

# Development of Solid Phase Micro Extraction Technique for Determination of Triazines in Water Samples Using Experimental Design

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A sensitive direct immersion solid phase microextraction technique coupled with gas chromatography-mass spectrometry (SPME-GC-MS) was optimized and validated for analysis of triazines in drinking water. Preliminary evaluation of fibre materials indicated that carbowax-divinylbenzene was better than poly-dimethyl siloxane-divinylbenzene thus it was selected for further work. Variables namely extraction times, extraction temperature, ionic strength and stirring rate were screened and optimized using a two-level full factorial design and central composite design. The proposed optimized analytical procedures was obtained using sample volume (10 mL), extraction time (50 min), extraction temperature (50 °C), ionic strength (20 % NaCl), stirring rate (240 rpm) and pH (6.5). Desorption time and temperature were 5 min and 250 °C respectively. Recoveries were achieved in the range of 77 to 91.6 % at fortified concentration of 2  $\mu$ g L<sup>-1</sup>. Linearity ranges from 0.02 to 10  $\mu$ g L<sup>-1</sup> (with r = 0.982 to 0.998). Detection and quantification limits were obtained in the range of 0.004 to 0.047  $\mu$ g L<sup>-1</sup> and 0.012 to 0.159  $\mu$ g L<sup>-1</sup> for all targeted compounds. An analysis of triazines in drinking water samples has managed to detect the presence of ametryn in the range of 0.026 to 0.240  $\mu$ g L<sup>-1</sup>.

Keywords: Microextraction, Herbicides, Response surface, Drinking water analysis.

#### **INTRODUCTION**

Triazines are group of organonitrogen pesticides usually applied as selective pre-and post-emergence herbicides for the control of weed in many agricultural crops like corn, wheat, barley, pineapple, sugarcane, cocoa and bananas as well as weed grow in railways, roadside verges and golf course [1-3]. Malaysian Pesticides Board has only registered several triazines namely atrazine, ametryn, hexazinone, metribuzin and terbutryn thus permitted to be used in Malaysia [4]. The maximum acceptable value ranging from 0.6 to 50 µg L<sup>-1</sup> depending on the specific triazines [5]. Atrazine, ametryn, prometryn, propazine, terbutryn and simazine have been short listed as few compounds to be endocrine-disrupting chemicals by the US Environmental Protection Agency [6].

Due to very low level of residual triazines in aquatic environment, many extraction techniques have been proposed to determine triazines in water. These include solid phase extraction (SPE) [7,8], solid phase microextraction (SPME) [9,10] and dispersive liquid-liquid microextraction (DLLME) [11] coupled to various chromatography detectors. Solid phase microextraction technology is preferable since it eliminates the use of organic solvent and has the advantages of simplicity, reusable and short time of analysis. In this study, carbowaxdivinylbenzene (CW-DVB) and poly-dimethyl siloxanedivinylbenzene (PDMS-DVB) fibers were evaluated since previous studies have shown that these fibers were more efficient than the polyacrilate or poly-dimethyl siloxane (PDMS) [10-13].

The aim of current work is to develop an optimized method of analysis of triazines and their degradation products in water samples. Main effects of experimental variables were evaluated using a two-level full factorial design (screening) before expanded to a central composite design (optimization). The developed method was applied to determine the presence of triazines in water from selected water treatment plant.

## EXPERIMENTAL

Mixture of standards containing (simazine, atrazine, propazine, terbuthylazine, sebuthylazine, desmetryn, metribuzin, ametryn, prometryn, terbutryn) and the internal standard (1-bromo-2-nitrobenzene) of purity above 99 % were purchased

SELECTED IONS FOR GC-MS IDENTIFICATION AND CONFIRMATION OF TARGET COMPONENTS								
Componente	Retention	Relative	Molecularion -	Selected characteristic ion $(m/z)$				
Components	time	retention time		Q	C1	C2	C3	
1-Bromo-2-nitrobenzene	10.295	-	201	155	157 (100)	201 (77)	203 (72)	
Simazine	25.657	2.492	201	201	186 (66)	173 (54)	202 (14)	
Atrazine	26.161	2.542	215	200	215 (53)	202 (32)	173 (30)	
Propazine	26.544	2.579	229	214	229 (58)	187 (34)	216 (32)	
Terbuthylazine	27.218	2.644	229	214	173 (45)	216 (32)	229 (25)	
Sebuthylazine	29.052	2.822	229	200	202 (32)	214 (14)	229 (11)	
Desmetryn	29.723	2.388	213	213	198 (68)	171 (28)	214 (15)	
Metribuzin	29.959	2.909	214	198	199 (44)	182 (11)	-	
Ametryn	30.65	2.977	227	227	212 (63)	185 (27)	228 (16)	
Prometryn	30.797	2.992	241	241	184 (108)	226 (60)	199 (28)	
Terbutryn	31.225	3.033	241	226	185 (91)	170 (77)	241 (55)	

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Q; quantitation ion, C1-C3; characteristic ions and values in bracket are the per cent of abundance ion to the quantitation ion

from Dr. Ehrenstorfer (GmBH, Germany). Stock solutions of mixed standards (100 mg L<sup>-1</sup>) and internal standard (10 mg L<sup>-1</sup>) were diluted to the required concentration with liquid chromatography grade of methanol and stored at 4 °C. Buffer solution was prepared by mixing appropriate amount of ammonium acetate, acetic acid and/or ammonium hydroxide depending on the required value of pH. Ultrapure Milli-Q water (Millipore) was used to daily prepare the working aqueous solution in the required working concentration range. SPME was performed using commercially available 65  $\mu$ m CW-DVB and 65  $\mu$ m PDMS-DVB coated fiber and housed in the appropriate manual holder (Supelco, Bellefonte, USA).

Chromatographic analyses were performed with a Shimadzu QP5050A gas chromatography-mass spectrometry with quadrupole mass filter and electron impact ionization (EI) at 70 eV as ionization source. The injector and detector temperatures were set at 250 and 280 °C, respectively. The split/splitless injector was maintained in splitless mode during 5 min of SPME fiber desorption. All compounds were separate in a DB-5MS column ( $30 \text{ m} \times 0.25 \text{ mm}$  ID  $\times 0.25 \text{ µm}$  thickness) using helium as carrier gas with a flow rate of 1.0 mL/min. The oven temperature programmed was as follows: initial temperature 60 °C (held 2 min), increased by 15 °C/min to 140 °C, increased to 158 °C (at 1 °C/min) and finally increased to 250 °C (at 10 °C/min) and held at this temperature for 8 min. The selected ion monitoring (SIM) mode was performed in which four ions for each compound except metribuzin (three ions) were chosen for the analysis. Table-1 shows the details of component identification and confirmation ions.

Selection of fiber: Extractions were performed by immersion of the fiber in 10 mL of Milli-Q water fortified with 200  $\mu$ g L<sup>-1</sup> mixture and internal standards in screw-cap vials with a PTFE-lined septum. Extraction procedure was set up at time (30 min), temperature (25 °C), stirring rate (80 rpm), pH (6.5-7.0) and desorption time was 5 min. The relative total ion chromatographic (TIC) area of the compound to the internal standard area was monitored as extraction efficiency. Extractions were repeated for 8 times. Significant difference in the extraction efficiency was evaluated by means of statistical t-test.

**Screening and optimization experiment:** Two level of Full Factorial Design, 2<sup>4</sup> was chosen involving 16 experiments, undertaken in random order. Quadruplicates of center point were included in this design to estimate experimental error.

All of experiments were carried out using 10 mL of ultrapure Milli-Q water fortified with 200  $\mu$ g L<sup>-1</sup> mixture of triazines and internal standards. The details of experimental variables and design matrix are shown in Table-2.

TABLE-2 DESIGN MATRIX OF FULL FACTORIAL DESIGN						
Variable	Level					
variable	Code	-1	0	+1		
Extraction time (min)	А	30	45	60		
Stirring rate (rpm)	В	80	160	240		
Ionic strength (% NaCl w/v)	С	10	15	20		
Extraction temperature ( $^{\circ}C$ )	D	40	50	60		

In the second stage of optimization, central composite design was carried out involving 30 experiments with rotateability satisfaction at  $\alpha = 1.682$ . Quadruplicates experiments also added in this stage to estimate experimental error. Design-Expert software version 6.0 was used for design of experiment and data processing. Table-3 summarized the code values given to each variables.

TABLE-3 DESIGN MATRIX OF CENTRAL COMPOSITE DESIGN							
Variable	Le				vel		
variable	Code —	-α	-1	0	+1	-0	
Extraction time (min)	А	20	30	45	60	70	
Ionic strength (% NaCl w/v)	С	5	10	15	20	25	
Extraction temperature (°C)	D	35	40	50	60	65	

**Selection of suitable pH:** The effect of sample pH to the extraction efficiency was evaluated separately from the experimental design. Three pH levels (4.0, 6.5 and 8.0) were selected and the experiments were carried out in three replicates for each level. The final decision of the optimum pH was chosen based on significant different in the statistical analysis of variance (ANOVA, p < 0.05).

**Method validation:** Analytical figure of merit namely recovery, linearity, precision, limit of detection and limit of quantitation were determined in this study. This step is necessary to evaluate suitability of the developed method SPME technique for routine analysis of triazines in drinking water. Linearity of all targeted compounds was tested with a series of mixture concentration ranging from 0.02 to 10  $\mu$ g L<sup>-1</sup> (n =

9). Recovery and precision were performed with fortified water samples at spiking levels of 2  $\mu$ g L<sup>-1</sup> and 5  $\mu$ g L<sup>-1</sup> (precision test only) respectively. Limit of detection and quantification were expressed from formula LOD =  $3S_a/b$  and LOD =  $10S_a/b$  where  $S_a$  is the standard deviation of the response and b is the slope of the calibration curve [14]. In this study, real drinking water samples were taken from seven water treatment plant intakes and outlets located in Perlis, northern state of Malaysia.

### **RESULTS AND DISCUSSION**

**Fiber screening:** Table-4 showed that there were significant differences (p < 0.05) in extraction efficiency for the fibers tested. The extraction efficiency was observed better for the CW-DVB fiber. The CW-DVB type fiber can extracts more polar triazines namely simazine and metribuzin which not extracted by the PDM-DVB type fiber. Therefore, CW-DVB fiber was selected for further extraction work. This result was in agreement with several previous studies on the analysis of triazine herbicides in water [11-13].

TABLE-4 EFFICACY OF EXTRACTION BETWEEN TWO TYPES OF FIBERS						
Compounds	Type of	fiber*	n voluo			
Compounds	PDMS-DVB	CW-DVB	p value			
Simazine	nd	0.150	nr			
Atrazine	0.141	0.272	0.000			
Propazine	0.274	0.402	0.000			
Terbuthylazine	0.497	0.761	0.000			
Sebuthylazine	0.297	0.636	0.000			
Desmetryn	0.102	0.338	0.000			
Metribuzin	nd	0.041	nr			
Ametryn	0.073	0.174	0.019			
Prometryn	0.288	0.663	0.000			
Terbutryn	0.319	1.315	0.000			
nd; not detected; nr; not relevant; *Peak area (standard/internal						

standard)

Screening and optimization of solid phase microextraction: Main effects of each variable on the extraction efficiency are shown in Table-5. Generally, all variables have showed positive effect on extraction efficiency, which explicate that extraction efficiency increases as the magnitude of variables increased. Extraction time seem to give the highest effect on

	TABLE-5					
MAIN EFFECT OF EACH VARIABLE ON						
THE EXTRACTION EFFICIENCY						
	Quantitation	Main effect (%)				

Compound	Quantitation					
Compound	ion $(m/z)$	Α	В	С	D	
Simazine	201	50.4	0.2	8.5	3.1	
Atrazine	200	34.7	1.2	15.9	27.5	
Propazine	214	39.6	0.2	13.3	29.1	
Terbuthylazine	214	35.9	3.0	13.5	22.4	
Sebuthylazine	200	40.7	1.7	14.7	22.5	
Desmetrin	213	42.0	0.6	16.4	18.4	
Metribuzin	198	23.0	1.2	20.0	8.5	
Ametryn	227	40.2	3.1	12.8	23.4	
Prometryn	241	36.1	2.5	9.6	21.7	
Terbutryn	226	44.7	4.6	4.5	21.3	

the extraction efficiency meanwhile stirring rate shows the lowest effect. The ionic strength show the second highest effect and particularly effective on the polar and low water solubility compounds such as simazine and metribuzin.

Statistical analysis of variance (ANOVA) at 95 % confidence level (Table-6), indicate that the effects of extraction time, extraction temperature and ionic strength on extraction efficiencies were significant for all triazines pesticides except simazine and terbutryn. In contrast, the effect of stirring rate was not significant for all compounds with the exception of sebuthylazine and ametryn. The observations may be ascribed to the fact that extraction work was carried out in immersion mode, therefore mobility into headspace was not relevant. Two ways interaction between variables namely extraction time (A) with ionic strength (C) and extraction time (A) with extraction temperature (D) appear to be statistically significant for most of targeted compounds.

Higher percent of sodium chloride (% w/v) was not considered in Full Factorial Design in order to prolong the fiber lifetime even though Pawliszyn [15] have stated that higher ionic strength are required for the extraction of polar compounds. Furthermore, Hernandez *et al.* [12] have found that very fast degradation of the fiber occurred when 30 % (w/v) of NaCl was used. In the case of stirring rate, there was no different in extraction efficiency between the stirring rate of 80 or 240 rpm. Therefore, the optimum stirring rate was set constant at 240 rpm in the next experimental works.

The second order polynomial equation obtained using coded values of optimized variables is given in eqn. 1 and response surface is expressed as total quantitation ion area.

TABLE-6 DESCRIPTIVE STATISTIC OF ANOVA TEST										
	Main effects						Intera	ctions		
	А	В	С	D	AB	AC	AD	BC	BD	CD
Simazine	0.002	0.684	0.038	0.139	0.201	0.021	0.713	0.238	0.029	0.200
Atrazine	0.000	0.050	0.001	0.000	0.099	0.002	0.011	0.007	0.577	0.030
Propazine	0.005	0.619	0.023	0.008	0.502	0.134	0.047	0.286	0.368	0.165
Terbuthylazine	0.003	0.144	0.018	0.008	0.614	0.060	0.043	0.106	0.484	0.171
Sebuthylazine	< 0.0001	0.029	0.006	0.000	0.577	0.006	0.003	0.007	0.730	0.016
Desmetryn	0.001	0.447	0.008	0.007	0.782	0.064	0.069	0.100	0.611	0.100
Metribuzin	0.005	0.330	0.007	0.035	0.469	0.010	0.217	0.028	0.026	0.259
Ametryn	0.0002	0.025	0.002	0.001	0.231	0.007	0.006	0.063	0.556	0.035
Prometryn	0.002	0.195	0.032	0.007	0.454	0.071	0.033	0.118	0.732	0.143
Terbutryn	0.003	0.097	0.097	0.010	0.391	0.103	0.033	0.085	0.574	0.744
37.1	~ <u>1</u> D	0.05								

Values are significant when P < 0.05

Response surface = 
$$24364 + 7778A + 4595C + 4331D + 2232AC + 2557CD$$
 (1)

In this equation, variables A (extraction time), C (ionic strength), D (extraction temperature) had positive linearity of fitted model. Figs. 1 and 2 illustrated the response surface for ametryn (expressed as quantitation ion area) as an example; generate using Design-Expert software. The optimum analytical procedures suggested is: extraction time (50 min), extraction temperature (50 °C), ionic strength (20 % NaCl), stirring rate (240 rpm) and pH (6.5). Other co-factor such as sample volume (10 mL), desorption time (5 min) and temperature (250 °C) were set constant in all experimental works.



Fig. 1. Response surface plot of extraction time vs. ionic strength

Comparison of means of extraction efficiencies using 15 % and 20 % NaCl showed that there were significant differences in extraction efficiencies for the more polar analyte namely simazine, atrazine and metribuzin. Therefore, ionic strength at 20 % (w/v) NaCl was selected as an optimum condition to cover all the targeted compounds. In selection of suitable pH, the statistical analysis ANOVA showed that there were no significant difference except for simazine and desmetryn (p = 0.014; 0.003). Simazine shows lower efficiency at pH 8.0 whereas desmetryn shows lower efficiency at pH 4.0. After comparing the extraction efficiency mean values between pH



Fig. 2. Response surface plot of extraction time vs. extraction temperature

6.5 and 8.0, all compounds has showed no significant different in extraction efficiency (p > 0.05), therefore the optimum pH was selected at 6.5. In fact, this pH value is also within the range of pH normally encountered in real drinking water samples.

**Method validation:** Analytical figure of merits obtained for the method validation was presented in Table-7. The calibration plots were prepared in the ranges of 0.02 to 10  $\mu$ g L<sup>-1</sup> for all targeted compounds (n = 9). Good linearity was obtained for all calibration curves with correlation coefficient, r values in the range of 0.982 to 0.998. Limits of detection (LOD) and limit of quantitation (LOQ) were achieved in the range of 0.004 to 0.047  $\mu$ g L<sup>-1</sup> and from 0.012 to 0.159  $\mu$ g L<sup>-1</sup>, respectively. Recovery at low spiking level was achieved in the range of 77.0 to 91.6 % for all targeted compounds. The precision of developed method was varied from 12.5 to 29.4 % RSD. Lowest value was obtained for simazine at 2  $\mu$ g L<sup>-1</sup> level meanwhile the highest RSD was obtained for ametryn at 5  $\mu$ g L<sup>-1</sup> spiking level.

**Analysis of real samples:** Twenty one samples consist of treated water from seven treatment plant intake and outlet waters, in state of Perlis were analyzed for multi residue of triazines. Only eight samples were found to contain residue of ametrin in the range of 0.026 to 0.240  $\mu$ g L<sup>-1</sup> (Table-8). None of other triazines residue were detected in treated water sample.

TABLE-7 ANALYTICAL FIGURE OF MERIT IN METHOD VALIDATION							
Compounds	Correlation		$LOO(u_{\pi}/L)$	Pecovery (%)	Precision (% RSD)		
Compounds	coefficient (r)	LOD (µg/L)	LOQ (µg/L)	Recovery (70)	2 μg L <sup>-1</sup>	5 µg L-1	
Simazine	0.993	0.026	0.085	89.1	12.5	18.7	
Atrazine	0.995	0.006	0.018	89.3	15.0	17.7	
Propazine	0.994	0.007	0.025	91.5	17.1	18.0	
Terbuthylazine	0.997	0.008	0.027	91.6	16.3	17.3	
Sebuthylazine	0.997	0.006	0.020	90.3	19.8	17.7	
Desmetryn	0.994	0.009	0.033	85.5	25.7	25.0	
Metribuzin	0.982	0.047	0.159	77.0	22.0	18.1	
Ametryn	0.998	0.004	0.012	91.1	29.1	29.4	
Prometryn	0.997	0.004	0.012	87.0	20.1	15.7	
Terbutiryn	0.998	0.004	0.013	88.3	24.1	21.0	

IABLE-8 ANALYSIS OF AMETRYN IN SELECTED WATER TREATMENT PLANT						
Location of treatment plant Total Positive Level sample ametryn (						
Timah Tasoh						
Intake point	3	3	0.134-0.240			
Outlet point	2	1	0.067			
Kaki Bukit						
Service reservoir outlet	1	0	Not detected			
Padang Besar						
Service reservoir outlet	1	0	Not detected			
Abi						
Service reservoir outlet	2	2	0.026-0.15			
Felda Chuping						
Outlet point	1	0	Not detected			
Guar Sanji	2	0	Not detected			
Intake point	2	0	Not detected			
Sungai Baru						
Intake point	4	1	0.153			
Outlet point	5	1	0.068			

Ametryn is known to be used in sugar cane plantation for weed control, therefore the presence of ametryn in Perlis water supply was not surprised since this state has extensive sugar cane plantation.

#### Conclusion

Carbowax-divinyl benzene (CW-DVB) type fiber was shown to be the best fiber for the solid phase micro extraction of multi residue triazines from water samples. The optimum condition for extraction was found to be as follow: extraction time (50 min), temperature (50 °C), ionic strength (20 % NaCl w/v) and stirring rate (240 rpm). Sample volume, desorption time and temperature were set constant at 10 mL, 5 min and 250 °C respectively. Good linearity and recovery were achieved during method validation. Low detection and quantification limit ( $\mu$ g L<sup>-1</sup>) were obtained for all targeted compounds. The developed method has proved to be suitable for routine analytical works and applicable for the monitoring of drinking water sources.

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