

A Simple and Fast Solid-Phase Extraction GC-ECD Method for the Routine Assessment of Atrazine Residues in Agricultural Produces

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Abstract

A simple and rapid solid-phase extraction (SPE) supported Gas Chromatographic-Electron Capture Detection (GC-ECD) method has been presented for the routine determination of atrazine in some common agricultural produces. Equivalent to USP G-42 GC stationary phase, DB-35 ms capillary column and ECD detector was selected on the basis of physical and structural characteristics of atrazine. Temperature programming of 20 minutes with 0.8 mL/min optimized gas flow gave maximum number of the theoretical plates ($N=10^4$) and high resolution ($R_s \sim 2.1$ min) with LOD and LOQ values of 0.001 $\mu\text{g/L}$ and 0.1 $\mu\text{g/L}$ respectively. LOQ achieved by the present method is almost below the maximum residue levels recommended by the European legislation. Satisfactory spiked recoveries of the atrazine were obtained from agricultural produces ranging from 82.2 to 97.8% with relative standard deviations below 3.8%. The minimum consumption of solvent (5 mL ethyl acetate) in SPE for residual workup in 5 minutes, with subsequent 20 minutes GC analysis makes the proposed method simple and fast having promise as an excellent economical alternative for the routine analysis of atrazine in environmental samples.

Keywords: Atrazine; Solid-phase extraction; GC-ECD; Residue analysis

Introduction

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine), structure shown in Figure 1, is one of the most heavily used and less expensive pre and post emergence herbicide and used against a broad spectrum of weeds on many crops including corn, potato, maize, wheat, apple, rice and forestry [1-4]. The residue due to spraying of high dosage of atrazine is one of the threats to human health [5]. Atrazine exhibits endocrine disruption both in human and animals and is responsible for adverse effects on reproductive system, hypothalamus, pituitary and thyroid glands [6,7]. Considerable concentration of this herbicide in agricultural produces is increasing and frequently exceeds the maximum residual limit (MRL) of 0.1 $\mu\text{g/L}$. To prevent adverse effects on health, assessment of atrazine residues in agricultural produces is required which necessitates the development of convenient, reliable and economic analytical method for screening its quantitative presence in agricultural produces viz. cereals, fruits, vegetables etc. Of the various methods viz. UV-V is spectrophotometry [8,9], electrochemical method [10,11], thin layer chromatography (TLC) [12,13], and immunoassay [14], the methods based on Gas chromatographic (GC) [15-19] and high performance liquid chromatographic (HPLC) [20-22] techniques are generally used for the determination of atrazine. GC methods however, are largely used for quantification studies due to thermal stability of atrazine [23]. Solid phase micro extraction (SPME) and gas chromatography with mass spectrometry (GC-MS) methods increase the sensitivity in the determination of atrazine but are relatively costlier

and time consuming in comparison to the methods employing other detectors like flame ionization detector (FID), nitrogen phosphorus detector (NPD) and thermal conductivity detector (TCD). The greater selectivity and sensitivity of electron capture detector (ECD) detector for halogenated compounds including atrazine compared with FID and TCD has not largely been reported in the determination of atrazine and thus prompted us to use it in the present work. The determination also involves extraction and clean up for matrix interference removal. Liquid-Liquid extraction has a long history for the purpose and is still useful. Solid phase extraction (SPE) involves minimum consumption of solvent and represents a convenient alternative to conventional extraction reported. A cost effective SPE (Silica based, 60 mesh~250 micron, 1.2 cm i.d. homogeneous glass column) with ethyl acetate elution at room temperature has been fabricated for residual workup [24,25] which completes the extraction process in 5 minutes. The greater selectivity of ECD detector compared with FID and TCD, with optimized temperature programming of 20 minutes makes the method rapid and sensitive.

Experimental

Reagents and materials

Standard Atrazine (Product No-34053, GC purity, 99.0%) was purchased from Sigma-Aldrich, Bangalore, India. Ethyl acetate and Methanol (HPLC Grade; Merck, Germany) were used. AR grade chemicals were used in the analysis.

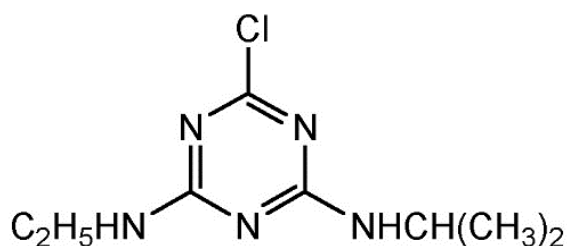


Figure 1: Structure of atrazine.

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Dilution Number	Concentration ($\mu\text{g/L}$)	Retention Time (Min)	GC Peak Area	Limits	S/N Ratio
1	10^3	14.8	19705		26.2
2	10^2	14.8	16837		23.8
3	10^1	14.9	14156		21.1
4	10^0	14.9	11340		17.2
5	10^{-1}	14.9	8569	LOQ	12.7
6	10^{-2}	14.9	5888		8.2
7	10^{-3}	14.9	3364	LOD	4.9
8	10^{-4}	14.9	608		2.7

Table 1: GC Quantization Limits for Standard Atrazine Solutions.

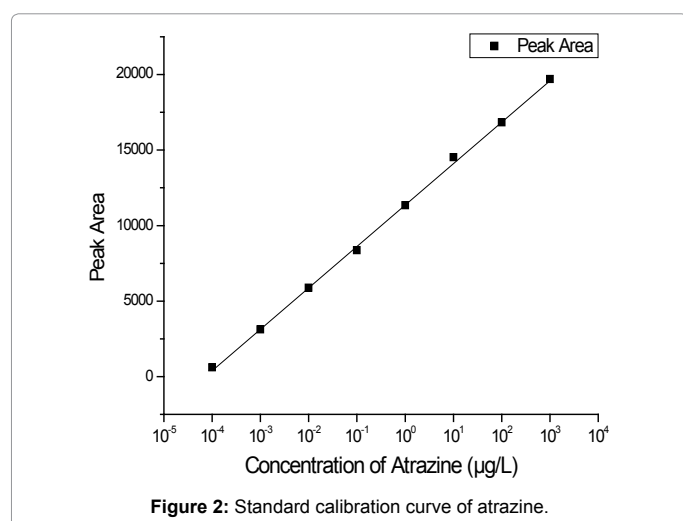


Figure 2: Standard calibration curve of atrazine.

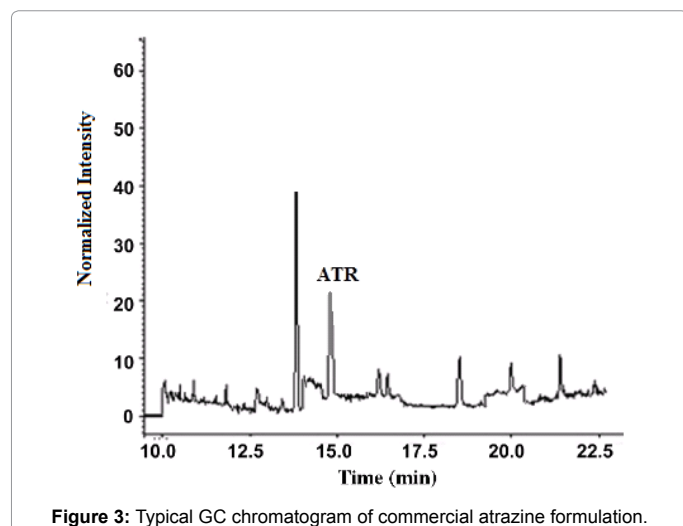


Figure 3: Typical GC chromatogram of commercial atrazine formulation.

Instrumentation and method

An Agilent 7890 A GC system (Germany) with Chemstation B.02.01 version software using ECD detector was used to perform GC analysis. The GC chromatographic Capillary Column DB-35 ms, consisted of 35%-phenyl-methyl polysiloxane, (length 25 m, i.d. 0.20 mm with 0.33 μm film thickness) and is equivalent to USP G-42, connected with the splitless injector with extended temperature 50-375°C with the injector liner of i.d.0.75 mm.

Career gas

Helium (99.9%) with Nitrogen as make up gas with constant flow of 0.8 mL/min was optimized and set.

Temperature gradient

The initial Temperature was 50°C (hold for 2 min) and then was raised to 230°C at a speed of 10°C/min; finally it was programmed to 370°C (hold for 2 min) at a speed of 10°C/min with initial injector injector temperature at 225°C and 1 μL (splitless) atrazine volume injected for each sample.

Column preparation for SPE

Glass column of 15 cm length and 1.2 cm in diameter was used, silica 8 g (Silica gel 60-120 mesh) for column chromatography (Sisco Research Laboratory Pvt. Ltd., Mumbai) was taken in beaker added 15 mL of ethyl acetate and column was filled with this slurry slowly so that silica spread homogeneously in the column [25].

Preparation of standard calibration curve

For the preparation of GC calibration curve standard $10^4 \mu\text{g/L}$ solution of atrazine was prepared in methanol and then diluted to $10^{-5} \mu\text{g/L}$ with the same solvent and calibration curve was built for quantitative measurements using the solutions prepared according to Table 1. GC analysis was performed in a 20 min run time method following above temperature gradient and 1 μL of atrazine sample was injected in each run and peak area was measured from ECD response peaks. Calibration curve was constructed by plotting peak area versus concentration of standard atrazine solutions as presented in Figure 2.

Formulation analysis

A wettable powder (WP) formulation of Atrazine purchased from Dhanuka Agritech Ltd, Gurgaon (An authorized dealer with State Govt. Haryana, India), containing 50% active atrazine ingredient was used. Solution of higher concentration was prepared in methanol and then solution of 100 $\mu\text{g/L}$ was prepared by dilution with same solvent. Aliquots of 100 $\mu\text{g/L}$ solution were taken and diluted to 5 ml with methanol and processed for GC analysis in the same manner as described above. The makers specification has also been established by a reference method [26].

Recovery experiment

Standard solution of 200000 $\mu\text{g/L}$ of atrazine was prepared in methanol. This solution was diluted to get the final concentration of 100 $\mu\text{g/L}$ with the same solvent and aliquots of this solution were added to 5 g portion of each grain material. The samples were mixed thoroughly and extracted with 10 mL of ethyl acetate and subsequent extracts was purified by passing through the silica column extractor at a flow rate of 0.7 mL/min. The eluates collected were dried with nitrogen gas drier and the remainder was dissolved in 10 ml methanol and analyzed with GC-ECD method developed above.

Results

Linearity of the method was investigated with correlation of

Atrazine Concentration (µg)	Recovery (% age) ^a				
	Maize	Rice	Wheat	Apple	Potato
0.10	95.49 ± 2.39	86.79 ± 2.90	91.67 ± 2.61	82.27 ± 2.25	89.04 ± 2.49
0.15	96.43 ± 2.80	88.95 ± 3.43	92.69 ± 2.36	83.15 ± 2.76	89.26 ± 1.26
0.20	96.61 ± 3.09	90.00 ± 3.04	94.43 ± 2.88	83.64 ± 2.63	90.22 ± 2.92
0.25	97.85 ± 2.33	91.54 ± 3.15	95.06 ± 2.79	84.34 ± 2.80	91.42 ± 2.43

^aValues are mean ± standard deviation for five determination.

Table 2: Recovery data of Atrazine from spiked grains, fruit, and vegetable matrices.

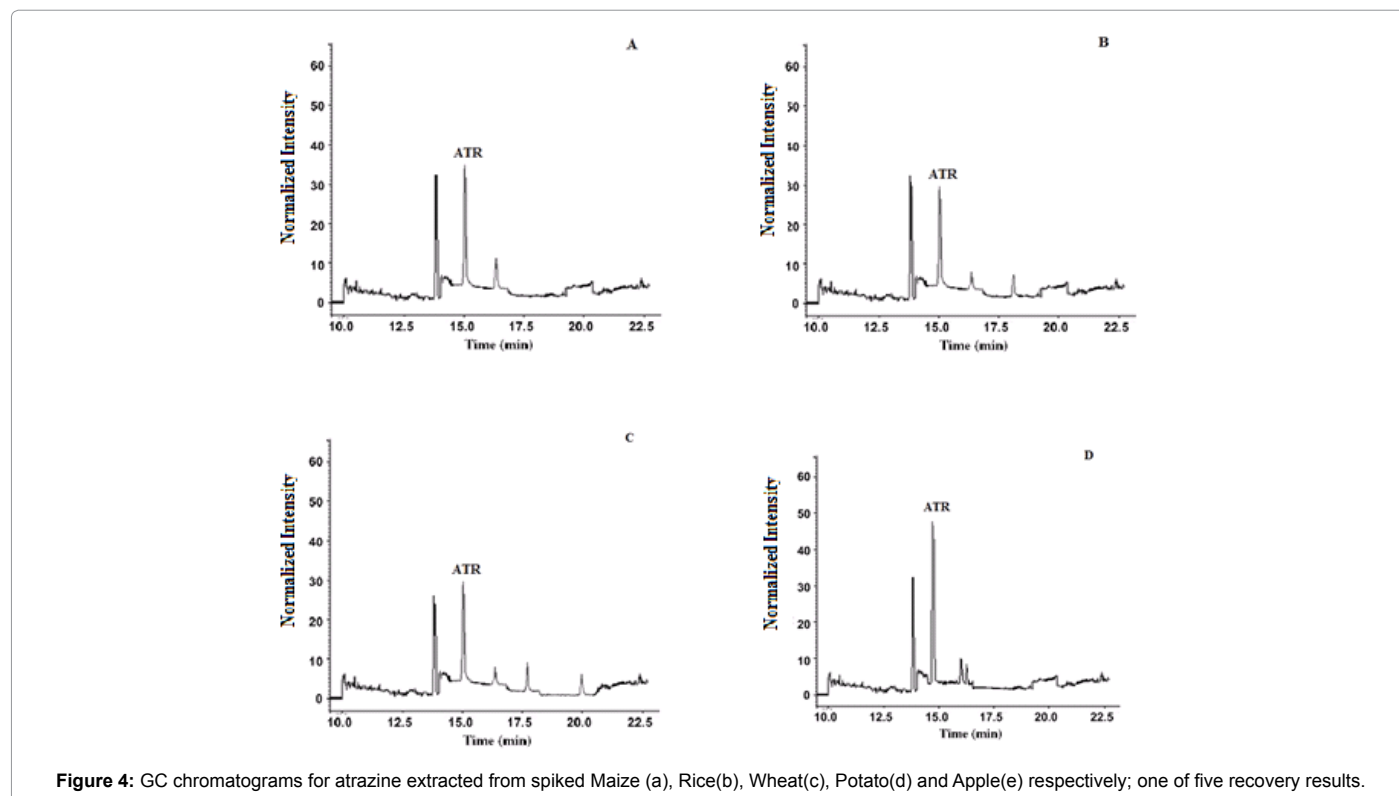


Figure 4: GC chromatograms for atrazine extracted from spiked Maize (a), Rice(b), Wheat(c), Potato(d) and Apple(e) respectively; one of five recovery results.

determination (r^2) values of 0.99 calculated from Regression equation $y=2721.65 \times +19584.16$. Temperature programming of 20 minutes with 0.8 mL/min optimized gas flow gave maximum number of the theoretical plates ($N=10^4$) and high resolution ($R_s \sim 2.1$ min) thus permitting the determination of atrazine below its maximum residual limit fixed by the European legislation (Council Directives 90/642/EEC, 1990) as 0.1 µg/L with the estimated LOD and LOQ ranged from 0.001 µg/L and 0.1 µg/L respectively. The method was applied to the analysis of a formulation of atrazine for its active ingredient content. The recovery of the atrazine (active ingredient) from the wettable powder (WP) formulation comprising 50% of standard active ingredient, has been found in the range 49.7-50.3% with relative standard deviation in the range 3.2% and its GC chromatogram is presented in Figure 3. A cost effective SPE column (Silica based, 60 mesh, 1.2 cm i.d homogeneous glass column) with ethyl acetate elution at room temperature has been fabricated for pre-concentration workup. The samples were extracted with ethyl acetate then purified with a silica SPE column, and finally, detected by GC-ECD. Recovery experiments of four fortified concentrations were carried out on maize, rice, wheat, potato and apple samples which did not contain the target compound. The recovery and precision data is shown in Table 2 and chromatograms are presented in Figures 4A-4D. Average recovery and maximum standard deviation of the analytical method applied were 82.27 to 97.85% of spiked amount and 3.43% respectively.

Discussion

Selection of Stationary Phase and ECD system was based on the high boiling point, structure with long hydrocarbon chain with chloride substituent and maximum residue levels (MRL) 0.1 µg/L values of atrazine. All data were subject to strict quality control procedures, including the analysis of procedural blanks and spiked samples with each set of samples analyzed. Though MS/MSⁿ detectors are more quantitative and sensitive over ECD, higher selectivity for halogenated analyte like atrazine and comparative low cost of ECD over Mass detectors expertise their preferred use for excellent Limit of Detection (LOD), Limit of Quantification (LOQ) and Limit of Linearity (LOL). Silica gel adsorbents are widely accepted as one of the best and cheaper adsorbents used in chromatography and the important criteria for opting for silica gel in the present study is that it remains neutral and does not interact with atrazine residues that are passed through it and also maintains its own stable structure throughout a process. Importantly, it can be regenerated or reused many times, cutting the costs of purification considerably. All it needs is to be heated to a specific temperature (about 150°C), when it releases all the substances absorbed by it. The method has successfully applied to the analysis of atrazine in its commercial formulation and residues in agricultural produces. Atrazine was not detected in the procedural blanks and

method performance was assessed using spiked food samples and the method was shown to have good precision and high recoveries. The former is essential not only to ensure the quality of marketed products but also to get reliable residue data.

Conclusion

A new simple and rapid SPE-GC-ECD method for the determination of atrazine in some agricultural samples has been described. The samples were extracted with ethyl acetate and purified with a cost effective SPE Silica based glass column with ethyl acetate elution at room temperature in five minutes, with subsequent 20 minutes GC-ECD detection makes the proposed method fast and simple and could be potentially extended to other matrices. The selectivity, linear range, recovery, precision, and limit of quantification were all evaluated and verified. The method is accurate, sensitive, and convenient. The limit of detection is 0.001 µg/L and keeps pace with the advances of international technology. LODs and LOQs obtained by this research can meet the European Union standards for MRLs of atrazine in agricultural stuffs and have a promising application ahead.

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