1 INTRODUCTION

1.1 Scope of the Method

JRA America Method AU-269R0 was developed to determine the residues of Aldicarb, Aldicarb Sulfone, and Aldicarb Sulfoxide in three water types: surface water, ground water, and drinking water using LC/MS/MS.

The validation was conducted using two fortification levels (0.1 and 1.0 µg/L) for all three water types. For each fortification at the LOQ 7 replicates were analyzed, and for the 10x LOQ five replicates were analyzed. Additionally, two replicates of unfortified samples were examined.

1.2 Principle of the Method

The residues of Aldicarb, Aldicarb Sulfone, and Aldicarb Sulfoxide are tested in 10mL of water either surface, ground or drinking by vortex and centrifugation. The final determination was conducted using LC-MS/MS in positive ion mode.

In all three water types: surface water, ground water, and drinking water the method has a limit of quantification of 0.1 µg/L for each analyte determined separately. The limit of detection for each analyte is set to 0.05 µg/L.

1.3 Specificity

Aldicarb, Alidcarb Sulfone, and Aldicarb Sulfoxide were identified and quantified as individual analytes.

2 MATERIALS AND METHODS

2.1 Test systems Matrices

The following test systems were considered in this study:

Surface water, ground water, and drinking water

The water characterization was done by AGVISE Laboratories.

Characterization	Drinking water(25790)	Surface water (104087)	Ground water (22485)
pH	6.5	8.1	7.4
Calcium	1.0ppm	33ppm	27ppm
Magnesium	Below 0.1ppm	10ppm	14ppm
Sodium	0.3ppm	102ppm	23ppm

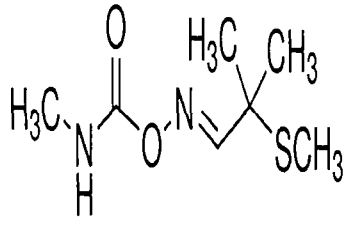
Hardness	Below DL	124mg Equ $CaCO_3$/L	127 Equ $CaCO_3$/L
Conductivity	0.03mmhos/cm	0.72mmhos/cm	0.37mmhos/cm
SAR	0.30	3.99	0.88
Total dissolved solid	26ppm	366ppm	194ppm
Turbidity	0.45 NTU	0.79NTU	0.25NTU

Agvise Laboratories: 604 highway 15 west P.O. Box 510 Northwood, ND 58267

2.2 Test and Reference Substances

2.2.1 Aldicarb

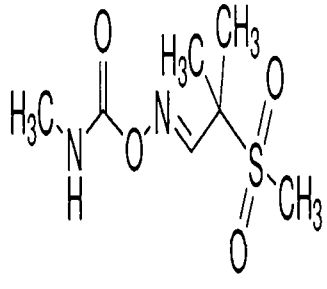
Chemical structure:



Molecular Formula	$C_7H_{14}N_2O_2S$
Molecular Weight	190.26
IUPAC Name	2-methy-2-(methythio)propanol O-(N-methylcarbamoyl)oxime
Batch No.	SZBC166XV
Purity (%)	99.9
Storage Advice	< 4 °C
GLP	Yes
Expiration Date	July 7th 2015

2.2.2 Aldicarb Sulfone

Chemical structure:

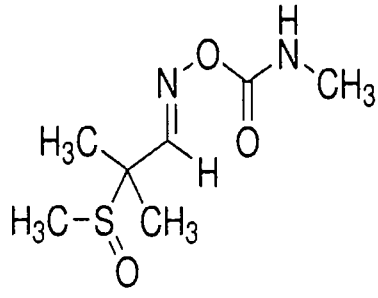


Molecular Formula	$C_7H_{14}N_2O_4S$
Molecular Weight	222.26
IUPAC Name	2-methyl-2-(methylsulfonyl)-propionaldehyde-O-(methylcarbamoyl)oxime
Batch No.	SZBB343XV
Purity (%)	99.5
Storage Advice	< 4 °C
GLP	Yes
Expiration Date	July 23 2015

2.2.3 Aldicarb Sulfoxide

Molecular Formula	$C_7H_{14}N_2O_3S$
Molecular Weight	206.26
IUPAC Name	(1E)-2-Methyl-1-(([methylamino)carbonyl]oxy)imino)-2-(methylsulfinyl)propane
Batch No.	SZBD049XV
Purity (%)	99.2
Storage Advice	< 4 °C
GLP	Yes
Expiration Date	24 July 2015

Chemical structure:



2.3 Materials and Methods

2.3.1 Equipment

Equipment	Size, Description	Manufacturer	Catalog No.
Balance, Analytical	Model AT200	Mettler Toledo	----
Beakers	Various Sizes	PYREX Brand ,VWR Scientific Products	13922-029
15 mL centrifuge tubes VWR	15 mL	VWR Scientific	
Bottle, Amber glass	Qorpak , 2 oz and 4 oz with Teflon®-lined screw cap	VWR Scientific Products Boston Round, Amber	----------
Volumetric, pipettes	Various Sizes	Eppendorf – Class A	----------
Cylinder, Graduated	Various sizes	Various Class A	----
Centrifuge	Allegra 6	Beckman Coulter	----
Ultrasonic Bath	Model FS 7652H	Fisher Scientific	
Volumetric Flask	Various sizes	VWR	----------
Vortex mixer	BenchMark	BenchMark Scientifc, Inc.	13112194
LC Vials	2 mL injection vials	Agilent	-------
LC-MS/MS	API 4000	AB Sciex	--------

2.3.2 Reagents

2.3.2.1 Chemicals

Chemical	Grade	Manufacturer/Supplier
Water	LC/MS	Omni
Ammonium acetate	LC/MS	Supelco/Sigma-Aldrich
Methanol	LC/MS	Omni

2.3.2.2 Solutions and Solvent Mixtures

Description	Code	Composition
Standard Preparation Solvent	**S1**	Water-Methanol (50:50, v/v) Add volumetrically 250 mL water and 250 mL methanol into a 500 mL Erlenmeyer flask and mix well to ensure a complete homogeneous solution.
HPLC mobile phase A	**LC1**	5mM Ammonium Acetate in Water and Weigh 0.385g of ammonium acetate in a 1L Volumetric flask
HPLC mobile phase B	**LC2**	100% Methanol in a 1L Volumetric Flask

Note: Equivalent reagents and chemicals from other suppliers may be substituted.

2.3.2.3 Stock Solutions Preparation

Stock Solutions

Preparation of Stock Solution

Prepare a 100 µg/mL stock solution individually by weighing an appropriate amount of each analyte into a volumetric flask. Dissolve with Acetonitrile as described below and dilute to mark.

For example, to prepare 100 mL of 100 µg/mL stock solution of Aldicarb in acetonitrile, weigh 10 mg Aldicarb into a 100 mL volumetric flask. Dissolve and dilute to mark with acetonitrile. Sonicate and vortex to ensure a complete homogeneous solution. The stock solutions for all other analytes are made in a similar fashion.

Note: Store all Aldicarb solutions in a refrigerator.

A correction for purity is done for all stock solution preparations.

Fortification Solutions

Fortifications Solution Preparation

A fortification solution was prepared by serial dilution of the stock solution with methanol. Volumetric flasks were used for all dilutions.

Analytes	Take solution (µg/mL)	Volume (mL)	Dilute with methanol to a final volume of (mL)	Concentration (µg/mL)
Aldicarb	125.9	0.397	50	1.0
Aldicarb Sulfoxide	105	0.476		
Aldicarb Sulfone	101.5	0.493		
Mix std	1	5	50	0.1

Calibration Standard Solutions

Calibration Standard Solutions Preparation

Take solution (ng/mL)	Volume (mL)	Dilute with S1 to a final volume of (mL)	Concentration (ng/mL)
100	0.1	10	1.0
1.0	5	10	0.5
0.5	5	10	0.25
0.25	4	10	0.1
0.1	5	10	0.05

All standard solutions were stored refrigerated when not in use.

Note: A different concentration scheme may be used and additional standards may be prepared as needed. The volume of solution prepared may be changed as necessary.

3. Analytical Procedure

3.1 Measurement and Fortification

The following scheme was used:

Sample Type	Sample Volume	Concentration of Spiking Solution	Volume of Spiking Solution	Level of Fortification
Control	10mL	-	-	0.00 µg/L

Fortification (LOQ)	10mL	100	µg/L	0.01mL	0.1 µg/L *
Fortification (10xLOQ)	10mL	100	µg/L	0.1mL	1.0 µg/L

* Limit of quantification

3.2 Extraction of Sample Material

Obtain the proper amount of source water and fortify according to method; following this vortex for 1 min, and centrifuge for 5 min at 3000rpm.

3.3 Preparation for Measurement

An aliquot is taken and placed in an HPLC vial for analysis.

Note: No color or particle may remain on the side of the tube after sonication and vortexing; this could affect the recoveries.

4. Instrumentation and Conditions

HPLC-MS/MS Conditions for Aldicarb, Aldicarb Sulfone, and Aldicarb Sulfoxide (Primary and Confirmatory Transitions)

	Parameter		
Chromatographic System	Shimadzu UFLC System		
Analytical-column	Nomura Develosil RPaqueous-3 150 x 2 mm 5µm		
Column Temperature	40°C		
Injection Volume	10 µL		
Mobile Phase A Mobile Phase B	5mM ammonium acetate, Methanol		
Flow Rate	450 µL/min		
Gradient (including wash and equilibration)	Time (min)	Phase A	Phase B
	0.01	95	5
	1.0	90	10
	1.5	75	25
	3.00	5	100
	6.00	95	5
	8.00	95	5
	8.01	95	5
Detection System	PE Sciex API 4000 Q Triple Mass Spectrometer		
Ionisation	Electrospray (ESI)		
Ionisation Temperature	450 °C		
Analyte	**Transitions**	**Polarity**	**Expected Retention Time**
Aldicarb	208 → 88.6*, 208 → 115.7	Positive	Approx. 3.80 min.
Aldicarb-sulfoxide	207 → 88.5*, 207 → 68.5	Positive	Approx. 3.04 min.
Aldicarb-sulfone	240 → 75.5*, 240 → 86.0	Positive	Approx. 3.14 min.

* Primary quantification transition. Either transition could be used for quantitation in case interference is observed at the same retention time.

4.1 Calibration Procedures

Calculation of results is based on peak area measurements using a calibration curve. The calibration curve is obtained by direct injection of the calibration standards containing known amounts of Aldicarb, Aldicarb Sulfone, and Aldicarb Sulfoxide in the range of 1.0 ng/mL to 0.05 ng/mL. Linear calibration functions were used for evaluation.

4.2 Calculation of Residues and Recoveries

Calculation of results is based on peak area measurements [µg/L]. The method requires that the sample volume be 10 ± 0.1 mL for fortification samples. The recovery is the percentage of the fortified amount (µg/L), which is recovered through the method, as shown in the equation below, during the final calculation step.

The residues of Aldicarb, Aldicarb Sulfoxide, and Aldicarb Sulfone are calculated as shown below:

I. Concentration [ng/mL] $= \dfrac{\text{Response} - \textit{Intercept}}{\textit{Slope}} = C_A$

II. Residue [µg/L] $= \textit{Calculated}.$

The recoveries of fortified compounds are calculated according to equation **III**:

III. Recovery % $= \dfrac{(\text{Residue in fortified sample} - \text{Residue in control}) \times 100}{\text{Amount of analyte fortified}}$

Example: Aldicarb, 208 → 88; Surface Water µg/L:

Concentration in the final volume [ng/mL]

Concentration [ng/mL] $= \dfrac{\text{Response} - \textit{Intercept}}{\textit{Slope}} = C_A$

Residue [µg/L]

$$\textbf{Recovery \%} = \frac{\text{Residue in fortified sample} - \text{Residue in control} \times 100}{\text{Amount of analyte fortified}}$$

The following values were used in this calculation:

Response of fortified sample	109159
Response of control sample	0
Slope:	942000
Intercept:	5150
Sample Volume (mL):	10
Final Volume (V_{end}):	10

$$\textbf{Concentration (ng/mL)} = \frac{109159 - 5150}{942000} = 0.110\ ng/ml$$

$$\textbf{Recovery \%} = \frac{(0.110\ \mu g/L - 0.00000\ \mu g/L) \times 100}{0.1\ \mu g/L} = 110\%$$

1 INTRODUCTION

Aldicarb is carbamate insecticide compound used as a selective pesticide that is currently utilized on potatoes and other vegetables to control thrips, and spider mites. It is also used to control soil born nematodes prior to growing potatoes.

Method Number AU-269R0 was successfully tested on surface water, ground water, and drinking water during the method development for determining the residues of Aldicarb and its metabolites using LC-MS/MS.

The method has a limit of quantitation (LOQ) of 0.1 µg/L (0.1 ppb) in multiple water matrices.

2 MATERIALS

2.1 Safety

The test and reference items, as well as the chemicals required for this analysis, should be handled in accordance with good industrial hygiene and safety practice. Avoid contact with the skin, eyes and clothing. Wearing of closed work clothing is recommended. Remove contaminated clothing.

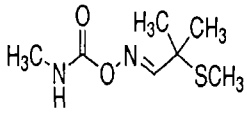
Store used work clothing separately. Keep away from food, drink and animal feed stuffs. No eating, drinking, smoking or tobacco use at the place of work. Hands and/or face should be washed before breaks and at the end of the shift. Details are given in the Safety Data Sheets (SDS) of the individual substances. All procedures involving organic solvents should be performed in a well-ventilated hood.

Disposal of samples and chemicals must be done in compliance with on-site safety policies and procedures.

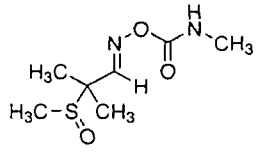
2.2 Test and Reference Items

Test and reference items should be stored according to the information provided in the certificate of analysis.

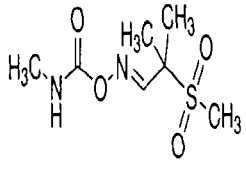
Common Name	Aldicarb
IUPAC Name	2-methy-2-(methythio)propanol O-(N-methylcarbamoyl)oxime
CAS-No.	116-06-3
Molecular Formula	$C_7H_{14}N_2O_2S$
Molecular Weight	190.26



Common Name	Aldicarb Sulfoxide
IUPAC Name	2-Methyl-2-(methylsulfinyl)propanalO-((methylamino)carbonyl)oxime
CAS-No.	1646-87-3
Molecular Formula	$C_7H_{14}N_2O_3S$
Molecular Weight	206.26



Common Name	Aldicarb Sulfone
IUPAC Name	2-methyl-2-(methylsulfonyl)-propionaldehyde-O-(methylcarbamoyl)oxime
CAS-No.	1646-88-4
Molecular Formula	$C_{11}H_7Cl_2NO_2$
Molecular Weight	222.26



2.3 Equipment

Equipment	Size, Description	Manufacturer	Catalog No.
Balance, Analytical	Model AT200	Mettler Toledo	----
Beakers	Various Sizes	PYREX Brand, VWR Scientific Products	13922-029
15 mL centrifuge tubes VWR	15 mL	VWR Scientific	
Bottle, Amber glass	Qorpak , 2 oz and 4 oz with Teflon®-lined screw cap	VWR Scientific Products Boston Round, Amber	----------
Volumetric, pipettes	Various Sizes	Eppendor	----------
Cylinder, Graduated	Various sizes	Various	----
Centrifuge	Allegra 6	Bechman Coulter	----
Ultrasonic Bath	Model FS 7652H	Fisher Scientific.	
Volumetric Flask	Various sizes, class A	VWR	----------
Vortex mixer	BenchMark	BenchMark Scientifc, Inc.	13112194
LC Vials	2 mL injection vials	Agilent	-------
LC-MS/MS	API 4000	AB Sciex	--------

Note: The equipment and instrumentation listed above may be substituted by that of similar specifications. The applicability is confirmed if the recoveries of the fortification experiments are in the expected concentration range.

2.4 Reagents

2.4.1 Chemicals

Chemical	Grade	Manufacturer/Supplier	Catalog No.
Water	LC/MS	EMD	WX000-01
Ammonium acetate	LC/MS	Supelco/Sigma-Aldrich	14267-25G
Methanol	LC/MS	EMD	MX0486-1

Note: Equivalent reagents and chemicals from other suppliers may be substituted.

2.4.2 Solutions and Solvent Mixtures

Description	Code	Composition
Standard Preparation solvent	S1	Water-Methanol (50:50, v/v) Add volumetrically 250 mL water and 250 mL methanol into a 500 mL Erlenmeyer flask and mix well to ensure a complete homogeneous solution.
HPLC mobile phase A	LC1	5mM Ammonium Acetate in Water Weigh in 0.385g of ammonium acetate in a 1L Volumetric flask
HPLC mobile phase B	LC2	100% Methanol in a 1L Volumetric Flask

Note: If necessary, the solutions may also be prepared in different volumes as long as the proportions are not modified.

2.4.3 Standard Solutions

Stock Solutions

Preparation of Stock Solution

Prepare a 100 µg/mL stock solution individually by weighing an appropriate amount of each analyte into a volumetric flask. Dissolve with Acetonitrile as described below and dilute to mark.

For example, to prepare 100 mL of 100 µg/mL stock solution of Aldicarb in acetonitrile, weigh 10 mg Aldicarb into a 100 mL volumetric flask. Dissolve and dilute to mark with acetonitrile. Sonicate and vortex to ensure a complete homogeneous solution. The stock solutions for all other analytes are made in a similar fashion.

Note: Store all Aldicarb solutions in a refrigerator. A different Scheme may be used to prepare solution.

A correction for purity is done for all stock solution preparations.

Fortification Solutions

Preparation of Fortification Solutions

Prepare mixed fortification standard solutions in volumetric flasks, by combining the solutions of each analyte that was prepared in Section 2.4.3, "Stock Solutions." Dilute volumetrically with appropriate solvents 50:50 methanol, water as described in the table below and ensure a complete homogeneous solution (e.g. , by sonication or vortexing).

Preparation of Fortification solutions:

Analytes	Take solution (µg/mL)	Volume (mL)	Dilute with methanol to a final volume of (mL)	Concentration (µg/mL)
Aldicarb, Sulfoxide, Sulfone	125.9	0.397	50	1.0
	105	0.476		
	101.5	0.493		
	1	5	50	0.1

Calibration Standard Solutions

Prepare calibration standard solutions for LC-MS/MS analysis, in volumetric flasks, by using the solutions that were prepared in Section Section 2.4.3, "Fortification Solutions". Dilute volumetrically with appropriate solvents as described in the table below and ensure a complete homogeneous solution (e.g. by sonication or vortexing).

Preparation of calibration standard solutions:

Take solution (ng/mL)	Volume (mL)	Dilute with S1 to a final volume of (mL)	Concentration (ng/mL)
100	0.1	10	1.0
1.0	5	10	0.5
0.5	5	10	0.25
0.25	4	10	0.1
0.1	5	10	0.05

Note: A different concentration scheme may be used and additional standards may be prepared as needed. The volume of solution prepared may be changed as necessary.

2.4.4 Stability of Standard Solutions

Stock and fortification solutions were assigned expiration dates as per our SOP: 6 months for stock and 3 months for fortification solutions and calibration standards.

3 ANALYTICAL PROCEDURE

3.1 Sample Preparation

Samples should be prepared as per description in this method.

3.2 Sample Storage

Samples are to be kept refrigerated until analysis or frozen for treated samples which will be stored for longer periods.

3.3 Measurement and Fortification

All matrices: Measured 10.0 ± 0.1 mL of water (control, fortification, and treated) into a 15 mL centrifuge tube.

The following fortification scheme may be used:

Sample Type	Sample Volume	Concentration of Spiking Solution	Volume of Spiking Solution	Level of Fortification
Control	10mL	-	-	0.00 µg/L
Fortification (LOQ)	10mL	100 µg/L	0.01mL	0.1 µg/L *
Fortification (10xLOQ)	10mL	100 µg/L	0.1mL	1.0 µg/L

*limit of quantification

3.4 Fortification and Preparation for Analysis

Obtain the proper amount of source water and fortify according to method, vortex for 1 min, and centrifuge for 5 min at 3000rpm.

3.5 Dilution

A. If necessary dilute with the S1 solvent solution.

3.6 Preparation for Measurement

An aliquot may be taken and placed in an HPLC vial for analysis.

Note:	No color or particles may remain on the side of the tube after sonication and vortexing; this could affect the recoveries.

3.7 Influence of matrix effects on analysis

The effects of matrix load on Aldicarb, aldicarb sulfoxide, and aldicarb sulfone recovery in drinking water, surface water, and ground water showed to be less than 20% indicating there is minimal or no matrix effect.

3.8 Stability of Extracts

Stability of Aldicarb in original extraction solution and in final volume solution was not investigated.

4 QUANTIFICATION AND CALCULATION

4.1 Set-up of the analytical run

A sequence for measurement generally consists of:

- Calibration standards
- Control samples
- Procedural recovery samples
- Unknown samples
- Instrument recovery sample

Reagent Blanks or blanks can also be injected if necessary. Each injection set should begin and end with an injection of a calibration standard. Standards should be interspersed with samples. Each calibration standard should be injected at least twice. At least 5 calibration levels need to be injected.

4.2 Instrumental analysis

	Parameter		
Chromatographic System	Shimadzu UFLC System		
Analytical-column	Nomura Develosil RPaqueous-3 C30 150 x 2 mm 5µm		
Column Temperature	40°C		
Injection Volume	10 µL		
Mobile Phase A Mobile Phase B	5mM ammonium acetate Methanol		
Flow Rate	450 µL/min		
Gradient (including wash and equilibration)	Time (min)	Phase A	Phase B
	0.01 1.0 1.5 3.00 6.00 8.00 8.01	95 90 75 5 95 95 95	5 10 25 100 5 5 5
Detection System	PE Sciex API 4000 Q Triple Mass Spectrometer		
Ionisation	Electrospray (ESI)		
Ionisation Temperature	450 °C		
Analyte	**Transitions**	**Polarity**	**Expected Retention Time**
Aldicarb	208 → 88.6*, 208 → 115.7	Positive	Approx. 3.80 min.
Aldicarb-sulfoxide	207 → 88.5*, 207 → 68.5	Positive	Approx. 3.04 min.
Aldicarb-sulfone	240 → 75.5*, 240 → 86.0	Positive	Approx. 3.14 min.

* proposed as quantification transition. Any of these transitions could be used for quantitation in case interference is observed at the same retention time or in case of any interaction between compounds

Example of parameter used for this method validation:

Aldicarb primary: DP 33, EP 10.3, CE 24, CXP 4

Aldicarb confirmatory: DP 33, EP 10.3, CE11, CXP 4

Aldicarb Sulfoxide primary: DP 55, EP 10, CE 20, CXP 4

Aldicarb Sulfoxide confirmatory: DP 55, EP 10, CE 23, CXP 4

Aldicarb Sulfone primary: DP 41, EP 10, CE 17, CXP 14

Aldicarb Sulfone confirmatory: DP 41.7, EP 9.20, CE 28.8, CXP 4

CAD: 10, CUR: 30, GS1: 40, GS2: 45, IS: 5500

These are optional to the user or optimized for the particular instrument if necessary

Note: Instruments with similar specifications may substitute the equipment listed above. The instruments used are applicable for analysis if the recoveries of the fortification experiments are in the acceptable range.

The ions used for Aldicarb and Aldicarb sulfone are the ammonium adducts. All compounds should be optimized in the presence of ammonium to obtain the proper Analyte.

Instrument conditions, e.g. injection volumes, columns, gradient steps or mass transitions may be modified, but any changes must be recorded in the raw data. Changes are acceptable when the recoveries of the fortification experiments are in the acceptable range.

Other parameters like gas flows and voltages are depended of the equipment used and therefore not listed. Those parameters may need to be adapted for the used instrument.

The same parameter may be tried if the instrument is of the same manufacture.

4.2.1 Calibration procedures

Calculation of results is based on peak area measurements using a calibration curve. At least 5 calibration levels need to be injected (e.g., required for enforcement). The calibration curve is obtained by direct injection of aldicarb standards containing a known amount in the range of 0.05 ng/mL to 1.0 ng/mL. In a given injection run, the same injection volume is used for all samples and standards.

Linear calibration functions are preferred, such as 1/x weighting.

4.2.2 Calculation of Residues and Recoveries

Calculation of results is based on peak area measurements [µg/L]. The method requires that the sample volume be 10 ± 0.1 mL for fortification samples. The recovery is the percentage of the fortified amount (µg/L), which is recovered through the method as shown in the equation below, during the final calculation step.

The residues of Aldicarb, Aldicarb sulfoxide, and aldicarb sulfone are calculated as shown below:

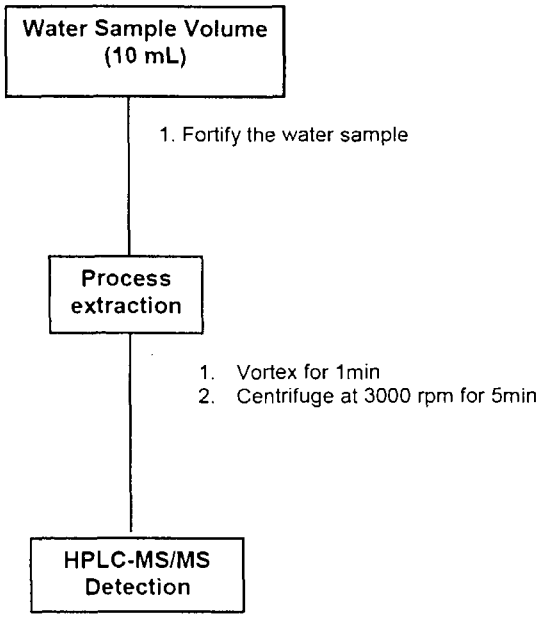
I. **Concentration [ng/mL]** $= \frac{\text{Response} - \textit{Intercept}}{\textit{Slope}} = C_A$

V_{end} = Final volume of the extract after all dilution steps [mL]
C_A = Concentration of analyte as read from the calibration curve [ng/mL]

The recoveries of fortified compounds are calculated according to equation III:

II. **Recovery %** $= \frac{(\text{Residue in fortified sample} - \text{Residue in control}) \times 100}{\text{Amount of analyte fortified}}$

5 FLOWCHART


Water Sample Volume (10 mL)
1. Fortify the water sample
Process extraction
1. Vortex for 1min
2. Centrifuge at 3000 rpm for 5min
HPLC-MS/MS Detection