrifuged at 13000 rpm for 15 min. Precipitated polymers were resuspended in water and methanol to remove un-reacted monomers and cross-linker. For template removal, GA-MIPs were washed several times with MeOH:HAc (80:20) and finally with water to remove acetic acid residue from GA-MIPs. After all washing, GA-MIPs and GA-NIPs were suspended in water at initial volume and weights of polymers were calculated before GA adsorption studies.

2.2.2. Characterization studies

UV-spectra of GA, VIM and pre-polymerization complex were scanned before polymerization. Functional groups of pre-polymerization complex, GA-MIPs, and GA-NIPs were analyzed by FTIR (Thermo Fisher Nicolet is50 FTIR). Particle size, size distribution and Zeta-potential values for GA-MIPs and GA-NIPs were determined by Zeta-sizer analysis (Zeta-Malvern Zetasizer Nano ZSP).

2.2.3. Adsorption studies

Many parameters such as adsorption medium, contact time, polymer amount, initial concentration, and temperature were identified for the optimization of GA optimization. Effect of solvent was determined by using MeOH, MeOH:H_2O mixture and universal buffers at pH 3.5, 4.0, 4.5 and 5.0 (adjusted with HCl and NaOH). Other parameters were determined as: time in the range of 10 and 120 min, polymer amount: in the range of 0.35 mg and 1.8 mg, initial concentration: in the range of 100 and 1500 ppm, and temperature: in the range of 5°C and 45°C.

At the end of all adsorption studies, adsorption media were centrifuged at 13000 rpm for 15 min and absorbance values of supernatant and initial solution were measured by UV-spectrophotometer at 270 nm. GA concentrations calculated from calibration equations and adsorption capacities according to these concentrations were calculated using equation below.

$Q = (C_i - C_{eq}) \times V / m$

Where, C_i and C_{eq} were the initial and equilibrium GA concentrations (ppm), V was the volume of adsorption solution (L) and m was the amount of polymer used (g).

2.2.4. Selectivity studies

Quantitative analysis of GA and its analogues were performed by HPLC with the method in the study of Hu et al. [22]. Chromatographic analysis of GA, HB, CA, and PCA were performed by Agilent 1290 infinity UHPLC system using Zorbax SB-phenyl (150 mm, internal diameter: 4.6 mm, 5 µm particle dimension, porous silica with 80Å pore diameter) as a stationary phase and mixture of 0.4% HAc in H_2O and 0.4% HAc in MeOH as a mobile phase. Linear gradient elution was performed such as 20-30 % B between 0 and 30 min at 0.8 mL/min flow rate. Spectrums were recorded at 260 nm for GA and HB, and at 312 nm for CA and PCA. Under this conditions, Rt values for GA, HB, CA, and PCA were 3.2, 6.7, 9.4 and 14.9 min, respectively. Selectivity coefficient (K) and relative selectivity (K') values were determined using dispersion coefficient (K_d) to determine the selectivity of GA-MIPs.

K_d (L/g)= Q (mg/g) / Ce (mg/L)

$K = K_d$ (GA)/ K_d (GA analogues)

K' = K(GA-MIP)/ K(GA-NIP)

2.2.5. GA separation from real samples with GA-MIPs

GA is found at various amounts in fruit and fruit juices, in alcoholic beverages such as wine and liqueur; in vegetables as cauliflower, eggplant, and succory; in herbs as green tea, garden sage, oregano, and clove; and some products as brewed tea, raw chestnut, and vinegar. Brewed tea and clove extract was selected as real samples due to extensive use in Turkey. Tea and clove samples were purchased from local markets in Aksaray, Turkey. 5 grams of tea was brewed with 50 mL boiling water for 5 min and used in adsorption experiments after filtration and cooling to room temperature. 5 grams of clove buds were grinded using ceramic mortar, mixed with 50 mL MeOH for 1 h and used in adsorption experiments after filtration. Adsorption experiments were performed at optimum conditions and GA concentration were analyzed with HPLC. Desorption of GA from GA-MIPs were conducted with 20 mL MeOH:HAc (80:20) and analyzed with HPLC (Agilent 1290 infinity UHPLC).

3. RESULTS

3.1. Characterization of pre-polymerization complex, GA-imprinted and non-imprinted polymers

UV spectra of GA, VIM and VIM-GA pre-polymerization complex and template removal solution were presented in Fig. 1. As seen in Fig 1., maximum absorbances were determined as 228 and 270 nm for GA and 230 nm for VIM.

Particle size and size distribution analysis showed that particle size of GA-MIPs was 2358 ± 45 nm with 0.31 ± 0.10 polydispersity index (PdI) and of GA-NIPs was 5348 ± 120 nm with 0.13 ± 0.02 PdI. PdI values vary between 0 and 1 and PdI values lower than 0.1 shows uniform-sized particle distribution [28]. Zeta potential values were measured as -3.29 ± 0.26 mV and +6.83 ± 0.53 mV for GA-MIPs and GA-NIPs, respectively. Conductivity values were measured as 0.048 ± 0.001 mS/cm and 0.049 ± 0.001 mS/cm for GA-MIPs and GA-NIPs, respectively.

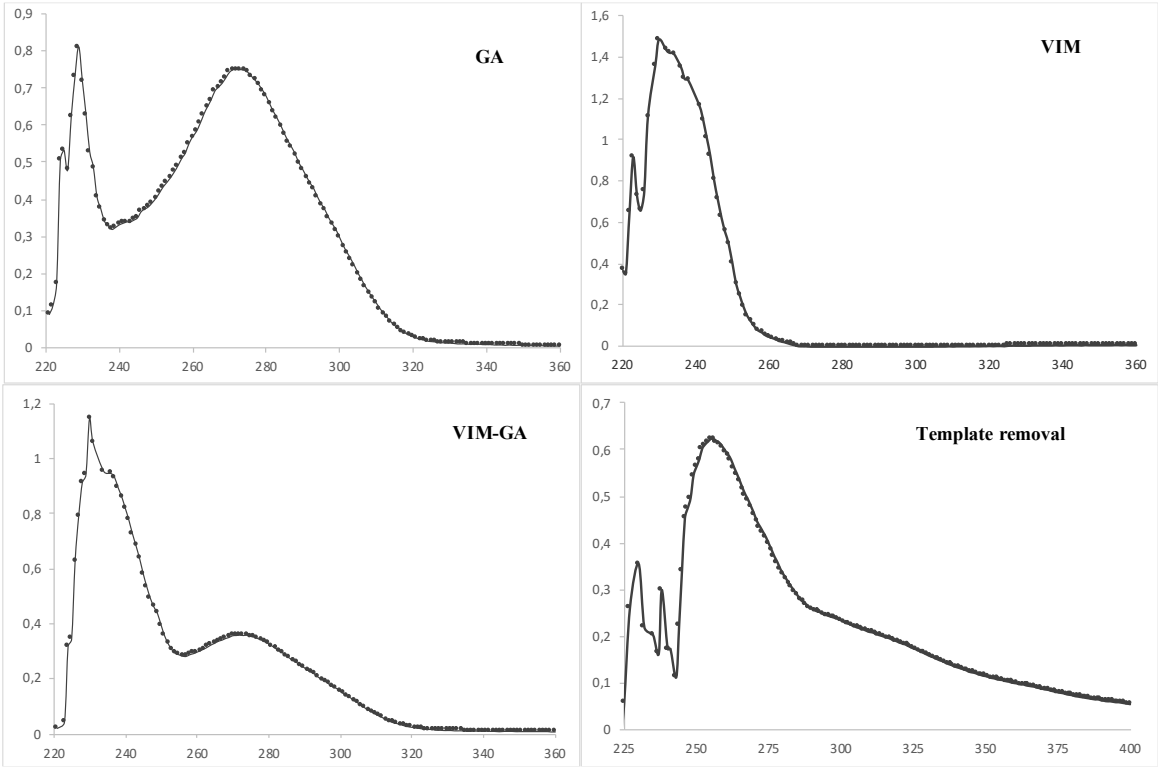


Fig. 1. UV spectra of GA, VIM and pre-polymerization complex and template removal solution

FTIR spectrum of VIM-GA pre-polymerization complex, GA-MIPs and, GA-NIPs were recorded and some fundamental FTIR frequencies were shown in Table 1.

VIM-GA pre-polymerization complex		
FTIR frequency	***Vibration type***	
3347 and 2976 cm^{-1}	H-bonded and free -OH stretching bands for GA	
2836, 2895 and 2937 cm^{-1}	-CH stretching bands CH and CH_2 groups for GA and VIM	
1651 cm^{-1} (with shoulder)	-C=O stretching band for GA and -C=N stretching band for VIM	
1501 and 1450 cm^{-1}	-CH bending and -C=C stretching bands of vinyl group for VIM	
1232 cm^{-1}	-C-N stretching band for VIM	
1046 cm^{-1}	-C-O stretching band for GA	
GA-MIPs and GA-NIPs		
FTIR frequency in GA-MIPs	***FTIR frequency in GA-NIPs***	***Vibration type***
2957 and 2991 cm^{-1}	2951 and 2900 cm^{-1}	-CH stretching bands
1720 cm^{-1}	1724 cm^{-1}	-C=O stretching band for EGDMA
1636 cm^{-1}	1636 cm^{-1}	-C=N stretching band for VIM
1454 cm^{-1} (with shoulder)	1460 cm^{-1} (with shoulder)	-CH bending bands of CH and CH_2 groups
1254 cm^{-1}	1269 cm^{-1}	-C-N stretching band for VIM
1149 cm^{-1}	1149 cm^{-1}	-C-O stretching band for EGDMA

Table 1. FTIR frequencies for VIM-GA pre-polymerization complex, GA-MIPs and, GA-NIPs

3.2. Optimization of GA adsorption onto GA-MIPs

Effect of adsorption medium on GA adsorption onto GA-MIPs was determined and results were represented in Fig. 2. As seen in Fig. 2, maximum adsorption was identified in H_2O (pH 3.5 without adjusting) as 1057.6 ± 101.4 mg GA/g MIPs. Imprinting factor (IF), one of indicator value for the selectivity of MIPs, expresses the ratio of Q(MIPs) to Q(NIPs). IF values for MeOH and H_2O solvents were calculated as 1.31 and IF values decreased with increasing pH values (Table 2).

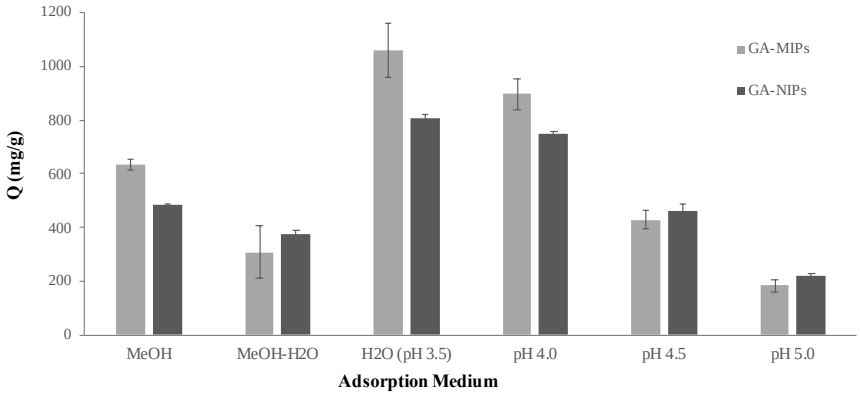


Fig. 2. Effect of adsorption medium on GA adsorption (1000 ppm GA, 25°C, 90 min)

Effect of contact time on GA adsorption was investigated in the range of 10 and 120 min and results were presented in Fig 3a. Amount of adsorbed GA increased by time till 75 min and then did not change significantly. All other adsorption studies were performed for 75 min. GA-MIPs were changed in the range of 0.45 and 1.8 mg for the determination of polymer amount effect. Results were represented in Fig. 3b and 0.9 mg MIPs were used in other studies. Effect of initial GA concentration was tested in the range of 100 and 1500 ppm onto both GA-MIPs and GA-NIPs (Fig. 3c). As seen in Fig. 3c, adsorption capacities were increased with increasing initial concentration up to 1000 ppm and then reached a plateau and maximum adsorption capacities were determined as 1012.4 ± 44.9 mg GA/g MIPs and 789.0 ± 40.1 mg GA/g NIPs. IFs at different GA concentrations were given in Table 2. Effect of temperature on GA adsorption was tested in the range of 5°C and 45°C since temperature is an important parameter on adsorption. Maximum adsorption was achieved at 25°C and results were represented in Fig. 3d. Adsorption capacity increased by 2.5-fold from 5°C to 25°C and decreased by 3.9-fold from 25°C to 45°C. IF values at different temperatures were calculated and given in Table 2.

IFs for different adsorption medium					
MeOH	MeOH:H_2O	H_2O (pH 3.5)	pH 4.0	pH 4.5	pH 5.0
1.31	0.82	1.31	1.20	0.93	0.83
Ifs for different GA concentration					
100 ppm	250 ppm	500 ppm	750 ppm	1000 ppm	1500 ppm
2.57	1.97	1.77	1.51	1.31	1.24
Ifs for different temperature					
5°C	15°C	25°C	35°C	45°C	
2.57	1.45	1.32	1.31	1.08	

Table 2. IFs for adsorption medium, GA concentration and temperature parameters

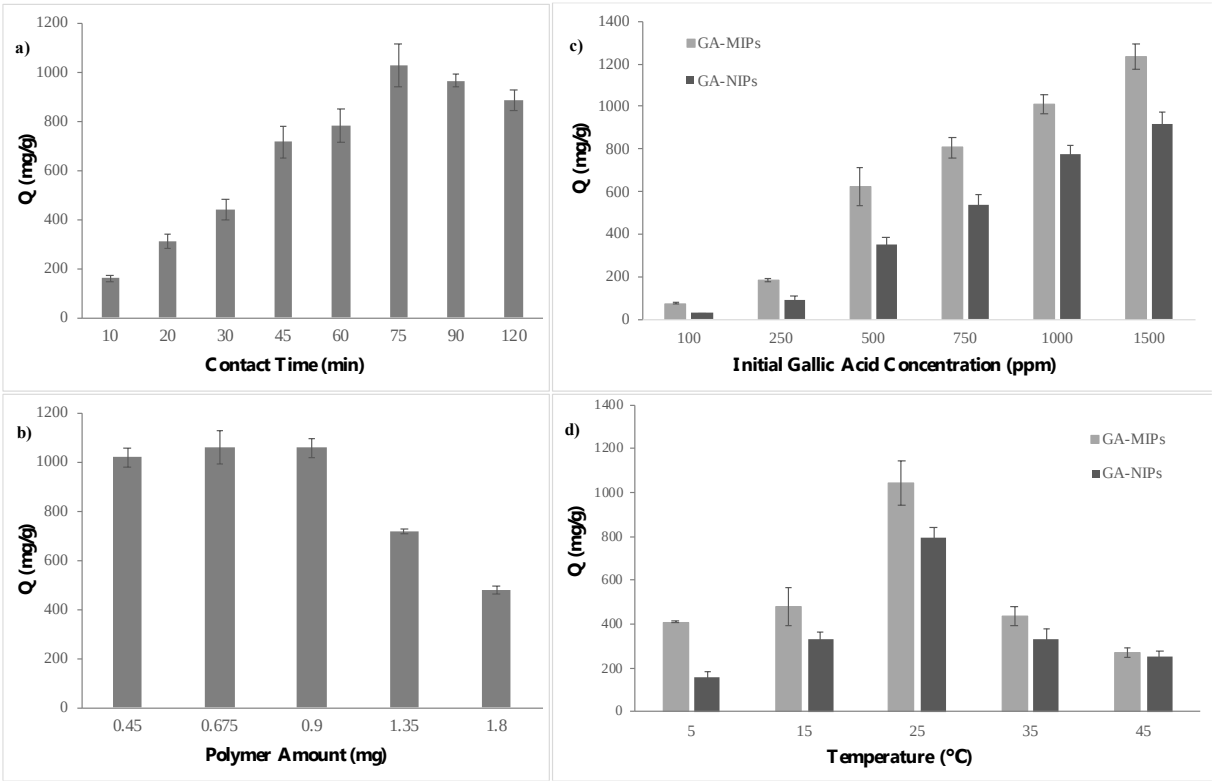


Fig. 3. Effects of contact time (a), initial concentration (b), polymer amount (c) and temperature (d) on GA adsorption onto GA-MIPs and GA-NI

3.3. Selectivity studies

Chromatograms for GA and HB at 260 nm were represented in Fig. 4a and for CA and PCA at 312 nm in Fig. 4b. In Fig. 4c, calibration equations for GA and its analogues in the range of 2.5 and 20 ppm were presented.

K_d values for all analogues were calculated from the results of competitive adsorption of GA with HB, CA and PCA. *K* values for GA-MIPs and GA-NIPs and *K*' values in parallel with these values were calculated and given Table 3.

	***K* (MIP)**	***K* (NIP)**	***K´***
GA-HB	1.65	0.59	2.77
GA-CA	1.36	0.4	5.60
GA-PCA	0.54	0.11	5.14

Table 3. Selectivity coefficient (*K*) for GA-MIPs and GA-NIPs and relative selectivity (*K´*)

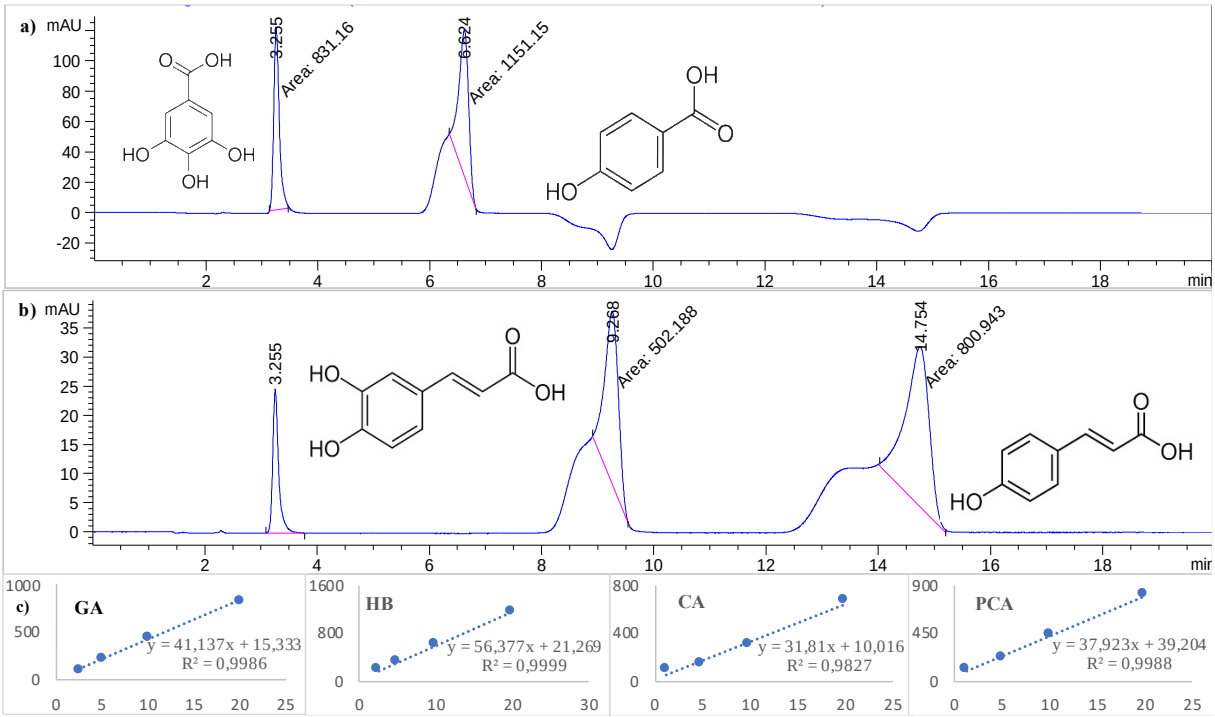


Fig. 4. Chromatograms for GA and HB at 260 nm (a), for CA and PCA at 312 nm (b) and calibration equations for GA and its analogues (c)

3.4. GA separation from real samples with GA-MIPs

Adsorption capacities from brewed tea and clove extracts were determined as 24.87 and 43.11 mg GA/g GA-MIPs. GA recoveries from GA-MIPs for brewed tea and clove extracts were specified as 87.39% and 94.51%, respectively. Chromatograms of brewed tea for before and after adsorption and desorption solutions were presented in Fig. 5.

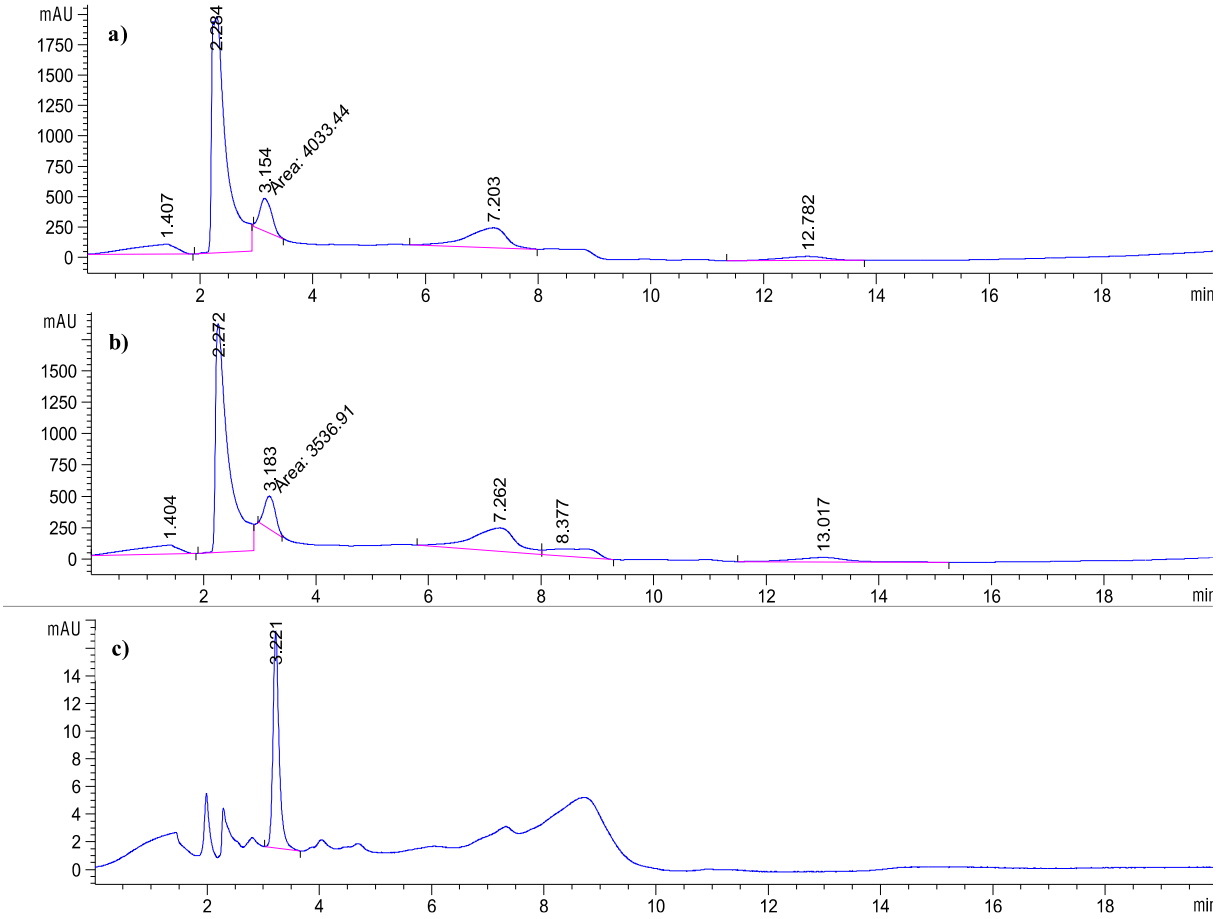


Fig. 5. Chromatograms of brewed tea for before and after adsorption and desorption solutions

4. DISCUSSION

In the UV spectra of VIM-GA pre-polymerization complex, peaks observed at 230 and 273 nm were thought to belong VIM and GA, respectively. A little shift was observed due to the complex formation between VIM and GA. The peak at 270 nm in the spectra of template removal solution belongs to GA and indicated successful template removal (Fig. 1)

OH stretching band of GA in the FTIR spectra of VIM-GA pre-polymerization complex was not seen in the FTIR spectra of GA-MIPs due to the template removal process. C=O and C-O stretching bands of EGDMA seen in the spectrum of GA-MIPs and GA-NIPs and absence of C=C stretching band of VIM indicated polymerization process (Table 1).

PdI value around 0.1 demonstrated partial uniform-sized particle distribution for GA-NIPs [28]. PdI value bigger than 0.1 for GA-MIPs can be explained by aggregations due to several centrifugation and re-suspension cycle during template removal studies [29]. Zeta potential for GA-MIPs in (-) value showed (-) surface potential and (+) value for GA-NIPs indicated (+) potential. Moreover, zeta potential values in the range of 0 and 5 mV both for (-) and (+) values demonstrate that coagulation rate of particles are high and particle stability is low [30]. According to this, GA-MIPs could be accepted as unstable and GA-NIPs as stable particles.

pKa value of GA is 4.40 and functional groups of GA get protonated below this pH. At pH 4.5 and 5.0 (above pKa), ionic interactions between GA and GA-NIPs increased and thus, adsorption capacity of GA-NIPs were determined higher than that of GA-MIPs. GA is negatively charged at pH 3.5 and 4.0 and interaction were higher between GA and GA-MIPs. Thus, IF values for pH 3.5 and 4.0 adsorption media were calculated as bigger than 1 (Fig. 2)

Maximum adsorption capacity was observed with 0.9 mg GA-MIPs for the effect of polymer amount test. Adsorption capacity decreased clearly with increasing polymer amount because the amount of GA adsorbed onto GA-MIPs was determined similar above 0.9 mg GA-MIPs (Fig. 3b)

IF values for the effect of initial concentration (Table 2) were calculated higher for lower GA concentrations and GA-MIPs adsorbed GA more selectively as compared to GA-NIPs. Thus, selectivity studies were performed using 100 ppm GA and 100 ppm its analogue.

Increase in the kinetic energy of molecules with increasing temperature may be cause to steric hinderance and decrease at interactions between GA and GA-MIPs (Fig 3d). Decline in IF values with increasing temperature may be explained by structural changes at imprinted parts and increased interactions between GA and GA-NIPs (Table 2).

K values calculated bigger than 1 for GA-MIPs expect PCA analogue showed that GA-MIPs adsorbed more GA as compared to HB and CA analogues. GA-MIPs adsorbed more PCA as far as GA and this result could be explained by more non-specific interactions. GA-NIPs adsorbed more GA analogues as compared to GA and thus, all *K* values for GA-NIPs were calculated lower than 1. Relative selectivity values demonstrated that GA-MIPs adsorbed more GA respect to all analogues as compared to GA-NIPs (Table 3).

In the HPLC chromatogram of desorption solution for brewed tea, the single peak at 3.2 min belongs to GA and this demonstrated selective GA recovery from GA-MIPs (Fig. 5c).

5. CONCLUSIONS

GA is a natural antioxidant and widely used in pharmaceutical and biomedical industries. Also, it is used in foods, drugs, and cosmetics due to its anti-bacterial, anti-viral, anti-oxidant, anti-inflammatory, anti-tumoral properties and protective effect to cardiovascular diseases. Demand of GA for each year is considerably getting higher, and therefore large amounts of chemicals are required for its manufacture and relevant toxic wastes come up during the manufacturing process. Even though GA is abundantly present in fruits and herbs, the selective extraction of GA is impossible due to the complexity of natural sources. Therefore, use of functional materials such as MIPs gain importance for selective isolation/separation of GA from natural sources. In this study, GA-MIPs prepared by easy and fast method and separated GA selectively is encouraging for industrial uses.

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