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Extraction phenols in walnut leaves and determination of using GC-FID

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Abstract
In recent years, emphasis has been placed on the use of natural materials in the control and treatment of various infections, as some chemically synthesized drugs have undesirable side effects. Beech forests play an important role in temperate in Golband area northern of Iran since they occupy infertile montane soils. In the last glacial maximum, Walnut (Juglans regia) leaves (beech) was confined to northern of Iran We sampled four different beech forest types .A simple method to pre-concentrated Phenols in Walnut (Juglans regia) leaves compounds on active carbon in column has been applied as stationary phase which is used to measure the concentration of Phenols in Walnut (Juglans regia) leaves compounds in leaf biophenols by means of solid-phase extraction. To measure 25 ml Phenols in Leaves of Juglans regia compounds sample and 250mg active carbon could be applied. Next step is to measure the Phenols in Walnut (Juglans regia) leaves compounds by injecting them to the gas chromatography with flame ionization. The advantages of applying gas chromatography–flame ionization detection (GC–FID) with SPE in presence Carbon active are high sensitivity, High speed transformation of Phenols in Leaves of Juglans regia compounds sample in the range of ppb or those with less than 10% of LOD. The quantity of extraction could be affected by sample's pH, amount of solvent, washing liquid type, solvent and flow rates of the sample solutions.

Keywords: solid phase extraction, phenols in leaves of juglans regia compounds sample, gas chromatography flame ionization detection (GC–FID)

1. Introduction
The genus *Juglans* (family Juglandaceae) comprises several species and is widely distributed throughout the world. Green walnuts, shells, kernels and seeds, bark, and leaves are used in the pharmaceutical and cosmetic industries. Phenols in Walnut (Juglans regia) leaves are involved in many in processes and frequently are released into the environment through industrial discharges. Moreover nitrophenols and chlorophenols occur in the environment as degradation products of the organophos phorus and chlorinated phenoxyalkanoic acid pesticides, respectively. Anilines also occur in the environment as degradation products of the phenyl urea and dinitroaniline herbicides. Phenols are per sistent in the environment and toxic at the low mg/l level [1]. In the 80/778/EEC directive of the European Union it is stated that the maximum admissible concentration for each individual phenol in drinking water should not exceed 0.1 mg/ l [2]. Anilines are also of toxicological importance and the monitoring of their levels in environmental waters is Break important for the protection of health and the environment [3]. The most common techniques for the analysis of anilines and phenols in environmental waters are gas (GC) and high-performance liquid chromatography (HPLC) [1]. The direct analysis of phenols by GC is difficult [4] and GC analysis is usually performed after a derivatization step [1, 5, 7]. Anilines are also thermolabile and polar compounds and a derivatization step is often required before GC analysis [3, 8, 11]. Most of the derivatization processes however are not straightforward and sometimes require handling of hazardous chemicals. Since in HPLC analysis there are no derivatization requirements, it appears to be a good alternative to GC analysis and nowadays has been widely accepted as the method of choice [1, 12].

A large number of procedures for the determination of phenolic compounds in water using SPE have been tested [13, 14, 18, 20]. Various types of solid-phase sorbents have been used, including C_{18} [21, 22], polystyrene–divinylbenzene-based polymers [22, 23], and various forms of carbon [18, 20]. A number of these sorbents show relatively low recovery for some phenolic compounds [13, 14, 7, 24, 25]. In this study, a β-cyclodextrin-bounded silica on active carbon in CBP column has been applied as stationary phase which is used to measure the concentration of Phenols in Walnut (Juglans regia) leaves compounds in leaf biophenols by means of solid-phase extraction.

2. Experimental
2.1 Reagents
Silica gel were purchased from Merck (Darmstadt, Germany). 3-Glycidoxypropyltrimethoxysilane (KH-560) and high-temperature epoxy resin of type 5203 were obtained from Huili Company (Jiangsu, China). Phenol (PN), cis-coniferin, taxifolin-xylopyranoside and cis-isoconiferin were obtained from Merck (Darmstadt, Germany). Standard solutions (2000 mg l^{-1}) from each individual compounds were prepared in methanol. A mixture of these phenolic compounds applied to different detection systems, was prepared weekly by diluting the standard solution with methanol, and more diluted working solutions were prepared daily by diluting these solutions with triple distilled water. The concentration of mixture to be analyzed by gas chromatography–flame

ionization detection (GC–FID) was made at a range of 100–200 mg l^{-1}. The commercial cartridges used were Carbon active Carbopack B from Merck.

One sites were intensively studied (insects collected, leaf phenolics determined and plant coverage measured) in Golband area northern of Iran. The study area is located on Parcel 206 Series 2, Jomand forestry plan in Golband forest. The average highest level of area1580 meters the, the average slope is 30 percent and the general direction is northeast. The rainy season, autumn, 633 mm with most rain and low rain seasons, spring rainfall is 134 mm. The average annual temperature is 16 degrees centigrade. The most cold month, January with temperatures 7 ° C and the hottest month of year is August with average temperature is 25.5 degrees Celsius. Along the northern slope of Alborz montains, 14 autochthonous beech populations (Walnut (Juglans regia) leaves) aged between 80 and 160 years were investigated. It was decided to select five locations along the distribution area of beech (in Hyrcanian zone) from Golband (Noshahr) and to establish three investigation stations in each regions (low, middle and high altitude of beech distribution range) to cover most of the geographical range of beech in Iran (Fig. 1). In each population beech twigs with dormant buds were sampled from 50 nonadjacent individuals (to avoid the sampling of related trees) chosen at random over a 3-4 ha area in a homogeneous environment.

Fig 1: Distribution of studied regions

2.2 Apparatus

A gas chromatograph model Varian Star 3800 equipped with a flame ionization detector and a split/splitless injector was used. Separations of Phenols in Leaves of Juglans regia compounds sample were carried out using a capillary column CBP 10 low bleed MS (25m×0.22mm I.D.) with 0.25µm film thickness. The injector and detector temperatures were set at 250 and 280 ˚C, respectively. The separation of Phenols in Leaves of Juglans regia compounds sample on (GC–FID) was performed by a temperature program as follows: 60 ˚C during 5 min, at 10 ◦Cmin^{-1} to 230 ˚C, 5 min hold at 230 ˚C. An aliquot of 1–2µl from each sample was introduced into the chromatographic columns using splitless mode injection. A Hewlett-Packard HP series gas chromatograph equipped with a split/splitless injector and a HP mass-selective detector was also used. The analytical column was a HP-5 MS 0.25 µm of 30m×250 µm I.D. The column temperature was programmed as follow: 80 ˚C for 3 min then was heated at 20 ˚Cmin^{-1} to 260 ˚C and 30 ˚Cmin^{-1} to 290 ˚C. The mass spectrometry was operated at electron energy of 70 eV. The injection and GC–MS interface temperature were set at 220 and 250 ◦C, respectively. The ion source temperature was set at 200 ˚C, and quadrupole temperature was set at 150 ˚C. The mass control system was programmed for a selected-ion monitor (SIM); the monitored ions were *m*/*z* at 96 for PN, *m*/*z* at 125 for 4- cis-isoconiferin, *m*/*z* at 130 for cis-coniferin and *m*/*z* at 110 for taxifolin-xylopyranoside. Chromatographic data were recorded using an HP Chemsation, which was controlled by Windows NT (Microsoft) and equipped with Wiley mass spectral library. Helium and nitrogen (99.999%) were used as carrier and make-up gas, respectively. The flow rate of carrier gas was adjusted at 1mlmin^{-1}. A JSM-6330F scanning electron micro analyzer (Japan Electronic Company) was used to investigate the CDS surface.

2.3 Solid-phase extraction equipment

A standard column 20mm glass vacuum filtration apparatus was utilised after being rebuilt according to The normal sintered piece of glass, acting as support for the glass fibre filters and SPE on active carbon in CBP, This construction facilitated and reduced the time for cleaning of the extraction equipment. The vacuum source used was a MZ 2C vacuum pump (Germany).

2.4 Sample preparation and derivatization

Prior to the preconcentration step, the pH of sample was adjusted to 1.5 with sulfuric acid. A known volume of Phenols in Leaves of Juglans regia compounds sample standards and was subsequently passed through a preconditioned SPE column at a flow-rate of 2–6 ml min^{-1}. When the sample had passed through, the cartridge was eluted with 2ml of methanol at the flow-rate of 0.2 ml min^{-1}. The cartridge was preconditioned by washing with 5ml of methanol and activated with 5ml of distilled water at pH 1.5. For those experiments where the pH effects were studied, citrate buffer (pH 1.5–5) was used for the adjustment. The derivatization procedure used was based on previous report by Rodr´ıguez et al. [31]. A volume of 2ml of a methanol solution containing Phenols in Leaves of Juglans regia compounds sample was mixed with 1ml of 5% K_2CO_3 and 2ml of *n*-hexane containing 200 µl of acetic anhydride and internal standard. The mixture was shaken for 1 min and the organic phase was allowed to be separate. The aqueous phase was then extracted with a further 1ml of *n*-hexane containing only internal standard. The two *n*-hexane portions were collected, mixed and dried over anhydrous sodium sulfate and injected into the GC system. To access lower detection limits in the sample solution at sub-ppb concentrations, the final extract was concentrated to 0.5 ml under a gentle stream of nitrogen.

3. Results and discussion

The CDS possesses a porous structure should significantly

increase the available surface area of it, and therefore, increase the extraction capacity.

3.1 Evaluation of sorbent

To evaluate the ability of the CDS for the extraction of Phenols in Walnut (Juglans regia) leaves compounds from leaf biophenols, a mixture of five phenolic compounds including PN, 4- cis-isoconiferin, cis-coniferin and taxifolin-xylopyranoside were used as model compounds. In general, phenols are amenable to GC without derivatization [17, 7, 9]. But at lower concentration, peak tailing and discrimination in the injector of capillary column might occur [9, 10], especially when environmental samples are analyzed. To overcome these problems, phenols could be derivatized with a suitable derivatizing reagent [11, 14]. Among the wide variety of derivatizing reagents used for this purpose, acetylating agents have been employed to the greatest extent [15, 17].

Effects of different parameters such as the sample pH, the sample volume, flow rate of sample (Table 1), the volume of eluting solvent, the capacity of sorbent and the linearity of recovery were evaluated using this sorbent. The sample pH is an important factor, which may effect on the recovery of Phenols in Leaves of Juglans regia compounds sample. To increase the extraction recovery of phenolic compounds by sorbents, it is necessary to acidify the sample [7]. At low pH, the acid–base equilibrium for the phenolic compounds shifts significantly toward the neutral forms, which have greater affinities toward the sorbent, and the extraction efficiencies are, therefore, increased. To study the effect of sample pH on the recovery of Phenols in Leaves of Juglans regia compounds sample, 12 ml samples with same concentration in the 200–300µg l^{-1} levels at different pH values (1.5, 3, and 5) were preconcentrated using CDS as a sorbent. Fig2 shows the recovery obtained at each pH and clearly, the maximum recovery is obtained at pH 1.5. Higher recovery results at low pH could indicate that the ion exchange interactions have little contribution in retaining mechanisms. The pronounced recovery decrease for phenolic compounds in comparison with phenol at higher pH, justifies the non-ionexchange interactions.

In order to determine the volume of the sample that can be concentrated with acceptable recoveries for all the compounds studied, it was necessary to obtain the breakthrough volumes. Different volumes (100, 300, 500, and 1000 ml) of distilled water, at pH 1.5, were spiked with a solution containing five phenolic compounds at the 200–500µg l^{-1} levels. Following the preconcentration step, the trapped analytes on the per column were eluted with 2ml of methanol. After derivatization and extraction with a total of 3ml of *n*-hexane, an aliquot of 2 µl was injected into the GC system. The recovery of phenolic compounds and the repeatability for the different volumes are given in Table 2. Good recoveries were obtained for all compounds studied using 250 ml sample volumes. Of course, when samples of 500 ml were preconcentrated, the recoveries were, still, acceptable, except for phenol. Further experiments revealed that, for less polar compounds, i.e. cis-coniferin breakthrough volumes higher than 600 ml was obtainable. It was also found that flow rates up to 7ml min^{-1} for leaf biophenols loading on the per column had no effect on the recovery percentage.

To find the required volume of methanol to elute all Phenols in Leaves of Juglans regia compounds sample from the cartridge, elution volumes up to 4ml were examined. It was found that a volume of 1ml was sufficient to desorb the trapped pollutants from the SPE per column; of course includes the volume of solvent to saturate the packed cartridge. This relatively low volume of methanol eluted all compounds from the cartridge easily and other solvents were, therefore, excluded from any further examination. The low consumption of desorbing solvent is a clear advantage of this sorbent, which would be far more useful in on-line applications. In order to study the capacity of the sorbent and the linearity of recovery, each compound was determined using Phenols in Leaves of Juglans regia compounds sample at much higher levels, i.e. 2–3 µg l^{-1}, by GC–MS. No significant differences were obtained, indicating that its capacity is sufficiently high. It also demonstrates that even the preconcentration of leaf biophenols with such levels of concentrations has no negative influence on the recovery results.

Table 1: Effect of flow rates of the sample solutions on the recovery percentage of Phenols in Walnut (Juglans regia) leaves compound.

Flow rate ml/min	Extraction%			
	phenol	cis-coniferin	4- cis-isoconiferin	taxifolin-xylopyranoside
0.5	80.97	50.25	30	60.5
1	75.12	33.24	28.60	28.42
1.5	57.74	27.0	25.5	21.2
2	8.23	22.95	21.37	10
3	8.0	5.27	12.27	7.02
4	2.2	0.64	5.2	3.0

Table 2: The extraction recoveries obtained for the studied Phenols in Walnut (Juglans regia) leaves compounds at different volume of sample solution (n =4)

							Volume (ml)	Compound
	1000		500		300		100	
RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	
5.9	22.3	6.2	97.4	5.5	80.5	5.0	85.2	PN
8.5	41.8	5.3	72.2	3.6	92.8	3.9	25.9	cis-coniferin
7.2	58.4	5.1	75.3	4.9	52.3	5.8	38.9	4- cis- isoconiferin
6.8	40.3	8.0	43.8	8.4	36.7	5.4	26.9	taxifolin- xylopyranoside

3.2 Comparison studies

In comparison with other reports, it appears that, these recoveries, at least for some, are better than those which obtained using some commercial sorbents such as C_{18} [4, 7, 23, 24], cyclohexyl [24] and monofunctional C_{18} (C_{18}/OH) [4]. In a report [23], 250 mg Amberchrom CG-161 was used for the preconcentration of 10 ml of water and recoveries lower than 75% for these phenolic compounds were obtained. Also, in another work [7] SPE of 250 ml of water sample by 250 mg active carbon, led to low recoveries, specially for cis-coniferin and taxifolin-xylopyranoside that were 40 and 48%, respectively. At the same time, another group [22] used cartridges of 500 mg of carbon active, cyclohexyl and PLRP-S, a styrene-divinyl benzene-based copolymer, for the preconcentration of Phenols in Leaves of Juglans regia compounds sample. Acceptable recoveries were obtained with these sorbents using different volumes of solution with and/or without use of ion-pair reagent for extraction of all compounds studied except cis-coniferin which had recoveries lower than 70% for all conditions applied. While some authors [4] have demonstrated that PLRP-S provides the best recoveries even for cis-coniferin was compared to other sorbents, in the previously described work [24] recoveries were reported to be lower than 70% for cis-coniferin. Generally, styrene–divinylbenzene-based polymers especially LiChrolut EN [33, 34, 42] because of its high surface area of 1200m2 g^{-1}, has shown satisfactory results.

Other sorbents such as Carbopack B and ENVI Chrom P were used by Pocurull et al. [3] for extraction of Phenols in Leaves of Juglans regia compounds sample with and without ion-pair reagent (tetrabutylammonium bromide). Recoveries higher than 90% were obtained for all compounds except pentachlorophenol which had a recovery value of about 75%. Comparing these related results using some common sorbents with the present work demonstrates that CDS has an enhanced performance for the extraction of phenolic compounds, especially cis-coniferin and taxifolin-xylopyranoside.

To have a better overview on the efficiency of the CDS, a comparison study was carried out using carbon active. carbon active is a well-known and widely used commercial sorbent and the other two polymers are relatively new and more efficient sorbents. Phenols in Leaves of Juglans regia compounds sample were passed through the cartridges. After elution and derivatization, an aliquot of 2µl was injected onto the GC. As Table 3 demonstrates, the recoveries of all compounds are less than 40% using 250 ml of leaf biophenols when carbon active was used.

3.3 Real sample

In order to study the effects of sample matrix on the performance of the sorbent, the recovery results were examined using real-life sample spiked with the phenolic compounds at two different concentration levels. A Phenols in Leaves of Juglans regia compounds sample was at 2–3 µg l^{-1} levels. After the SPE and derivatization step, an aliquot of final extraction was injected into the GC–MS system. The TIC traces obtained from SPE of 20 ml of Phenols in Leaves of Juglans regia compounds sample when CDS was used revealed that, in this case, the clean up process was more efficient. The capacity of CDS for retaining Phenols in Walnut (Juglans regia) leaves compounds were 50–125 mg g^{-1}, while for phenol was 30mg g^{-1}. Fig. 3 shows the gas chromatograms of the Phenols in Leaves of Juglans regia compounds sample with a standard solution of phenolic compounds. The limits of detection using 20 ml of Phenols in Leaves of Juglans regia compounds sample were calculated based on a signal-to-noise ratio of 3 and were in the range of 15–120 ng l^{-1}, using TIC mode (Table 3).

Table 3: The extraction recoveries obtained for the studied Phenols in Walnut (Juglans regia) leaves at 20 ml sample in the range between 2 and 3 µg1−1 using carbon active a.

Carbon active			
LOD (%)	**Recovery (%)**	**LOD (%)**	**RSD (%)**
144	10.4	51	3.5
158	20.2	85	5.8
128	38.3	74	4.9
153	30.7	69	8.6
191	35.9	131	9.4

a The relative standard deviations (RSD) between 3.5–9.4% (n = 4).

4. Conclusions

The developed Using carbon active CDS as a SPE sorbent method was capable of handling various leaf biophenols samples with a reduced sample preparation time and solvent consumption compared to classical LLE The capability of this sorbent to extract Phenols in Walnut (Juglans regia) leaves has been compared with the results obtained for commercial sorbents and this laboratory-made with a relatively small specific surface area, showed comparable breakthrough volumes for the studied compounds. A CDS sorbent was prepared and investigated with three Phenols in Walnut (Juglans regia) leaves. It could be used more than 150 times. It exhibited fast equilibrium in the extraction for the porous structure of silica particles.

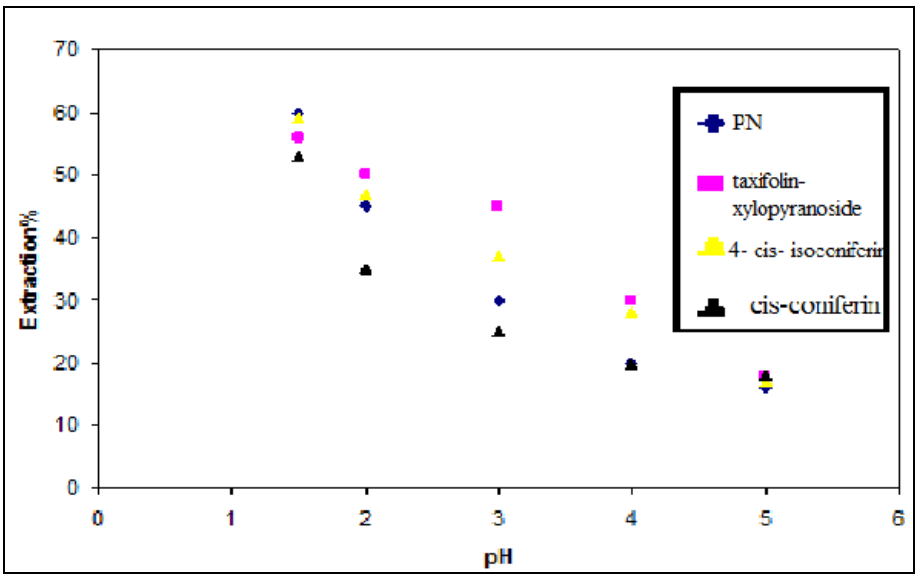

Fig 2: The extraction recoveries obtained for the studied Phenols in Walnut (Juglans regia) leaves compounds at different sample pH

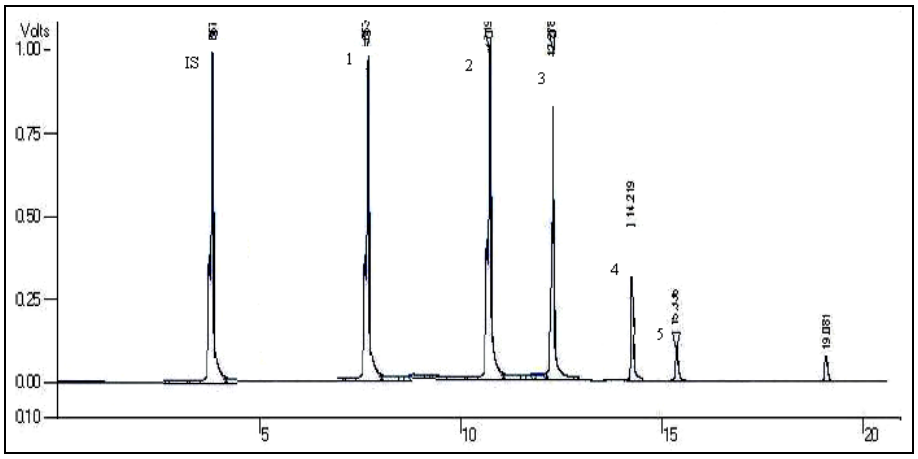

Fig 3: Gas chromatograms of the Phenols in Walnut (Juglans regia) leaves compounds 20 ml sample with 2–3 µg l^{-1} of a standard solution of Phenols in Walnut (Juglans regia) leaves compounds. Internal standard (I.S.), (1) PN, (2) 4- cis-isoconiferin, (3) taxifolin-xylopyranoside, and (4,5) cis-coniferin.

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6. References

1. Taher MA, Mostafavi A, Afzali D, Rezaeipour E, Bull. Korean Chem. Soc., 2004; 25:1125-1129.
2. Moghimi A. Journal of Chemical Health Risks. 2014; 4(2):15-22.
3. Moghimi A. Russian Journal of Physical Chemistry A, 2013; 87(11):1851-1858.
4. Sharafati-Chaleshtori R. Sharafati-Chaleshtori F. Rafieian M. Turk J Biol, 2011; 35:635-639.
5. Hart AP, Dasgupta A. J Forensic Sci., 1997; 42:693.
6. Heberer T, HJ Stan, Anal. Chim. Acta, 1997; 341:21.
7. Nick K, Scholer HF, Fresenius J Anal. Chem., 1992; 343:304.
8. Dasgupta A, Jagannath C. Ther. Drug Monit. 1999; 21:238.
9. Haas R, Smidt TC, Steinbach K, von Low E. Fresenius J Anal. Chem., 1997; 359:497.
10. Longo M, Cavallaro A, J Chromatogr. A, 1996; 753:91.
11. Lee HB, Chromatogr J, 1988; 457:267.
12. Patsias J, Papadopoulou-Mourkidou E. J AOAC Int., 1999; 82:968.
13. Pocurull E, Calull M, Marc´e RM, Borull F. Chromatographia, 1996; 719:105.
14. Puig D, Barcel´o D. Chromatographia, 1995; 40:435.
15. Landzettel WJ, Hargis KJ, Caboot JB, Adkins KL, Strein TG, Veening H, Becker HD. J Chromatogr. A, 1995; 718:45.
16. Kawamura K, Kaplan IR. Environ. Sci. Technol.. 1983; 17:497.
17. Turnes MI, Rodr´ıguez I. MC Mejuto, R. Cela, J. Chromatogr. A, 1994; 683:21.
18. Rodr´ıguez I, Turnes MI, Mejuto MC, Cela R. J Chromatogr. A, 1996; 721:297.
19. Hennion MC, Trends Anal. Chem., 1991; 10:317.

20. Rodr´ıguez I, Turnes MI, Mejuto MC, Cela R. J Chromatogr. A, 1997; 786:285.
21. Jung MW, Lee DW, Rhee JS, Paeng KJ. Anal. Sci. Int., 1996; 12:981.
22. Pocurull E, Marc´e RM, Borrull F. Chromatographia, 1995; 41:521.
23. Brouwer ER, Th UA. Brinkman J Chromatogr. A, 1994; 678:521.
24. Mußmann P, Levsen K, Radeck W. Fresenius J Anal. Chem., 1994; 348:654.
25. Pocurull E, Calull M, Marc´e RM. Borrull F. Chromatographia, 1994; 38:579.
26. Feng YQ, Xie MJ, Da SL, Anal. Chem. Acta, 2000; 403:187.
27. Tang Y, Zukowwski J, Armstrong DW, Chromatogr J. A, 1996; 743:261.
28. Lee SH, Berthold A, Armstrong DW. J Chromatogr A, 1992; 603:82.
29. Fan Y, Fenge YQ, Da SL, Anal. Chim. Acta., 2003; 484:145.
30. Li G, Zheng Y, Hu Y, Anal. Sci., 2004; 20:667.
31. Erickson K, Erni R, Lee Z, Alem N, Gannett W. A Zettl, Adv. Mater, 2010; 22:4467-4472.
32. Bagheri H, Saraji M. J Chromatogr A, 2001; 910:87.
33. Bagheri H, Saraji M, Chromatogr J A, 2003; 986:111.
34. Rodr´ıguez I, Llompart MP, Cela R. J Chromatogr A, 2000; 885:291.
35. Ali Moghimi. Oriental Journal of Chemistry. 2006; 22(3):527.
36. Moghimi A. Journal of Chemical Health Risks, 2014, 4(2):15-22.
37. Moghimi A. Russian Journal of Physical Chemistry A, 2013; 87(11):1851-1858.
38. Moghimi A. Oriental Journal of Chemistry, 2006; 22(3):527.
39. Nayebi P. Moghimi A. Oriental Journal of Chemistry. 2006; 22(3):507.
40. Moghimi A. Chinese Journal of Chemistry, 2007; 25:640.
41. Moghimi A. Chinese Journal of Chemistry. 2007; 25(10):1536.
42. Moghimi A. Russian Journal of Physical Chemistry A. 2013; 87(7):1203-1209.