DETERMINATION OF SIMAZINE IN FISH AND SHRIMP SAMPLES USING ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETER (UPLC-MS/MS)

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A RESEARCH PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF CHEMISTRY, FACULTY OF SCIENCE, UNIVERSITY OF MALAYA, IN PARTIAL FULFILMENT FOR THE DEGREE OF MASTER OF SCIENCE (ANALYTICAL CHEMISTRY AND INSTRUMENTAL ANALYSIS)

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ABSTRACT

Ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method was successfully developed and validated for the analysis of simazine in fish and shrimp samples at trace levels to meet European Union requirements and import requirements from certain countries. This method also utilized a simple, fast and inexpensive extraction technique called QuECheRS (quick, easy, cheap, rugged and safe). Matrix-matched calibration standard are used to construct a calibration curve and Ethoprophos is used as internal standard. At optimal conditions, the chromatographic separation was achieved in less than 10 minutes with the total run time of 13.5 minutes. The mean recoveries for fish matrix ranged from 101 % to 107 % while shrimp matrix shows the range from 97 % to 101 %. Limit of detection (LOD) for simazine in fish and shrimp were 1.18 ng/g and 1.06 ng/g respectively. The limits of quantification (LOQ) were reported as 3.52 ng/g in fish matrix and 3.19 ng/g in shrimp matrix. Results from this study showed that the concentration of simazine obtained in fish and shrimp samples were below the limit of quantification and it is reported as not detected in the sample.

ABSTRAK

Kaedah Ultra Prestasi Cecair Kromatografi dengan Spektrometri Jisim (UPLC-MS/MS) telah berjaya dibangun dan di validasi bagi analisis simazine dalam sampel ikan dan udang di peringkat surih dan ia bertujuan memenuhi syarat Kesatuan Eropah dan keperluan import dari negara-negara luar. Kaedah ini juga telah menggunakan teknik pengekstrakan mudah, cepat dan murah yang dikenali sebagai QuECheRS (cepat, mudah, murah, tahan lasak dan selamat). Padanan matrik dengan tentukuran piawai digunakan bagi tujuan membina lengkuk penentukuran dengan piawai dalaman yang digunakan adalah Ethoprophos. Pada keadaan optimum, pemisahan kromatografi dicapai dalam tempoh kurang dari 10 minit dengan jangka masa keseluruhan analisis adalah 13.5 minit. Keputusan perolehan semula bagi sampel ikan berjulat dari 101 % hingga 107 % manakala sampel udang menunjukkan julat perolehan semula dari 97 % hingga 101 %. Had pengesanan bagi simazine dalam sampel ikan dan udang masing-masing 1.18 ng/g dan 1.06 ng/g. Had kuantifikasi dilaporkan sebagai 3.52 ng/g dalam sampel ikan dan 3.19 ng/g dalam matriks udang. Hasil daripada kajian ini menunjukkan bahawa nilai kepekatan simazine yang diperolehi dalam sampel ikan dan udang berada di bawah had kuantifikasi dan ia dilaporkan sebagai tidak dikesan dalam sampel.

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In the name of Allah the Most Compassionate and Merciful, selawat and salam to our prophet Muhammad s.a.w and his companions. First of all I would like to express my gratitude to Allah s.w.t for enabling me to complete this project.

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LIST OF ABBREVIATIONS

QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
MeCN	Acetonitrile
HAc	Acetic acid
$MgSO_4$	Magnesium sulfate
NaAc	Sodium acetate
SPE	Solid-phase extraction
IS	Internal standard
UPLC-MS/MS	Ultra Performance Liquid Chromatography Tandem Mass Spectrometer
GC	Gas chromatography
ESI	Electrospray Ionization
ELS	Early Life Stage
QC	Quality Control
RSD	Relative Standard Deviation
DL	Detection limit
IDL	Instrument detection limit
LOD	Limit of detection
LOQ	Limit of quantification
SN	Signal-to-noise ratio
CCS	Calibration curve slope
LFB	Laboratory fortified blank
UWL	Upper warning limit
LWL	Lower warning limit

UCL	Upper control limit
LCL	Lower control limit
MU	Measurement Uncertainty

CHAPTER 1

INTRODUCTION

1.1 Herbicide

Herbicides which belong to the class of pesticide have increased dramatically during the last two decades in agriculture areas. Most of the agricultural herbicides applied before or during planting in order to maximize crop productivity by minimizing other vegetation. Besides, they also may be applied to crops in the fall, to increase harvesting.

There are allegations related herbicides that have caused numerous adverse effects on human health ranging from skin rashes to death. Some other effects of this pesticide are improper direct contact with field workers, the inhalation of air sprays, consumption of contaminated food and contact with the contaminated soil waste. Besides that, these herbicides can also be transported via surface runoff to contaminate distant surface water and hence another pathway of ingestion through extraction of those surface waters for drinking. Some of the herbicides decompose rapidly in soils and other types have more persistent characteristics with longer environmental half-lives.

Herbicides that have been classified under the group 5 which are Atrex (atrazine), Velpar (hexazinone), Sinbar (terbacil), Princep Nine-T (simazine) are Photosynthetic inhibitors at Photosystem II Site A. These chemicals can disrupt the process of photosynthesis of the plant. Therefore, the carbohydrates would not be produced from plants and cause plant death. These groups of chemicals have activity when applied to

leaf tissue but they are typically used as per-emergent applications, as they are taken up by the roots of newly emerging weeds.

1.2 Simazine

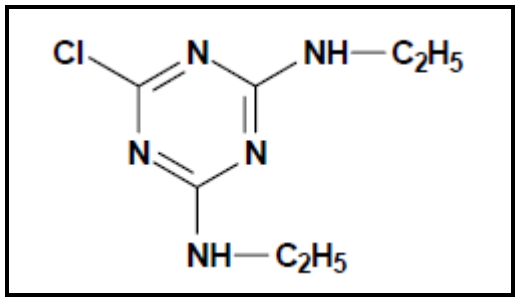

Figure 1: Chemical structure of Simazine

Simazine (2-chloro-4,6-bis(ethylamino)-*s*-triazine) as in **Figure 1** is a chlorinated triazine herbicide, which also includes the pesticide of atrazine and propazine. This synthetic chemical is widely used as an herbicide in modern agriculture to control the growth of weeds, annual grasses in field and ornamental crops. It also used to control submerged weeds and algae in large aquariums, fish hatcheries and become contaminant in marine ecosystems. Simazine has a relatively low stability in water. This chemical is formulated as granules, pellets or tablets, dry flowable concentrates, wettable powders, liquid and granular formulation.

At high levels, simazine is classed as toxic to wildlife, particularly aquatic organisms. Simazine that present in the atmosphere is usually deposited onto soils or water bodies and the remaining is broken down within a matter of hours. Simazine can persevere in soils and waters for a considerable time and it has been found far from its point of

release. Due to this reason, simazine pollution is of concern at a global as well as local level.

1.3 Impacts of Simazine towards aquatic organism

Simazine is categorized as slightly (>10 to 100 mg/L) to moderately (>1 to 10 mg/L) toxic to freshwater fish on an acute exposure basis. This chemical of herbicide has a relatively low solubility in water (0.025 mM) [7], as well as a low sorption coefficient [14]. Aquatic life such as fish and shrimp that are still in Early Life Stage test (ELS) is a species that is considered to be faster and more cost-effective bioassay for testing the toxicity of chemicals and the environment samples. Experience shows that these developmental stages are often the most sensitive to toxic effects [12].

The effects of periodic applications of simazine on the growth of *Tilapia nilotica* swim-up fry (< 12 mm in length) in circular fiberglass pools (4.12 m^2) with an average depth of 45 cm was studied by [11]. The results indicated that, from the study there is a reduction of approximately 32% due to the reduction of natural food (phytoplankton) from simazine activity in treated pool. However, there is an additional reduction of about 20% in revenue is due to a combination of direct effects simazine and poor water quality. It is unclear, however, the percentage of reduction in yield due to the impact of the indirect effects (ie, loss of fish due to reduced phytoplankton diet of simazine application) and toxic effects of simazine.

1.4 QuEChERS Technique

Recently, the selection of the latest techniques and quick in carrying out analytical analysis has become the focus of the researchers. But the quality of analysis could not be ignored in any analysis method used.

For the purpose of analysis involves analyte pesticides, QuEChERS method was selected which is an acronym of **Qu**ick, **E**asy, **Ch**eap, **E**ffective, **R**ugged and **S**afe. In a relatively short time after the publication of the original QuEChERS method by Anastassiades *et.al*. [13], QuEChERS has experienced widespread acceptance around the world and today may be the approach taken in the sample preparation is the most widely used primarily in the analysis of pesticide residues in the whole world.

The technique that involved in this study are the extraction of the sample with acetonitrile (MeCN) containing 1 % acetic acid (HAc) and simultaneous liquid-liquid partitioning formed by adding anhydrous magnesium sulfate ($MgSO_4$) plus sodium acetate (NaAc) followed by a simple cleanup step known as dispersive solid-phase extraction (SPE).

QuEChERS method has several advantages over the traditional methods of analysis and was listed as follows [9]:

- High recoveries (>85 %) are achieved for a wide polarity and volatility range of pesticides, including notoriously difficult analytes.

- The results obtained are very accurate (true and precise) due to an internal standard (IS) that used to correct for commodity to commodity water content differences and volume fluctuations.
- High sample throughput of about 10-20 pre-weighed samples in ≈ 30-40 min are possible.
- Small amount of solvent usage and waste, yet no chlorinated solvents are used.
- A single person can perform the method without much training or technical skill.
- Not much glassware is used throughout the analysis.
- This method is quite rugged because extract cleanup is done to remove organic acids.
- Need little bench space thus the method can be done in a small mobile laboratory if needed.
- The solvent involves in this technique such as MeCN is added by dispenser to an unbreakable vessel that is immediately sealed, thus minimum exposure of solvent to the worker.
- Inexpensive cost involved for the reagent usage.
- Only few devices are needed to carry out sample preparation.

1.5 Ultra Performance Liquid Chromatography Tandem Mass Spectrometer (UPLC-MS/MS)

Most developed countries now have chosen LC-MS/MS which rapidly becoming an indispensable tool in the analysis of chemical analysis mainly involves monitoring of pesticides residue. In addition, most modern pesticides are not Gas Chromatography

(GC) amenable, and if they do not fluoresce or contain a strong chromophore for UV/vis absorption, the LC-MS/MS is the only way to detect chemicals in its underivatized form.

In the past, the analytes that require derivatization technique will be analyzed using GC, but such methods are usually problematic to develop and implement in practice, and they do not lend themselves to the multiclass, multiresidue applications [7]. On the other hand, LC-MS/MS setup has higher compatibility detecting polar compounds such as organic acids, organic amines, nucleosides, ionic species, nucleotides, and polyamines compared to a GC.

Most of the literature review are using Electrospray Ionization (ESI) for which is an ionization technique that involves sample solution is sprayed into a strong electric field in the presence of nitrogen to help desolvation. Then, the formed droplets will evaporate in an area that is maintained at a vacuum resulted in causing the charge to increase on the droplets. The multiply charged ions will then entering the analyzer. The most obvious feature of the ESI spectrum is that the ion carries a variety of charges, which reduces the ratio of mass to their charge against the singly charged species. This allows mass spectra to be obtained for large molecules. [20].

There are several advantages of using UPLC-MS/MS quantification analysis and is listed as follows:

- MS provides an exceptionally clean product (fragment) ion chromatogram for quantification purposes.

- Useful for rapid screening of complex samples in which the analytes of interest are known.
- MS/MS can be used to verify the compound identity based on MS product ion scan mode.
- Classified the compound of interest by detecting specific product ion (precursor ion mode) or charged fragments resulting from loss of neutral (neutral loss mode).

1.6 Objectives of this study

A simple and sensitive method was developed to detect the simazine in fish and shrimp sample using liquid chromatography tandem mass spectrometry (LC-MS/MS). So as to ensure that the result obtained from the laboratory is accurate, the validation of method must be done before routine analysis can be carried out.

This study was carried out to fulfill the following objectives:

i. To identify and quantify the simazine in fish and shrimp samples by Ultra Performance Liquid Chromatography Tandem Mass Spectrometer (UPLC-MS/MS).

ii. To validate the method before it is to be implemented for routine samples analysis.

CHAPTER 2

METHODOLOGY

2.1 Principle of Method

This method utilizes a simple and fast extraction technique called QuECheRS (Quick, Easy, Cheap, Rugged and Safe). Simazine is extracted from fish and shrimp sample using acetonitrile, followed by liquid-liquid partitioning by adding anhydrous magnesium sulfate and sodium acetate. After centrifugation, the extract is decanted into a tube containing primary secondary amine (PSA), carbon 18 (C18) and magnesium sulfate which constitutes a cleanup procedure called dispersive solid-phase extraction (dispersive SPE). Then, the acetonitrile extract is filtered, diluted 10 times with water and analyzed by UPLC-MS/MS. Matrix-matched calibration standards are used to construct a calibration curve and Ethoprophos is used as the internal standard.

2.2 Apparatus

a. Ultra Performance Liquid Chromatography Tandem Mass Spectrometer Detector (UPLC-MS/MS) with triple quadrupole analyzer.
b. Chopper and mixers
c. Centrifuge
d. Liquid dispensers
e. Analytical balance
f. Vials and vessels

g. Vortex mixer

h. Teflon Fluorinated Ethylene Propylene (FEP) Centrifuge tube and dispersive-SPE tube.

2.3 Chemical Standards

Pesticide reference standard and internal standard which were Simazine and Ethoprophos were purchased from Dr. Ehrenstorfer (Augsburg, Germany) with the purity 98.5% and 92% respectively. All these reference standards were provided with its Certificate of Analysis (COA) and Material Safety Data Sheet (MSDS) as references.

2.4 Reagents

The chemical reagents that involved in this experiment must have higher purity level for analysis purposes which includes Acetonitrile and Methanol (LCMS grade). These organic solvents were obtained from Fisher, USA. Other chemicals are Glacial Acetic Acid, Magnesium Sulfate anhydrous and Sodium Acetate were purchased from Merck Germany, Solid phase extraction (SPE) sorbent – Primary Secondary Amine (PSA) sorbent from Varian and Supelclean ENVI-18 (C18) was obtained from Supelco.

2.5 Procedure

2.5.1 Preparation of Standard Stock Solution

a) Standard Stock Solution for Calibration

i) Standard stock solution for Calibration – 1000 µg/mL

About 10 mg of simazine standard was weighed accurately and make up to volume with methanol in 10 mL of volumetric flask.

ii) Standard solution for standard calibration – 50 µg/mL

0.5 mL of standard stock solution (2.5.1-a-i) was pipetted into 10 mL volumetric flask and make up to volume with acetonitrile.

iii) Standard solution for standard calibration – 5 µg/mL

1 mL of 50 µg/mL standard solution was pipetted into 10 mL volumetric flask and make up with acetonitrile.

b) Internal Standard Solution (IS)

i) Internal standard solution – 1000 µg/mL

9.93 µL of standard Ethoprophos (92 % purity) was pipetted into 10 mL volumetric flask and make up to volume with methanol.

ii) Intermediate internal standard solution – 100 µg/mL

1 mL of 1000 µg/mL internal standard solution (2.5.1-b-i) was pipetted into 10 mL volumetric flask and make up to volume with acetonitrile.

iii) Internal standard solution – 50 µg/mL

5 mL of 100 µg/ml internal standard solution was pipetted into 10 mL volumetric flask and make up to volume with acetonitrile.

iv) Internal standard solution – 5 µg/mL

1 mL of 50 µg/mL internal standard solution was pipetted into 10 mL volumetric flask and make up to volume with acetonitrile.

2.5.2 Preparation of Matrix-matched Calibration Standard

a) Five calibration standards were prepared with concentration of 5, 10, 25, 50 and 100 ng/mL respectively. An appropriate amount of 5 µg/mL standard solution (as in **Table 1**) and 150 µL of internal standard solution 5 µg/mL (2.5.1-b-iv) were pipetted into 1.5 mL auto sampler vial containing 1mL of matrix blank extract.

b) Appropriate volume of acetonitrile was pipetted to give consistent final volume of 1.5 mL for each calibration standard and the solution was mixed well.

c) 0.1 mL of each of the calibration standard solution were then pipetted into appropriately labeled auto sampler vials and make up each vial with 0.9 mL distilled water to give concentration as in **Table 1**.

Table 1: Matrix-matched calibration standard solution

Calibration standard Solution	Standard solution 5 µg/mL	Internal Standard Solution (IS) 5 µg/mL	Amount in sample matrix (ng/g)
1	15 µL	150 µL	5
2	30 µL	150 µL	10
3	75 µL	150 µL	25
4	150 µL	150 µL	50
5	300 µL	150 µL	100

2.5.3 Sample Collection

In this study, the fish and shrimp samples were received from fisheries biosecurity whole Malaysia. The size and species of the two samples have been ignored because it will not be taken into account for the purpose of establishing the data but these samples are used to determine the performance of the method. Most of the samples were received in the frozen condition and it will keep frozen by stored in the freezer at the laboratory at temperature around -15 ℃ until the analysis is carried out.

2.5.4 Sample Pretreatment

Before the analysis can be carried out, the samples were defrosted at room temperature. Then, the samples were isolated the body and head, cleaned, chopped and finally blended it for homogenized purposes. About 20 samples of fish and 10 samples of

shrimp were received from period of February 2014 until April 2014 for the purpose of analysis of simazine.

2.5.5 Preliminary Steps and Sample Comminution

All weighing of samples and chemicals involved were made on a digital scale B3002-S (Mettler Toledo).

6.00 ± 0.15 g of anhydrous magnesium sulfate and 1.50 ± 0.15 g of sodium acetate were weighed (in advance), cap and store at room temperature. Then, sufficient number of dispersive-SPE tube containing 0.75 g of PSA, 0.375 g of C18 and 2.25 g of anhydrous magnesium sulfate were prepared prior to the analysis. Large chopper was used to comminute the sample and blend it to ensure the subsequent subsample is homogenized and representative.

2.5.6 QuEChERS Extraction and Cleanup

The homogenized samples were weighed about 15.00 ± 0.05 g into a 50 ml FEP centrifuge tube. Then, the 15 mL of the ratio 99:1 of acetonitrile and acetic acid was added into each tube using the solvent dispenser. All samples were added with 150 µL of the 50 µg/mL Internal Standard Solution (IS). Mixture was then capped and shaken vigorously by hand for 45 seconds. After that, the lid was opened and 6 g of magnesium sulfate and 1.5 g of sodium acetate was added. The tube were recapped, shaken vigorously and centrifuged at 5000 rpm for 1 minute.

After the centrifuge, the organic layer was poured into the tube-dispersive SPE which have been prepared previously containing 0.75 g and 2.25 g PSA anhydrous magnesium sulfate and 0.375 g C18. After that, the tube was lid and shaken vigorously for 45 seconds by hand. Than the tube were centrifuged for the second time at 5000 rpm for 1 minute.

The organic upper layer was filtered through the syringe filter of 0.2 µm pore size into 12 mL vial. Finally, the 0.10 mL of extractant was then transferred to the 2 mL auto sampler vial and 0.90 mL of distilled water was added. A quality control sample was analysed with every batch of samples. The layers of final extract in tube during the cleanup process were deciphered in **Figure 2**.

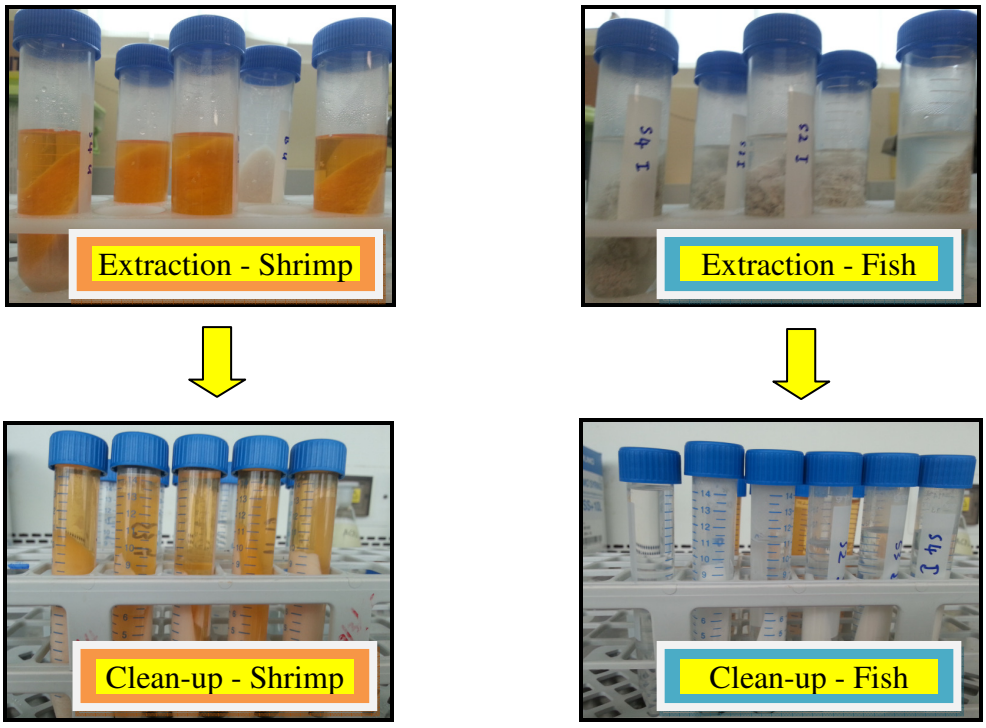

Figure 2: Sample extraction and clean-up process for fish and shrimp matrices

2.5.3 Operation procedure for Instrument of Ultra Performance Liquid Chromatography with Tandem Mass Spectrometry Detector

The samples were analysed using Ultra Performance Liquid Chromatography (UPLC) with triple quadrupole analyzer and mass spectrometry system was Waters® Micromass® Quattro Micro™ from Waters, UK. (**Figure 3**).

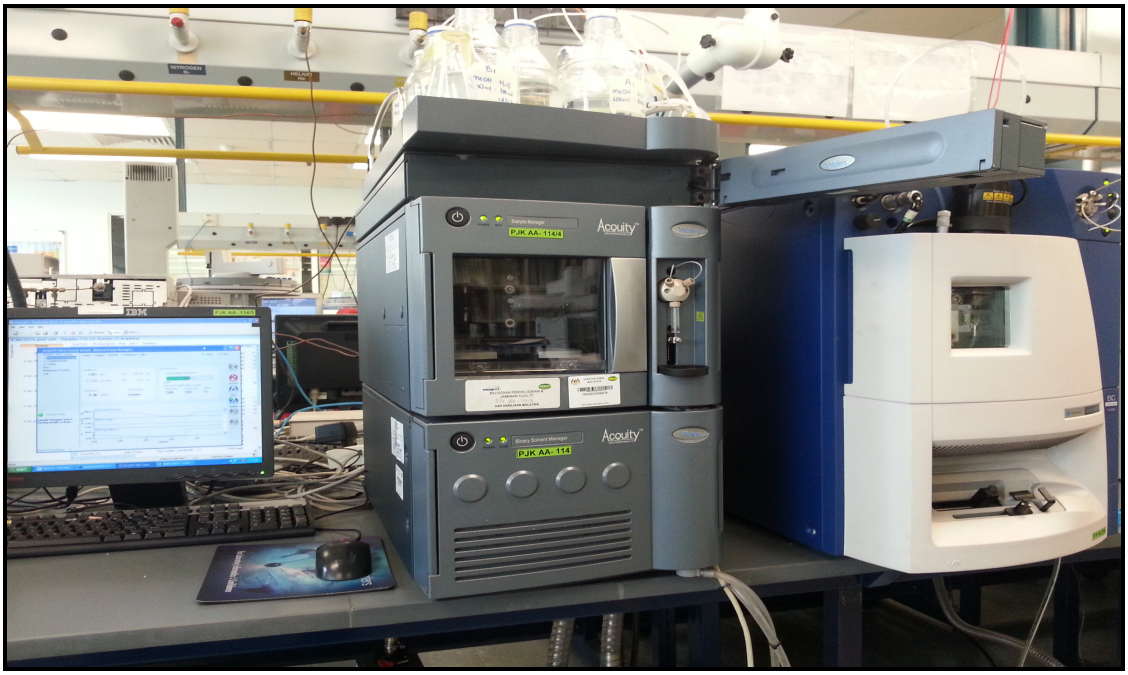

Figure 3: Ultra Performance Liquid Chromatography Tandem Mass Spectrometry

The separation was achieved using Waters Acquity UPLC™ BEH C18 column with the packing material size of 2.1 x 100 mm, 1.7 µm and eluted gradiently at a flow rate 0.3 mL/min. All the data obtained were recorded in the Multiple Reaction Monitoring (MRM) mode using Waters Masslynx 4.0 software as shown in **Table 2**.

Table 2: MRM conditions for pesticide standards

No.	Standard	Transition	Collision Energy (eV)	Cone Voltage (V)
1.	Simazine	202.05 > 123.95	18	30
		202.05 > 131.92	19	30
2.	Ethoprophos	243.1 > 130.8	23	20
		243.1 > 96.8	23	30

There are advantages in using UPLC with 1.7 µm particle system which provide significantly more resolution while reducing run times and yet improving the sensitivity for the analysis of trace levels compared to single MS analyzer. The high technology for column used in this system also gives good respond for the separation of compound and can reduce the solvent consumption thereby reducing the column backpressure. To ensure the separation of the targeted compound and the internal standard, gradient mode has been introduced in this experiment.

2.6 Quality control protocols

A typical quality control was analysed which include matrix blank and reagent blank for every batch of sample. Suitable matrix that has been tested free from any pesticide residue was used and follows the QuEChERS extraction and cleanup procedure.

To ensure the quality of the analysis is maintained, each QC sample will be analyzed for every batch of sample (typically 10 samples). The result obtained from control sample must falls within the control limit and if it beyond these limits, new QC solution should

be made again and the analysis will be repeated. The investigation steps must be carried out if the same situation still happened.

This experiment was designed by using spiked QC sample which contains 25 ng/g of simazine.

2.7 Calculation

The TargetLynx software in UPLC-MSMS was used for calibration and estimating the simazine concentration in the samples. The area of the Internal Standard Solution (Ethoprophos IS) and simazine standard peaks was measured by calculate the ratio of the simazine standard peak area to the Ethoprophos (IS) peak area to give the response value. It can be expressed as a formula below:

$$\text{Response} = \frac{\text{Area Standard}}{\text{Area Internal Standard}}$$

A calibration graph for the standards was constructed by plotting response values against the concentration (ng/g) of the simazine standard and the slope of the calibration curve from the graph was identified.

The final concentration of simazine in sample was expressed in ng/g which the unknown value obtained from the slope then times the dilution factor of 10 to give the

concentration simazine in the unknown samples. Concentration of simazine was given by:

$$\text{Amount simazine in sample, ng/g} = \frac{\text{response}}{\text{slope}} \times 10$$

$$= \text{LC Reading}$$

CHAPTER 3

RESULTS AND DISCUSSIONS

3.1 Results and Performance Characteristic Data

Determination and validation of analytical methods require a precise correlation between estimated through mathematical and response, as measured by the concentration anayte. This continuity is extended to the measurement by instruments used to obtain accurate analysis results and reliable. Therefore, the selection of calibration methods is very important and it depends on the type of analytical method used, type of sample, and the required accuracy of the analyte concentration range studied.

For an analysis of the quality, performance characteristics and criteria need to be set. These include sensitivity, selection, limit of detection, limit of determination, and basic background, repeatability, reproducibility, and also concern with the obtained results. Besides, the interpretation of each analytical results also depend on the existence of error, precision, standard deviation, systematic errors and the accuracy which diverted into statistical form for the purpose of issuing a final decision.

The performance characteristic in this study was carried out in Department of Chemistry Malaysia and supervised by Section head of Research and Quality Assurance.

3.2 Validation

3.2.1 Matrix-matched Calibration

In carrying out the method validation process, the matrix effect is the most important part that should be assessed. They are notoriously variable in occurrence and intensity but some techniques are particularly prone to them. If the techniques used are not inherently free from such effects, calibration should be matrix-matched routinely, unless an alternative approach can be shown to provide equivalent or superior accuracy [5]. The most effective ways to negate matrix effects are calibrations by standard addition and isotope dilutions with the isotope-labelled internal standard being added at any stage of the procedure prior to measurement.

Therefore, in this study a series of matrix-matched calibration standard of 5 ng/g, 10 ng/g, 25 ng/g, 50 ng/g and 100 ng/g were prepared from 1000 µg/mL of simazine standard stock solution using a blank matrix. The solution preferably freshly prepared for each analysis period. Then, a calibration curve from the series of these working standards is constructed.

The calibration graph of interest analyte was constructed by plotting the response value against the concentration (ng/g). The calibration curve gave a good linearity with correlation coefficient (r) is more than 0.99 as shown in **Figure 4** and **Figure 5**. The graph is used to determine the amount of analyte in the unknown samples.

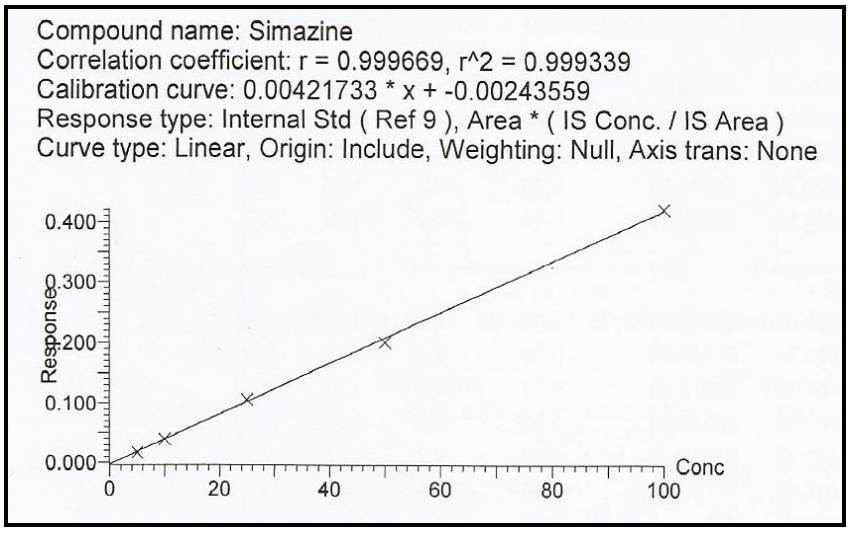

Figure 4: Calibration curve of Simazine

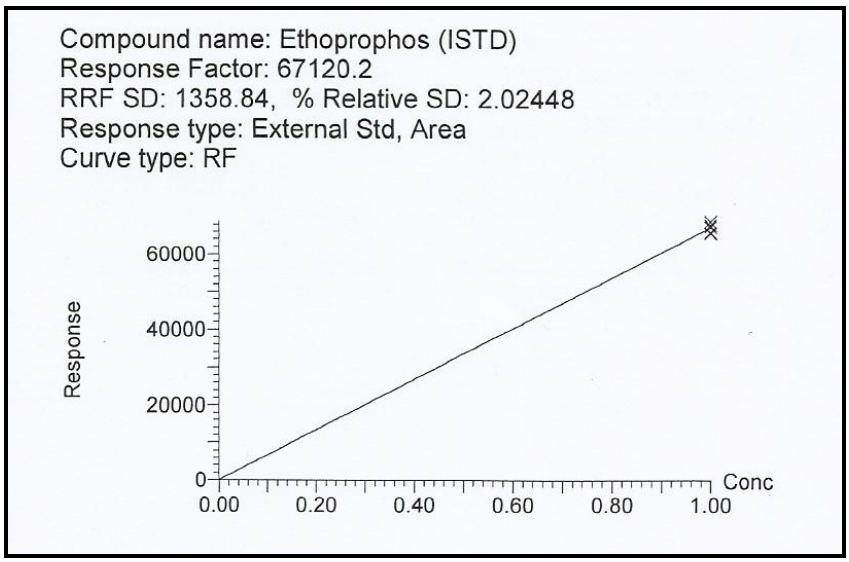

Figure 5: Calibration curve of Internal Standard - Ethoprophos

Since the concentration tested on real samples showed that the concentration of simazine was in the range of 5 to 100 ng/g, this calibration curve was used throughout of this study.

3.2.2 Precision and Accuracy

The quality of a measurement can be determined by the level of precision in analysis. The precision of the method is a statement of the closeness of agreement between mutually independent test results and is usually stated in terms of standard deviation [4]. Precision can be derived and determined using two different ways which are under repeatability and reproducibility condition. Repeatability is a type of precision that relate to the repeated condition which are using the same method, same matrices, same operator, analysis was carried in the same laboratory and it is made in narrow time period. While the reproducibility data was obtained from different operator, different laboratories, different equipment, need longer period of time but use the same method.

To perform the precision data, blank sample of fish and shrimp was spiked which were contained 10 ng/g. The results are shown in **Table 3** and **Table 4**.

Accuracy can be defined as a combination of the bias and precision of an analytical procedure, which reflects the closeness of a measured value to a true value. This quantitative analytical method should be demonstrated at initial and extended validation as being capable of providing mean recovery values at each spiking level and for at least one representative commodity from each relevant group within the range 70 to 120 % [5]. Typically with multiresidue methods, value of recoveries which falls outside this range may be accepted.

Table 3: Results for fish sample spiked with simazine at concentration of 10 ng/g

Replicates	**Blank Matrix (Fish) 10 ng/g**
1	9.7
2	10.0
3	10.6
4	10.3
5	10.4
6	10.2
7	9.7
8	9.8
Mean, x	10.1
Standard Deviation, s	0.34
Variability, 2s	0.68
Variability, 3s	1.02
% Relative Standard Deviation, = (s/x)*100	3.37

Table 4: Results for shrimp sample spiked with simazine at concentration of 10 ng/g

Replicates	**Blank Matrix (Shrimp) 10.0 ng/g**
1	9.7
2	9.9
3	9.9
4	10.9
5	9.9
6	10.4
7	10.4
8	10.2
Mean, x	10.2
Standard Deviation, s	0.39
Variability, 2s	0.79
Variability, 3s	1.18
% Relative Standard Deviation, = (s/x)*100	3.82

Table 5: Summary of results for simazine in different matrices

Sample Type/matrix	Expected value, ng/g	Observed Mean, ng/g	s	2s	3s	RSD %
Spiked on blank solvent	5	5.0	0.08	0.15	0.23	-
Spiked on fish	10	10.2	0.34	0.68	1.02	3.37
Spiked on shrimp	10	10.1	0.39	0.79	1.18	3.28

As can be seen from the RSD results shown in **Table 5**, the percentage of relative standard deviations (% RSD) or CV ranging from 3.28 to 3.37 were obtained for the above stated spiked concentration.

Meanwhile, accuracy is often calculated as percentage recovery of the analysis and determined at known level of spiking. In order to prove the validity of the method, the simazine recoveries were analysed in two different types of blank samples which were spiked with 5 ng/g of simazine. The results were depicted as in **Appendix A-B** and recovered in the range of 101 % to 107 % for fish matrix and 97 % to 101 % for shrimp matrix.

3.2.3 Selectivity

Multiple Reaction Monitoring (MRM) mode is the one of the selective technique that be introduced in this study. It can identify and differentiate this triazine group including simazine.

Selectivity can be defined as a method which can determine particular analyte(s) in a complex mixture without interference from the other components in the mixture (**Appendix G**) [4]. To prove that this method is selective, two types of sample matrix were introduced. In each case, the performances of the recovery of analyte were determined in a good range (**Appendix A** and **B**).

3.3 Detection Limits

Detection Limits (DLs) or also known as limit of detection (LOD) are estimates of concentrations at which we can be fairly certain that the compound is present. Concentrations below this limit may not be detected. Concentrations above this limit are almost certainly detected in the analysis. Therefore, with the result shows Not Detected (ND) indicates that the analyte may be present at below the value of LOD.

This LOD can be determined by repeat analysis of a blank test portion and is the analyte concentration of the response of which is equivalent to the mean blank response plus 3 standard deviations [4]. The values establish is likely to be different for each types of sample matrix.

Limit of quantification (LOQ) can be calculated based on the lowest concentration of analyte that can be determined with an acceptable level of uncertainty [4]. LOQ can be derived via three different methods which includes signal-to-noise ratio (SN), calibration curve slope (CCS) and laboratory fortified blank (LFB). The most often used for determine the LOQ is 10 times the signal-to-noise ratio. In this study, the signal-to-noise ratio shows approximates to the standard deviation of the blank matrix, so the

LOQ was calculated by 10 times of the standard deviation of the blank matrix. The average of LOD and LOQ in these two matrices was calculated and summarized as in **Table 6**.

Table 6: LOD and LOQ for each sample matrix

Matrix	Fish		Shrimp	
	LOD (ng/g)	LOQ (ng/g)	LOD (ng/g)	LOQ (ng/g)
Simazine	1.18	3.52	1.06	3.19

3.3.1 Instrument Detection Limit, IDL

Another parameter that should be considered is Instrument Detection Limit (IDL). It can be determined due to capability of instrument used for detecting the lowest concentration of compound. This was done on the blank solvent which has not gone through any sample preparation steps. The IDL should always be below the method detection limit (MDL), and is not used for compliance data reporting, but may be used for statistical data analysis and comparing the attributes of different instruments for the validation purposes. In this study, the blank solvent was fortified with known concentration of simazine standard. The datas for IDL were collected from replicates analysis of blank solvent and the results was calculated as in **Table 7**.

Table 7: Results of blank solvent for Instrument Detection Limit

Replicates	Analytical Results (ng/g)
1	5.0
2	5.1
3	4.9
4	5.0
5	5.0
6	5.1
7	4.9
8	5.0
Mean, x	5.0
Standard deviation, s	0.08
Variability, 2s	0.15
3s	0.23

Instrument detection limit, IDL = 3s

= 0.23 ng/g

3.4 Data for Quality Control

In order to maintain the level of quality for each analysis conducted in the chemical laboratory, they must comply with the quality protocol. The clause of Quality control (QC) in the international standard ISO/IEC 17025 indicates that any laboratory shall establish, implement and maintain a quality system appropriate to the scope of its activities. The use of control charts in the quantitative analysis is the most important activity in internal QC for monitoring and controlling the routine analysis conducted. Each laboratory must maintain records to document the quality of data produced [1].

The QC sample must be put through the whole analytical method which is analysed at specified interval (every 10 samples). Control charts were used for monitoring the variability and to provide a graphical display of statistical control. In this study, the spiking level of simazine which is equivalent to 25 ng/g was selected for plotting QC charts. A total of 20 datas were used to calculate the mean, standard deviation, upper control limit (UCL), lower control limit (LCL), upper warning limit (UWL) and lower warning limit (LWL) (see **Table 8**).

Table 8: Data for Quality Control

Entry No.	Analytical Result, (ng/g)	Entry No.	Analytical Result, (ng/g)
1	24.3	11	23.8
2	25.4	12	23.5
3	24.5	13	23.9
4	25.5	14	23.7
5	24.7	15	24.5
6	24.2	16	23.9
7	24.8	17	25.2
8	25.6	18	24.8
9	24.9	19	24.6
10	25.4	20	23.8

No. of data	20	% RSD	2.91
Mean	24.59	Upper warning limit	26.01
Standard deviation, s	0.71	Lower warning limit	23.16
Variability, 2s	1.43	Upper control limit	26.73
3s	2.14	Lower control limit	22.44

For routine analysis, on-going QC data should be acquired and the validity of the method should be periodically reassessed.

Example of QC chart which plotted data was taken from November 2013 to April 2014 as shown in **Figure 6**.

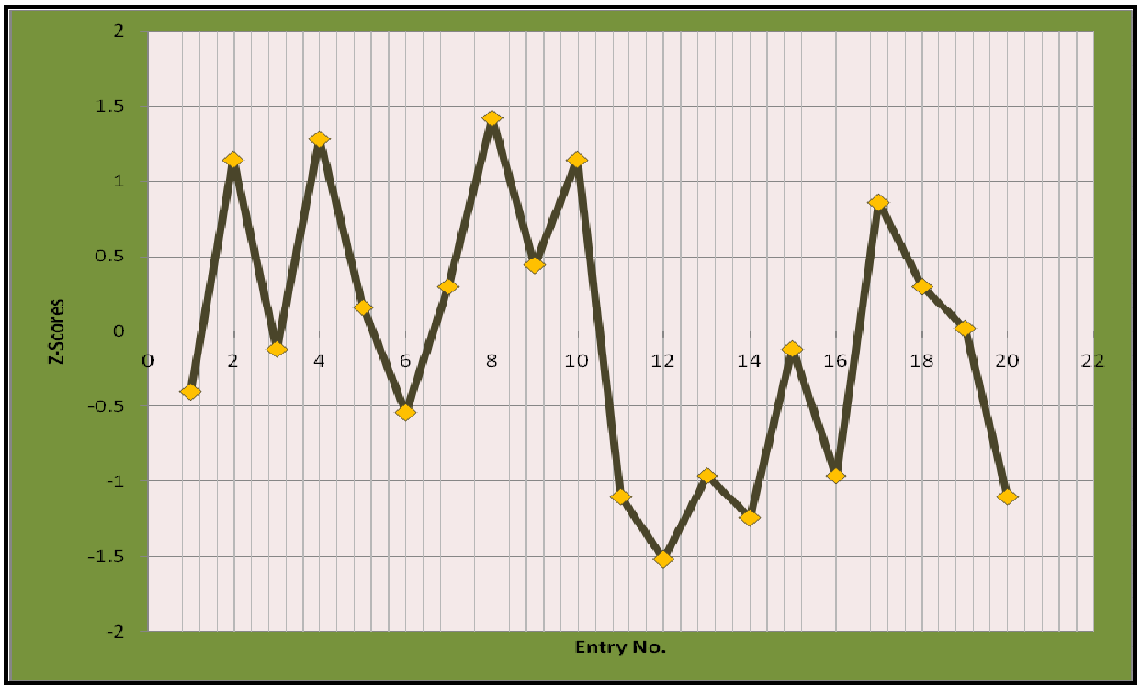

Figure 6: QC chart for Simazine

The centerline represents the average, or expected value. The use of control charts in quality assurance is based on the assumption that the results obtained are normally distributed. For a normal distribution with mean (x), and standard deviation (s), the control limits are ± 3s, where 99.7 % of the data should lie. If results obtained fall outside the control limits, the readjustment is necessary to ensure that the process is under control. The upper limit of the control (UCL) and lower control limit (LCL) are the values for which measurements should fall.

The data obtained for QC chart was expressed as *z*-scores, where *z* is given by equation below:

Z-score = (xi – x)/s

Where;

xi = QC reading

x = Mean

s = Standard deviation

From the results obtained and plotted in the control chart showed that it is under statistical control within the warning limit. Therefore, the analytical result can be acceptable.

3.5 Simazine content in fish and shrimp

In this study, the concentration of simazine in the fish and shirimp sample were identified. Both types of samples used in this study and it is fresh samples were analyzed based on performance characteristic shown in previous validation methods. Refers to one of the objectives of this study, the content of simazine in fish and shrimp samples were summarized in the **Table 9**.

Table 9: Simazine content in fish and shrimp samples

Month	**Simazine content (ng/g)**	
	Fish sample	**Shrimp sample**
February	1.4 ~ (ND)	2.4 ~ (ND)
Mac	1.7 ~ (ND)	1.7 ~ (ND)
April	1.8 ~ (ND)	2.0 ~ (ND)

The value summarized in the **Table 9** was monthly average value from 20 samples of fish and 10 samples for shrimp. The raw datas were attached in the **Appendix C** and **Appendix D**. The results indicated that, the concentration value of simazine in fish and shrimp samples were bellowed the quantification limit (LOQ) which reported as not detected (ND). This condition may be caused by the sampling source of fish and shrimp are not tainted with any high concentration of pesticide residue. At the same time, simazine values for both samples comply with EU regulation. Based on the EU No 212/2013 the maximum permitted limit (MPL) for simazine in tissue was 0.01 mg/kg or 10 ng/g.

3.6 Measurement Uncertainty

Measurement uncertainty is a parameter associated with the result of a measurement, that characterizes the dispersion of the values that could reasonable be attributed to the measurand. There are several sources identified the cause of a measurement made is uncertain and this includes the resources from systematic effect such as reference standard, measuring instrument, item being measured, environment, operator and others. In order to decide whether a result indicates compliance or non-compliance with a specification, it is necessary to take into consideration of the measurement uncertainty associated with the result.

In this study, the overall measurement uncertainty can be calculated for two different matrices which are fish and shrimp. The budget uncertainty table for both matrices can be seen in **Appendix E** and **Appendix F**.

Upon consideration of the complete procedure from sample preparation to instrumental determination, the expanded uncertainty for simazine in fish sample under study was found to be at any concentration $0.10[C_{Simazine}]$ where $C_{Simazine}$ represent for concentration of simazine in sample. While expended uncertainty for simazine in shrimp sample was found to be $0.08[C_{Simazine}]$.

CHAPTER 4

CONCLUSION

A simple, rapid and inexpensive analysis method for the determination of simazine in fish and shrimp samples has been successful validated. As a finding, this method has provided good linearity for certain level of concentration of simazine with correlation coefficient is more than 0.99. The method is able to analyse the simazine with limit of detection (LOD) shows the value of 1.18 ng/g and LOQ is 3.52 ng/g for fish sample while LOD and LOQ for shrimp sample gives 1.06 ng/g and 3.19 ng/g respectively.

There are some challenges for the detection of simazine at the first stage due to matrix effect. But after trying several times, eventually the problem can be solved successfully. So, the change should be done especially in sample preparation, which focuses in the part of extraction and cleanup of samples for the purpose of obtaining reliable analytical result.

The concentration of simazine in fish and shrimp has been determined using validated method. The results indicated that none of the samples were found to contain simazine and all reported as not detected. Therefore, it meets the requirement regulated by EU.

It can be concluded that the objectives of this study was attained. The validity for measuring simazine is absolutely depends on the precision and accuracy of the method used in laboratory. Further study is recommended to determine simazine in animal feed for the purpose of data collection that can fit in the Malaysian Feeds Act.

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Appendix A

Results for 10 ng/g spiked on fish sample

Replicates	1st Batch			2nd Batch		
	Expected Results ng/g	**Analytical Results ng/g**	**Recovery %**	**Expected Results ng/g**	**Analytical Results ng/g**	**Recovery %**
1	5	5.2	104.0	5	5.3	106.0
2	5	5.1	102.0	5	5.4	108.0
3	5	5.1	102.0	5	5.2	104.0
4	5	5.3	106.0	5	5.2	104.0
5	5	5.2	104.0	5	5.5	110.0
6	5	4.9	98.0	5	5.5	110.0
7	5	4.8	96.0	5	5.4	108.0
8	5	4.9	98.0	5	5.1	102.0
% Mean Recovery = Mean Value *100 / Ref. Value	101			107		

Appendix B

Results for 10 ng/g spiked on shrimp sample

Replicates	1st Batch			2nd Batch		
	Expected Results ng/g	**Analytical Results ng/g**	**Recovery %**	**Expected Results ng/g**	**Analytical Results ng/g**	**Recovery %**
1	5	4.8	96.0	5	5.2	104.0
2	5	5.2	104.0	5	5.0	100.0
3	5	4.5	90.0	5	4.9	98.0
4	5	5.0	100.0	5	4.7	94.0
5	5	4.7	94.0	5	5.3	106.0
6	5	4.9	98.0	5	5.4	108.0
7	5	4.7	94.0	5	5.2	104.0
8	5	4.8	96.0	5	4.9	98.0
% Mean Recovery = Mean Value *100 / Ref. Value	97			101		

Appendix C

Raw data for simazine in fish samples

Sample ID	LC Reading (ng/g)
February	
FF1-F_14	1.2
FF2-F_14	1.3
FF3-F_14	1.4
FF4-F_14	2.1
FF5-F_14	1.0
Average	1.4 ~ ND
Mac	
FF1-M_14	2.2
FF2-M_14	2.3
FF3-M_14	1.6
FF4-M_14	1.5
FF5-M_14	1.8
FF6-M_14	0.9
FF7-M_14	2.2
FF8-M_14	2.0
FF9-M_14	1.3
FF10-M_14	1.1
Average	1.7 ~ ND
April	
FF1-A_14	2.2
FF2-A_14	2.5
FF3-A_14	1.0
FF4-A_14	1.8
FF5-A_14	1.6
Average	1.8 ~ ND

Appendix D

Raw data for simazine in shrimp samples

Sample ID	LC Reading (ng/g)
February	
S1-F_14	2.6
S2-F_14	2.5
S3-F_14	2.2
Average	2.4 ~ ND
Mac	
S1-M_14	1.7
S2-M_14	1.6
S3-M_14	1.9
Average	1.7 ~ ND
April	
S1-A_14	2.4
S2-A_14	2.6
S3-A_14	1.5
S4-A_14	1.4
Average	2.0 ~ ND

Appendix E-1

Recovery study

No.	Fish 5 ng/g
1	5.3
2	5.4
3	5.2
4	5.2
5	5.5
6	5.5
7	5.4
8	5.1
Mean	5.33
Mean Recovery	1.0650
std Dev	0.1488
RSD	0.0279
n	8

Mean Observation, C obs :	5.33
Recovery, Rm :	1.0650
Standard Deviation, s :	0.1488
bias (1-Rm)	0.0650

Appendix E-2

The uncertainty μRm due to method recovery Rm can be calculated from this formula:

$$uR_m = R_m \times \sqrt{\left(\frac{s^2}{n \times C_{obs}{}^2}\right) + \left(\frac{\mu C_{spike}}{C_{spike}}\right)^2}$$ → Ignored

μ(Rm)	0.010522
Significant testing :	
Calculated t-value, I1-RmI/μ(Rm)	6.177483
The calculated t-value is more than 2, the coverage factor. Therefore, Rm is significant different from 1 Correction of the expected value is necessary, μ(Rm)" is =	0.034161

Data for sample matrix effect

No.	Fish 5 ng/g	Shrimp 10 ng/g
1	5.2	9.2
2	5.1	9.2
3	4.5	9.0
4	4.8	9.0
5	4.5	10.0
6	5.1	9.6
7	4.9	9.6
8	4.9	10.0
Mean	4.88	9.45
Mean Recovery	0.98	0.95
Std Dev, u(Rs)	0.0212	
n	8	8

	standard relative uncertainty
μ(Rm)	0.03416
μ(Rs)	0.0212
combined relative uncertainty	0.0402

$$uR = \sqrt{u(Rm)^2 + u(Rs)^2}$$

$$SD_{pooled} = \sqrt{\left(\frac{(n_1 - 1) \times SD_1^{\,2} + (n_2 - 2) \times SD_2^{\,2} + \ldots}{(n_1 - 1) + (n_2 - 1) + \ldots} \right)}$$

Appendix E-4

Budget Uncertainty for fish sample

Parameter	Description	Value, x	Std uncertainty, μ(x)	Rel Std uncertainty(μx/x)
P	Precision	1	0.0300	0.0300
Rec	Recovery	1	0.0402	0.0402
Expanded uncertainty (k)	K	2		
Combined relative uncertainty				0.05

$\mu[C_{Simazine}]/[C_{Simazine}]$ = 0.05 (Relative value)

The uncertainty of Simazine = $\mu [C_{Simazine}] = 0.05 \times [C_{Simazine}]$ = _____

At 95 % Confidence level k=2 therefore expanded uncertainty, $\mathbf{U [C_{Simazine}]} = \mu[C_{Simazine}] \times 2$ = _____

Example at 10 ng/g = <u>10.00 ± 0.10</u>

Appendix F-1

Recovery study

No.	**Shrimp 5 ng/g**
1	5.2
2	5.0
3	4.9
4	4.7
5	5.2
6	5.4
7	5.2
8	4.9
Mean	5.0625
Mean Recovery	1.0125
std Dev	0.2264
RSD	0.0447
n	8

Mean Observation, C obs :	5.06
Recovery, Rm :	1.0125
Standard Deviation, s :	0.2264
bias (1-Rm)	0.0125

Appendix F-2

The uncertainty μRm due to method recovery Rm can be calculated from this formula:

$$uR_m = R_m \times \sqrt{\left(\frac{s^2}{n \times C_{obs}{}^2}\right) + \left(\frac{\mu C_{spike}}{C_{spike}}\right)^2}$$

→ Ignored

μ(Rm)	0.016008
Significant testing :	
Calculated t-value, I1-RmI/μ(Rm)	0.780869
The calculated t-value is more than 2, the coverage factor. Therefore, Rm is significant different from 1 Correction of the expected value is necessary, μ(Rm)" is =	0.017185

Data for sample matrix effect

No.	Fish 5 ng/g	Shrimp 10 ng/g
1	5.2	9.2
2	5.1	9.2
3	4.5	9.0
4	4.8	9.0
5	4.5	10.0
6	5.1	9.6
7	4.9	9.6
8	4.9	10.0
Mean	4.88	9.45
Mean Recovery	0.98	0.95
Std Dev, u(Rs)	0.0212	
n	8	8

	standard relative uncertainty
μ(Rm)	0.03416
μ(Rs)	0.0212
combined relative uncertainty	0.0402

$$uR = \sqrt{u(Rm)^2 + u(Rs)^2}$$

$$SD_{pooled} = \sqrt{\left(\frac{(n_1 - 1) \times SD_1^{\,2} + (n_2 - 2) \times SD_2^{\,2} + \ldots}{(n_1 - 1) + (n_2 - 1) + \ldots} \right)}$$

Appendix F-4

Budget Uncertainty for Shrimp sample

Parameter	Description	Value, x	Std uncertainty, μ(x)	Rel Std uncertainty(μx/x)
P	Precision	1	0.0329	0.0329
Rec	Recovery	1	0.0273	0.0273
Expanded uncertainty (k)	K	2		
Combined relative uncertainty				0.04

$[C_{Simazine}]/[C_{Simazine}]$ = 0.04 (Relative value)

The uncertainty of Meth = $\mu [C_{Simazine}] = 0.04 \times [C_{Simazine}]$ = _____

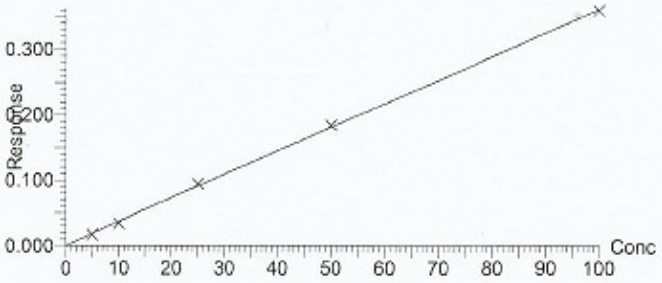
At 95 % Confidence level k=2 therefore expanded uncertainty, $\mathbf{U [C_{Simazine}]} = \mu[C_{Simazine}] \times 2$ = _____

Example at 10 ng/g = <u>10.00 ± 0.08</u>

Compound name: Simazine

#	Sample Text	Type	RT	Area	IS Area	Response	ppb	1° Ratio (A...	%Rec
1	Std 5 ppb simazi...	Standard	4.47	591.979	35266.320	0.017	4.6	0.964	92.0
2	Std 10 ppb sima...	Standard	4.47	1260.161	37436.473	0.034	9.3	1.242	92.5
3	Std 25 ppb sima...	Standard	4.45	3487.478	37126.199	0.094	25.9	1.162	103.4
4	Std 50 ppb sima...	Standard	4.45	6072.578	32883.887	0.185	50.9	1.094	101.7
5	Std 100 ppb sim...	Standard	4.45	11021.313	30534.789	0.361	99.4	1.058	99.4
6	Mobile phase	Blank							
7	Blank Spl ikan	Analyte			36130.410				
8	Spl 1 - ikan	Analyte	4.42	921.941	26258.471	0.035	9.7	1.029	96.5
9	Spl 2 - ikan	Analyte	4.42	1044.593	28679.475	0.036	10.0	1.140	100.1
10	Spl 3 - ikan	Analyte	4.45	1113.579	28837.484	0.039	10.6	1.426	106.2
11	Spl 4 - ikan	Analyte	4.42	994.564	26551.084	0.037	10.3	1.024	103.0
12	Spl 5 - ikan	Analyte	4.42	1058.966	27943.223	0.038	10.4	1.289	104.2
13	Spl 6 - ikan	Analyte	4.40	1047.099	28220.959	0.037	10.2	1.144	102.0
14	Spl 7 - ikan	Analyte	4.40	910.297	25812.900	0.035	9.7	1.043	96.9
15	Spl 8 - ikan	Analyte	4.40	992.345	27865.367	0.036	9.8	1.181	97.9

Compound name: Simazine
Correlation coefficient: r = 0.999826, r^2 = 0.999651
Calibration curve: 0.00362859 * x + 9.351e-005
Response type: Internal Std (Ref 8), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None



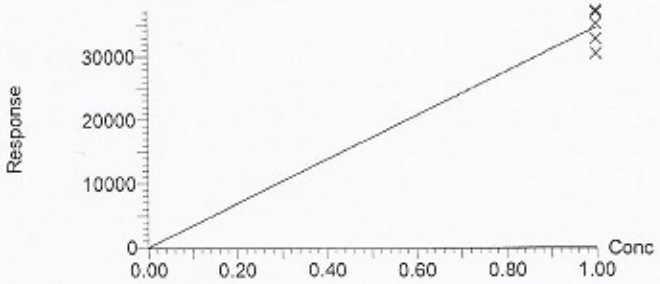
Compound name: Ethoprophos (ISTD)

#	Sample Text	Type	RT	Area	IS Area	Response	ppb	1° Ratio (A...	%Rec
1	Std 5 ppb simazi...	Standard	7.24	35266.320		35266.320	1.0	1.420	101.8
2	Std 10 ppb sima...	Standard	7.25	37436.473		37436.473	1.1	1.426	108.0
3	Std 25 ppb sima...	Standard	7.24	37126.199		37126.199	1.1	1.471	107.1
4	Std 50 ppb sima...	Standard	7.24	32883.887		32883.887	0.9	1.385	94.9
5	Std 100 ppb sim...	Standard	7.22	30534.789		30534.789	0.9	1.369	88.1
6	Mobile phase	Blank							
7	Blank Spl ikan	Analyte	7.22	36130.410		36130.410	1.0	1.464	104.3
8	Spl 1 - ikan	Analyte	7.22	26258.471		26258.471	0.8	1.367	75.8
9	Spl 2 - ikan	Analyte	7.22	28679.475		28679.475	0.8	1.443	82.8
10	Spl 3 - ikan	Analyte	7.22	28837.484		28837.484	0.8	1.425	83.2
11	Spl 4 - ikan	Analyte	7.21	26551.084		26551.084	0.8	1.416	76.6
12	Spl 5 - ikan	Analyte	7.21	27943.223		27943.223	0.8	1.521	80.6

Compound name: Ethoprophos (ISTD)

#	Sample Text	Type	RT	Area	IS Area	Response	ppb	1° Ratio (A...	%Rec
13	Spl 6 - ikan	Analyte	7.19	28220.959		28220.959	0.8	1.421	81.4
14	Spl 7 - ikan	Analyte	7.19	25812.900		25812.900	0.7	1.371	74.5
15	Spl 8 - ikan	Analyte	7.19	27865.367		27865.367	0.8	1.342	80.4

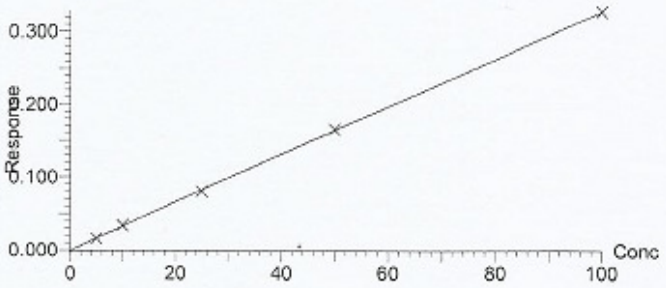
Compound name: Ethoprophos (ISTD)
Response Factor: 34649.5
RRF SD: 2929.59, % Relative SD: 8.45492
Response type: External Std, Area
Curve type: RF



Compound name: Simazine

#	Sample Text	Type	RT	Area	IS Area	Response	ppb	1º Ratio (A...	%Rec
1	Std 5 ppb simazi...	Standard	4.40	360.444	23049.816	0.016	4.9	0.896	97.4
2	Std 10 ppb sima...	Standard	4.38	824.212	24372.191	0.034	10.4	1.100	104.0
3	Std 25 ppb sima...	Standard	4.38	1897.388	23833.545	0.080	24.3	0.932	97.3
4	Std 50 ppb sima...	Standard	4.35	3918.442	23739.137	0.165	50.3	1.122	100.6
5	Std 100 ppb sim...	Standard	4.38	7962.331	24248.943	0.328	100.0	1.105	100.0
6	Mobile phase	Blank							
7	Blank Spl ikan	Analyte			25090.660				
8	Spl 1 - ikan	Analyte	4.38	789.576	23094.121	0.034	10.5	1.237	105.1
9	Spl 2 - ikan	Analyte	4.42	737.624	22571.230	0.033	10.1	1.264	100.5
10	Spl 3 - ikan	Analyte	4.38	755.551	21978.025	0.034	10.6	1.417	105.7
11	Spl 4 - ikan	Analyte	4.38	816.395	23998.574	0.034	10.5	1.635	104.6
12	Spl 5 - ikan	Analyte	4.38	817.471	24080.785	0.034	10.4	1.218	104.4
13	Spl 6 - ikan	Analyte	4.38	752.139	22872.658	0.033	10.1	1.161	101.1
14	Spl 7 - ikan	Analyte	4.38	798.170	22919.383	0.035	10.7	1.184	107.1
15	Spl 8 - ikan	Analyte	4.35	747.811	23032.000	0.032	10.0	1.562	99.9

Compound name: Simazine
Correlation coefficient: r = 0.999949, r^2 = 0.999897
Calibration curve: 0.00328804 * x + -0.000373609
Response type: Internal Std (Ref 8), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None



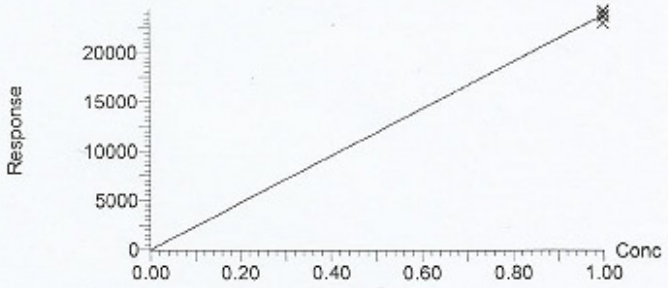
Compound name: Ethoprophos (ISTD)

#	Sample Text	Type	RT	Area	IS Area	Response	ppb	1º Ratio (A...	%Rec
1	Std 5 ppb simazi...	Standard	7.18	23049.816		23049.816	1.0	1.326	96.7
2	Std 10 ppb sima...	Standard	7.19	24372.191		24372.191	1.0	1.434	102.2
3	Std 25 ppb sima...	Standard	7.18	23833.545		23833.545	1.0	1.410	99.9
4	Std 50 ppb sima...	Standard	7.18	23739.137		23739.137	1.0	1.402	99.5
5	Std 100 ppb sim...	Standard	7.19	24248.943		24248.943	1.0	1.348	101.7
6	Mobile phase	Blank							
7	Blank Spl ikan	Analyte	7.18	25090.660		25090.660	1.1	1.531	105.2
8	Spl 1 - ikan	Analyte	7.18	23094.121		23094.121	1.0	1.343	96.8
9	Spl 2 - ikan	Analyte	7.18	22571.230		22571.230	0.9	1.443	94.6
10	Spl 3 - ikan	Analyte	7.18	21978.025		21978.025	0.9	1.310	92.2
11	Spl 4 - ikan	Analyte	7.19	23998.574		23998.574	1.0	1.407	100.6
12	Spl 5 - ikan	Analyte	7.19	24080.785		24080.785	1.0	1.379	101.0

Compound name: Ethoprophos (ISTD)

#	Sample Text	Type	RT	Area	IS Area	Response	ppb	1° Ratio (A...	%Rec
13	Spl 6 - ikan	Analyte	7.18	22872.658		22872.658	1.0	1.378	95.9
14	Spl 7 - ikan	Analyte	7.19	22919.383		22919.383	1.0	1.328	96.1
15	Spl 8 - ikan	Analyte	7.18	23032.000		23032.000	1.0	1.374	96.6

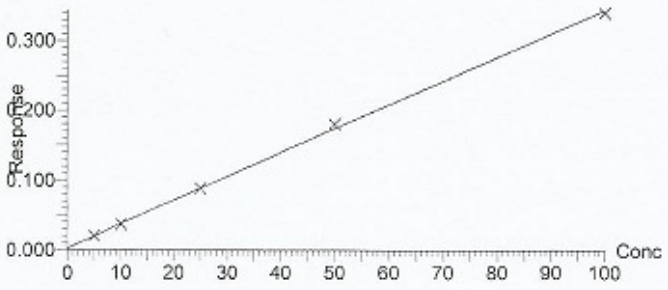
Compound name: Ethoprophos (ISTD)
Response Factor: 23848.7
RRF SD: 520.741, % Relative SD: 2.18352
Response type: External Std, Area
Curve type: RF



Compound name: Simazine

#	Sample Text	Type	RT	Area	IS Area	Response	ppb	1° Ratio (A...	%Rec
1	Mobile phase	Blank							
2	Std 5 ppb simazi...	Standard	4.35	455.789	23252.955	0.020	5.0	1.495	100.3
3	Std 10 ppb sima...	Standard	4.35	839.139	23621.010	0.036	9.7	1.394	96.6
4	Std 25 ppb sima...	Standard	4.38	2120.167	24045.709	0.088	25.0	1.075	100.1
5	Std 50 ppb sima...	Standard	4.38	4051.367	22468.018	0.180	51.9	1.111	103.9
6	Std 100 ppb sim...	Standard	4.38	8271.913	24211.064	0.342	99.1	1.119	99.1
7	Mobile phase	Blank							
8	Blank Spl udang	Analyte			23852.373				
9	Spl 1 - udang	Analyte	4.35	855.090	23258.658	0.037	10.0	1.197	100.3
10	Spl 2 - udang	Analyte	4.38	849.496	24217.906	0.035	9.5	1.352	95.3
11	Spl 3 - udang	Analyte	4.35	808.201	22301.400	0.036	9.9	1.430	98.7
12	Spl 4 - udang	Analyte	4.38	883.773	24703.219	0.036	9.7	1.479	97.4
13	Spl 5 -udang	Analyte	4.35	878.262	23299.523	0.038	10.3	1.389	103.0
14	Spl 6 -udang	Analyte	4.35	820.732	23235.477	0.035	9.6	1.135	96.0
15	Spl 7 - udang	Analyte	4.38	855.936	24064.682	0.036	9.7	1.377	96.8
16	Spl 8 - udang	Analyte	4.38	826.712	24738.857	0.033	9.0	1.477	90.5
17	Mobile phase	Blank							

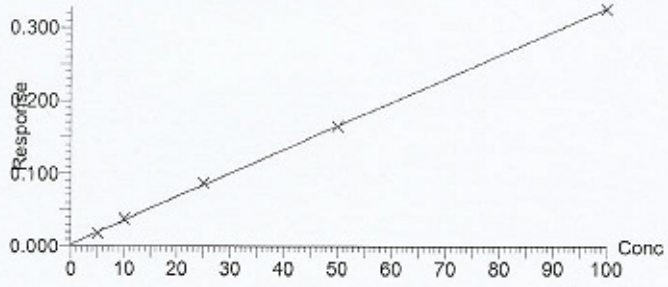
Compound name: Simazine
Correlation coefficient: r = 0.999635, r^2 = 0.999270
Calibration curve: 0.00342473 * x + 0.00242914
Response type: Internal Std (Ref 8), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None



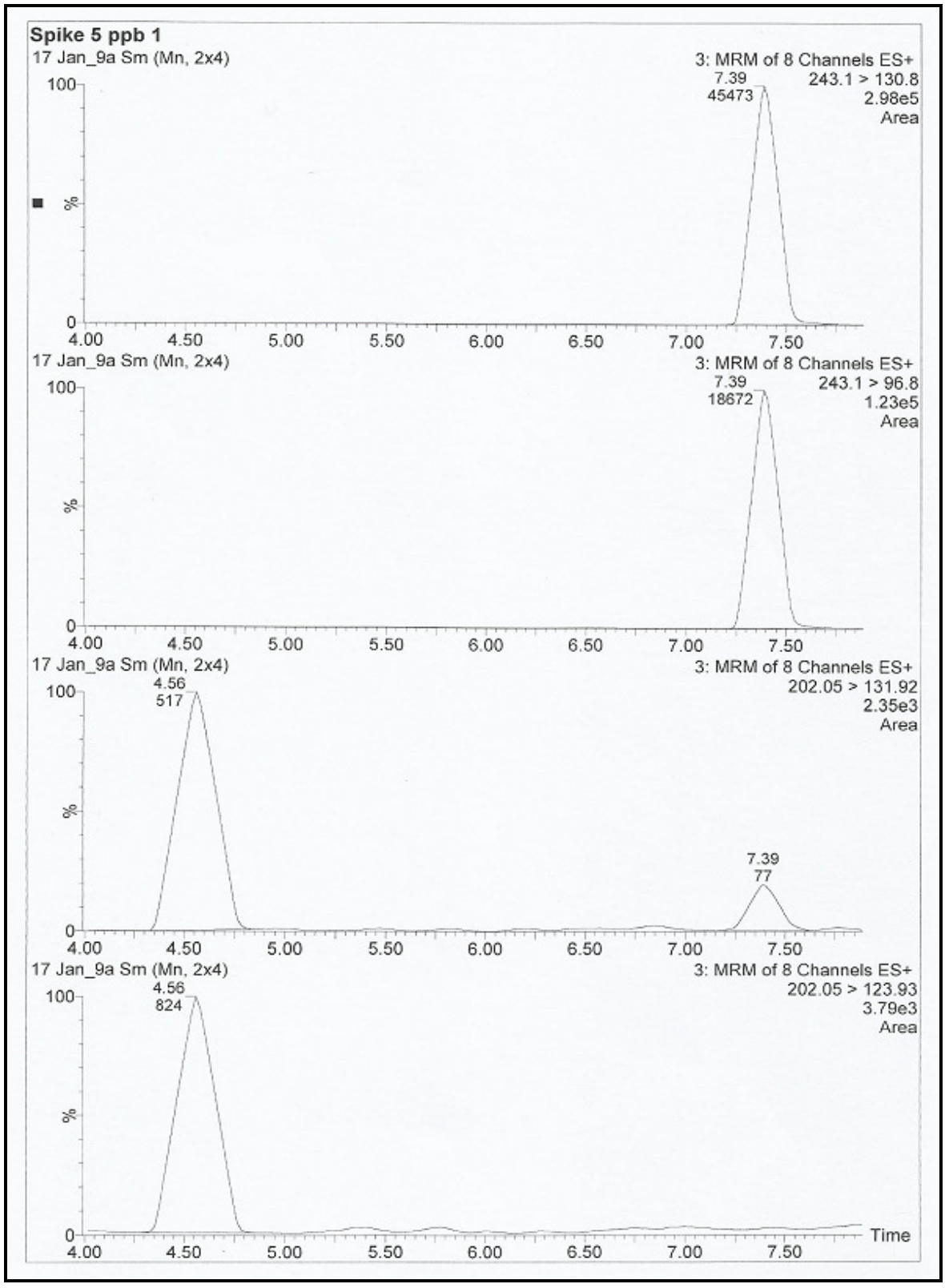
Compound name: Simazine

#	Sample Text	Type	RT	Area	IS Area	Response	ppb	1° Ratio (A...	%Rec
1	Mobile phase	Blank							
2	Std 5 ppb simazi...	Standard	4.49	588.400	33592.977	0.018	4.6	1.060	91.2
3	Std 10 ppb sima...	Standard	4.47	1269.753	33814.582	0.038	10.7	1.112	107.2
4	Std 25 ppb sima...	Standard	4.47	2856.493	32871.820	0.087	25.9	1.020	103.6
5	Std 50 ppb sima...	Standard	4.47	5696.216	34554.695	0.165	49.9	1.058	99.7
6	Std 100 ppb sim...	Standard	4.49	9700.338	29643.580	0.327	99.8	1.043	99.8
7		Blank							
8	Blank Spl udang	Analyte			36492.047				
9	Spl 1 - udang	Analyte	4.49	939.256	27545.746	0.034	9.7	1.042	96.6
10	Spl 2 - udang	Analyte	4.47	991.284	28478.482	0.035	9.9	1.141	98.8
11	Spl 3 - udang	Analyte	4.49	1015.780	29038.359	0.035	9.9	1.008	99.3
12	Spl 4 - udang	Analyte	4.49	1115.635	29178.531	0.038	10.9	1.177	109.3
13	Spl 5 -udang	Analyte	4.47	983.895	28109.021	0.035	9.9	1.152	99.4
14	Spl 6 -udang	Analyte	4.47	1051.693	28773.477	0.037	10.4	1.007	104.1
15	Spl 7 - udang	Analyte	4.47	1068.958	29295.199	0.036	10.4	0.906	103.9
16	Spl 8 - udang	Analyte	4.47	1094.628	30493.029	0.036	10.2	0.904	102.1
17	Mobile phase	Blank							

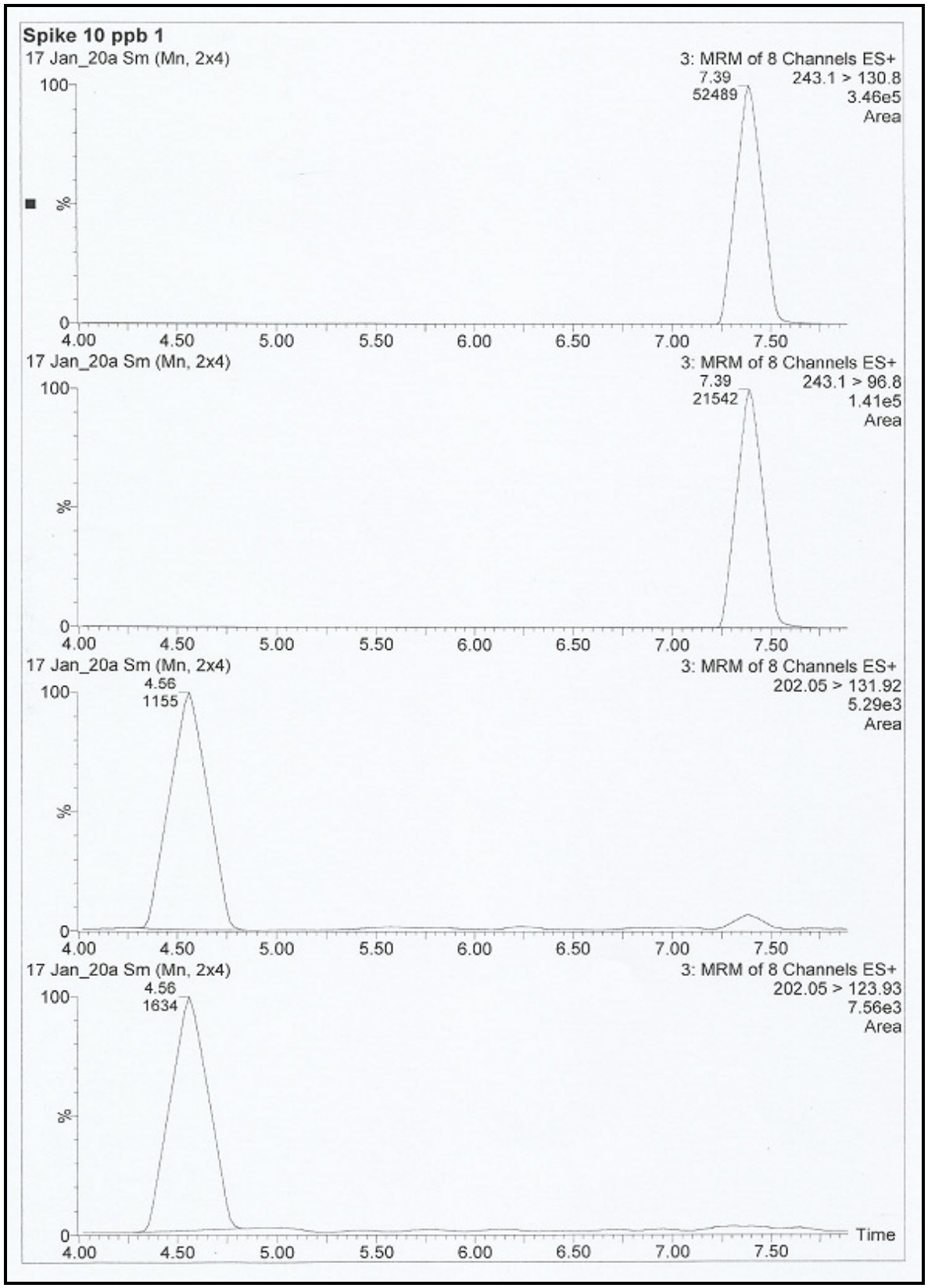
Compound name: Simazine
Correlation coefficient: r = 0.999844, r^2 = 0.999688
Calibration curve: 0.00325204 * x + 0.00269261
Response type: Internal Std (Ref 8), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None



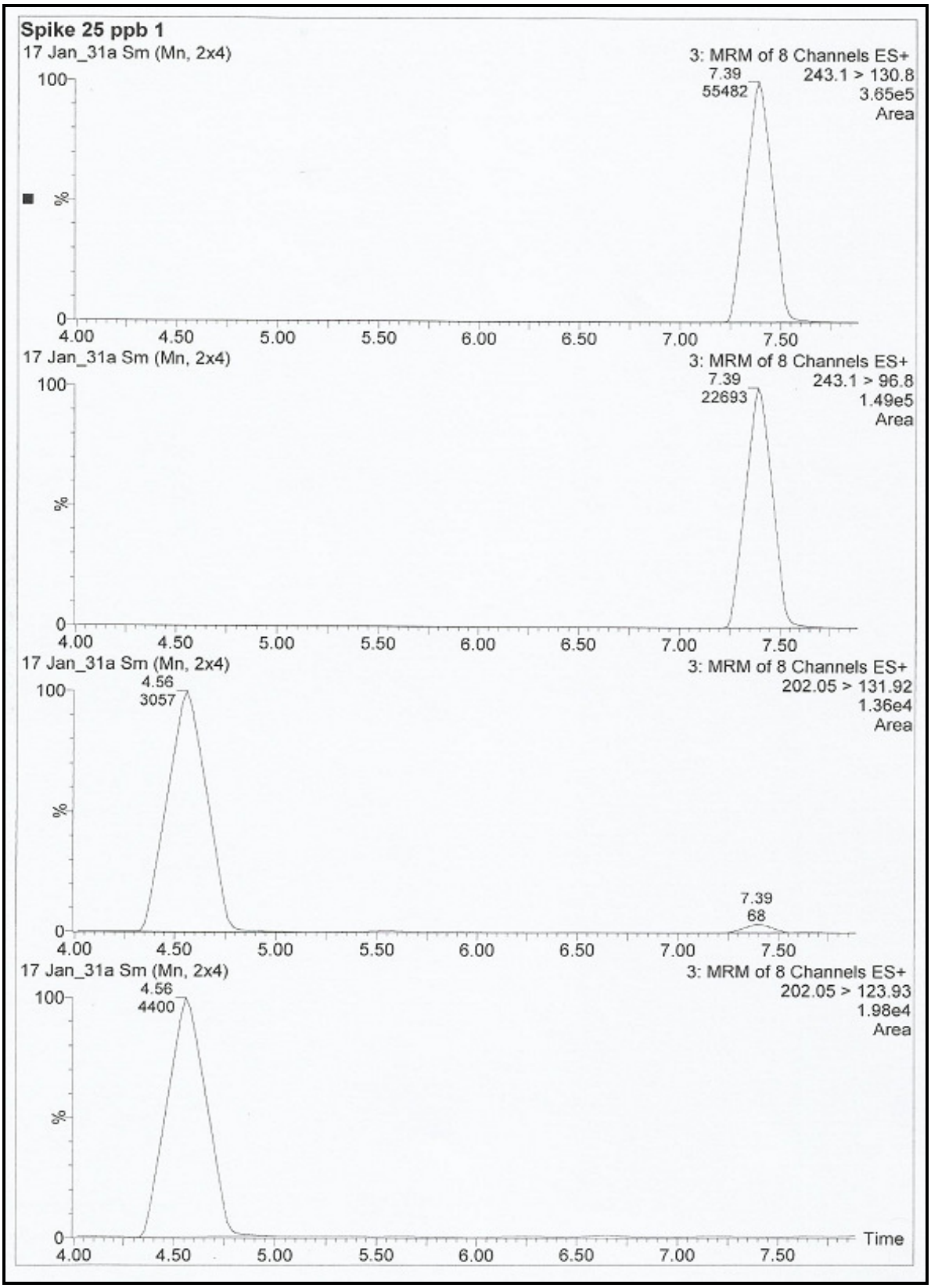
Appendix G-7


Spike 5 ppb 1
17 Jan_9a Sm (Mn, 2x4)
3: MRM of 8 Channels ES+
243.1 > 130.8
7.39
45473
2.98e5
Area
17 Jan_9a Sm (Mn, 2x4)
3: MRM of 8 Channels ES+
243.1 > 96.8
7.39
18672
1.23e5
Area
17 Jan_9a Sm (Mn, 2x4)
3: MRM of 8 Channels ES+
202.05 > 131.92
4.56
517
7.39
77
2.35e3
Area
17 Jan_9a Sm (Mn, 2x4)
3: MRM of 8 Channels ES+
202.05 > 123.93
4.56
824
3.79e3
Area
100
%
0
4.00
4.50
5.00
5.50
6.00
6.50
7.00
7.50
Time

Appendix G-8


Spike 10 ppb 1
17 Jan_20a Sm (Mn, 2x4)
3: MRM of 8 Channels ES+
7.39
52489
243.1 > 130.8
3.46e5
Area
17 Jan_20a Sm (Mn, 2x4)
3: MRM of 8 Channels ES+
7.39
21542
243.1 > 96.8
1.41e5
Area
17 Jan_20a Sm (Mn, 2x4)
3: MRM of 8 Channels ES+
4.56
1155
202.05 > 131.92
5.29e3
Area
17 Jan_20a Sm (Mn, 2x4)
3: MRM of 8 Channels ES+
4.56
1634
202.05 > 123.93
7.56e3
Area
100
%
0
4.00
4.50
5.00
5.50
6.00
6.50
7.00
7.50
Time

Appendix G-9


Spike 25 ppb 1
17 Jan_31a Sm (Mn, 2x4)
3: MRM of 8 Channels ES+
243.1 > 130.8
7.39
55482
3.65e5
Area
100
%
0
4.00
4.50
5.00
5.50
6.00
6.50
7.00
7.50
17 Jan_31a Sm (Mn, 2x4)
3: MRM of 8 Channels ES+
243.1 > 96.8
7.39
22693
1.49e5
Area
100
%
0
4.00
4.50
5.00
5.50
6.00
6.50
7.00
7.50
17 Jan_31a Sm (Mn, 2x4)
3: MRM of 8 Channels ES+
202.05 > 131.92
4.56
3057
1.36e4
Area
7.39
68
100
%
0
4.00
4.50
5.00
5.50
6.00
6.50
7.00
7.50
17 Jan_31a Sm (Mn, 2x4)
3: MRM of 8 Channels ES+
202.05 > 123.93
4.56
4400
1.98e4
Area
100
%
0
Time
4.00
4.50
5.00
5.50
6.00
6.50
7.00
7.50