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Improvement of a 'SSQuEE' Method for Recovery and Preconcentration of Pesticides from Environmental Samples

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A simple, sensitive, quick, easy and efficient (SSQuEE) analytical technique based on cloud point extraction (CPE) has been developed for the determination of different pesticides present 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 non-ionic surfactant can effectively extract imidacloprid (insecticide), flusilazole (fungicide) and atrazine (herbicide) at cloud point temperature at 67 ºC, 82 ºC and 62 ºC, respectively. To reach the optimum extraction efficiency, different experimental parameters like surfactant concentration, salt type and its concentration, equilibrium time and temperature, pH were observed. At the optimum conditions, linear regression coefficient of the standard curves was greater than 0.9924. The limit of detection of imidacloprid, flusilazole and atrazine were 0.10 μg L^{-1}, 0.24 μg L^{-1}, 0.15 μg L^{-1} and recovery percent are 99.71 %, 88.1% and 89.74%, respectively.

Keywords: Pesticides, Environmental samples, Surfactants, Cloud point extraction.

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 physico-chemical 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)-*N*-nitroimidazolidin-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 [4,8], single-drop micro extraction [9,10], hollow fiber-based liquid-phase micro extraction [11], dispersive liquid-liquid micro extraction [12], *etc*.

It is, therefore, of great importance to develop sensitive and efficient analytical methods to detect pesticides from multimedia. Several analytical methods have been reported including gas chromatography [13], high performance liquid chromato-

graphy [14] and capillary electrophoresis [15]. Now, cloud point extraction (CPE) [16,17] is simple, sensitive, quick, efficient, easy, environmental friendly route using different surfactants, which has hydrophobic in nature [18]. The cloud point extraction is a process where at an optimum temperature two distinct phases is separated like surfactant-rice and an aqueous [19]. 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 [20,21], polycyclic aromatic hydrocarbons (PAHs) [22], vitamins [23,24], estrogens [25] and proteins [26]. With the use of non-ionic 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 cloud point extraction (CPE), like types and concentration of surfactant, temperature, ionic strength, time of incubation and pH of the solution.

EXPERIMENTAL

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, India), Tween 80 (CAS no. 9005-65-6, Merck, India) and Triton X-100 (Batch no. 005A-2602-13, Product no. 40632, sd. fine-chem. Ltd., India). 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, USA). All the solvents were filtered through 0.45 µm membrane filter.

Sample preparation: The stock solutions of all the three pesticides (0.1 µg/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 ultrapure 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 × 4.60 m: 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 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 100 µL 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 (E_p) and recovery parameter (R_p) 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).

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

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

$$R_P = \frac{W_{sed}}{W_o} \times 100 = \frac{C_{sed} V_{sed}}{C_o V_o} \times 100 \quad (2)$$

where, V_{sed} and V_o are the sediment phase volume and aqueous phase volume, respectively; W_{sed} and W_o are the amount of solute in sediment and aqueous phase, respectively. Eqns. 1 and 2 on combining, enrichment parameter (E_p) and recovery parameter (R_p) can be related as:

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

RESULTS AND DISCUSSION

Surfactant selection: Selecting an appropriate surfactant is important for target analyte extraction. Various nonionic surfactants were employed for pesticide analyte cloud-point extraction. Three surfactants, namely Tween 80, Tween 20 and Triton X-100 were evaluated as extraction solvents. Triton X-100 played a role superior to the Tween pair for pesticide extraction (Fig. 1). Therefore, for pesticide extraction, Triton X-100 was selected as the efficient surfactant. The enrichment parameter was high for Triton X-100. For imidacloprid, the surfactant concentration was 3.5% (w/v); temperature was 98 ºC, 98 ºC and 76 ºC for Tween 20, Tween 80 and Triton X-100, respectively and the extraction time was 6 min. For flusilazole, the surfactant concentration was 2.5% (w/v); temperature was 96 ºC, 96 ºC and 92 ºC, Tween 20, Tween 80 and Triton X-100, respectively; and the extraction time was 6 min. For atrazine, the surfactant was 2.5% (w/v); temperature was 82 ºC, 82 ºC and 80 ºC for Tween 20, Tween 80 and Triton X-100, respectively and the extraction time was 12 min.

Role of Triton X-100 concentration: During cloud-point extraction, the theoretical maximum enrichment and extraction efficiency mainly depended on surfactant concentration. Triton

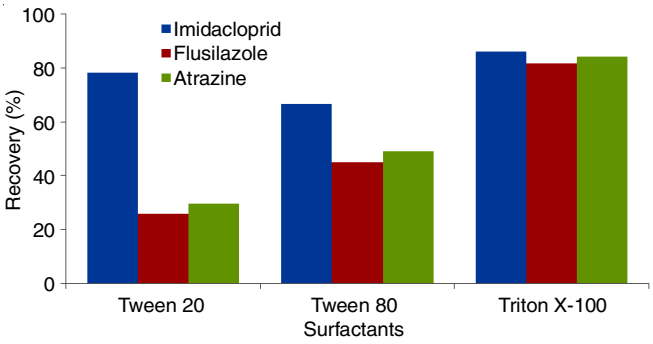

Fig. 1. Effect of type of surfactant on recovery (%)

X-100 concentration presents a considerable effect (Fig. 2). The target compound extraction efficiency drastically increased when the Triton X-100 concentration increased from 0.5% to 2.0% (w/v) and remained constant when this concentration was 2.5%-5 % (w/v). The extraction efficiencies of flusilazole, imidacloprid and atrazine attained the maximum levels. The Triton X-100 concentration increased up to 3.5%, 2.5% and 2.5% (w/v). Without salt addition, the extraction efficiencies of flusilazole, imidacloprid and atrazine were 81.64%, 86.16% and 84.15%, respectively. With an increase in water solubility, the analyte extraction efficiency decreased. Triton X-100 concentrations of 3.5%, 2.0% and 2.5% (w/v) were employed in the subsequent studies. For imidacloprid, flusilazole and atrazine, cloud-point temperature (CPT) was 76 ºC, 92 ºC and 77 ºC, respectively, and the extraction time was 6 min, 6 min and 12 min, respectively.

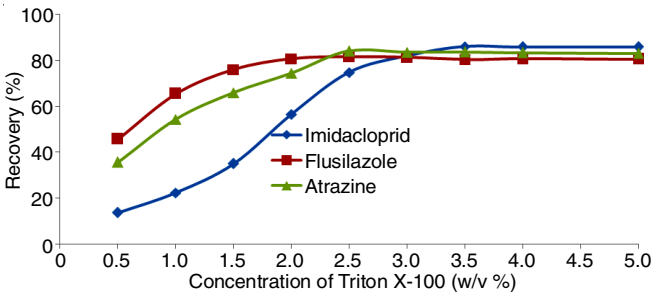

Fig. 2. Effect of Triton X-100 (w/v) % concentration on recovery (%)

Effects of ionic salt and its concentration: In non-ionic surfactants, the presence of salts may lead to an increase in pesticide extraction recovery and hydrophobic compounds can be partitioned easily in the surfactant phase. To study the influence of salts, in the sample solutions, different Na_2SO_4 concentrations of 0.5-4.0 (w/v)% were added. The extraction efficiency increased with an increase in Na_2SO_4 salt concentration to 1.5% (w/v). This efficiency remained constant when Na_2SO_4 concentration was 1.5%-4.0% (w/v) (Fig. 3). The sediment obtained at the bottom of a centrifuge tube was the surfactant-rich phase.

The influence of ionic salts, such as KCl, NaCl and Na_2SO_4, on target compound extraction was investigated. Na_2SO_4 has the highest ionic strength among the studied salts. The high ionic strength can lead to an increase in analyte solubility in Triton X-100. When Na_2SO_4 was used, the surfactant activity

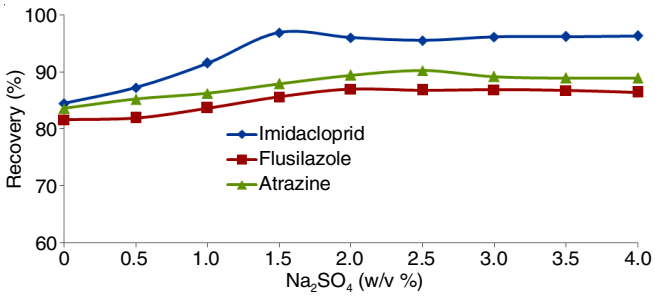

Fig. 3. Effect of concentration of Na_2SO_4 on recovery (%)

increased and the phase separation time decreased. Thus, 2.0%, 1.5%, 2.5% (w/v) of Na_2SO_4 were employed in the further studies of flusilazole, imidacloprid and atrazine, respectively. In imidacloprid, Triton X-100 concentration and extraction time were 3.5% (w/v) and 6 min, respectively at 67 ºC. For flusilazole, Triton X-100 concentration and extraction time were 2.5% (w/v) and 6 min, respectively at 82 ºC. For atrazine, Triton X-100 concentration and extraction time were 2.5% (w/v) and 12 min, respectively at 62 ºC.

Equilibration temperature and incubation time: To enhance phase separation, equilibration temperature and incubation time were analysed. At temperatures less than cloud-point temperature (CPT), two phases could not be formed. At equilibration temperature of 50-92 ºC, the extraction efficiency was analyzed (Fig. 4). The time required to attain equilibrium phase separation was 0-20 min (Fig. 5). For all analytes, the extraction efficiency highly increased at equilibration temperature. For imidacloprid, atrazine and flusilazole, temperature increased from 50 ºC to 67 ºC, 50 ºC to 62 ºC and 50 ºC to 82 ºC, respectively and then stabilized. For imidacloprid, flusilazole and atrazine, extraction times of 6 min, 6 min and 12 min were sufficient. For imidacloprid, Triton X-100 concentration, Na_2SO_4 concentration and extraction time were 3.5% (w/v),

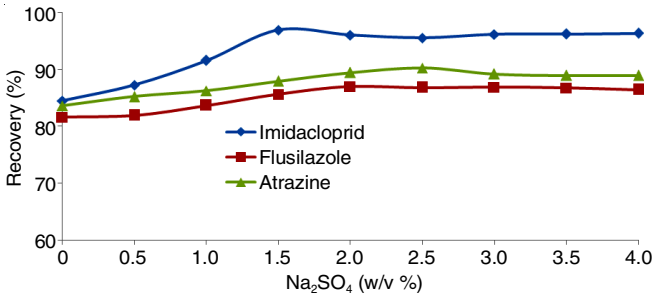

Fig. 4. Effect of temperature (ºC) on recovery (%)

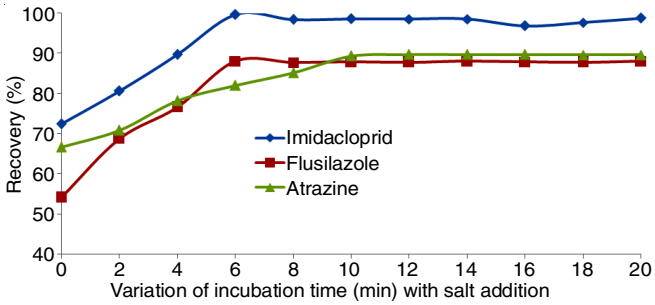

Fig. 5. Effect of incubation time (min) with salt addition on recovery (%)

1.5% (w/v) and 6 min, respectively; for flusilazole, those were 2.5% (w/v), 2% (w/v), and 6 min, respectively and for atrazine, those were 2.5% (w/v), 2.5% (w/v) and 12 min, respectively.

Role of pH: With an increase in pH, pesticide extraction first increased, then reached the maximum and finally decreased (Fig. 6). Under the optimum conditions, for imidacloprid, flusilazole and atrazine extractions, Triton X-100 concentration, Na_2SO_4 concentration and extraction time were 3.5% (w/v), 1.5% (w/v) and 6 min; 2.5% (w/v), 2% (w/v) and 6 min; and 2.5% (w/v), 2.5% (w/v) and 12 min; respectively, at pH 6.13, 10.22 and 5.2, respectively.

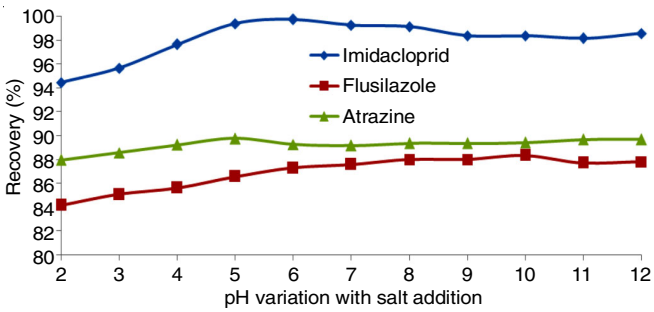

Fig. 6. Effect pH on recovery percent with salt addition

Analytical performance: Table-1 presents the analytical properties of the technique, including limit of detection (LOD), linear range, RSD % and correlation coefficient (r^2), acquired under the optimized conditions.

Soil and water sample analysis: Cloud-point extraction was applied for the recovery and preconcentration of the pesticides in water and soil samples (Table-2). Fig. 7 shows the chromatograms of the three standard pesticides. For HPLC analysis, a mobile phase of acetonitrile:water [(90:10, v/v)] was injected for 10 min at a flow rate 1 mL/min. The detector was set to the injection volume of 20 µL and maximum wavelength of 280 nm. At the retention time, the standard solute solution exhibited sharp peaks at approximately 1:33.4, 1:47.0, and 1:51.5 min for imidacloprid, atrazine and flusilazole, respectively, under the working conditions of HPLC.

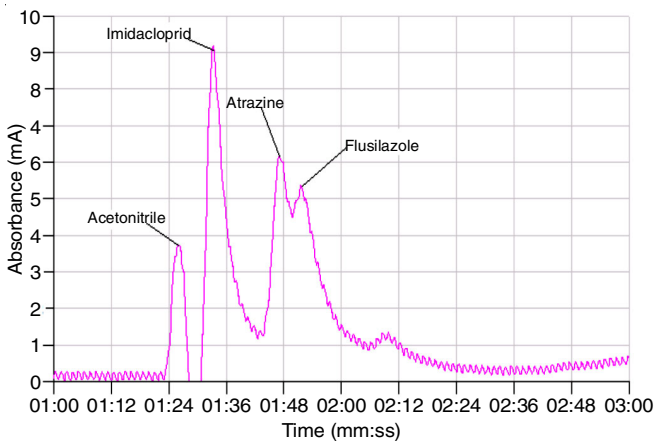

Fig. 7. Chromatogram of three standard chemicals without CPE and spiked water sample extracted by Triton X-100

Conclusion

On the basis of cloud point extraction, a sensitive, simple, rapid, efficient and easy extraction technique was developed for pesticide preconcentration of environmental samples. In this study, the non-ionic surfactant (Triton X-100) was utilized as the extraction solvent. Different experimental parameters, such as surfactant concentration, extraction efficiency, ionic salts and their concentrations, temperature, equilibrium time, and pH were optimized. The recovery percentages were 99.71%, 89.74% and 88.1% for imidacloprid (insecticide), atrazine (herbicide), and flusilazole (fungicide), respectively.

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TABLE-1
ANALYTICAL PERFORMANCE OF THE PROPOSED METHOD

Analyte	Linear range (µg L^{-1})	Correlation coefficient (r^2)	Recovery (%)		Precision (RSD%, n = 3)	LOD (µg L^{-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-2
RECOVERY OF IMIDACLOPRID, FLUSILAZOLE AND ATRAZINE FROM SOIL AND WATER

Pesticides	Spiked level (µg L^{-1})	Soil			Water		
		Found (µg L^{-1})	Recovery (%)	RSD% (n = 3)	Found (µg L^{-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

CONFLICT OF INTEREST

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

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