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A sensitive spectrophotometric determination of atrazine in micellar medium and its application in environmental samples

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Abstract A sensitive analytical procedure based on solid phase extractive-spectrophotometry has been established for the determination of the widely used herbicide atrazine .The method is based on the Konig reaction in which atrazine reacts with pyridine reagent to form a quaternary pyridinium halide, which further forms glutaconic aldehyde in the presence of alkali. Glutaconic aldehyde is subsequently coupled with 4-aminoacetanilide in the micellar medium of anionic surfactant sodium dodecyl sulphate to give a yellow-orange dye. The produced dye was enriched on a C_{18} cartridge and is measured spectrophotometrically at 460 nm. The sensitivity and selectivity of the method was largely enhanced in the micellar media and SPE on the C_{18} cartridge and avoids the use of toxic solvents. Beer's law was obeyed in the range 0.012–0.12 μg mL^{-1}. Molar absorptivity and Sandell's sensitivity were found to be 1.52×10^6 L mol^{-1} cm^{-1} and 0.0002 μg cm^{-2}, respectively. The limit of detection and quantification were 0.001 and 0.003 μg mL^{-1}, respectively. The proposed method was applied successfully for the determination of atrazine in environmental and biological samples with a recovery range of 96–101 %. The method was found to be free from interference of a large number of foreign species. The accuracy and reliability of the method was further established by parallel determination by the reference method, and by recovery studies.

Keywords Spectrophotometry · 4-Aminoacetanilide · Sodium dodecyl sulphate · Solid phase extraction

Abbreviations
CPC Cetyl-pyridiniumchloride
CTAB Cetyl-trimethylammoniumbromide

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SDS Sodiumdodecylsulphate
SPE Solid phase extraction

Introduction

Atrazine [2-chloro-4-(*N*-ethyl amino)-6-(*N*-isopropyl amino)-1,3,5-*s*-triazine] is a human-made chlor-triazine herbicide that is used worldwide to control broadleaf and grassy weeds. As one of the most heavily used pesticides, it can be present in parts per million in agricultural runoffs [1], which exceeds the U.S. Environmental Protection Agency's maximum allowable contaminant level of 3 ppb in groundwater and surface waters [2]. It is a selective, pre- and post-emergence herbicide, and is persistent in soil [3], persisting for longer than 1 year under dry or cold conditions [4]. Atrazine has been classified as a restricted use pesticide (RUP) due to its potential for groundwater contamination [5]. Recently, it has been considered as an endocrine-disrupting chemical [6]. The structure of atrazine is shown in Fig. 1.

Atrazine is slightly to moderately toxic to human and other animals. It can be absorbed orally, dermally, and by inhalation. Symptoms of poisoning include abdominal pain, diarrhea and vomiting, eye irritation, irritation of mucous membranes, and skin reactions. At very high doses, rates show excitation followed by depression, slowed breathing, incoordination, muscle spasms, and hypothermia [7]. Recent studies have shown that atrazine causes sexual abnormalities in frogs [8–11], and prostate cancer in workers at an atrazine-manufacturing factory [12].

Atrazine has phototoxic effects which include decomposition of chloroplast, inhibition of water photolysis, diminishing the carbohydrate content, suppression of tissue respiration, change in enzyme activity, and blocking of Hill reaction [13]. These studies suggest that there is cause for concern about atrazine residues in soil, groundwater, and surface waters. Due to its wide application and high toxicity, numerous instrumental methods have been reported for the determination of atrazine such as high-performance liquid chromatography (HPLC) [14], gas chromatography (GC) [15], liquid chromatography-mass spectrometry (LC-MS) [16], gas chromatography-mass spectrometry (GC-MS) [17], thin-layer chromatography (TLC) [18], electrochemical methods (EC) [19], and immunoassay [20]. The reported methods like HPLC, GC and GC-MS analyses are restricted to laboratory facilities, are time-consuming and expensive due to analytical cost, and require highly qualified laboratory persons, while spectrophotometry is considered the most convenient analytical technique because of its simplicity, low cost, and wide availability in most laboratories. Simple spectrophotometric methods based on the

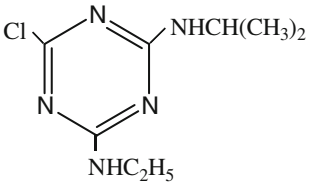


Fig. 1 The chemical structure of atrazine

Konig reaction have been applied to determine the herbicide. Kesari and Gupta [21] reported a method for spectrophotometric determination of atrazine in different samples, based on the formation of a yellow-orange polymethine dye with *p*-aminoacetophenone in the presence of pyridine in hydrochloric acid medium.

The proposed method is based on a modified Konig reaction in which atrazine reacts with the pyridine reagent to form glutaconic aldehyde which forms a yellow-orange polymethine dye with 4-aminoacetanilide in micellar medium of SDS. The use of a surfactant makes the reaction rapid, and increases color stability, sensitivity and selectivity by specific micelle interaction [22]. The chromogen is extracted on bonded C_{18} cartridges [23] and the colored product is eluted with methanol and measured at 460 nm.

Experimental

Apparatus and reagent solutions

A Systronics spectrophotometer 166 was used for spectral measurements. pH measurements were made with Systronics digital pH meter 335. All chemicals used were of analytical grade, and double-distilled deionized water has been used throughout the experiment.

Atrazine (Nagarjuna Agrichem, Hyderabad, India) stock solution (1 mg mL^{-1}) was prepared in *n*-propanol. A working standard was prepared by appropriate dilution of stock solution. Pyridine reagent was prepared by mixing 3 mL of concentrated hydrochloric acid with 18 mL of freshly distilled pyridine (Merck, Mumbai, India) to which 12 mL of double-distilled water was added [21]. A 1.0 % (w/v) aqueous solution of 4-aminoacetanilide (Merck) was prepared. A 2.0 mol L^{-1} aqueous solution of sodium hydroxide (Loba Chemie, Mumbai, India) and an aqueous 1.4×10^{-3} mol L^{-1} solution of sodium dodecyl sulphate (SDS) (Loba Chemie) was prepared. C_{18} cartridge (Alltech, Deerfield, IL, USA) was used for solid phase extraction. Methanol (Merck) was used for elution.

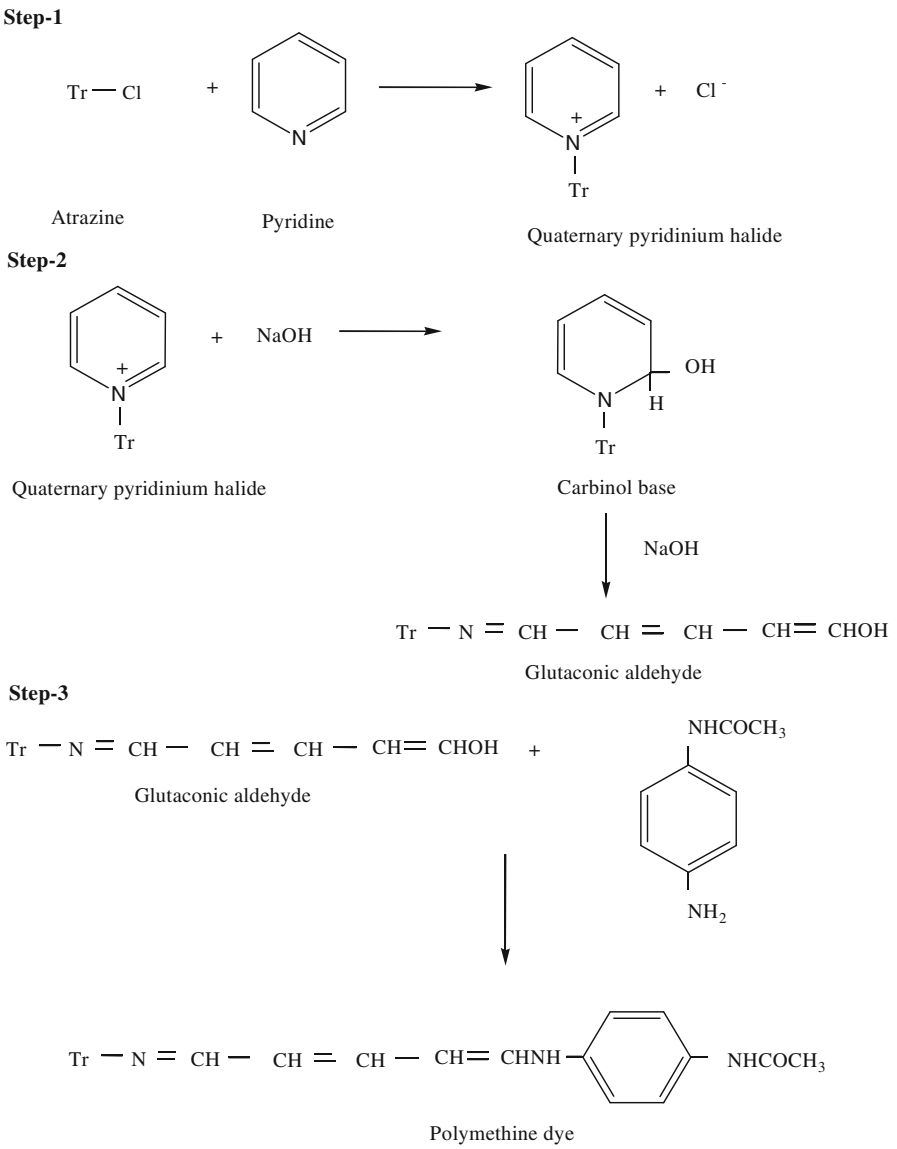
General procedure

An aliquot of the standard solution containing 0.012–0.12 μg mL^{-1} of atrazine was taken into a series of 20-mL graduated tubes mixed with 0.2 mL pyridine reagent and kept in a boiling water bath at 70 °C for 15 min. It was allowed to cool to room temperature, after which 1 mL of 2 mol L^{-1} sodium hydroxide solution, 2 mL of 1% 4-aminoacetanilide solution and 2 mL of 4 mol L^{-1} HCl were added and immediately the solution was made up to the mark with 1.4×10^{-3} mol L^{-1} SDS solution and kept for 5 min for complete color development. The resulting yellow-orange product was extracted by passing 20 mL of this solution through a C_{18} cartridge that was preconditioned by passing in sequence 3 mL methanol and 3 mL water. The colored product was eluted from the cartridge by passing 5 mL methanol and measured at 460 nm against a reagent blank that gave negligible absorbance at this wavelength.

Results and discussion

The probable reaction is shown in Scheme 1 and can be explained mainly in three steps:

1. Atrazine reacts with pyridine and forms quaternary pyridinium halide.



Scheme 1 Color reaction of atrazine

2. Pyridinium halide further undergoes addition of hydroxyl group in the presence of alkali to form a carbinol base, which further undergoes breaking of the heterocyclic ring forming glutaconic aldehyde.
3. Glutaconic aldehyde couples with 4-aminoacetanilide in an acidic and surfactant medium to form a yellow-orange polymethine dye.

Effect of reagent concentration

Under the proposed reaction conditions, it was found that 0.2 mL of pyridine reagent (Fig. 2), 1 mL of 2 mol L^{-1} NaOH (Fig. 3), 2 mL of 1% 4-aminoacetanilide (Fig. 4), and 2 mL of 4 mol L^{-1} HCl (Fig. 5) were required for complete color development.

Effect of temperature, time and pH

Under the optimum conditions, the final absorbance was measured at pH 1.5–2.5. Maximum color intensity was observed when the solution containing a mixture of atrazine and pyridine reagent was heated for 15 min in a boiling water bath at 70 °C and allowed to cool to room temperature (Fig. 6). The colored product was found to be stable for about 24 h in the temperature range of 15–35 °C, while 5 min time was sufficient for complete color development after addition of the reagent and SDS.

Effect of nature and concentration of surfactant

The influence of the nature and concentration of the surfactants on the absorbance of the final product was studied with anionic (SDS), cationic (CPC, CTAB) and nonionic (Triton-X-100) surfactants. The larger enhancement of the sensitivity was observed in case of the anionic surfactant (SDS) (Fig. 7). This can be explained by the fact that at the pH of the reaction both the coupling reagent 4-aminoacetanilide and polymethine dyes were protonated and thus they were strongly bound to the

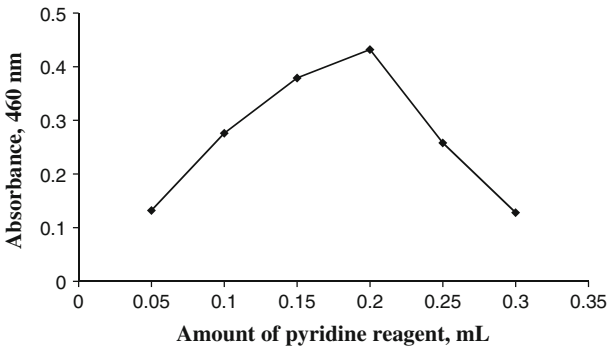


Fig. 2 Effect of pyridine reagent

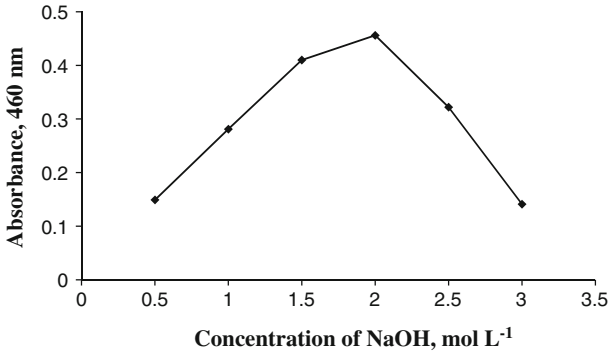


Fig. 3 Effect of NaOH concentration

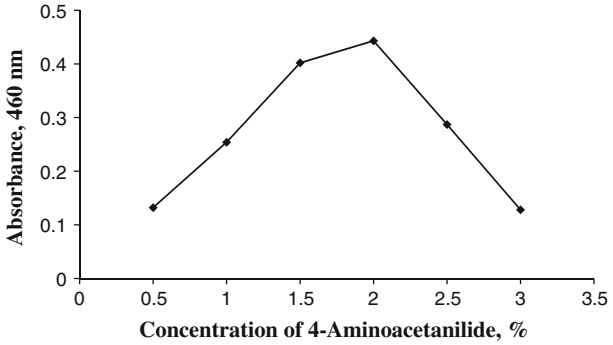


Fig. 4 Effect of concentration of 4-aminoacetanilide

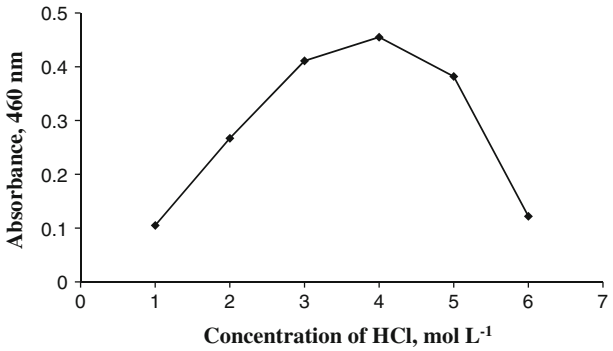


Fig. 5 Effect of concentration of HCl

anionic SDS micelles. The sensitivity enhancement produced by SDS was due to the increase of both the reaction yield and the molar absorptivity of the polymethine dye.

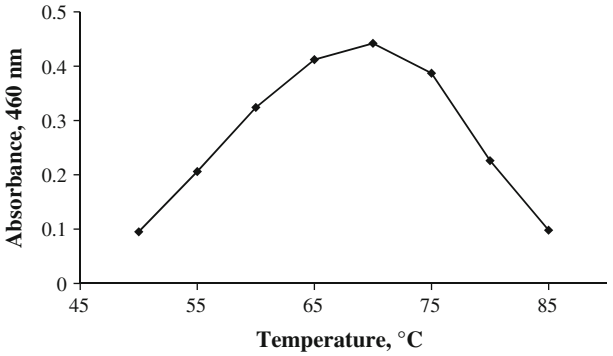


Fig. 6 Effect of temperature on sensitivity

A series of experiments were performed to investigate the effect of the presence of surfactants on the hydrolysis and coupling reactions by adding the surfactants at different steps of the reaction. Most of the enhancement in the absorbance was produced by an increase in the reaction yield. This enhancement occurred when the surfactant was added before the hydrolysis of the pyridine derivative and also when it was added during the coupling reaction. Once the coupling reaction was finished, the addition of the surfactant does not affect the absorbance of the solution. Thus, the step largely affected by the presence of SDS is the coupling of glutaconic aldehyde to form polymethine dye.

The effect of SDS concentration was studied with optimized reaction conditions. The results show that, by increasing the SDS concentration up to 1.4×10^{-3} M, the sensitivity increases, whereas a greater amount decreases the sensitivity. Thus, 1.4×10^{-3} M SDS was selected throughout the study

Effect of foreign species

The effect of foreign species and other pesticides, which are likely to interfere in the determination of atrazine, was studied. Known amounts of foreign species and

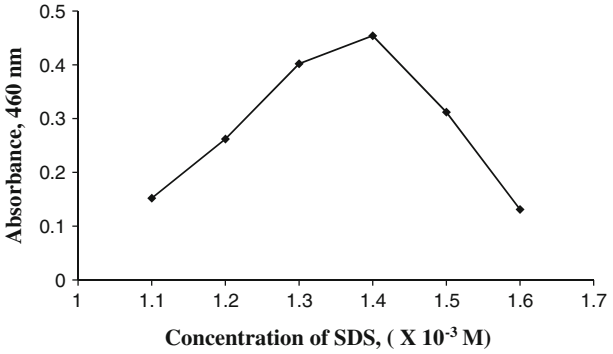


Fig. 7 Effect of SDS concentration

Table 1 Effect of diverse ions (concentration of atrazine 0.1 μg mL^{-1})

Foreign species	Tolerance level[a] (μg mL^{-1})
Diquat	800
Paraquat	750
Mg^{2+}, Ca^{2+}	700
Al^{3+}, Cd^{2+}	300
Trichloroacetic acid	300
NO_3^-	200
CCl_4	5
Ethanol	4
$CHCl_3$	3.5
Phenol	2.5

[a] Tolerance limit causes ±2% variation in absorbance value

pesticides were added to the standard solution containing 0.1 μg mL^{-1} and then analyzed by the proposed method (Table 1).

Spectral characteristics and method validation

The proposed method involves the formation of a yellow-colored product having λ_{max} 460 nm. The reagent blank had negligible absorption at this wavelength. The absorption spectrum of the colored product and the corresponding reagent blank are given in Fig. 8. Beer's law was obeyed over the concentration range of 0.012–0.12 μg mL^{-1} (Fig. 9). The molar absorptivity and Sandell's sensitivity are given in Table 2. The slope, intercept, and the correlation coefficient were calculated by least squares regression analysis (Table 2). The detection limits (DL = 3.3 σ/S) and quantitation limits (QL = 10 σ/S), where 'S' is the slope of the calibration curve and 'σ' is standard deviation of blank, and standard deviation (SD) of the slope and intercept calculated are given in Table 2. The precision of the

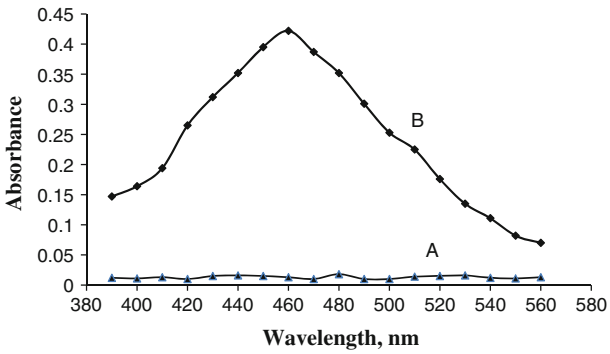


Fig. 8 Absorption spectra of colored product

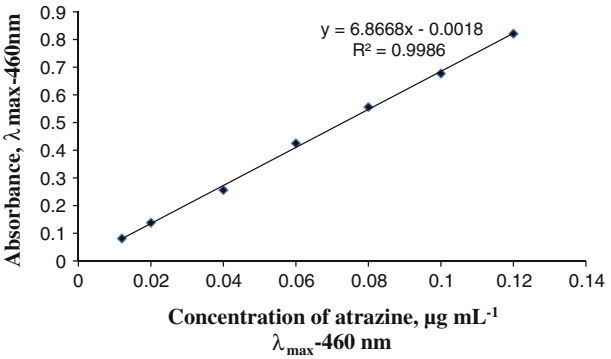


Fig. 9 Calibration data for the determination of atrazine

method was calculated in terms of intermediate precision (intra-day and inter-day). Three different concentrations of atrazine (within the working limits) were analyzed in seven replicates during the same day (intra-day precision) and seven consecutive days (inter-day precision). The RSD (%) (relative standard deviation) values of the ranges of intra-day and inter-day studies showed that the precision was good for the method (Table 2).

Applications

The proposed method has been applied for the determination of atrazine in environmental and biological samples.

Table 2 Spectral characteristics, precision and accuracy of the proposed method

Parameters	Results
λ_{max} (nm)	460
Stability of color	~24 h
Range of Beer's law (µg mL^{-1})	0.012–0.12
Molar absorptivity (L mol^{-1} cm^{-1})	1.52×10^6
Sandell's sensitivity (µg cm^{-2})	0.0002
Relative standard deviation (%)	
Intra-day	0.737–2.130
Inter-day	0.817–2.254
SD of slope	0.074
SD of intercept	0.002
Limit of detection (µg mL^{-1})	0.001
Limit of quantification (µg mL^{-1})	0.003
Regression equation ($Y = bx^2 + a$)	
Slope (b)	6.87
Intercept (a)	−0.001
Correlation coefficient	0.998

[2]Concentration in µg mL^{-1}

Determination of atrazine in vegetable, biological, soil and water samples

Different samples of fruits and vegetables, free from atrazine were taken and spiked with known amount of atrazine and kept for one day. Weighed samples were crushed and washed with 50 mL of water, from which atrazine was extracted by 10 mL of ethanol. The extract was evaporated to dryness and residue dissolved in 10 mL of *n*-propanol and analyzed by the proposed and reported method [21] (Table 3).

The synthetic biological samples were prepared by adding known amount of atrazine in blood and urine samples. The samples were deproteinsed with trichloroacetic acid and extracted with 2 × 5 mL of ethanol evaporated and residue was dissolved in 10 mL of *n*-propanol [24]. Aliquots were analyzed as described above and result was confirmed by the analysis using the reported method [21] (Table 3). A known amount of atrazine was sprayed on various soil and water samples. The soil samples were weighed and washed with 10 mL of *n*-propanol and water samples were also extracted in 10 mL *n*-propanol. The samples were analyzed by the proposed and reported methods [21] (Table 3).

Determination of atrazine in real environmental samples

To check the validity as well as to compare the proposed method with the reported method, various samples of vegetables and agricultural waste water were collected from different fields where atrazine had been sprayed as insecticide. Samples were weighed, macerated with *n*-propanol, and filtered through a thin cotton cloth, and the filtrate was centrifuged at 1,850*g* for 10 min. The filtrate, which was greenish yellow due to the presence of organic matter from plants, was passed through a silica gel column (10 × 1 cm) to remove chlorophyll and other interfering materials. The column was washed with 10 mL of *n*-popanol. Washings were collected and analyzed by the proposed as well as the reported methods. The results are shown in Table 4.

Conclusion

The proposed method is both sensitive and selective for the determination of atrazine. The method was found to be free from interference of a large number of foreign species. It was found that the use of the anionic surfactant SDS and the solid phase extraction technique increased the sensitivity of the method. It was successfully applied to soil, water, vegetable, and biological samples.

Table 3 Determination of atrazine in different vegetable, soil, water, and biological samples

Sample	Atrazine added ($\times 10^{-2}$ μg)	Proposed method		Reported method [21]		*t* test[a]	*F* test[b]
		Atrazine found ($\times 10^{-2}$ μg mL^{-1} ± SD)[c]	Percentage recovery	Atrazine found ($\times 10^{-2}$ μg mL^{-1} ± SD)	Percentage recovery		
Potato (20 g)	4	3.84 (0.037)	96.00	3.83 (0.045)	95.75	0.54	1.48
	8	7.86 (0.027)	98.25	7.83 (0.049)	97.88	1.41	3.29
	10	10.02 (0.033)	100.2	9.96 (0.057)	99.60	1.97	2.98
Pineapple (20 g)	4	3.93 (0.033)	98.25	3.89 (0.025)	97.25	2.24	1.74
	8	7.90 (0.032)	98.75	7.87 (0.059)	98.38	0.93	3.39
	10	9.89 (0.057)	98.90	9.92 (0.051)	99.20	0.82	1.25
Corn (20 g)	4	3.90 (0.035)	97.50	3.85 (0.045)	96.25	2.27	1.65
	8	8.01 (0.030)	100.13	7.99 (0.064)	99.88	0.45	4.55
	10	10.10 (0.068)	101.0	10.03 (0.038)	100.3	2.00	3.20
Soil (10 g)	4	3.89 (0.035)	97.25	3.86 (0.032)	96.50	1.41	1.19
	6	5.96 (0.049)	99.33	5.88 (0.064)	98.00	2.00	1.71
	8	7.96 (0.057)	99.50	7.91 (0.069)	98.88	1.25	1.47
Water (40 mL)	4	3.96 (0.064)	99.00	3.94 (0.068)	98.50	0.48	1.13
	6	6.01 (0.075)	100.10	5.98 (0.039)	99.67	0.42	3.69
	8	7.94 (0.045)	99.25	7.92 (0.048)	99.00	0.81	1.14
Blood (2 mL)	4	4.04 (0.043)	101.00	3.99 (0.034)	99.75	1.49	1.59
	6	5.91 (0.046)	98.50	5.85 (0.046)	97.50	2.14	1.00
	8	7.98 (0.034)	99.75	7.97 (0.052)	99.63	0.43	2.34
Urine (2 mL)	4	3.95 (0.035)	98.75	3.90 (0.032)	97.50	2.35	1.19
	6	6.03 (0.032)	100.50	5.99 (0.024)	99.83	1.89	1.78
	8	7.97 (0.044)	99.63	7.98 (0.037)	99.75	0.62	1.41

[a] Tabulated *t* value for 8 degree of freedom at 95% confidence level is 2.31

[b] tabulated *F* value for (4,4) degree of freedom at 95% confidence level is 6.39

[c] Mean ± standard deviation (n = 5)

Table 4 Application of the method to real samples

Samples	Atrazine found, μg[a]	
	Present method[a]	Reported method [21]
Vegetable sample[b]		
Potato	3.24 (0.040)	2.98 (0.036)
	4.50 (0.034)	4.49 (0.017)
	2.77 (0.032)	1.51 (0.039)
Pineapple	5.93 (0.026)	5.17 (0.034)
	4.55 (0.037)	4.21 (0.031)
	3.45 (0.020)	3.14 (0.026)
Corn	2.86 (0.033)	2.51 (0.046)
	2.23 (0.027)	2.06 (0.036)
	3.53 (0.019)	3.27 (0.024)
Agricultural waste water[c]		
A	2.81 (0.023)	2.52 (0.039)
B	3.03 (0.029)	2.76 (0.032)
C	2.75 (0.034)	2.43 (0.030)

[a] Mean of five replicate analyses (±SD)

[b] Amount of vegetable samples (from fields where atrazine was sprayed): 20 g

[c] Volume of the aliquot of sample, after treatment described in procedure: 10 mL

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