# Polyaniline/Graphene Nanocomposite as a Promising Sorbent for Dispersive Solid Phase Extraction of Avermectins from Citrus Fruit Juice

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A solid phase extraction sorbent based on polyaniline/graphene nanocomposite is presented. The structure and morphology of the synthesized nanocomposite were investigated by Fourier transform infrared spectroscopy, field emission scanning electron microscopy, X-ray diffraction and thermal gravimetric analysis. The dispersive solid phase extraction was employed to the isolation and preconcentration of avermectins insecticide (mixture of $B_{1a}$ and $B_{1b}$). The extraction procedure was investigated by high performance liquid chromatography-UV detection. The sorbent demonstrated a favorable analytical performance for avermectins detection with reasonable linear ranges (1.5-1000 µg l$^{-1}$ and 5.0-1000 µg l$^{-1}$ for $B_{1a}$ and $B_{1b}$ in order) and acceptable detection limits (0.5 µg l$^{-1}$ for $B_{1a}$ and 2.5 µg l$^{-1}$ for $B_{1b}$) under optimized conditions. The extraction efficiency of polyaniline/graphene nanocomposite in the extraction of avermectins was compared with graphene, polyaniline, carbon nanotube and C18 sorbents. Moreover, the applicability of proposed method was assessed for the extraction of analyte from citrus fruit juice.

**Keywords:** Polyanilline/graphene nanocomposite, Dispersive solid phase extraction, Avermectins, High performance liquid chromatography, Citrus fruit juice

## INTRODUCTION

Nowadays, pest control and quality enhancement of agricultural products is commonly performed using chemical pesticides. Avermectins are one of these pesticides which widely used to control insects and mites in vegetables and fruits by acting on nervous system [1,2]. Avermectins (Fig. 1) are present in two forms of $B_{1a}$ (80%) and $B_{1b}$ (20%). However, they could cause adverse health effects, and the exposure potential to their residues in the diet is also threatening. Thus, development of a sensitive method for the determination of avermectins in fruit juices and fresh fruit at trace levels is increasingly demanding.

High performance liquid chromatography-UV detection (HPLC-UV), HPLC-fluorescence detection and Liquid

chromatography-mass spectrometry (LC-MS) are mainly used for determination of avermectins [3-5]. Liquid-liquid extraction (LLE) and solid-phase extraction (SPE) have also been used for the isolation, enrichment and cleanup of avermectins from animal tissues, fruit juice, and vegetables [6,7]. However, LLE requires the use of large amount of highly-purified solvents, which are frequently hazardous and leads to the production of toxic laboratory waste. In addition, it is tedious and time consuming. Although SPE technique is likely to be promising and needs less organic solvent, the commercial SPE cartridges are rather expensive. Thus there is an increasing tendency to the synthesis of less expensive efficient sorbents [8].

In recent years the novel member of carbon family, graphene (Gr), has been attracted great attention. This could be due to its outstanding charge transport, thermal, optical, and mechanical properties. Extensively large surface area, π-electron-rich structure, and thermal and chemical stability

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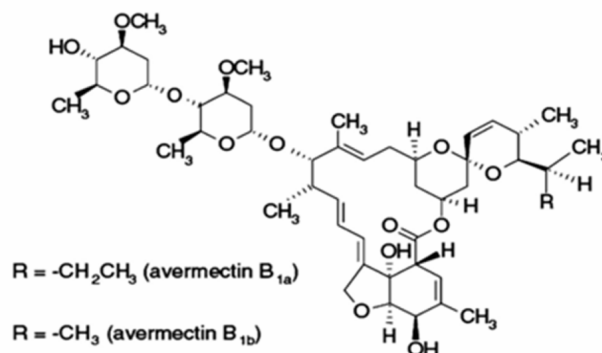*Corresponding author. E-mail: faezeh.khalilian@yahoo.com

**Fig. 1.** Chemical structure of avermectins.

makes this material a promising adsorbent in solid phase-based extraction techniques such as dispersive solid phase extraction (DSPE) [9-13]. Simple chemical methods of synthesis [14], accessibility of both surfaces of the planar sheet and consequently its larger surface area are significant advantages of graphene [15,16]. Furthermore, flexibility of graphene sheets makes it possible to be attached simply to other structures. This property is particularly beneficial in preparing adsorbents such as conductive polymers-based composite. Among conductive polymers, polyaniline (PANI) in particular, has been corroborated a big attraction as solid phase extraction sorbent due to their multifunctional properties such as their hydrophobicity, acid-base character, π-π interaction, polar functional groups, ion exchange property, hydrogen bonding, and their electroactivity [17-24].

In this work, graphene is chemically synthesized from graphite and then chemical polymerization of aniline is performed in a suspension of graphene in water. In the following, the potential of PANI/Gr composite in DSPE of avermectins from fruit juice samples is investigated.

## EXPERIMENTAL

### Reagents and Chemicals

Natural flake graphite (99.9%) was obtained from Merck (Darmstadt, Germany). Sulfuric acid, nitric acid, hydrochloric acid, potassium chlorate, hydrazine hydrate (80%), ammonium peroxydisulfate (APS) and sodium hydroxide were all purchased from Merck (Darmstadt, Germany). Aniline, acetonitrile, acetone, methanol and ethanol were of analytical grade and obtained from Merck. HPLC grade water was purchased from Caledon (Ontario, Canada). Avermectins powder (containing 80% $B_{1a}$, 20% $B_{1b}$) was obtained from Sigma-Aldrich (St. Louis, USA). A stock standard solution containing 100 mg $l^{-1}$ of avermectins was prepared by dissolving an appropriate amount of powder in methanol. Standard solutions of avermectins were prepared by diluting the stock solution with methanol and were stored at 4 ºC.

### Apparatus

An Agilent 1200 series HPLC system including a quaternary pump and a UV detector were used for separation and determination of the analytes. The separation was performed on Zorbax Eclipse XDB-$C_{18}$ (150 mm × 4.6 mm ID, 5 µm) column. The chromatographic data were collected and recorded using ChemStation software. The direct sample introduction was carried out using a Rheodyne manual injector (Rohnert Park, CA, USA) with a 20 µl loop. Column temperature was kept constant at 25 °C using a thermostatted column compartment. Chromatographic separations were carried out using the isocratic elution with the mixture of acetonitrile and water (80:20). The flow rate was 1.4 ml $min^{-1}$ and detection was performed at 254 nm [8].

### Synthesis of Polyaniline/Graphene Composite

Graphene oxide was prepared according Staudenmaier method [25]. Briefly, an amount of 1 g graphite was carefully added to a mixture containing 18 ml sulfuric acid and 9 ml nitric acid, placed in an ice bath. After proper

dispersion of graphite powder, an amount of 11 g potassium chlorate was added slowly to this solution. The mixture was stirred at room temperature for 120 h. After completion of the reaction, the resulting product was washed with deionized water until obtaining neutral pH. Graphite oxide was dried at 60 °C in a vacuum oven.

Dispersing graphite oxide in water using ultrasonic bath caused to unfold graphite oxide sheet and resulted in formation of graphene oxide. So, an amount of 350 mg graphite oxide was added to 350 ml of water and it was placed under ultrasonic condition. Then, the volume of 5 ml hydrazine hydrate (80%) was added and the mixture was stirred for 4 h at 100 °C. The mixture was centrifuged and rinsed with deionized water. The resulting product was dried for 24 h in a vacuum oven at 50 °C [26].

To synthesize polyaniline/graphene composite, an amount of 100 mg graphene and 0.1 ml distilled aniline were added into 40 ml HCl solution (0.2 M). After sonication of this mixture, a 20 ml solution containing 0.125 g APS and 0.2 M HCl was rapidly added. Polymerization reaction was performed for 12 h at room temperature. The resulting dark green precipitate was washed several times with water and ethanol. This product was dried in a vacuum oven at 80 °C for 24 h [27].

## DSPE Procedure

To perform DSPE process, a volume of 10 ml sample containing avermectins at concentration level of 100 $\mu$g l$^{-1}$ and 5% (m v$^{-1}$) NaCl was transferred into a test tube to perform extraction. In the following, 100 mg polyaniline/graphene composite was added and the mixture was placed in an ultrasonic bath for dispersion of sorbent in solution. The extraction was performed for 5 min at room temperature while stirring at 1500 rpm with a magnetic stirrer. Then, the mixture was centrifuged and after washing the sorbent, desorption process was carried out using 800 $\mu$l of ethanol in 2 min. The eluent was dried using a gentle flow of N$_2$ gas. The residue was dissolved in 50 $\mu$l methanol and then injected into the HPLC system.

## RESULTS AND DISCUSSION

## Characterization of Polyaniline/Graphene Composite

XRD patterns for both Gr and PANI/Gr composite (Fig.

2) showed peaks at $2\theta = 26.53$ degrees, which are in accordance with the literature [28,29]. Despite the similarity between X-ray diffraction patterns for composite and graphene, the peak intensities of the composite are slightly lower. This reduction in intensities corroborates the coverage of graphene sheets by polymer.

FT-IR spectra of PANI, Gr and PANI/Gr composite are shown in Fig. 3. The appearance of peaks at 1573 and 1489 cm$^{-1}$ are attributed to the C=C stretching of the quininoid and benzenoid rings, respectively. Moreover, the band at 3443 cm$^{-1}$ is due to the N-H stretching of PANI. The characteristic bands of graphene for C-O, C=C, C=O, C-H and O-H are appeared at 1049, 1639, 1738, 2920 and 3421 cm$^{-1}$, respectively. The presence of polyaniline in PANI/Gr composite is responsible for the shifts to 1649 and 3457 cm$^{-1}$ which is due to the $\pi$-$\pi$ interaction and hydrogen bonding, in order [29,30].

Figure 4 display FE-SEM images of Gr and PANI/Gr composite. According to the images, the thickness of graphene sheets, after coverage, was in the range of 23-50 nm. These images show a rather high surface area and also indicate coverage of graphene sheets by polyaniline and confirm formation of nano-sized composite.

To estimate the amount of polyaniline in nanocomposite, a thermal gravimetric analysis (TGA) was performed. According to the TGA analysis (Fig. 5), the portion of conductive polymer in this structure was estimated 22.16%, in accordance with the weight loss after 400 °C.

## DSPE Optimization Process

The effect of influential parameters on DSPE method including sorbent amount, extraction time, ionic strength, type and volume of desorption solvent, desorption time were studied. Since both avermectins are neutral compound, no further optimization was performed for pH adjustment and all experiments were accomplished in neutral medium. A univariate approach was employed to optimize the influential factors. Moreover, the peak area was used to assess the extraction efficiency under investigated condition.

Sorbent amount is one of the parameters that influence the extraction capacity and sensitivity of DSPE method. The extraction efficiency was increased up to 100 mg and afterward a steadiness was observed for both avermectins.
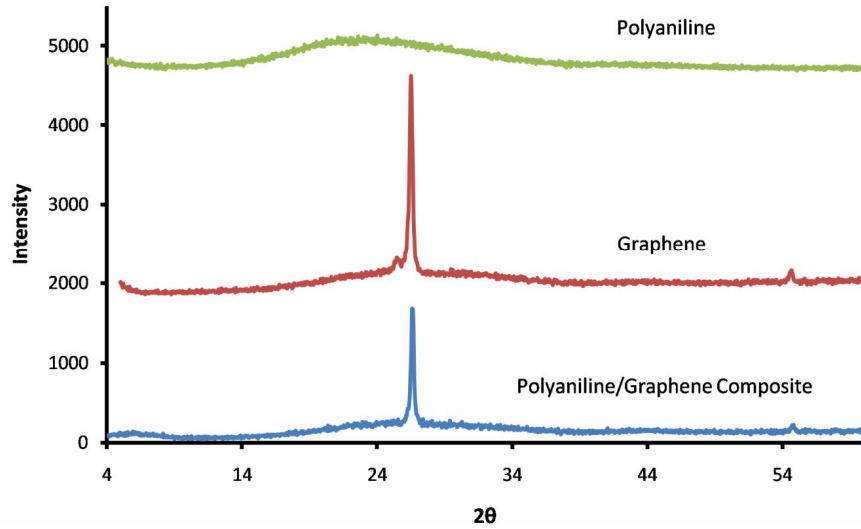
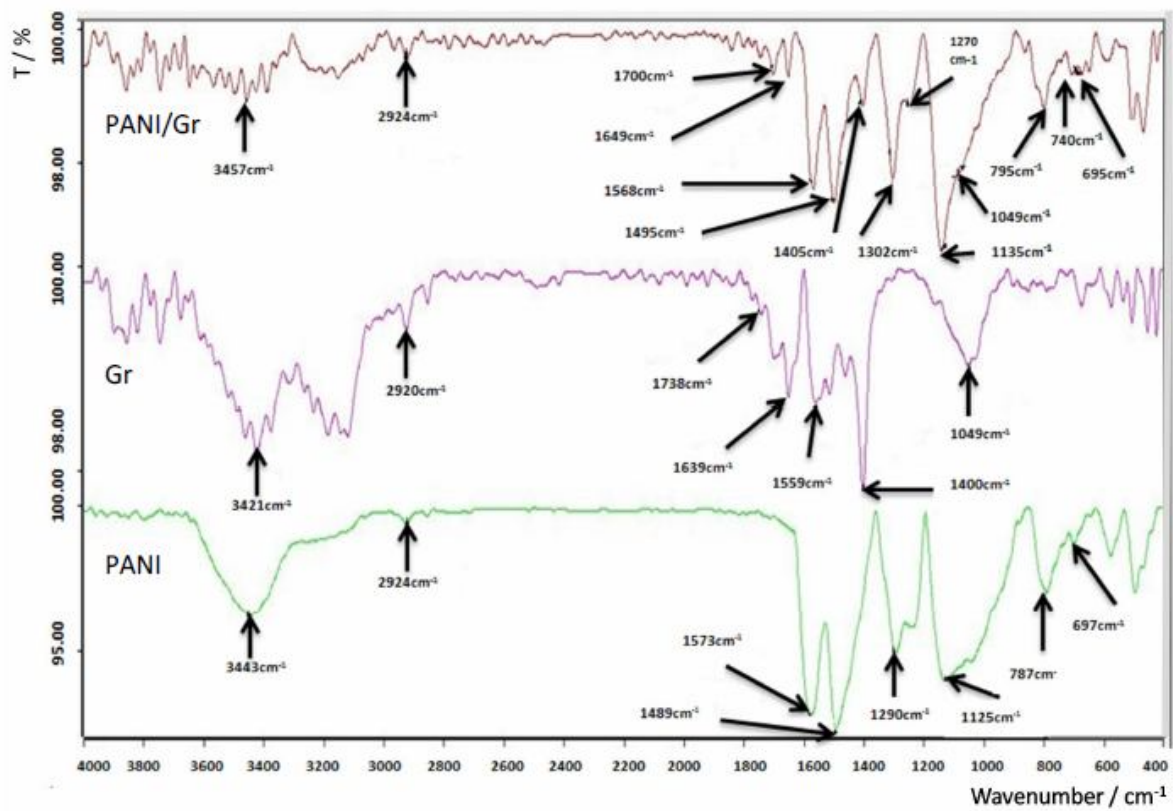**Fig. 2.** X-ray diffraction patterns of a) polyaniline, b) graphene, and c) polyaniline/graphene composite.

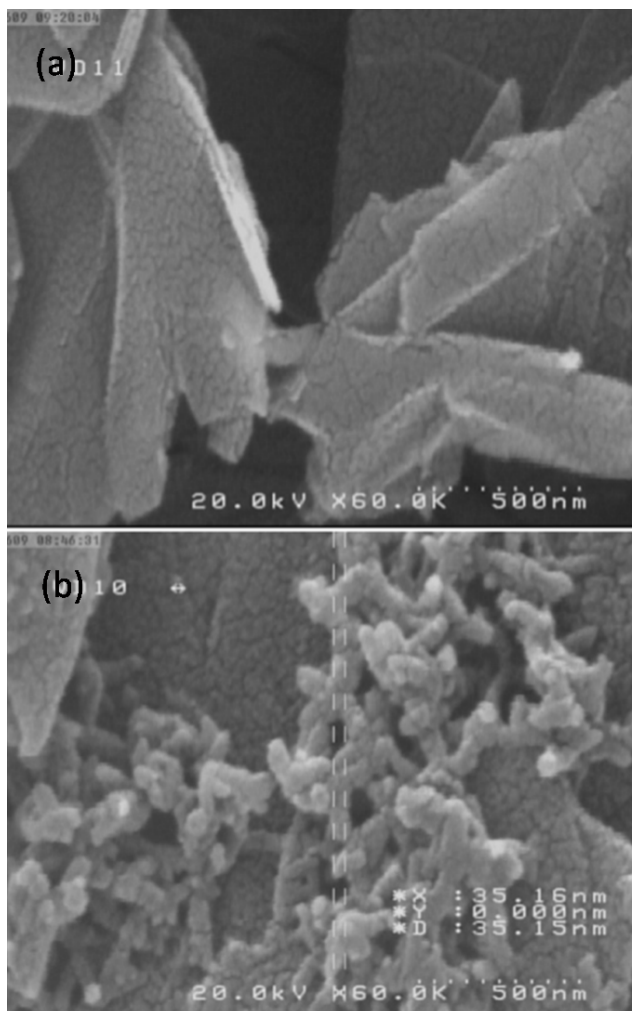**Fig. 3.** FT-IR spectra of PANI, Gr and PANI/Gr.

**Fig. 4.** FE-SEM images of a) graphene and b) polyaniline/graphene.

So, an amount of 100 mg sorbent was used as the optimum value and it was used in further experiments.

Dispersive solid phase extraction process was investigated in the range of 1 to 25 min. As demonstrated in Fig. 6a, there has been a sharp rise for two compounds up to 5 min. After a decrease, a steady decrease was observed. This gradual drop could be assigned to the agglomeration of sorbent particles causing lower available surface area for the extraction process [31]. Hence, an extraction time of 5 min was used as the optimized time.

Study on salt amount was investigated in the range of 0-30% (m v$^{-1}$) of NaCl. According to the results (Fig. 6b), the best DSPE recoveries were obtained at 15%. Generally,

engagement of water molecules in the hydration spheres around the salt ions would decrease solubility of organic solutes in the aqueous solution resulting in more extraction efficiencies up to 15%. However, increasing solution viscosity could bring about lower extraction recoveries afterwards.

To select the most suitable desorption solvent for desorption process in DSPE, acetone, acetonitrile, ethanol and methanol were utilized. The best results were obtained from ethanol that could be attributed to greater solubility of the analytes in this solvent. Subsequently, the volume of ethanol as desorption solvent were studied in the range of 200-1100 µl. According to Fig. 6c, desorption process was
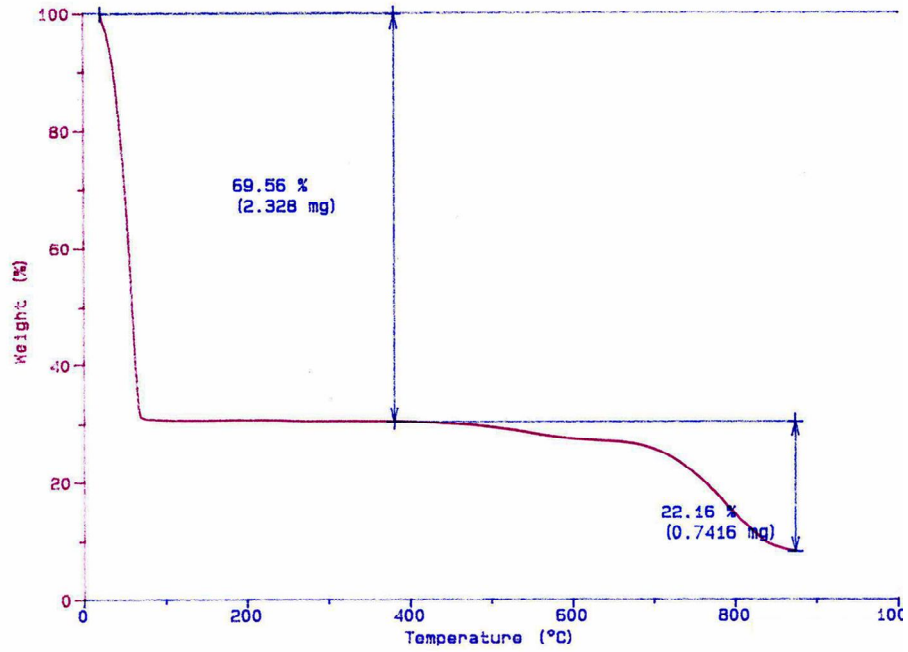
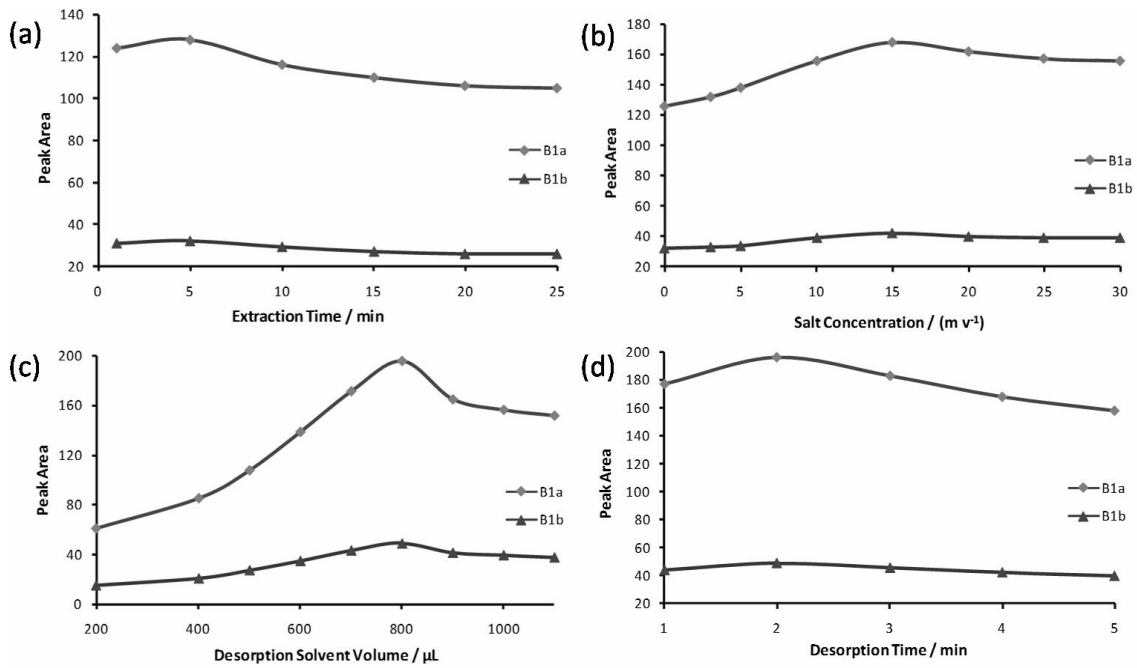**Fig. 5.** TGA curve of polyaniline/graphene composite.



**Fig. 6.** (a) Solid phase extraction time profiles of avermectins $B_{1a}$ and $B_{1b}$; (b) ionic strength effect; (c) desorption solvent volume influence; (d) desorption time profile.

**Table 1.** The analytical data obtained from DSPE/HPLC-UV of avermectins using PANI/Gr

| Compound | LDR ($\mu$g L$^{-1}$) | $R^2$ | LOD ($\mu$g L$^{-1}$) | RSD% (n=5) | EF |
|---|---|---|---|---|---|
| B$_{1a}$ | 1.5-1000 | 0.995 | 0.5 | 1.1 | 120 |
| B$_{1b}$ | 5.0-1000 | 0.992 | 2.5 | 2.7 | 74 |

carried out using 800 $\mu$l of ethanol.

Desorption time were also studied in the range of 1-5 min. As demonstrated in Fig. 6d, desorption time of 2 min is quite suitable. Agglomeration of nanoparticles and therefore trapping of the analytes in the middle space of sorbent particles could be responsible for this significant decrease [31].

**Polyaniline/Graphene Sorbent Lifetime**

By performing repeated extractions under optimized experimental conditions, the sorbent lifetime was studied. The sorbent regeneration was performed using ethanol after each extraction step. It was proved that PANI/Gr sorbent could be used for about 5 extraction/desorption cycles with no noticeable drop in efficiency.

**Method Validation**

Considering the optimized parameters, quantitative analysis was assessed for the determination of the target analytes. As demonstrated in the Table 1, wide linear dynamic ranges (LDRs) along with good $R^2$ values were acquired for B$_{1a}$ and B$_{1b}$, respectively. Reproducibility of the method based on RSD% (n = 5) values were assessed. These values were acquired 1.1 and 2.7% for B$_{1a}$ and B$_{1b}$ at the concentration level of 100 $\mu$g l$^{-1}$, respectively. The amounts of limit of detection (LOD) based on the signal to noise ratio of 3 were obtained 0.5 $\mu$g l$^{-1}$ for B$_{1a}$ and 2.5 $\mu$g l$^{-1}$ for B$_{1b}$. Since the lowest MRL for avermectins is 10 $\mu$g l$^{-1}$, the LOD amounts shows the enough sensitivity of the developed method to trace avermectins residues in citrus fruit juice. In addition, enrichment factor (EF) of the proposed method was obtained 120 for B$_{1a}$ and 74 for B$_{1b}$, respectively.

The ability of PANI/Gr nanocomposite in the extraction of avermectins was compared with various novel and classic sorbents including Gr, PANI, carbon nanotubr (CNT) and C18. Nanocomposite was shown 1.8, 1.4, 4.3 and 3.1 times enhancement in recoveries compared to these sorbents, respectively. As expected, C18, regarding to its low polarity, showed less efficiency in comparison to other sorbents. Although characteristic structures of carbon nanotubes also allow interaction with organic molecules via non-covalent forces [32], surface area plays a key role compared with graphene. The large delocalized $\pi$-electron system of graphene, and its ability in contribution of $\pi$-$\pi$ interaction with avermectins along with its large surface area [12] have been effective in extraction process. PANI also provides high signal due to the multifunctional properties mentioned before [17-24]. According to the experiments, synergic effect resulted in best extraction efficiency of PANI/Gr in comparison with PANI and Gr.

Comparing proposed method with our previous work in extraction of avermectins, magnetic SPE-dispersive liquid liquid microextraction-HPLC-UV [8], proves comparable LODs without using dispersive liquid liquid microextraction as a coupled method. Without using a sensitive detector such as FLD and MS-MS our method shows lower limit of detection and wider linear dynamic range in comparison with ultrasonic assisted extraction-HPLC-FLD [33] and solvent extraction-HPLC-ESI-MS-MS [34].

To evaluate the applicability of the proposed method, experiments were performed using an orange juice sample. The sample was diluted two times and then was extracted under optimized conditions. The chromatograms (Fig. 7) were confirmed the absence of avermectins in fruit juice in non-spiked samples. The amounts of relative recoveries for orange juice sample were satisfactory and they were obtained 97.0 and 95.6% for B$_{1a}$ and B$_{1b}$, respectively.
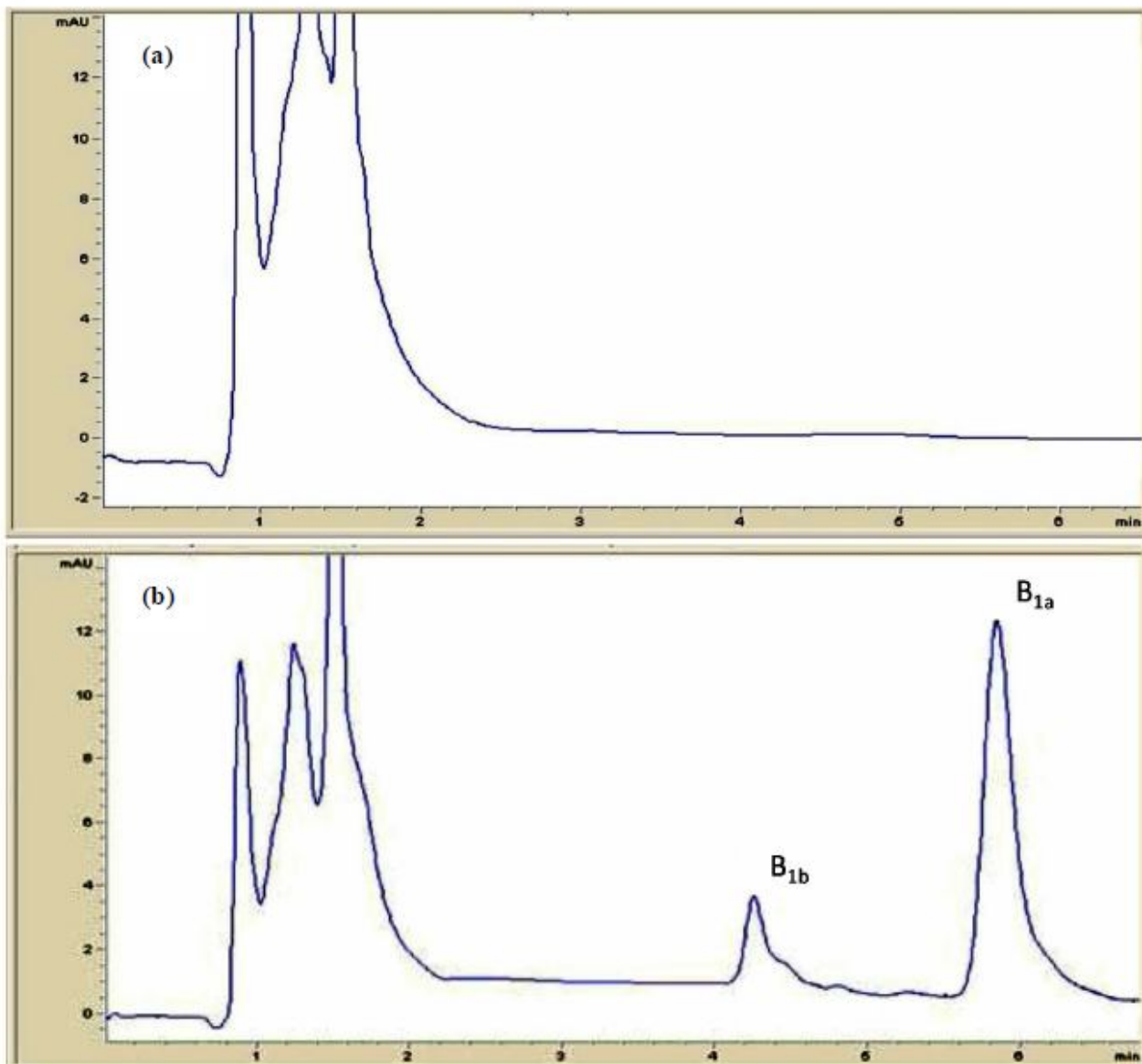
**Fig. 7.** Chromatograms obtained from DSPE/HPLC-UV of avermectins from orange juice sample (a) before and (b) after being spiked with avermectins at concentration level of 10 μg l$^{-1}$.

## CONCLUSIONS

The principal aim of this study was to evaluate the potential of graphene-based material as a sorbent in sample preparation field. Regarding to this objective, the synthesized nanocomposite was used as a sorbent in dispersive solid phase extraction of avermectins. Method validation proved good sensitivity and reproducibility along with an appropriate enrichment factor for both avermectins. Furthermore, relative recoveries data obtained from the extraction of avermectins from fruit juices reveal robustness and feasibility of graphene-based sorbents in sample preparation field. Comparing extraction of avermectins with different sorbents was corroborated superiority of

synthesized nanocomposite. Possessing high specific surface area and consequently high loading capacity of nanocomposite along with capability of the chemical structures of both graphene and polyaniline for contribution in π-π interaction between the analytes and the sorbent, makes the PANI/Gr composite suitable for extracting compounds having aromatic moiety.

## ACKNOWLEDGEMENTS

## REFERENCES

[1]    Q. Wang, J.A. Chen, Z.M. Liu, S.G. Wu, X.P. Zhao, C.X. Wu, Insect. Sci. 12 (2005) 109.

[2]    J.L. Shipp, K. Wang, G. Ferguson, Biol. Control 17 (2000) 125.

[3]    A.I. Valenzuela, D.S. Popa, M.J. Redondo, J. Chromatogr. A 918 (2001) 59.

[4]    B. Roudant, Analyst 123 (1998) 2541.

[5]    L. Kolar, J. Kuzjner, N.K. Erzjen, Biomed. Chromatogr. 18 (2004) 117.

[6]    G.L. Bienvenida, J.F. Garcia-Reyes, M.D. Antonio, Talanta 79 (2009) 109.

[7]    X. Feas, J.A. Seijas, M.P. Vazquez-Tato, P. Regal, A. Cepeda, C. Fente, Anal.

[8]    Chim. Acta 631 (2009) 237.

[9]    F. Khalilian, M. Rezaee, M. Kashani Gorgabi, Anal. Methods 7 (2015) 2182.

[10]   V. Singh, D. Joung, L. Zhai, S. Das, I. Saiful, S. Seal, Prog. Mater. Sci. 56 (2011) 1178.

[11]   Q. Liu, J. Shi, G. Jiang, Trends Anal. Chem. 37 (2012) 1.

[12]   F. Khalilian, S. Ahmadian, J. Sep. Sci. 39 (2016) 1500.

[13]   N. Li, J. Chen, Y. Shi, Talanta 141 (2015) 212.

[14]   A. Speltini, M. Sturini, F. Maraschi, L. Consoli, A. Zeffiro, A. Profumo, J. Chromatogr. A 1379 (2015) 9.

[15]   S. Park, R.S. Ruoff, Nat. Nanotechnol. 4 (2009) 217.

[16]   M.D. Stoller, S.J. Park, Y.W. Zhu, J.H. An, R.S. Ruoff, Nano Lett. 8 (2008) 3498.

[17]   K.P. Loh, Q.L. Bao, G. Eda, M. Chhowalla, Nat. Chem. 2 (2010) 1015.

[18]   P.R. Teasdale, G.G. Wallace, Analyst 118 (1993) 329.

[19]   S.B. Adeloju, G.G. Wallace, Analyst 121 (1996) 699.

[20]   A.G. MacDiarmid, Angew. Chem. Int. Ed. 40 (2001) 2581.

[21]   H. Shirakawa, Angew. Chem. Int. Ed. 40 (2001) 2574.

[22]   U. Lange, N.V. Roznyatovskaya, V.M. Mirsky, Anal. Chim. Acta 614 (2008) 1.

[23]   H. Bagheri, N. Alipour, Z. Ayazi, Anal. Chim. Acta 740 (2012) 43.

[24]   H. Bagheri, Z. Ayazib, M. Naderi, Anal. Chim. Acta 767, 12 (2013) 1.

[25]   L. Liu, H. Liu, Y. Li, X. Wang, X. Du, Anal. Method. 6 (2014) 3467.

[26]   L. Staudenmaier, Ber. Dtsch. Chem. Ges. 31 (1898) 1481.

[27]   H. Hu, X. Wang, J. Wang, F. Liu, M. Zhang, C. Xu, Appl. Surf. Sci. 257 (2011) 2637.

[28]   Y. Li, H. Peng, G. Li, K. Chen, Eur. Polym. J. 48 (2012) 1406.

[29]   G. Wang, S. Zhuo, W. Xing, Mater. Lett. 69 (2012) 27.

[30]   G. Wang, W. Xing, S. Zhuo, Electrochim. Acta 66 (2012) 151.

[31]   W. He, W. Zhang, Y. Li., X. Jing, Synth. Met. 162 (2012) 1107.

[32]   F. Khalilian, S. Amiri Hanzaki, M. Yousefi, J. Sep. Sci. 38 (2015) 975.

[33]   K. Pyrzynska, Sep. Purif. Rev. 37 (2008) 372.

[34]   X. Xie, X. Wang, L. Zhao, Food Anal. Methods 4 (2011) 203.

[35]   O.J. Pozo, J.M. Marin, J.V. Sancho, F. Hernandez, J. Chromatogr. A 992 (2003) 133.