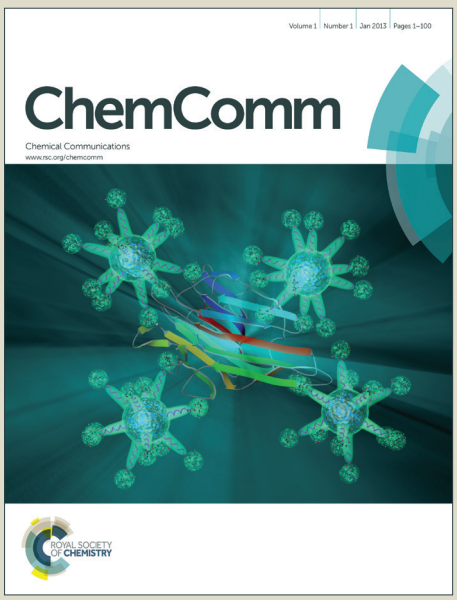
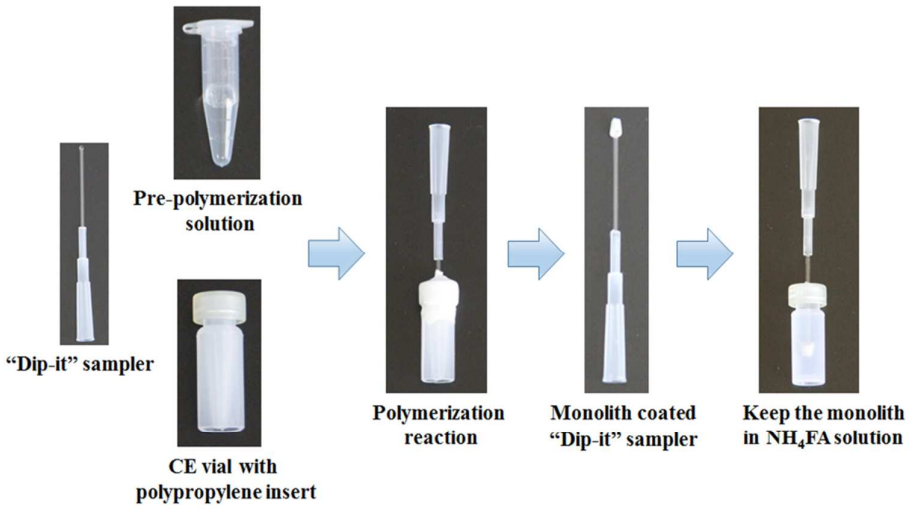
ChemComm

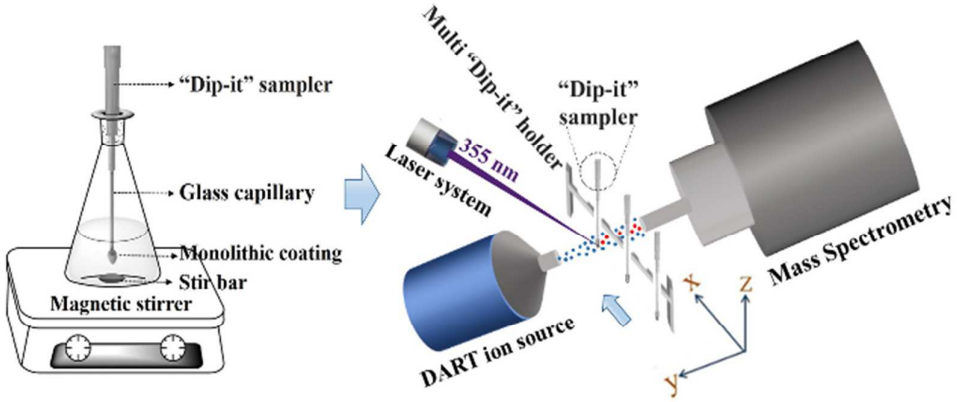
Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm





Polymer monolith microextraction coupled to plasma assisted laser desorption ionization mass spectrometry for rapid and organic solvent-free trace analysis.

Chemical Communications RSCPublishing

COMMUNICATION

Just dip it: online coupling of "Dip-it" polymer monolith microextraction with plasma assisted laser desorption ionization mass spectrometry

Cite this: DOI: 10.1039/xoxxoooooox

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

www.rsc.org/

Xin Wang, Xianjiang Li, Yu Bai* and Huwei Liu*

A polymer monolith microextraction (PMME) procedure coupled to plasma assisted laser desorption ionization mass spectrometry (PMME-PALDI-MS) was developed for rapid and organic solvent-free trace analysis. The extraction device used "Dip-it" sampler coated with MWNT incorporated monolith, and the analytes adsorbed on monoliths were effectively desorbed by laser, improving detection sensitivity.

The determination of trace residues and contaminants in complicated matrix often requires extensive sample preparation prior to instrumental analysis.[1] Usually, sample preparation is the bottleneck in a whole analytical procedure, and minimized preparation steps is highly desired to reduce both time and sources of error.[2] In recent years, polymer monolith has been considered as an attractive material for sample pretreatment,[3, 4] because its advantages such as easy preparation, high permeability, satisfactory loading capacity, large surface area and good control of porosity. Nowadays, a variety of monomers and crosslinkers are available, so diverse biocompatible and pH-stable polymer monoliths can be tailored by introducing different functional groups.[5, 6] The biocompatibility of polymer monolith allows the direct analysis of samples in complicated matrix after extraction, with no manipulations other than dilution or centrifugation, greatly simplifying the entire sample pretreatment procedures.[7] On the other hand, most analytical methods rely on the separation by liquid chromatography (LC) or gas chromatography (GC), which make the entire method complicated and time-consuming. Ambient mass spectrometry (AMS), that the ionization takes place in open air under ambient conditions, is frequently used for the rapid determination or screening of analytes without the need of chromatography separation in some cases.[8]

Direct analysis in real time (DART)[9] ionization source, developed by Cody *et al*., is operated by exposing sample to a gas stream of helium or nitrogen. Ionization of analytes is then performed by the interactions of excited metastable gas with analytes and the atmospheric gases.[10] DART-MS provides a rapid way to analyze various samples, including explosives,[11] dyes,[12] toxic industrial chemicals[13] and organometallic compounds.[14] However, the sensitivity of DART-MS is often lower than traditional GC-MS or LC-MS methods. Two strategies have been proposed to solve this issue. The first one is to use suitable sample pretreatment method before DART-MS analysis for the cleanup of matrix and enrichment of the desired analytes at the same time.[15, 16] The other strategy is conducted by introducing laser desorption into DART-MS to construct an ambient plasma assisted laser desorption ionization mass spectrometry (PALDI-MS) system.[17] With the laser desorption process, more analyte molecules will be desorbed and the signal intensity will be definitely improved.[18]

Herein, we report our initial efforts to combine these two strategies together to fabricate a novel polymer monolith for microextraction followed by PALDI-MS detection (Fig. 1 and Fig. S1). The PALDI-MS system is composed of four parts: DART ion source, ToF mass spectrometry, laser system and Multi "Dip-it" holder (Fig. 1B). In our previous publication,[17] sample was introduced by the thin layer plate, so the analytes were firstly desorbed by laser and then ionized by DART. While in this experiment, in order to realize the online coupling of PMME with PALDI-MS, the analytes were extracted onto the "Dip-it" sampler and then *in situ* desorption and detection were performed at the same time. In the experiment, polymer monolith was prepared on the external surface of the glass capillary of "Dip-it" sampler; the monolith coated sampler was then applied for microextraction (Fig. 1A). After that, the "Dip-it" samplers were placed to the Multi "Dip-it" holder and the movement of the holder was controlled by software. With the movement of the holder, the "Dip-it" samplers with monolithic coating passed through DART ion source, and laser beam was focused on the polymer monolith when the "Dip-it" sampler was placed in the middle between the DART outlet and MS inlet. So the analytes would be desorbed from monolith by the laser beam, ionized by the DART and detected by the MS simultaneously. Since the desorption and detection were conducted at the same time, this method was truly "real time" and the analytes were fast quantified within only 30 s for each sample. Moreover, no organic solvent was needed in the desorption procedure, so the proposed method was environmental friendly and laboursaving.

Multi-wall carbon nanotubes (MWNT) incorporated monolith was used in this experiment since carbon nanomaterials can act as

medium to transfer the energy of laser beam to the analytes during the ionization step.[19, 20] The polymer monolith was synthesized by *in situ* thermally initiated polymerization using methacrylic acid (MAA) as functional monomer and ethylene dimethacrylate (EDMA) as the crosslinker (poly(MAA-EDMA-MWNT) monolith). To make MWNT fully disperse in the pre-polymerization solution, oxidative cutting of MWNT was proceeded using the previously reported method.[15, 21] The key point for preparing the monolith coated "Dip-it" sampler is to protect the coating from damage. So the polymerization reaction was proceeded in an Agilent CE vial with 250 μL polypropylene insert, and the glass capillary of "Dip-it" sampler was derivatized with 3-(trimethoxysilyl) propyl methacrylate before use to make the monolithic coating attach tightly to the "Dip-it" sampler. The details of preparing monolith coated "Dip-it" sampler are illustrated in the Supporting Information (Fig. S2).

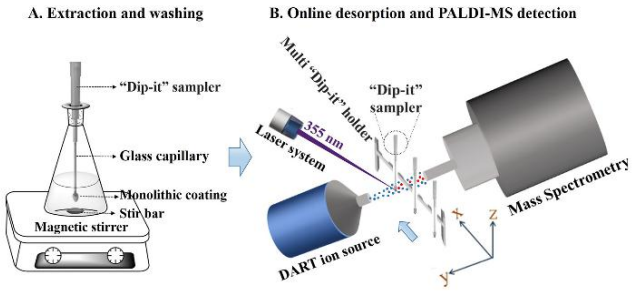


Fig. 1 Experimental set up and configuration for "Dip-it" PMME (A) and desorption/ionization using PALDI-MS system (B).

The morphology of the poly(MAA-EDMA-MWNT) monolith was examined by SEM, and a typical micrograph was illustrated in Fig. 2. Cross section of the homogeneous micro-globules could be clearly observed. Moreover, the micro-globules interconnected to form large clusters, which resulted in uniform polymer-based monolithic matrix with micrometer-sized through-pores. The through-pore properties of the monolith were further measured by mercury porosimeter (Fig. S3). The results showed that the monolithic coating contained through-pores of approximately 1.6 μm with a narrow size distribution, which could lead to good permeability and favorable mass transfer during the extraction applications. The specific surface area and mesopore size distribution of the polymer monolith were determined by nitrogen sorption experiments. The surface area of the poly(MAA-EDMA-MWNT) monolith was 212 m^2/g and the average mesopore size was 3.51 nm. The existence of mesopores and high specific surface area would ensure satisfactory extraction capacity of the prepared monolithic coating. Moreover, thermogravimetric analysis of the monolith was performed and the results were illustrated in Fig. S4, showing that the monolith was stable at the temperature below 235 ℃.

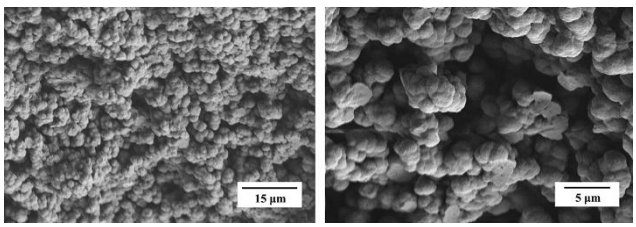


Fig. 2 Scanning electron microscope images of the poly(MAA-EDMA-MWNT) monolithic coating.

Seven triazines were selected as analytes to evaluate the extraction performance of the monolithic coating. Several parameters affecting the extraction efficiency were optimized, including the pH, salt concentration, organic solvent content of the sample loading solution and the extraction time (Fig. 3). The pH of sample loading solution is very important in the extraction process. The change of pH results in the change of the charge status for both monolith and analytes, so the extraction efficiency is expected to be pH-dependent. As shown in Fig. 3A, the highest extraction efficiency was reached when pH was between 5.0 and 7.0. Under this condition, the triazines existed in protonated forms and interacted with the ionized carboxyl groups on monolithic coating via ion-exchange interaction. The effect of inorganic salt concentration on extraction efficiency was illustrated in Fig. 3B. The results indicated that the competing adsorption[22] and salting-out effect[23] were all involved in the experiment. These results were consistent with the previous study.[15] The influences of organic solvent content on extraction efficiency are mainly based on two mechanisms. Proper addition of ACN makes the monolith swell, exposes more carboxyl groups and increases the extraction efficiency. However, extraction efficiency also decreases with ACN content increase because of the elution of analytes.[3] Fig. 3C demonstrated that the elution ability of ACN played a major role in this experiment, so the extraction efficiency was severely down with the increase of ACN content. Consequently, the conditions for optimum extraction efficiency of triazines from aqueous media were pH 7.0 of sample loading solution, no inorganic salt and organic solvent were used in the experiment.

To assess the extraction ability of the poly(MAA-EDMA-MWNT) monolithic coating, the equilibrium extraction time profile was investigated by increasing the extraction time from 0.5 h to 4 h. As shown in Fig. 3D, the extracted amounts of the triazines increased rapidly with prolonged extraction time from 0.5 h to 2.5 h, showing great enrichment ability of the monolithic coating. The extraction equilibrium was obtained after 2.5 h, so the signals of analytes did not increase anymore. As a result, 2.5 h was chosen in the further experiments. In order to evaluate the enrichment ability of the monolith, enrichment factors were calculated by comparing the peak areas obtained by PMME-DART-MS with these by direct DART-MS without PMME for the analysis of standard sample solution containing 20 ng/mL triazines[3]. Fig. S5 shows the MS signals of the analytes obtained after PMME (Peak A), and obtained by direct DART-MS without extraction (Peak B). The enrichment factors of seven triazines were found to be 92-258, indicating the significant sensitivity improvements after PMME.

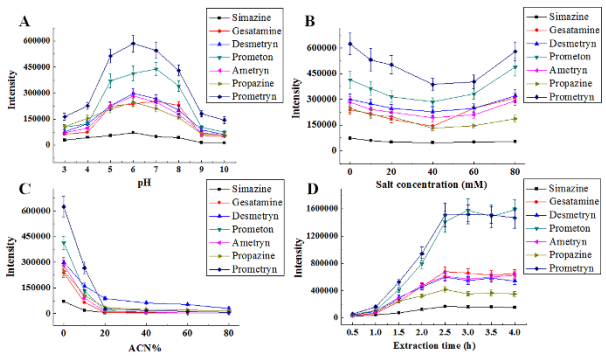


Fig. 3 Effect of pH value (A), salt concentration (B), organic solvent content (C) of sample solution on extraction efficiency; and the equilibrium extraction time profile of triazines for "Dip-it" PMME (D).

In the current study, in order to establish a rapid and totally organic solvent-free method, *in situ* laser desorption and MS detection were performed at the same time. The operating parameters of the DART including plasma heating temperature, the distance between the DART outlet and MS inlet and the laser energy were optimized to obtain the best detection sensitivities of triazines. As shown in Fig. 4A, no MS signals of the analytes were observed when the plasma heating temperature was lower than 300 ℃; when the temperature was higher than 300 ℃, significant signals of the triazines were observed, indicating that thermal desorption mechanism was involved in the experiment. Lastly, 400 ℃ of plasma heating temperature was selected in the present work. It should be noted that 400 ℃ was the plasma temperature when it was generated at DART ion source. When the temperature was set as 400 ℃, the practical temperature of plasma measured at the DART outlet was much lower than 400 ℃ (only 195 ℃). So the monolith was stable enough in the whole experiment. We also optimized the distance between the DART outlet and MS inlet. Fig. 4B shows that the detection sensitivity of triazines decreased with the distance increase. However, bifurcate peaks of some analytes were obtained when the distance between the DART outlet and MS inlet was less than 1.0 cm (Fig. S6). In order to ensure the accuracy of quantitative analysis, the distance of 1.0 cm was selected for further experiments.

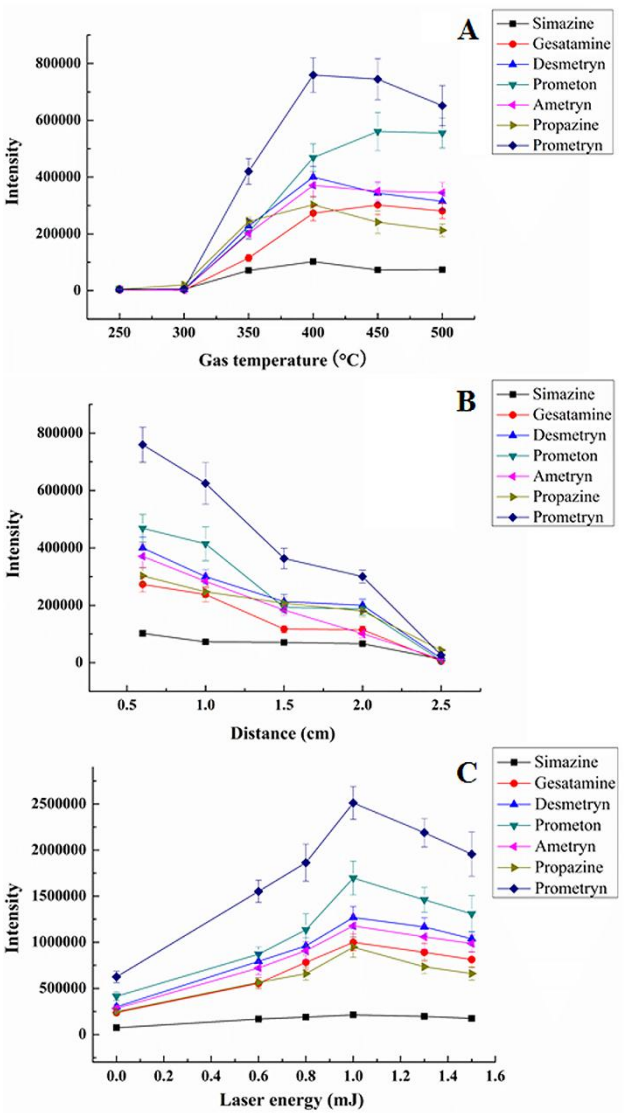


Fig. 4 The optimization of PALDI-MS parameters affecting the detection sensitivities of seven triazines: (A) the plasma heating temperature, (B) the distance between the DART outlet and MS inlet, and (C) the laser energy.

Laser desorption was applied to improve the desorption efficiency, and the laser energy was also optimized systematically. As shown in Fig. 4C, significant signal increase was observed after using laser and the desorption efficiency increased with the increase of laser energy when the laser energy was lower than 1.0 mJ, which suggested that it was very beneficial for laser desorption to be involved in this online system. This might be because the MWNT in monolith can act as medium to transfer the energy of laser beam to the analytes during the desorption step. However, when the laser energy increased from 1.0 mJ to 1.5 mJ, the desorption efficiency decreased gradually and the monolithic coating tended to crack because of the high laser energy. Considering all these factors, 1.0 mJ of laser energy was used in the current study.

Next, an attempt was made to demonstrate that quantitation can be realized using this online "Dip-it" PMME-PALDI-MS method with the help of isotope-labelled internal standard (Atrazine-d5). Under the optimized conditions, validation of the online method was proceeded (Table S1). The matrix-free standard curves were determined by plotting analytes signal intensities versus concentration. To minimize the influence of the monolithic coating quality, relative signal intensities to the isotope-labelled internal standard was used. The linear regression correlation coefficients (R^2) of the calibration curves for triazines were between 0.9957-0.9997 with the relative standard deviations (RSDs) lower than 13%, which was sufficient for quantitative analysis. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated as the concentration corresponding to signal-to-noise ratios of 3 and 10, respectively. The LODs and LOQs of seven triazines were found to be 0.002-0.029 ng/mL and 0.008-0.097 ng/mL, which were lower than the maximum residue levels established by the European Union (EU) for these analytes.[24] From the above, a sensitive and reproducible online "Dip-it" PMME-PALDI-MS method was established, which is promising for the analysis of triazines in complicated real samples.

Lastly, the proposed online "Dip-it" PMME-PALDI-MS method was successfully applied to trace analysis of the triazines in soil sample, and the results were summarized in Table S2. Only desmetryn and prometryne were detected, at the concentration of 1.30 ng/mL and 0.02 ng/mL, respectively. To assay the accuracy of the method, recoveries were investigated by spiking soil samples with target analytes at the concentrations of 1.0 and 10.0 ng/mL. As listed in Table S2, the method recoveries were in the range of 86.2 to 111.0% with RSDs below 13%, indicating that the present method was applicable to routine analysis. Due to the matrix-free calibration curves used in the experiment, the relative recoveries of this method can be used to evaluate the matrix effect.[25] Because the recoveries were between 85 and 115%, the matrix effect of this newly established method was negligible and the method was suitable for the analysis of real sample with complicated matrix.[26] When compared to the reports on triazines analysis published previously,[15, 27-29] this online "Dip-it" PMME-PALDI-MS method provided better sensitivity within a shorter analytical time. These results indicated that even though chromatographic separation was not involved in the experiment, the detection sensitivity of this online method was still satisfactory because of the "Dip-it" PMME and laser desorption procedures.

To further explain the role of MWNT, we have designed a control experiment for the analysis of seven triazines by the online "Dip-it" PMME-PALDI-MS method using poly(MAA-EDMA) monolith, and compared the results with that obtained using poly(MAA-EDMA-MWNT) monolith. As shown in Fig. S7, by using MWNT incorporated monoliths, the signal intensities for the sample containing triazines at 1 ng/mL have increased by 3.0-5.5 fold, compared to those obtained by using the poly(MAA-EDMA) monolith. We further measured the LOQs of seven triazines by using the poly(MAA-EDMA) monolith. The LOQs were found to be 0.044-0.394 ng/mL when the poly(MAA-EDMA) monolith was used, while that were 0.008-0.097 ng/mL when the poly(MAA-EDMA-MWNT) monolith was employed. These results indicated that the carbon nanotubes incorporated monolith improved the detection sensitivity of the online "Dip-it" PMME-PALDI-MS method for triazines analysis. Two factors might be responsible for the positive effects of MWNT on sensitivity: one is the ion-exchange interaction between the amino groups of triazines and the carboxyl groups modified on the tips of nanotubes by the oxidative cutting procedures[21], as well as the hydrophobic and π-π interactions between the MWNT and triazines. Besides, during the desorption step, the laser beam was focused on the monolithic coating and impinged the surface of monolith with quasicircular focal spots. The MWNT on the laser contact surface of monolith may act as medium to transfer the energy of laser beam to the analytes. So, by using the poly(MAA-EDMA-MWNT) monolith, more analyte molecules will be desorbed and the signal intensity will be improved.

In conclusion, a novel poly(MAA-EDMA-MWNT) monolith coated "Dip-it" sampler was prepared in this study and used for polymer monolith microextraction. Based on the proposed extraction device, online coupling of "Dip-it" PMME with PALDI-MS was achieved for the first time and applied in the determination of triazines in soil samples. Compared with traditional LC-MS or GC-MS method, this newly established method was more rapid because *in situ* desorption and detection of analytes were proceeded simultaneously and sample was analyzed without the need of chromatographic separation. In addition, the method did not suffer the negative matrix effect problems, and was environmental friendly since no organic solvent was involved in the entire experimental procedures. For these advantages, this proposed online "Dip-it" PMME-PALDI-MS method provides a new strategy for rapid and sensitive analysis of triazines, and could be useful in many fields, such as pharmaceutical, food and environmental analysis.

We gratefully acknowledge the financial support from the National Natural Science Foundation of China (Grant Nos. 21275012 and 21175008) and the Ministry of Science and Technology of China (Grant Nos. 2013YQ510391 and 2012YQ090194-9).

Notes and references

Beijing National Laboratory for Molecular Sciences, Key Laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education, Institute of Analytical Chemistry, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, P. R. China. Tel.: 86-10-62754976; Fax: 86-10-62751708; E-mail: hwliu@pku.edu.cn

† Electronic supplementary information (ESI) available: Experimental details, additional figures and tables. See DOI: 10.1039/c000000x/

1. W. W. Buchberger, *Anal. Chem. Acta*, 2007, **593**, 129-139.
2. M. M. Moein, R. Said, F. Bassyouni and A. Abdel-Rehim, *J. Anal. Methods Chem.*, 2014, DOI: 10.1155/2014/921350.
3. X. Wang, Q. Ma, M. Li, C. L. Chang, Y. Bai, Y. Q. Feng and H. W. Liu, *J. Chromatogr., A*, 2013, **1317**, 121-128.
4. T. Nema, E. C. Y. Chan and P. C. Ho, *Journal of Pharmaceutical and Biomedical Analysis*, 2014, **87**, 130-141.
5. K. C. Saunders, A. Ghanem, W. B. Hon, E. F. Hilder and P. R. Haddad, *Anal. Chem. Acta*, 2009, **652**, 22-31.
6. Y. C. Liu, L. B. Ren and Z. Liu, *Chem. Commun.*, 2011, **47**, 5067-5069.
7. H. Kataoka and K. Saito, *Journal of Pharmaceutical and Biomedical Analysis*, 2011, **54**, 926-950.
8. M. E. Monge, G. A. Harris, P. Dwivedi and F. M. Fernandez, *Chem. Rev.*, 2013, **113**, 2269-2308.
9. R. B. Cody, J. A. Laramee and H. D. Durst, *Anal. Chem.*, 2005, **77**, 2297-2302.
10. J. H. Gross, *Anal. Bioanal. Chem.*, 2014, **406**, 63-80.
11. F. Rowell, J. Seviour, A. Y. Lim, C. G. Elumbaring-Salazar, J. Loke and J. Ma, *Forensic Sci. Int.*, 2012, **221**, 84-91.
12. C. S. Deroo and R. A. Armitage, *Anal. Chem.*, 2011, **83**, 6924-6928.
13. J. Schurek, L. Vaclavik, H. Hooijerink, O. Lacina, J. Poustka, M. Sharman, M. Caldow, M. W. F. Nielen and J. Hajslova, *Anal. Chem.*, 2008, **80**, 9567-9575.
14. D. L. G. Borges, R. E. Sturgeon, B. Welz, A. J. Curtius and Z. Mester, *Anal. Chem.*, 2009, **81**, 9834-9839.
15. X. Wang, X. J. Li, Z. Li, Y. D. Zhang, Y. Bai and H. W. Liu, *Anal. Chem.*, 2014, **86**, 4739-4747.
16. G. A. Gomez-Rios and J. Pawliszyn, *Chem. Commun.*, 2014, **50**, 12937-12940.
17. J. L. Zhang, Z. G. Zhou, J. W. Yang, W. Zhang, Y. Bai and H. W. Liu, *Anal. Chem.*, 2012, **84**, 1496-1503.
18. A. R. Venter, K. A. Douglass, J. T. Shelley, G. Hasman and E. Honarvar, *Anal. Chem.*, 2014, **86**, 233-249.
19. X. M. He, G. T. Zhu, J. Yin, Q. Zhao, B. F. Yuan and Y. Q. Feng, *J. Chromatogr., A*, 2014, **1351**, 29-36.
20. J. L. Zhang, Z. Li, C. S. Zhang, B. S. Feng, Z. G. Zhou, Y. Bai and H. W. Liu, *Anal. Chem.*, 2012, **84**, 3296-3301.
21. C. Samori, R. Sainz, C. Menard-Moyon, F. M. Toma, E. Venturelli, P. Singh, M. Ballestri, M. Prato and A. Bianco, *Carbon*, 2010, **48**, 2447-2454.
22. Y. B. Luo, Q. A. Ma and Y. Q. Feng, *J. Chromatogr., A*, 2010, **1217**, 3583-3589.
23. Q. W. Yu, X. Wang, Q. Ma, B. F. Yuan, H. B. He and Y. Q. Feng, *Anal. Methods*, 2012, **4**, 1538-1545.
24. J. B. Sass and A. Colangelo, *Int. J. Occup. Env. Heal.*, 2006, **12**, 260-267.
25. A. Van Eeckhaut, K. Lanckmans, S. Sarre, I. Smolders and Y. Michotte, *J. Chromatogr., B*, 2009, **877**, 2198-2207.
26. L. Wang, C. Duan, D. Wu and Y. Guan, *J. Chromatogr., A*, 2014, **1359**, 44-51.
27. M. B. R. Cerqueira, S. S. Caldas and E. G. Primel, *J. Chromatogr., A*, 2014, **1336**, 10-22.
28. X. F. Dong, Z. H. Shi, S. X. Liang and H. W. Sun, *Anal. Methods*, 2014, **6**, 6939-6947.
29. D. B. G. Williams, M. J. George, R. Meyer and L. Marjanoyic, *Anal. Chem.*, 2011, **83**, 6713-6716.

