Development of a 'SSQuEE' method for recovery and preconcentration of pesticide from environmental samples

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Abstract— **A simple sensitive quick and easy and efficient (SSQuEE) analytical techniue based on cloud point extraction (CPE) has been developed for the determination of different class of pesticides in soil and water with High Performance Liquid Chromatography separation and ultraviolet detection. The environmentally friendliness surfactant like Triton X -100, compared to Tween series of nonionic surfactant can effectively extract imidacloprid (insecticide), flusilazole (fungicide) and atrazine (herbicide) at cloud point temperature at 67^{o}C, 82^{o}C and 62^{o}C respectively. To reach the optimum extraction efficiency, different experimental parameter like surfactant concentration, salt type and its concentration, equilibrium time & temperature, pH were observed. At the optimum conditions linear regression coefficient of the standard curves was grater than 0.9924. The limit of detection of imidacloprid, flusilazole and atrazineare are 0.10, 0.24, 0.15µgL^{-1}and recovery percent are 99.71%, 88.1% and 89.74% respectively.**

Index Terms— **Pesticides, Environmental samples, Surfactants, CPE, HPLC-UV-VIS.**

I. INTRODUCTION

Humans are exposed to pesticides as a consequence of their applications in farming as well as their persistence in different environmental components viz air, water, soil and plant system. The interaction of pesticide with environmental factors may result in alteration of their physicochemical properties. Trace amount of pesticides in water and soil compartment together with residue analysis sometimes become challenging in terms of compatibility with the determination tool. To increase the production of vegetable the application of agro chemicals for agriculture as well as for plant protection and animal health has converted the problem of environmental pollution into national and international issues [1]. Sorption is one of the most important factors that affects the fate of pesticides in the soil and determines their distribution in the soil/water environment, which is widely used to describe the process of a pesticide partitioning between water solution and soil [2]. Imidacloprid [1-(6-chloro-3-pyridylmethyl) -Nnitroimidazolidin-2-ylideneamine], flusilazole [1-((bis(4-fluorophenyl)methylsilyl)methyl)-1H-1,2,4-triazole], atrazine [1-Chloro-3- ethylamino-5- isopropylamino-2,4,6-triazine] are systemic insecticide, fungicide and herbicide respectively which were used with different mode of action. These pesticides were used as seed-dressing, soil treatment and foliar treatment in different crops and extensively used in agricultural areas. It is necessary to drawn attention to the pesticides [3]. The transport; retention, mode of action and transformation are more and more of a public concern. This pesticide residue is highly persistent and can survive many years in soils, waters, and organisms [4]. Migration of the pesticides into groundwater via soil layers has therefore become one of the primary approaches leading to the widespread contamination to ecosystems [5]. The massive accumulation of pesticides in ecosystems not only affects the quality of crops which are directly exposed to the pesticides, but also serves as a food chain to pose a threat to human health [6] .Thus far, the extraction and analysis of pesticide residues have been established using liquid to liquid [7], solid-phase [8,9], single-drop micro extraction [10,11], and hollow fiber-based liquid-phase micro extraction [12], Dispersive liquid- liquid micro extraction [13] etc.

It is therefore of great importance to develop sensitive and efficient analytical methods to detect pesticides from multi-media. Several analytical methods have been reported including gas chromatography [14], high performance liquid chromatography [15] and capillary electrophoresis [16]. Now, Cloud point extraction (CPE) [17-18] is simple, sensitive, quick, easy, efficient, environmental friendly route using different surfactants which has hydrophobic in nature [19]. In cloud point extraction is a process where at an optimum temperature two distinct phases is separated like surfactant-rice and an aqueous. [20]. Proper Surfactants can form micelles and become turbid when heated to the particular temperature. The organic solutes enclosed in the micelles of surfactants and separate from the bulk, water solvent. The cloud point extraction method is applied for the determination of different organic and inorganic molecule or ions [21, 22], polycyclic aromatic hydrocarbons (PAHs) [23], vitamins [24, 25], and estrogens [26] and proteins [27]. With the use of nonionic surfactant cloud point extraction procedure can be improved the enrichment of pesticides residue in environmental sample like soil, water and vegetable with the use of HPLC combined with ultraviolet-visible spectrophotometer. There are many several factors affecting on the CPE, like types and concentration of surfactant, temperature, time of incubation, ionic strength and pH of the solution.

II. EXPERIMENTAL

Reagent and materials

Imidacloprid (CAS no 138261-41-3), flusilazole (CAS no 85509-19-9) and atrazine (CAS no 1912-24-9) obtained from sigma Aldrich (St Louis, MO, USA). Tween 20 (Cas no 9005-64-5, Merck Mumbai.), Tween 80 (Cas no 9005-65-6, Merck Mumbai.) and Triton X-100 (batch no 005A-2602-13,

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product no-40632, sd. fine-chem. Limited, Mumbai.). HPLC grade solvents such as acetonitrile and methanol were purchased from Merck, India. All the other reagents used in the experiment were of the highest grade commercially available. At laboratory temperature the pesticides were detected by HPLC instrument, acetonotrile: water (90:10, v/v) used as mobile phase at flow rate 1.0 ml/min for 10 min,with a λ_{max} 280 nm wavelength. The pH was monitored with 0.01(N) HCl or NaOH. Water was purified by using a Milli-Q system (Millipore, Bedford, MA, USA). All the solvents were filtered through 0.45 µm membrane filter.

Sample preparation:

The stock solutions of three pesticides (0.1µg/L) were prepared by using minimum volume of methanol, which diluted with deionized water adjusting the working concentration. The stock solutions stored at room temperature. The collected field sample filtered through a 0.45 µm membrane filter and diluted with equal volume of ultra-pure water for CPE procedure with the minimum time delay.

Instrumentation:

Shimadzu model UV-2401 PC UV-Vis recording spectrophotometer with quartz cells was used for recording absorbance spectra. All spectral measurements were performed using the blank solution as a reference. A Rotofix centrifuge was used to accelerate the phase separation process. Adjustment of pH of solution was done by Systronic digital pH meter. A Cecil (CE 4201) model HPLC coupled with UV-Vis detector, detected on a column type, Hyper-clone 5µ ODS (C_{18}) 120A [150 X 4.60m: particle size 5µ] was used for analysis of the analytes. The Power Stream software was used for the analysis of chromatogram.

Extraction procedure:

In the present extraction operation 5.0 ml of aqueous sample was taken in 10 ml screw cap graduated centrifuge glass test tube with conical bottom. By adding known volume of Triton X-100 with known concentration added to test tubes. Then heating the test tubes in a thermostatic bath at optimum temperature and time were observed for different pesticides. Then separation Phase was also accelerated by centrifugation at 4000 rpm for 5 min. After the phase separation the bulk aqueous phase was removed. A 100µL volume of surfactant rich phase was transferred with the HPLC syringe and this solution diluted with 100 µL acetonitrile. A 20 µL volume of the diluents surfactant-rich phase analyte was injected at flow rate 1.0 ml/min for 10 min into HPLC for analysis.

Enrichment parameter (Ep) and Recovery parameter (Rp) calculation:

The ratio of concentration of analyte in the sediment phase (C_{sed}) to the initial concentration of the analyte (C_o) is the enrichment parameters (E_P).

$$E_P = C_{sed}/C_o \quad (1)$$

Now, the recovery parameter (R_P) is as the fraction of solute transferred to the sediment phase, is expressed in percentage as,

$$R_P=(W_{sed}/W_o)\times 100 = (C_{sed}V_{sed}/C_oV_o) \times 100 \quad (2)$$

Where, V_{sed} and V_o are the sediment phase volume and aqueous phase volume, respectively. Where W_{sed}, W_o are the amount of solute in sediment and aqueous phase respectively. Equation (1) and (2) on combining, E_P and R_P can be related as,

$$R_P = E_P \times (V_{sed}/V_o) \times 100 \quad (3)$$

Surfactant selection:

The choice of a proper surfactant is crucial for extraction of target analyte. Here different nonionic surfactants are used for the cloud-point extraction of pesticide analytes. Three surfactants, such as Tween 20, Tween 80 and Triton X-100 were examined as extraction solvents. In Fig. 1, Triton X-100 showed excellent role for the extraction of pesticides compared with Tween pair. Therefore, Triton X-100 select as efficient surfactant for extraction of pesticide. It also observed, enrichment parameter is high for this particular surfactant. For imidacloprid, the concentration of surfactant:3.5% (w/v); temperature: 98°C for Tween 20; 98°C for Tween 80; 76°C for Triton X-100; extraction time:6 min. In case of flusilazole - concentration of surfactant:2.5% (w/v); temperature: 96°C for Tween 20; 96°C for Tween 80; 92°C for Triton X-100; extraction time:6 min and in atrazine - concentration of surfactant:2.5% (w/v); temperature: 82°C for Tween 20; 82°C for Tween 80; 77°C for Triton X-100; extraction time:12 min.

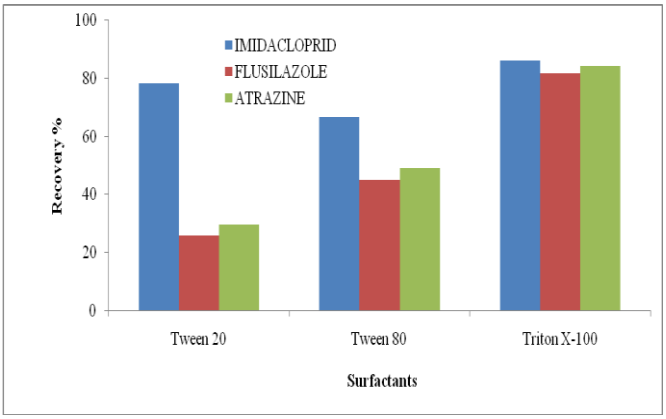

Fig. 1. Effect of type of surfactant on recovery percent.

Role of Triton X-100 concentration:

At the time of cloud point extraction, the extraction efficiency and theoretical maximum enrichment depended mainly upon the concentration of surfactant. Thus, it is necessary to optimize the surfactant concentration for maximum extraction of the target analytes, Fig. 2 shows that the concentration of Triton X-100 has a considerable effect. The extraction efficiency of the target compound increased sharply when the concentration of Triton X-100 increased from 0.5% (w/v) to 2.0 % (w/v) and were constant when concentration of Triton X-100 in between 2.5% (w/v) and 5.0 % (w/v). The extraction efficiency of imidacloprid, flusilazole and atrazine reached upto maximum level. The concentration of Triton X-100 increased up to 3.5 % (w/v), 2.5% (w/v) and 2.5% (w/v) the recovery respective pesticides, maximum. The extraction efficiency of imidacloprid, flusilazole and atrazine are 86.16 %, 81.64% and 84.15% respectively, without salt addition. With increasing water solubility, extraction efficiency of the analyte extraction decreased is examined from experiment. The concentration of Triton X-100 3.5% (w/v), 2.0% (w/v) and 2.5% (w/v) were used for the further study. In case of imidacloprid cloud point temperature: 76°C extraction time: 6 min, for flusilazole, cloud point temperature: 92°C extraction time: 6 min and in atrazine cloud point temperature: 77°C, extraction time is 12 min.

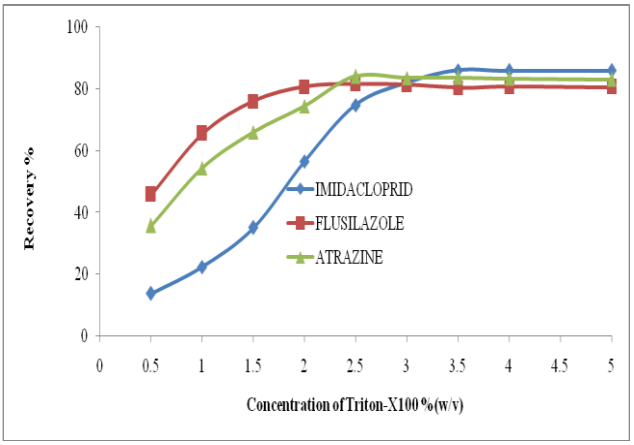

Fig. 2. Effect of Triton X-100 % (w/v) on recovery.

Effect of ionic salt and its concentration:

Concentration of ionic salt is also important role in CPE. For the use of nonionic surfactants, the appearance of salts may increase the extraction recovery of pesticide, with hydrophobic compounds being easily partitioned into the surfactant phase. To study the effect of the salt, different concentrations of Na_2SO_4 in a range of 0.5–4.0wt% were added to the sample solutions. It is examined that in Fig. 3 indicate the extraction efficiency increased when the Na_2SO_4 salt concentrations increased to 1.5 (w/v) % and then kept constant, when the Na_2SO_4 concentrations were in between 1.5% (w/v) and 4.0% (w/v). The surfactant-rich phase was sediment at the bottom of centrifuge tube. NaCl, KCl, Na_2SO_4 was also investigated, the extraction of target compound. Na_2SO_4 has higher ionic strength than NaCl and KCl. Due to highest ionic strength could increase the solubility of analytes in the Triton X-100 phase. When Na_2SO_4 used, the activity of surfactant increase, the time of phase separation was shorter. Therefore, 1.5% (w/v), 2.0% (w/v), 2.5% (w/v) of Na_2SO_4 were selected for further study of imidacloprid,flusilazole and atrazine. In imidacloprid ,concentration of Triton X-100 is 3.5 % (w/v) where time of extraction is 6 min when temperature is 67°C, for flusilazole, the concentration of Triton X-100 is 2.5 %(w/v) when extraction time 6 min and temperature reaches at 82°C and in case of atrazine concentration of Triton X-100 is 2.5 % (w/v), extraction time is 12 min at temperature 62°Cis observed.

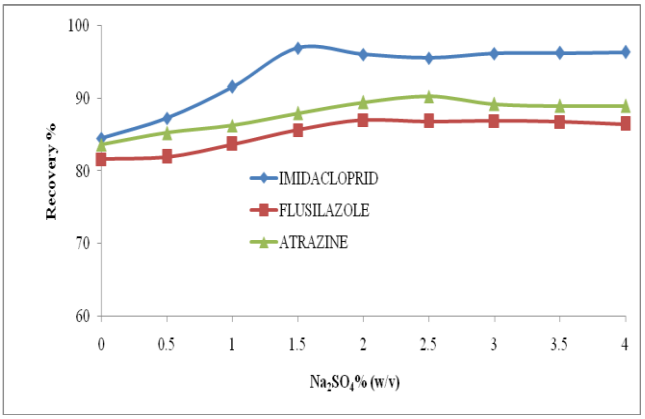

Fig. 3. Effect of concentration of Na_2SO_4 on recovery.

Incubation time and Equilibration temperature:

To optimize efficient phase separation minutely observed incubation time and equilibration temperature. Two phases cannot be formed at temperatures below the cloud point temperature (CPT). Extraction efficiency was tested at equilibration temperature of 50–92°C in Fig. 4.

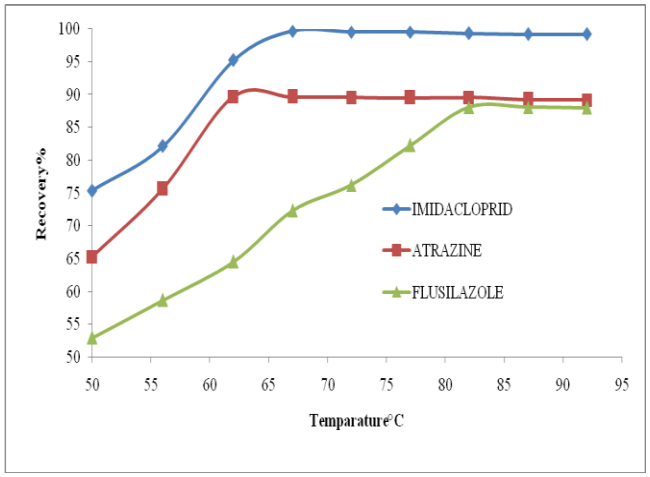

Fig. 4. Effect of Temperature (°C) on recovery.

To reach an equilibrium phase separation the observed time ranges 0–20 min. which shown in Fig. 5. The extraction efficiency of all analytes increased significantly at an equilibration temperature. It is observed that temperature increase from 50 to 67°C then was unchanged for imidacloprid, 50 to 82°C for flusilazole and 50 to 62°C for atrazine. Extraction times 6 min for imidacloprid, flusilazole and 12 min for atrazine were sufficient for analysis. In case of imidacloprid concentration of Triton X-100 used 3.5 % (w/v), extraction time, 6 min; Na_2SO_4:1.5% (w/v). For flusilazole, concentration of Triton X-100 is 2.5 % (w/v), extraction time, 6 min; Na_2SO_4 is 2.0% (w/v). Now in case of atrazine, Triton X-100 concentration is 2.5% (w/v); extraction time, 12 min and Na_2SO_4 concentration 2.5% (w/v).

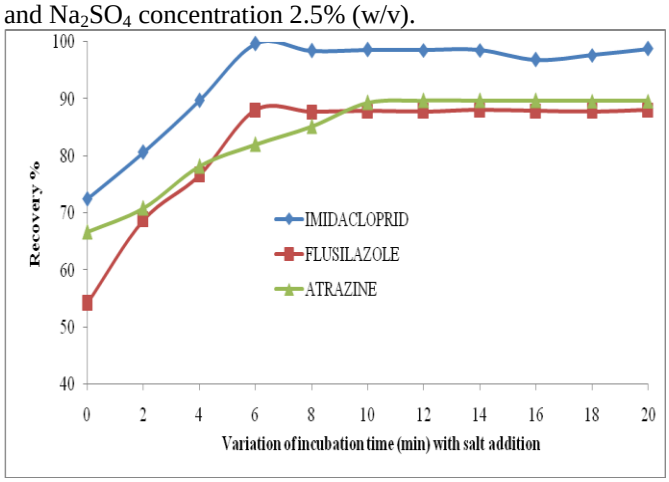

Fig. 5. Effect of incubation time (min) on recovery

Role of pH:

The important key parameters are pH which governs the extraction of target analyte from the sample solution. They are (a) solubility and (b) stability of the solute due to ionization. It is found in Fig. 6. With increase in pH the extraction of pesticides increases, reaches a maximum and again decreases. Finally, at an optimum condition for extraction of imidacloprid, the concentration of Triton X-100 is 3.5%(w/v), extraction time, 6 min, concentration Na_2SO_4, 1.5% (w/v) at pH 6.13. In case of flusilazole concentration of Triton X-100 is 2.5%(w/v) the extraction time, 6 min the Na_2SO_4 salt concentration, 2.0%(w/v) at pH 10.22. IN case of atrazine concentration of Triton X-100 is 2.5% (w/v), extraction time is 12 min, and concentration of Na_2SO_4 is 2.5% (w/v) at pH 5.2.

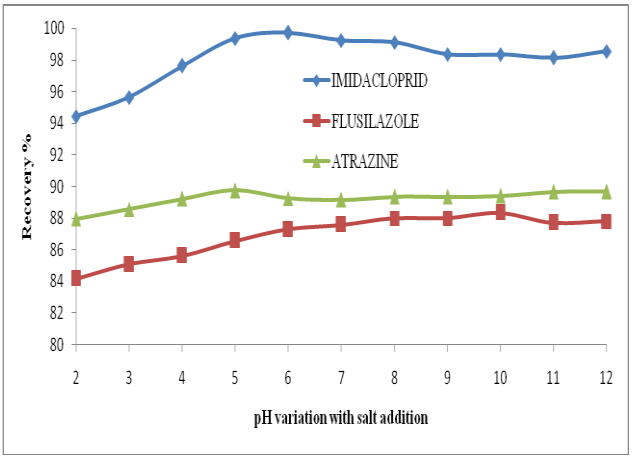

Fig. 6. Effect pH on recovery percent with salt addition.

Analytical performance:

At the optimized condition the experimental condition the analytical characteristics of the method such as linear range, limit of detection (LOD), correlation coefficient (r^2), RSD % are shown in Table 1.

Analyte	***Linear range (μgL^{-1})***	***Correlation coefficient (r^2)***	***Recovery (%)***		***Precision (RSD%, n=3)***	***LOD (μgL^{-1})***
			soil	***water***		
Imidacloprid	0.1-100	0.9924	95.35	99.71	3.16	0.10
Flusilazole	0.1-100	0.9981	81.64	88.10	5.38	0.24
Atazine	0.1-100	0.9944	84.12	89.74	4.57	0.15

TABLE -1 ANALYTICAL FEATURE OF THE PROPOSED METHOD.

Application soil and water samples:

The cloud point extraction method was applied for preconcentration, recovery of studied pesticide in soil and water samples. The results are shown in Table 2. The Chromatogram of three standard chemicals of pesticides is simultaniously shown in Fig.7, without CPE. Mobile phase was used acetonitrile : water [(90:10, v/v)] was injected at flow rate 1.0 ml/min for 10 min into HPLC for analysis. The detector set at λ_{max} =280 nm wavelength, injection volume: 20µL, standard solute solution showed sharp peak at retention time near about 1:33.4, 1:51.5, 1:47.0 respective minute for imidacloprid, flusilazole and atrzine respectively under HPLC working condition.

TABLE-2 RECOVERY OFPESTICIDES FROM SOIL AND WATER SPIKED SAMPLES.

Pesticides	***Spiked level (μgL^{-1})***	***Soil***			***Water***		
		Found (μgL^{-1})	***Recovery %***	***RSD% (n=3)***	***Found (μgL^{-1})***	***Recovery %***	***RSD% (n=3)***
Insecticide (Imidacloprid)	5	4.32	86.40	5.73	4.52	90.40	4.85
	10	8.65	86.50	5.74	9.51	95.10	4.89
	100	88.35	95.35	5.75	99.71	99.71	4.90
Fungicide (Flusilazole)	5	3.89	77.80	2.38	4.10	82.00	3.62
	10	7.99	79.90	2.39	8.41	84.10	3.65
	100	81.64	81.64	2.41	88.10	88.10	3.66
Herbicide (Atrazine)	5	4.11	82.20	1.38	4.23	84.60	2.61
	10	8.28	82.80	1.39	8.81	88.10	2.62
	100	84.12	84.12	1.40	89.74	89.74	2.63

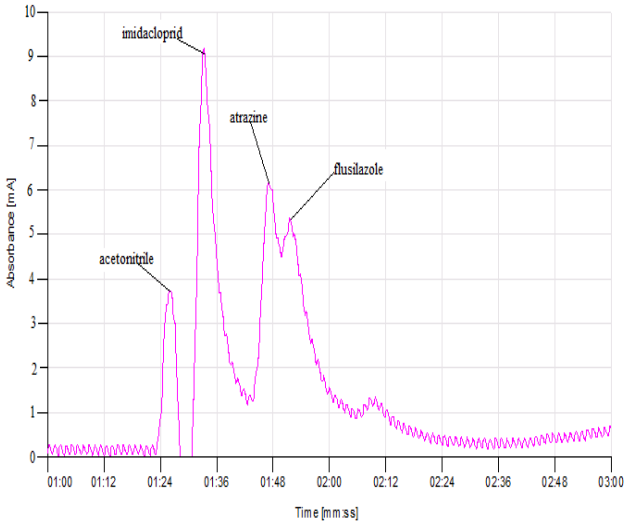

Fig. 7. Chromatogram of the imidacloprid, atrazine and flusilazole, 20 µL volume of analyte solution was injected at flow rate 1.0 ml/min for 10 min. Chromatographic conditions specified in the text.

CONCLUSION:

The study allowed to development of a simple, sensitive, quick, easy and efficient extraction method. Here the nonionic surfactant used as extraction solvent in cloudpoint extraction procedure for determination of three pesticide from environmental sample. Among the surfactant Trition X-100 is versatile; provide good enrichment factors, simple and efficient separation. It is very much safer, a small amount of the surfactant is used which is low toxic. In instrumental step, small amount volume of sample is used. The procedure is also environmentally friendly which is further used for extraction of environmental sample.

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CONFLICT OF INTEREST:

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. P. D. Capel, L. Ma, B. R. Schroyer, S. J. Larson, and T. A. Gilchrist, *Environ. Sci. Technol.*, **29**, 1702 (1995); https://doi.org/10.1021/es00006a039
2. B. M. Gawlik, N.Sotiriou, E. A. Feicht, S. Schulte-Hostede, and A. Kettrup, *Chemosphere.* **34**, 2525 (1997); https://doi.org/10.1016/S0045-6535 (97)00098-2
3. T. L. McTier, N. A. Evans, M. Martin-Short, and K. Gration, *Vet Parasitol.*, **116**, 45 (2003); https://doi.org/10.1016/S0304-4017 (03)00163-8
4. J. H. Tan, S. F. Jin, and H. Yang, *CLEAN–Soil, Air, Water.*, **41**, 510 (2013); https://doi.org/10.1002/clen.201200359
5. M. Arias-Estévez, E. López-Periago, E. Martínez-Carballo, J. Simal-Gándara, J. C. Mejuto, and L. García-Río, *Agric. Ecosyst. Environ.*, **123**, 247 (2008); https://doi.org/10.1016/j.agee.2007.07.011
6. P. Fantke, R. Charles, L. F. D. Alencastro, R. Friedrich, and O. Jolliet, *Chemosphere.*, **85**, 1639 (2011); https://doi.org/10.1016/j.chemosphere.2011.08.030
7. H. Sabik, and R. Jeannot, *J. Chromatogr. A.*, **818**, 197 (1998); https://doi.org/10.1016/S0021-9673 (98)00555-X
8. J. H. Tan, S. F. Jin, and H. Yang, *CLEAN–Soil, Air, Water.*, **41**,510 (2013); https://doi.org/10.1002/clen.201200359
9. F. Liu, G. Bischoff, W. Pestemer, W. Xu, and A. Kofoet, *Chromatographia.*, **63**, 233 (2006); https://link.springer.com/article/10.1365/s10337-006-0725-x
10. T. Kumazawa, X. P. Lee, K. Kondo, K. Sato, H. Seno, K. Watanabe-Suzuki and O. Suzuki, *Chromatographia.*, **52**, 195 (2000); https://doi.org/10.1007/BF02490456
11. L. Xu, C. Basheer, and H. K. Lee, *J. Chromatogr. A.*, **1152**, 184 (2007); https://doi.org/10.1016/j.chroma.2006.10.073
12. G. Shen, and H. K. Lee, *Anal. Chem.*, **74**, 648 (2002); https://doi.org/10.1021/ac010561o
13. R. Joarder, D. Santra, S. Marjit, and M. Sarkar, *Eur. Chem. Bull.*, **3**, 612 (2014); DOI: 10.17628/ECB.2014.3.612
14. J. Zhu, *Environ. Int.*, **30**, 135 (2004); https://doi.org/10.1016/S0160-4120 (03)00168-5
15. F. Kamarei, H. Ebrahimzadeh, and Y. Yamini, *J. Haz. Mater.*, **178**, 747 (2010); https://doi.org/10.1016/j.jhazmat.2010.01.148
16. K. Pecková, J. Musilová, and J. Barek, *Crit Rev Anal Chem.*, **39**, 148 (2009); https://doi.org/10.1080/10408340903011812
17. R. Kachangoon, J. Vichapong, R. Burakham, Y. Santaladchaiyakit, and S. Srijaranai, *Molecules.*, **23**, 1165 (2018); https://doi.org/10.3390/molecules23051165
18. R. de Prá Urio, and J. C. Masini, *Analytical Letters.*, **50**, 1065 (2017); https://doi.org/10.1080/00032719.2016.1212203
19. Y. Takagai, and W. L. Hinze, *Anal. chem.*, **81**, 7113 (2009); https://doi.org/10.1021/ac9009963
20. C. D. Stalikas, *Trends Anal. Chem.*, **21**, 343 (2002); https://doi.org/10.1016/S0165-9936 (02)00502-2
21. M. B. Gholivand, A. Babakhanian, and E. Rafiee, *Talanta.*, **76**, 503(2008); https://doi.org/10.1016/j.talanta.2008.03.057
22. M. Ghaedi, A. Shokrollahi, K. Niknam, E. Niknam, A. Najibi, and Soylak, M. *J. Haz. Mater.*, **168**, 1022 (2009); https://doi.org/10.1016/j.jhazmat.2009.02.130
23. K. C. Hung, B. H. Chen, and L. E. Yu, *Sep. Purif. Technol.*, **57**, 1 (2007); https://doi.org/10.1016/j.seppur.2007.03.004
24. K. Seebunrueng, Y. Santaladchaiyakit, P. Soisungnoen, and S. Srijaranai, *Anal Bioanal Chem.*, **401**, 1703 (2011); https://doi.org/10.1007/s00216-011-5214-x
25. H. Abdollahi, and L. Bagheri, *Anal. Chim. Acta.*, **514**, 211 (2004); https://doi.org/10.1016/j.aca.2004.03.048
26. L.Wang, Y. Q. Cai, B. He, C. G. Yuan, D. Z. Shen, J. Shao, and G. B. Jiang, *Talanta.*, **70**, 47 (2006); https://doi.org/10.1016/j.talanta.2006.01.013
27. A. S. Lopes, J. S. Garcia, R. R. Catharino, L. S. Santos, M. N. Eberlin, and M. A. Z. Arruda, *Anal. Chim. Acta.*, **590**, 166 (2007); https://doi.org/10.1016/j.aca.2007.03.043