

Solid Phase Extraction Method for the Determination of Atrazine and Cyanazine in Water Samples

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Triazine is one of the herbicides group that is widely used in agriculture that acts as an inhibitor for the growth of unwanted weeds in plants. The use of this herbicide on plants is absorbed by the soil and flows into a nearby water system. This research focused on two types of triazines, namely atrazine and cyanazine. This research aims to extract this type of triazine herbicides and to determine their concentration in water samples. It was quantified by using gas chromatography-electron capture detector (GC-ECD). Solid phase extraction (SPE) method was applied to extract herbicides from water samples. The results indicate that all the samples contained atrazine and cyanazine. Studies in the range of 0.5 - 25 mg/L achieved good linearity with good correlation of determination, r^2 value of 0.9922 - 0.9982 mg/L. Relative standard deviations (RSD) for triplicate analysis of the samples were less than 10.0%. The limit of detection (LODs) and limit of quantification (LOQs) of cyanazine and atrazine were found, ranging from 3.33 – 6.67 µg/L and 11.09 – 20.10 µg/L, respectively. The recoveries of the triazine herbicides studied in water samples ranged from 82.5% to 107.6%. The developed method exhibited excellent clean-up capability and was successfully applied for determining triazine herbicide residues in water samples.

Keywords: triazine herbicides; atrazine; cyanazine; solid phase extraction; gas chromatography

I. INTRODUCTION

In the stream of development in technology, especially in the plantation sector may lead to the rises of the environmental pollution. One of the factors is because of the contamination of herbicides. Herbicides are widely used in agriculture to increase the quality of crops (Jose *et al.*, 2013) at the same time have led to major environmental problems when they are discharged to the environment.

This phytotoxic chemical that is mainly used for destroying various weeds and hindering their growth. It can be classified into different groups based on their chemical nature and mechanism of action (Gupta, 2014). Herbicides are mobile and soluble in water and can be absorbed by the soil. According to Solomon *et al.* (2013), the excess concentration

of herbicides in water is toxic to other forms of organisms such as animals and aquatic life. It can also affect human health due to the water that is exposed to the herbicides and consumed as drinking water.

Atrazine and cyanazine are the most commonly used herbicides for weed control in rice fields of Peninsular Malaysia (Jose *et al.*, 2013; Mohd *et al.*, 2018; Zhou & Gao, 2014). Due to the widespread and prolonged use, high chemical stability and water solubility, the residues of triazines herbicides would enter surface water and other environmental water through the ecological chain (Amelin *et al.*, 2013; Gang *et al.*, 2003). It has been reported that triazines and its degradation products are very toxic, they can disturb and damage endocrine and reproductive systems of

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animals, and cause birth defects, weight loss of embryos and even multiple kinds of cancer (An *et al.*, 2010).

Few analytical instruments have been applied for the analysis of trace triazines in environmental samples. Herrera *et al.* (2015), have evaluated that gas chromatography (GC) is the most favourable method for separating and analysing the triazine herbicides. It is used to separate and analyse the complex compounds in analytical chemistry without decompose the compound itself. GC is capable of dealing with volatile analytes. Moreover, GC method is extensively used for quantification studies by the reason of thermal stability of these compounds during analysis (Sharma *et al.*, 2017; Tankiewicz *et al.*, 2010). Various selective detectors are used in trace analysis of herbicides. The electron-capture detector (ECD) was first used for the determination of halogen-containing compounds or halogenated derivatives due to its high sensitivity for these compounds. Therefore, GC-ECD is one of the instruments that can provide accurate analysis in determining the triazine herbicides, and this technique is the most preferable and recommended to determine the halogen-containing organic compounds such as herbicides in water samples (Tandon *et al.*, 2015).

According to Tankiewicz *et al.* (2010), prior to analysis by GC, the water samples need to undergo separation technique to determine the trace component of triazines in water samples. Determination of triazines in real samples is challenging because of their low concentration and the complexity of real sample matrix (Philisiwe & Precious, 2019). Sample pre-treatment techniques are usually needed prior to instrumental measurement. The most favourable technique that is widely used is solid phase extraction (SPE). In SPE, cartridges or disk form is used to separate one or more compounds in a liquid mixture by passing the liquid which is known as the mobile phase through a solid that acts as the stationary phase. The separation is due to their different physical or chemical interactions, called hydrophobic interaction where the mobile phase is polar and the stationary phase is non-polar. It is also known as reversed phase of extraction in SPE. SPE has been proven as the best and commonly used technique of extracting target analyte from water samples due to its ability to clean up interferences with low consumption of solvents and time (Oluseyi *et al.*, 2011).

Thus, the aim of this study is to determine the concentrations of atrazine and cyanazine in water samples prior to the analysis by using gas chromatography with electron capture detector (GC-ECD).

II. MATERIALS AND METHOD

A. Chemicals and Reagent

All reagents and solvents used were of analytical grade or HPLC grade. Methanol, ethyl acetate and triazine standard solution of atrazine and cyanazine were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). All compounds had a purity of higher than 97%. Deionized water was prepared in-house using a Millipore Milli-Q system (USA).

1. Preparation of standards and sample solutions

Stock solutions in 1000 mg/L of atrazine and cyanazine compounds were prepared in methanol and stored in freezer at 4°C. All working solutions were prepared by serial dilution concentration of stock of 0.5, 1, 5, 15 and 25 mg/L.

2. Gas Chromatography – Electron Capture Detector (GC-ECD) procedure

An Agilent Technologies 7820 A system gas chromatography equipped with an electron capture detector (ECD) and 30 m x 250 µm HP5-MS capillary column (Agilent Technologies, CA, USA) was used for separations. Nitrogen gas has been set up as make up gas with constant flow of 1.5 mL/min. The initial temperature was set as 80°C and hold for 1 min. Afterwards, the temperature was increased by 20°C/min until it reached 200°C. The final temperature has been programmed to 250°C and hold for 1 min at a speed of 10°C/min. The overall run time was 15 min. The temperature of initial injector that has been used in this study is 225°C and the detector temperature was at 280°C with a flowrate of 1.5 mL/min and 1 µL of triazine standards was injected into injection port using splitless mode.

3. Sampling and pre-treatment of samples

Water samples were collected from rice field at Jalan Bukit Kepis-Juasseh, Kampung Sawah Lebar, Negeri Sembilan. The water samples were taken from three different sampling points; point A, point B and point C. Point A was from the rice

field area (stagnant water), point B was from water stream a (run-off from nearby area) and point C was from water stream b (conventional irrigation supply). Water samples (1 L) were taken from the upper 50 cm surface of the water using polyethylene buckets that had been pre-cleaned and rinsed with water samples. The samples were then transferred to high density polyethylene (HDPE) bottles and labelled as A, B, and C. Water samples were filtered by using 0.45 μm pore size membrane to discard any suspended particles from the samples. Samples were kept in refrigerator at temperature of 4°C until analysis.

4. Solid phase extraction (SPE) procedure

The SPE cartridges were conditioned by using 5 mL of methanol for 30 seconds to wet and activate the sorbent surface. Then, the cartridges were rinsed with 10 mL of distilled water without applying vacuum. Filtered water sample (50 - 100 mL) was added to pre-condition column using a vacuum at flow rate of 40-45 mL/min. Then, the column was dried for 15 min by using vacuum. Next, the absorbed residue was eluted with 5 mL of ethyl acetate and evaporated under a gentle stream of nitrogen gas at room temperature to make it concentrated to 1 mL prior to GC analysis.

5. Method validation

The calibration standards (five levels) were prepared in mobile phase following concentration ranges: 0.5, 1, 5, 15, and 25 mg/L for every individual analyte. Linearity was assessed by plotting peak arrangement concentration of the analyte against response peak area using linear regression analysis. Precision and repeatability were determined by analyzing triplicate of each sample for five days interval followed by the calculation of the percentage relative standard deviation for each compound.

Recovery was assessed by analyzing the concentration of each analyte in triplicate aliquots of spiked and unspiked samples. Each aliquot of sample was spiked to give an added concentration similar to the second level of the calibration range used in this study. The on-column limit of detection was estimated as $3 \times$ signal/noise ratio and limit of quantitation as $10 \times$ signal/noise ratio for each compound.

III. RESULTS AND DISCUSSION

A. Chromatographic Separation

Temperature programming of 12 mins with 1.5 mL/min optimized gas flow gave maximum number of the theoretical plates ($N=156$) and high resolution ($R_s \sim 10.2$ min) thus permitting the elution of atrazine and cyanazine. Figure 1 shows representative chromatographic profile atrazine and cyanazine elution time at 25.0 mg/L.

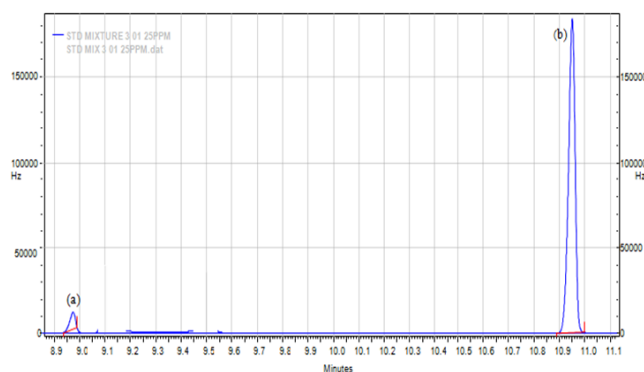


Figure 1. Chromatogram of (a) atrazine and (b) cyanazine at 25.0 mg/L

B. Method Validation

Table 1 shows the data of correlation coefficients (r^2) of the calibration curves are 0.9922 for atrazine and 0.9982 for cyanazine over the range of 0.5 – 25.0 mg/L indicating good linearity. The linear range used throughout does not exceed 25 mg/L to avoid column degradation and to increase the lifespan of the column. Furthermore, lower concentration will provide more precise result. However, this result is slightly varied with previous study which reported different linearity of atrazine and cyanazine at 0.9991 and 0.9995, respectively (Sanagi, 2012).

Table 1. The regression equation, correlation coefficients (r^2), and linear range data

Triazines	Regression equation	Correlation coefficient (r^2)	Linear range (mg/L)
Atrazine	$y = 77565x + 117926$	0.9922	0.5 – 25.0
Cyanazine	$y = 10006x + 139806$	0.9982	0.5 – 25.0

The detection limit (LOD) was calculated by comparing the signal-to-noise ratio (S/N) of the lowest detectable concentration to a S/N=3. A S/N of 10 was applied for the calculation of the quantification limit (LOQ). Based on the result, the LODs for atrazine and cyanazine were 0.00667 mg/L and 0.00333 mg/L, respectively. The LOQs were calculated at signal-to-noise ratio of 10 (S/N ~ 10) for atrazine and cyanazine were 0.0201 mg/L and 0.01109 mg/L, individually. These LODs and LOQs that were obtained in our study is higher than the LOD and LOQ that has been stated in Environmental Protection Agency (EPA). EPA reported that LOD are 0.03 µg/L for atrazine and 0.05 µg/L for cyanazine while LOQ recommended by EPA are 3.00 µg/L for atrazine and 2.50 µg/L for cyanazine (Environmental Protection Agency, 2009).

The differences of LODs and LOQs that were obtained in our study would be due to some modification of the methods or should be explored further.

1. Precision

Precision and repeatability were determined by analysing triplicate of each sample for five days interval under the same operating conditions and calculated as between-day RSD of peak areas. As can be seen in Table 2, the results obtained were satisfactory with relative standard deviation (RSD) values below 10% for both compounds.

2. Extraction Recovery

To figure out the accuracy of SPE procedure, recovery tests were performed by spiked samples with known concentration. Accordingly, 1.00 mg/L of standard solution was spiked into 50 mL sample. The spiked and non-spiked samples were extracted simultaneously by the optimized extraction and concentration of each triazine was determined in triplicate. Table 2 shows the analytical results obtained for the recovery test of triazine herbicides. The acceptable range for most of the triazine herbicides for recovery test were 75 – 110% (Ueji & Inao, 2014). It shows that the recoveries of atrazine and cyanazine were within the acceptable range with SPE using C18 cartridge. Recovery of the targeted analytes was calculated in Equation (1) as follows:

$$\text{Percentage recovery} = \frac{C_s - c}{s} \times 100 \quad (1)$$

where C_s was stand for concentration of triazines in spike sample, c was the concentration of triazines in non-spike sample and s was the known concentration that had been spiked in the sample.

C. Analysis of Triazine Herbicides in Rice Field Waters

Rice field water samples were collected and analyzed to support the method development and validation. Surface water samples were taken from three different sampling points namely point A, point B and point C. The collected samples were analyzed by the present method.

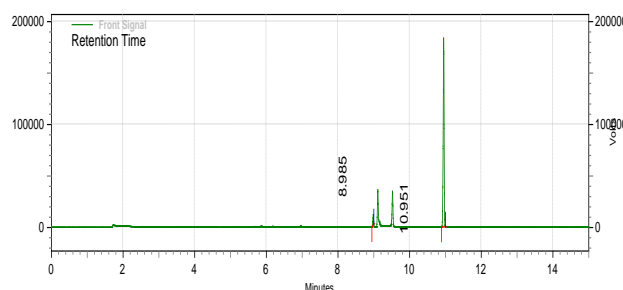


Figure 2. Chromatogram obtained from rice field water sample (sample B) spiked with 1.00 mg/L mixture of atrazine ($t_R = 8.985$) and cyanazine ($t_R = 10.961$)

Atrazine and cyanazine were detected in water samples from all sampling points. The concentration of cyanazine was much higher than atrazine in rice field water samples. The concentration levels of herbicide residues in the rice field water were hardly affected by the herbicide and the volume of standing water at application time when sampling was done (Ismail *et al.*, 2015). The mean level of atrazine and cyanazine for water samples in our study were higher than recommended value by WHO (2010) which is 0.006 mg/L and 0.001 mg/L, respectively.

Nevertheless, our results provide information of possible contamination by the agrochemical which is triazine herbicides in rice field water at Jalan Bukit Kepis-Juasseh, Kampung Sawah Lebar, Negeri Sembilan and suggest that cleanup and control procedures should be conducted.

Table 2. Summary of method validation of atrazine and cyanazine

Triazine	Type of sample	Relative Standard		Recovery (%)	LOD (mg/L)	LOQ (mg/L)	Concentration in water sample (mg/L)±SD
		Deviation (%)					
		Day 5 (mg/L)	Day 10 (mg/L)				
Atrazine	A	0.059	0.033	82.5	0.00667	0.0201	0.520±0.013
	B	0.042	0.015	85.9			0.383±0.006
	C	0.051	0.025	83.7			0.213±0.004
Cyanazine	A	0.273	0.159	98.7	0.00333	0.01109	0.760±0.014
	B	0.083	0.042	107.6			0.806±0.017
	C	0.154	0.079	88.6			0.244±0.005

IV. CONCLUSION

In summary, the level of triazines reported could be related to abundant used on crops that may carry a potential health issue for humans, animals and aquatic life. Although it has been successfully determined for two triazine herbicides using Gas Chromatography Electron Capture Detector (GC ECD), it is suggested to improve the sample preparation by using Magnetic Solid-Phase Extraction (MSPE) method. This method offers lower sensitivity with the exploration of different solvent such as elution. A method will be established

with more rapid efficiency and reduce the number of organic reagents used during analysis.

V. ACKNOWLEDGEMENT

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