JCHR (2023) 13(2), 259-266

Journal of Chemical Health Risks

www.jchr.org

ORIGINAL ARTICLE

# Preparation of Efficient Kit for the Semi-Quantitative Determination of Sarcosine as a cancer marker by Grafting Molecularly Imprinted-Stationary on Glass Plate

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*(Received: 5 May 2020 Accepted: 20 November 2020)*

**KEYWORDS**

Imprinted polymer;
Sarcosine;
Kit;
Grafting

**ABSTRACT:** This paper presents a novel, rapid, and simple method for the determination of sarcosine. The surface of a glass plate was modified with 3-(methacryloxy) propyltrimethoxysilane. Then, a sarcosine-imprinted polymer was grafted on the glass plate by copolymerization of the vinyl end groups with a functional monomer and a cross-linking agent. The synthesized polymers were characterized by Fourier transform infrared spectroscopy and scanning electron microscopy. In the subsequent step, the determination of sarcosine was conducted using the synthesized kit in optimized conditions. The synthesized grafted plate was able to absorb sarcosine selectively in the presence of other amino acids, showing that the proposed method enabled the rapid determination of sarcosine.

## INTRODUCTION

Prostate cancer is the most prevalent kind of tumor disease in males [1]. A better treatment will be achieved by early diagnosis of prostate cancer [2]. Therefore, the findings of new markers, such as proteins, nucleic acids, or amino acids, are highly interesting. Prostate-specific antigen (PSA) is generally utilized as the main biomarker of this type of cancer. It is possible to detect this marker only in blood and it has a sensitivity of about 80%. Besides, this protein cannot be used in the diagnosis of the early stages of the disease. Presently, no test is available to diagnose prostate cancer's early stages. Therefore, a potential is assigned to the non-protein amino acid sarcosine produced by glycine-N-methyltransferase in its biochemical cycle. The incidence of keyword sarcosine is increasing. It has been recognized as a metabolite critically is increased when the metastatic process and prostate cancer progresses and it can be found in urine [3].

All twenty amino acids have a similar basic backbone structure and differ only in the structure of the side chain or R group. There are no reagents to specifically determine amino acids in the complicated matrix and preliminary separation of amino acids is required. For example, ninhydrin, as the most sensitive reagent for amino acids, produces color with all of the peptides, proteins, amino acids, and many other ninhydrin-positive compounds [4].

Several different methods have been applied for the separation of sarcosine. In most investigations, sarcosine has been separated in a chromatographic system [5-9]. Most of these methods have a high degree of accuracy and reproducibility, but they are often complicated,

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DOI: 10.22034/jchr.2022.693893

laborious, and require rather expensive technical equipment [10]. Nevertheless, inexpensive and simple approaches are required for routine determination of sarcosine in large clinical sample sets since simple methods for the determination of amino acids such as sarcosine are not found in the literature.

Molecular imprinting is an attractive and promising method to develop specific sorbents. In molecular imprinting, polymers are prepared with predesigned recognition areas, which have the right shape and functionality to capture template molecules specifically [8, 11-14].

However, there are still challenges regarding molecularly imprinted polymers (MIPs) developed by conventional imprinting techniques, like poor site accessibility, slow binding kinetics, and low binding capacity. These problems are due to the recognition sites that are deeply embedded in cross-linked polymer networks [15, 16].

Numerous studies have investigated novel imprinting strategies. For example, it is possible to use grafting to apply molecular imprinting on the carbon nanotube surfaces [17, 18], silica [19, 20], and $Fe_3O_4$ [21, 22] nanoparticles. The advantages of the The advantages of the obtained MIP composites include quicker mass transfer and more accessible binding sites in comparison to the MIPs developed by traditional bulk polymerization methods.No studies grafting MIP on surface glass plates have been reported. Some works on the application of MIP as the stationary phase of thin layer chromatography have been conducted but discontinued possibly because of the difficulties of adhesion of synthesized polymers to the surface of support [23-25]. These studies were conducted using CaSO4 but resulted in disordered holes. The bonding of MIP is very weak with support, in samples without an adsorption agent [26].

In this work, a sarcosine-imprinted polymer was grafted on the surface of the glass plate after silanization with 3-(methacryloxy)propyltrimethoxysilane (MPTS). Then, the synthesized glass plate, which was grafted with sarcosine–MIP, was successfully applied to obtain the semiquantitative determination of sarcosine in urine samples.

## MATERIALS AND METHODS

### *Reagents and standards*

All reagents and chemicals were of analytical grade and employed with no further purification. Merck provided 2,2′ -azobisisobutyronitrile (AIBN), methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), ninhydrin, MPTS, methanol, sodium hydroxide, hydrochloric acid, sulfuric acid, acetic acid, ethanol, potassium dihydrogen phosphate, potassium hydroxide, phosphoric acid, acetonitrile, and H2O2 (30% (w/w). Sarcosine, sarcosine, valine, lysine, and alanine were provided by Sigma-Aldrich. As support, we used microscopic lam (75×26Chance Propper Ltd, UK). A Bunsen burner was used for flame-treatment of microscope slides. For this purpose, the slides were passed over the flame four times. Then, after cooling the slides at ambient temperature for three minutes, they were put in a glass Coplin jar that contained an aqueous solution of hydrogen peroxide (8.4 N) and hydrochloric acid (4.7 N). Then, the slides were sonicated for one hour in the jar. Subsequently, the slides were rinsed twice with deionized water (25 mL). Prior to silanization with MPTS, the cleaned slides were kept in DI water. The slides were dried for 10 s with a stream of nitrogen.

### *Chemical modification of the glass plate*

0.5 mL of MPTS was pipetted onto the slides that were warmed for half an hour at 100°C under vacuum. Afterward, they were cooled and placed in a clean Coplin jar that contained toluene (60 mL) and sonicated for 30 min. Each of the silylated slides was washed with 30 mL methanol and kept in methanol at 4°C before being grafted [27].

### *Imprinting of sarcosine molecules on the surface of MPTS–microscope slides*

Before polymerization, we prepared a solution by dissolving 4 mmol MAA and 1 mmol sarcosine in acetonitrile (50 mL) and kept it for 12 h in the dark. Each of the MPTS-microscope slides was put in toluene-acetonitrile (25 mL) (4/1, v/v) via ultrasonic vibration. Afterward, the pre-determined solution, 0.16 mmol

AIBN, and 16 mmol EGDMA were melted in the solution. The resulting solution was purged for 10 min with high-purity nitrogen and was cooled in an ice bath. The process was continued by conducting a three-phase temperature polymerization reaction at 300 rpm in an incubating shaker. Firstly, pre-polymerization was performed for 6 h at 50 °C, followed by completing polymerization at 60 °C for 24 h. The temperature was subsequently elevated to 70 from 60 °C at 0.25 °C min−1 within one hour. Then, the products were aged at 75 °C for 6 h so that high cross-linking density is obtained. In the following, the obtained glass grafted to sarcosine-MIP was detached. Also, the preparation of non-imprinted polymer (NIP) followed the previously described procedure, but the template was not added. Lastly, the resulting grafted glass was cleaned ultrasonically with methanol-acetic acid (9/1, v/v) so that the template is removed. Then, methanol was used for rinsing, and it was vacuum dried at ambient temperature [28].

***Semiquantitative determination of sarcosine with polymer grafted on the surface of the microscope slides***

A 25 mL of Standard solutions containing sarcosine (5 mg $L^{-1}$) was placed into beakers (50 mL), followed by adjusting the pH to 5.0 by citrate buffer (0.01 mol $L^{-1}$). Then, microscope slides were placed horizontally on each beaker, where they remained for 30 min so that sarcosine adsorption onto the imprinted polymer is facilitated. Sarcosine was adsorbed on the imprinted polymer grafted on the microscope slide. Then, the microscope slide was immersed in a solution that contained 5 mg ninhydrin, glycerol (6/4 v/v), and 0.35 mol L−1 citrate buffer, after which it was placed in a domestic microwave oven for 60 s. The beaker was cooled, and the purple color of the slide was considered. A schematic diagram of grafting of sarcosine imprinted polymer grafted on the surface of the microscope slides and determination of sarcosine was shown in Figure 1.

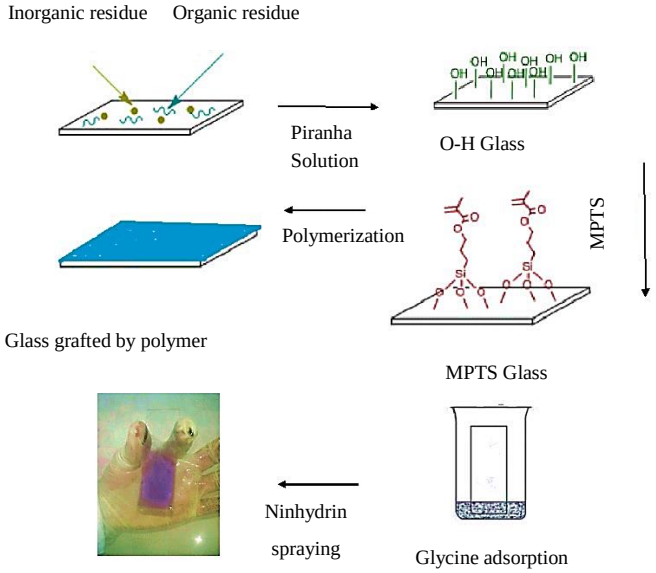



**Figure 1.** Schematic diagram of grafting of molecular imprinted polymer grafted on the surface of the microscope slides and determination of sarcosine

## RESULTS

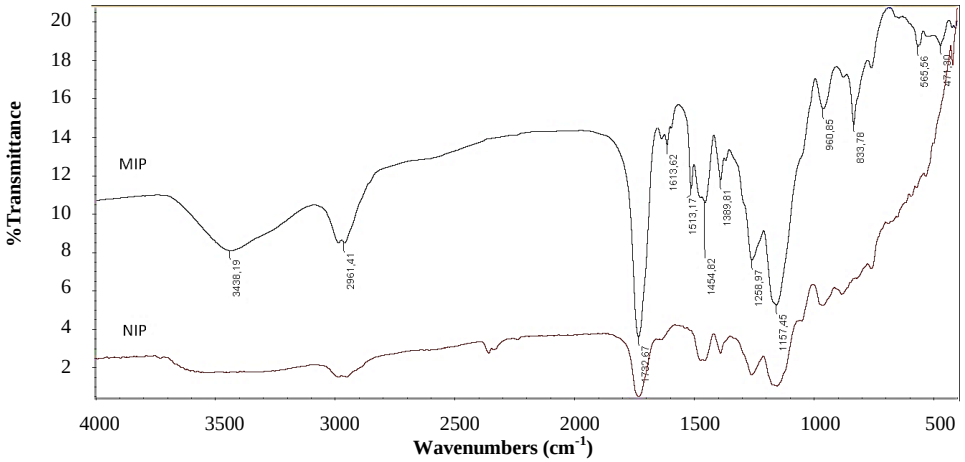
***Characterization of polymer grafted on the surface of the microscope slides***

Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FTIR) spectra were carried out by using Nicolet 6700 instrument (Nicolet Instrument, Thermo Company, USA) in the range of 4000-600$cm^{-1}$ with a resolution of 4 $cm^{-1}$.

The FTIR spectra of MIP and NIP grafted on the surface of the microscope slides are shown in Figure 1.

The polymer grafted on the surface of the microscope slides was also assessed through field emission scanning electron microscopy (FE-SEM). FE-SEM images were obtained on a HITACHI S-4160. Figures 2, 3 and 4 show the FE-SEM images of MIP and NIP in 10 and 100 kx magnification.

These images confirm the formation of uniform polymer on the surface of microscope slides. Uniform grafting of polymer on the surface of microscopic lam and porous structure of both MIPs and NIPs could be observed by SEM images in Figures 3 and 4; Both NIP and MIP have similar morphology.



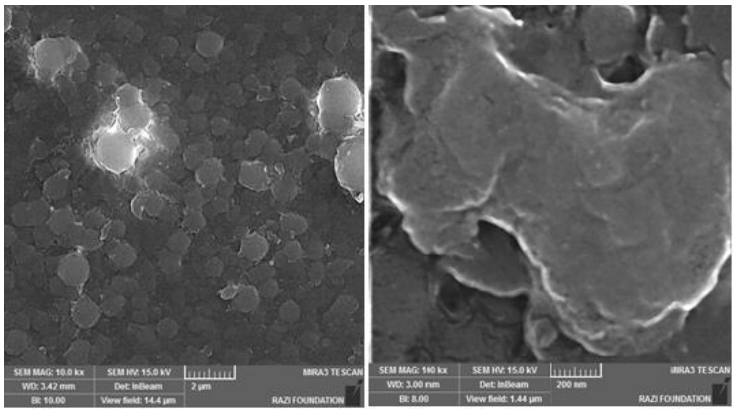



**Figure 3.** FE-SEM images of MIP grafted on the surface of the microscope slides (A) ×10 kx magnification (B) ×100 kx magnification.

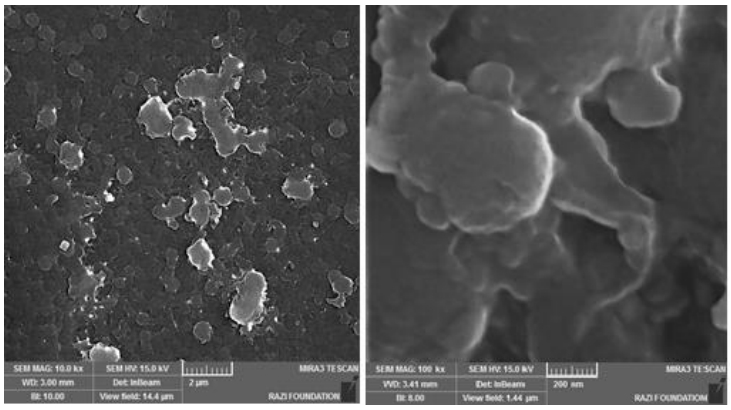


**Figure 4.** FE-SEM images of NIP grafted on the surface of the microscope slides (A) ×10 kx magnification (B) ×100 kx magnification.

***Optimization of the adsorption conditions of sarcosine on polymer***

***Effect of pH on the adsorption of sarcosine***

The effect of varying pH values on sarcosine uptake was investigated. Six portions of standard solutions (25mL) containing sarcosine (5mg $L^{-1}$) were transferred into 50 mL beakers. Then, the microscope slides were placed horizontally on each beaker, and the pH value was adjusted in a range of 3–8 with 0.001 M, $HNO_3$ or NaOH. Sarcosine was adsorbed onto the imprinted polymer grafted on the microscope slide. Then adsorbed sarcosine was reacted with ninhydrin according to the mentioned procedure.

***Effect of time on the adsorption of sarcosine***

Six portions of standard or sample solutions (25mL) containing sarcosine (5mg $L^{-1}$) were transferred into 50 mL beakers and the pH was adjusted to 5.0 by using 0.01 mol $L^{-1}$ citrate buffer. Then, the microscope slides were placed horizontally on each beaker, where they remained for 10, 20, 30, 60, 90, 120, 150, and 180 min to facilitate the adsorption of sarcosine onto the imprinted polymer. Sarcosine was adsorbed onto the imprinted polymer grafted on the microscope slide. Then for semiquantitative determination of sarcosine, it was reacted with ninhydrin according to the mentioned procedure.

***Semiquantitative determination of sarcosine and the effect of sarcosine concentration***

Three portions of standard or sample solutions (25mL) containing sarcosine (2, 5, and 20mg L−1) were transferred into 50mL beakers and the pH was adjusted to 5.0 by using 0.01 mol $L^{-1}$ citrate buffer. Then, the microscope slides were placed horizontally on each beaker for 30 min. Sarcosine was adsorbed on the imprinted polymer grafted on the microscope slide. Then for semiquantitative determination of sarcosine, it was reacted with ninhydrin according to the mentioned procedure. The established colors are shown in Figure 5.

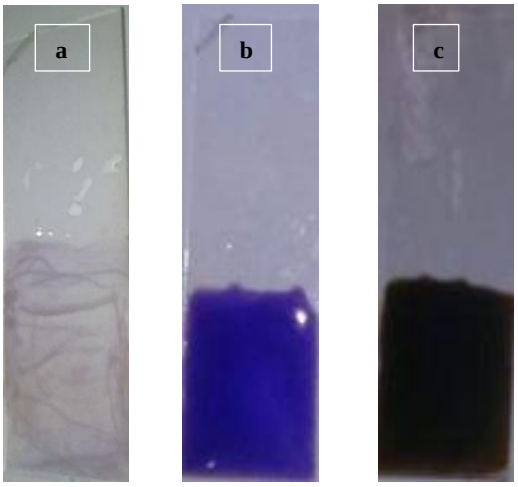



**Figure 5.** The colors established at different sarcosine concentrations (2, 5, and 20mg $L^{-1}$).

***Chemical stability of polymer grafted on the surface of the microscope slides***

The prepared polymer grafted on the surface of the microscope slides was individually immersed in NaOH (1.00 mol $L^{-1}$), HCl (1.00 mol $L^{-1}$), toluene, hexane, methanol, chloroform, 10% acetic acid in methanol, as well as distilled water for investigating chemical stability. The solutions were kept for 2 h at ambient

temperature, and then, the MIP coating showed an acceptable surface quality without cracking, swelling, or desquamating. The plates were then utilized in order to extract sarcosine in spiked water solutions. No significant degradation of extraction ability or formed color was observed. Notably, the proposed polymer grafted on the surface of the microscope slides showed chemical stability in strong base, acid, and organic solvents. Moreover, the results indicated that MIP has superior characteristics and performance.

***Comparison of MIP and NIP adsorption***

Extracts of MIP and NIP were assessed in separate water samples under optimal conditions. Standard or sample solutions (25 mL) containing sarcosine (5 mg $L^{-1}$) were transferred into 50 mL beakers and the pH was adjusted to 5.0 by using 0.01 mol $L^{-1}$ citrate buffer. Then, MIP and NIP microscope slides were placed horizontally on each beaker, where they remained for 30 min. The microscope slides were immersed in ninhydrin solution before being placed in a domestic microwave oven for 60 s. The beaker was cooled, and the color of the slide was considered. The extraction recovery of NIP was considerably lower than that of MIP and colors were not produced in NIP because of the lower capacity of NIP for extraction of sarcosine than that of MIP.

***Interference effects***

After evaluating the efficiency of grafting a polymer on the surface of a microscope slide, the selectivity of such grafted polymers was investigated. In particular, the performance of MIP was evaluated. Glycine, alanine, valine, lysine, and histidine were investigated in this experiment. The polymers grafted on the microscope slides were separately contacted by 10 mg $L^{-1}$ of each amino acid in an aqueous solution. The obtained data showed that none of these amino acids could form a visible color on the surface of modified lam. After derivitization by ninhydrin. This confirms the high selectivity of the modified lam for sarcosine.

***Determination of sarcosine from spiked human urine***

Sarcosine in spiked urine samples was determined with the synthesized polymer grafted on the surface of the microscope slide. The urine sample (5mg $L^{-1}$) was spiked with sarcosine, and the presented method was applied for the determination of sarcosine. The obtained results showed a good recovery of sarcosine in spiked samples. Meanwhile, in the unspiked sample, no color was formed. These results indicated that sarcosine was determined in the presence of interfering compounds, showing that imprinted polymers grafted on the surface of the microscope slides are highly efficient for the semiquantitative determination of sarcosine in real samples.

## DISCUSSION

In this study, a simple kit was designed and it was used for the semiquantitative determination of sarcosine.

Two separation methods; solid phase extraction and thin layer chromatography (TLC) are combined in designing this kit, by using the advantages of molecularly imprinted polymer a specific stationary phase was prepared. The isolated sarcosine produces purple color with ninhydrin on the surface of modified microscope slides. The proposed kit has great potential for commercial uses, especially in the medicine area.

FTIR spectroscopy was used for the characterization of the MIP grafted on the glass surface. Similar IR spectra were observed in NIP and MIP, which indicates their similar backbone structure. Si–O vibrations resulted in the band at about 529 cm−1. The Si–O–Si band at approximately 1,100 $cm^{-1}$ showed overlap with the C–O band. A strong C=O peak was apparent at 1,730 $cm^{-1}$. Absorbance values were related to the methyl (or methylene) groups at 2,800 $cm^{-1}$ to 3,000 $cm^{-1}$. The IR spectra of NIPs and MIPs presented almost identical characteristic peaks. Also, the formation of uniform polymer on the surface of the glass was confirmed by SEM images.

The condition of sarcosine adsorption on the surface of the modified glass was optimized and the effect of interferences was investigated.

The obtained results show that the intensity of purple color increased with pH, and the maximum adsorption occurred at pH 5.0. Therefore, pH 5.0 was selected for this experiment because the adsorption capacity of the polymer decreases beyond this pH level. Also, the optimum time for sarcosine adsorption was 30 min, after which color was constant.

Investigations on the selectivity of modified glass demonstrate the excellent selectivity of synthesized MIPs as each investigated amino acid did not produce any colors. This finding indicates the potential analysis of sarcosine in complicated matrices with the presence of an interfering agent by glass grafted MIP.

The use of polymers grafted on the microscope slides as a kit could be an alternative to other more time-consuming techniques normally considered instrumental for the determination of sarcosine. The presented method offers several advantages, including low cost, ease of use, and excellent extraction efficiency. The presented method also provides a selective, simple, and practical strategy for sarcosine determination. The above-presented method is simple and could be performed on a large series of samples in most laboratories, and the method requires a minimum of technical equipment.

## ACKNOWLEDGEMENTS

We are grateful to the Laboratory Complex of Damghan Branch, Islamic Azad University and Pars Material Research and Testing for their valuable technical assistance.

### *Conflict of interest*

The authors declare that they have no conflict of interest

## REFERENCES

1. Sboner A., Demichelis F., Calza S., Pawitan Y., Setlur S.R., Hoshida Y., Perner S., Adami H.O., Fall K., Mucci L.A., 2010. Molecular sampling of prostate cancer: a dilemma for predicting disease progression. BMC Medical Genomics. 3(1), 1-12.

2. Cuzick J., Thorat M. A., Andriole G., Brawley O. W., Brown P. H., Culig Z., Eeles R.A., Ford L.G., Hamdy F.C., Holmberg L., 2014. Prevention and early detection of prostate cancer. The Lancet Oncology. 15(11), e484-e492.

3. Salciccia S., Capriotti A.L., Laganà A., Fais S., Logozzi M., De Berardinis E., Busetto G.M., Di Pierro G.B., Ricciuti G.P., Del Giudice F., 2021. Biomarkers in Prostate Cancer Diagnosis: From Current Knowledge to the Role of Metabolomics and Exosomes. International Journal of Molecular Sciences. 22(9), 4367.

4. Friedman M., 2004. Applications of the ninhydrin reaction for analysis of amino acids, peptides, and proteins to agricultural and biomedical sciences. Journal of Agricultural and Food Chemistry. 52(3), 385-406.

5. Meyer T.E., Fox S.D., Issaq H.J., Xu X., Chu L.W., Veenstra T.D., Hsing A.W., 2011. A reproducible and high-throughput HPLC/MS method to separate sarcosine from α-and β-alanine and to quantify sarcosine in human serum and urine. Analytical Chemistry. 83(14), 5735-5740.

6. Chen S.E., Hu J., Yan P., Sun J., Jia W., Zhu S., Zhao X.E., Liu H., 2021. 12-Plex UHPLC-MS/MS analysis of sarcosine in human urine using integrated principle of multiplex tags chemical isotope labeling and selective imprint enriching. Talanta. 224, 121788.

7. Yamkamon V., Yee P.P., Yainoi S., Eiamphungporn W., Suksrichavalit T., 2018. Simultaneous determination of sarcosine and its related metabolites by gas chromatography-tandem mass spectrometry for prostate cancer diagnosis. EXCLI Journal. 17, 965.

8. Hashemi-Moghaddam H., Hagigatgoo M., 2015. Nonderivatized Sarcosine Analysis by Gas Chromatography after Solid-Phase Microextraction by Newly Synthesized Monolithic Molecularly Imprinted Polymer. Chromatographia. 78(19-20), 1263-1270.

9. Hashemi-Moghaddam H., Rahimian M., Niromand B., 2013. Molecularly imprinted polymers for solid-phase extraction of sarcosine as prostate cancer biomarker from human urine. Bulletin of the Korean Chemical Society. 34(8), 2330-2334.

10. Montero L., Conradi S., Weiss H., Popp P., 2005. Determination of phenols in lake and ground water samples by stir bar sorptive extraction–thermal desorption–gas chromatography–mass spectrometry. Journal of Chromatography A. 1071(1), 163-169.

11. Hashemi-Moghaddam H., Rahimian M., Niromand B., 2013. Molecularly Imprinted Polymers for Solid-Phase Extraction of Sarcosine as Prostate Cancer Biomarker from Human Urine. Bull. Korean Chem Soc. 34(8), 2331.

12. Hashemi-Moghaddam H., Alaeian M.R., 2014. Synthesis of molecularly imprinted polymer for removal of effective impurity (benzhydrol) from diphenhydramine hydrochloride drug. Journal of the Chinese Chemical Society. 61(6), 643-648.

13. Hashemi-Moghaddam H., Abbasi F., 2015. Synthesis of Molecularly Imprinted Polymers Coated on Silica Nanoparticles for Removal of P-Nitrophenol from Crude Pharmaceuticals. Pharmaceutical Chemistry Journal. 49(4), 280-286.

14. Hashemi-Moghaddam H., Jedi D.J., 2015. Solid-phase microextraction of chlorpyrifos in fruit samples by synthesised monolithic molecularly imprinted polymer fibres. International Journal of Environmental Analytical Chemistry. 95(1), 33-44.

15. Wackerlig J., Lieberzeit P.A., 2015. Molecularly imprinted polymer nanoparticles in chemical sensing–Synthesis, characterisation and application. Sensors and Actuators B: Chemical. 207, 144-157.

16. Svec F., Bleha M., Tennikova T. B., Belenkii B.G., 1990. Google Patents.

17. Hashemi-Moghaddam H., Hosseni M., Mohammadhosseini M., 2017. Preparation of molecularly imprinted polymers on the surface of optical fiber for HS-solid-phase microextraction of phenol. Separation Science and Technology. 1-9.

18. Hirayama K., Sakai Y., Kameoka K., Noda K., Naganawa R., 2002. Preparation of a sensor device with specific recognition sites for acetaldehyde by molecular imprinting technique. Sensors and Actuators B: Chemical. 86(1), 20-25.

19. Tang A.N., Duan L., Liu M., Dong X., 2016. An epitope imprinted polymer with affinity for kininogen fragments prepared by metal coordination interaction for cancer biomarker analysis. Journal of Materials Chemistry B. 4(46), 7464-7471.

20. Iida H., Takayanagi K., Nakanishi T., Osaka T., 2007. Synthesis of $Fe_3O_4$ nanoparticles with various sizes and magnetic properties by controlled hydrolysis. Journal of Colloid and Interface Science. 314 (1), 274-280.

21. Sun S., Zeng H., 2002. Size-controlled synthesis of magnetite nanoparticles. Journal of the American Chemical Society. 124(28), 8204-8205.

22. Hashemi-Moghaddam H., Ahmadifard M., 2016. Novel molecularly-imprinted solid-phase microextraction fiber coupled with gas chromatography for analysis of furan. Talanta. 150, 148-154.

23. Suedee R., Saelim J., Thavornpibulbut T., Srichana T., 1999. Chiral determination of various adrenergic drugs by thin-layer chromatography using molecularly imprinted chiral stationary phases prepared with α-agonists. Analyst. 124(7), 1003-1009.

24. Suedee R., Songkram C., Petmoreekul A., Sangkunakup S., Sankasa S., Kongyarit N., 1999. Direct enantioseparation of adrenergic drugs via thin-layer chromatography using molecularly imprinted polymers. Journal of Pharmaceutical and Biomedical Analysis. 19(3), 519-527.

25. Fischer L., Mueller R., Ekberg B., Mosbach K., 1991. Direct enantioseparation of. beta.-adrenergic blockers using a chiral stationary phase prepared by molecular imprinting. Journal of the American Chemical Society. 113(24), 9358-9360.

26. Gao F., Hu Y., Chen D., Li-Chan E. C., Grant E., Lu X., 2015. Determination of Sudan I in paprika powder by molecularly imprinted polymers–thin layer chromatography–surface enhanced Raman spectroscopic biosensor. Talanta. 143, 344-352.

27. Chen J.J., Struk K.N., Brennan A.B., 2011. Surface Modification of Silicate Glass Using 3-(Mercaptopropyl) trimethoxysilane for Thiol–Ene Polymerization. Langmuir. 27(22), 13754-13761.

28. García-Galán M.J., Díaz-Cruz M.S., Barceló D., 2008. Identification and determination of metabolites and degradation products of sulfonamide antibiotics. TrAC Trends in Analytical Chemistry. 27(11), 1008-1022.