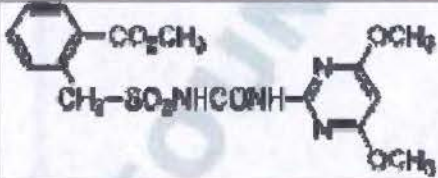## 2.   EXPERIMENTAL PROCEDURE

2.1   Test Item

Details of the test item provided by the Sponsor (Ref. TIDS) are as follows:

Test Item Name	Bensulfuron methyl technical
IUPAC Name	methyl α-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl)-*o*-toluate
C.A. Name	methyl 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)-amino]carbonyl] amino] sulfonyl]methyl]benzoate
CAS Number	83055–99–6
Molecular Formula	$C_{16}H_{18}N_4O_7S$
Molecular Weight	410.4
Molecular Structure	
Batch/Lot Number	AUG12SHF31
Analysed Purity	98.93% (Certificate of Analysis, Refer Appendix 3)
Manufactured by	DuPont, China
Supplied to JRF by	RiceCo LLC, USA
Date of Manufacture	August 2012
Date of Expiry	January 2016
Appearance	White colored powder
Other Characteristics (pH, Solubility, Specific gravity)	pH: 6.4 – 6.9, Solubility: 67 mg/L (in water at pH-7 and 25 °C)
Storage Condition (at JRF)	As per the instruction received from the Sponsor on storage of the test item, the test item was stored: Storage Temperature: Room Temperature Storage Container : In original container as supplied by the Sponsor Storage Location : Test Item Control Office, JRF

Source: IUPAC Name, CA Name, CAS Number, Molecular weight and Molecular structure were obtained from e-Pesticide Manual, fifteenth edition, Version 5.1 (2010).

2.2 Reference Standard

Details of reference standard are provided below:

Reference Standard	Bensulfuron methyl
IUPAC Name	methyl α-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl)-o-toluate
C.A. Name	methyl 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)-amino]carbonyl] amino] sulfonyl]methyl]benzoate
Molecular Weight	410.41
Molecular Formula	$C_{16}H_{18}N_4O_7S$
CAS N°	83055–99–6
Lot N°	20503
Certification Date	September 18, 2012
Expiry Date	September 17, 2016
Purity	98.0% (Certificate of Analysis, Refer Appendix 4)
Source	Dr. Enhenstrofer , Germany
Storage Condition	Refrigerator
JRF Entry Number	PC-2978

2.3 Equipments/Instruments

Sr. N°	Instrument/Apparatus	Model	Make / Supplier
1	Analytical Balance	XP26	Mettler Toledo
2	MS-MS	4000Q Trap	AB MDS Sciex Applied Biosystem
3	HPLC	Nexera X2	Shimadzu
4	Cyclo Mixer	CM 101 DX	Remi
5	Variable Micropipette	-	Eppendorf
6	Millipore Water Purification System	Milli Q	Millipore
7	Refrigerator	Eon	Godrej

2.4 Solvents and Chemicals

Sr. N°	Name	Grade	Source
1	Acetonitrile	HPLC	J.T baker
2	Formic Acid	MS Grade	Fluka
3	Milli-Q water	Type-I	Millipore water purification system

2.5　　**Principle**

The residues of Bensulfuron methyl in drinking water, ground water and surface water are determined using LC-MS-MS.

2.6　　**Test System**

A]　**Surface Water**

Sampling Location　:　Daman Ganga River, Near N.H. No.8, Near: Jai Research Foundation, Vapi, Gujarat, India.

B]　**Ground Water**

Source　　　　　:　Jai Research Foundation, Vapi, Gujarat, India.

C]　**Drinking Water**

Source　　　　　:　Bisleri International Pvt. Ltd.

Batch number　　　: 210

Manufacturing date　: 29/07/2014

Expiry date　　　　: 28/01/2015

2.7　　**Validation of Analytical Method**

The analytical method for the determination of residues of Bensulfuron methyl in drinking water, ground water and surface water was validated using LC-MS-MS. The validation covered the aspects viz., specificity, linear dynamic range (LDR), limit of detection (LOD), limit of quantification (LOQ), precision (% RSD) and accuracy (% Recovery) at LOQ and 10 x LOQ level.

2.7.1　　**Preparation of 0.1 % Formic Acid in Water**

A volume of 2.0 mL formic acid was transferred into volumetric flask of 2000 mL capacity containing 500 mL milli Q water and the volume was made upto the mark with milli Q water. The content was mixed thoroughly.

2.7.2　　**Specificity**

2.7.2.1　　**Preparation of Bensulfuron methyl Reference Standard Stock Solution**

Name of Reference standards	Purity %	Weight of Reference standard (mg)	Capacity of volumetric flask (mL)	Volume made up with	Concentration obtained (mg/L)	Reference standard stock solution identification
Bensulfuron methyl	98.0	5.10	10.00	Acetonitrile	499.80 or 499800.00 (µg/L)	A

2.7.2.2　Preparation of Bensulfuron methyl Reference Standard Working Solution

Reference Standard Solution Identification	Solution Taken (mL)	Final volume (mL) with Acetonitrile	Obtained Concentration Solution (µg/L)	Reference Standard Solution
A	0.10	10	4998.00	B
B	0.01	10	5.00	C
C	0.20	10	0.10	D

2.7.2.3　Preparation of Bensulfuron methyl Test Item Stock Solution

Name of Test Item	Purity %	Weight of Test Item (mg)	Capacity of Volumetric Flask (mL)	Volume made up with	Concentration Obtained (mg/L)	Test Item stock solution identification
Bensulfuron methyl Technical	98.93	5.05	10.00	Acetonitrile	499.60 or 499600.00 (µg/L)	TIA

2.7.2.4　Preparation of Bensulfuron methyl Test Item Working Solutions

Test Item Stock Solution Identification	Solution Taken (mL)	Final Volume (mL)	Volume made up with	Obtained Concentration Solution (µg/L)	Test Item working solution
TIA	0.10	10		4996.00	TIB
TIB	0.01	10	Acetonitrile	5.00	TIC
TIC	0.20	10		0.10	TID

2.7.2.5　Preparation of Reference Standard and Test Item Working Solutions

Identification of Solution	Solution Taken (mL)	Final Volume (mL)	Volume made up with	Obtained Concentration (µg/L)	Identification of Solution
C	0.20	10	Acetonitrile	0.10	D
	0.20	10	Drinking Water	0.10	TIDW
TIC	0.20	10	Ground Water	0.10	TIGW
	0.20	10	Surface Water	0.10	TISW

The acetonitrile, mobile phase, solution D, TID, blank drinking water, ground water, surface water, TIDW, TIGW and TISW were injected onto LC-MS-MS in accordance with **section 2.7.12**

2.7.3   **Working Solution Preparation for Limit of Detection (LOD) and Limit of Quantification (LOQ)**

Test Item Stock Solution	Solution Taken (mL)	Final volume (mL)	Obtained Concentration (µg/L)	Sample Identification
	0.20	10	0.10	01
	0.10	10	0.05	02
	0.04	10	0.02	03
	0.02	10	0.01	04
	0.20	10	0.10	05
	0.10	10	0.05	06
TIC	0.04	10	0.02	07
	0.02	10	0.01	08
	0.20	10	0.10	09
	0.10	10	0.05	10
	0.04	10	0.02	11
	0.02	10	0.01	12

above prepared samples 01 to 04, 05 to 08 and 09 to 12 were diluted with drinking water, ground water and surface water, respectively and injected onto LC-MS-MS in accordance with **section 2.7.12.**

2.7.4   **Working Solution Preparation for Linear Dynamic Range**

Working Solution (µg/L)	Solution Taken (mL)	Final volume (mL) in Acetonitrile	Obtained Concentration (µg/L)	Working Solution I.D.
	0.008	10	4.00	SS1
	0.020	10	10.00	SS2
4998.00 (B)	0.040	10	19.99	SS3
	0.100	10	49.98	SS4
	0.160	10	79.97	SS5
	0.200	10	99.96	SS6

2.7.5   **Linear Dynamic Range (LDR) for Surface Water**

Working Solution (µg/L)	Solution Taken (mL)	Final volume (mL) in surface water	Obtained Concentration (µg/L)	Sample Identification
4.00 (SS1)	0.01	1.00	0.04	SWSTD 1
10.00 (SS2)	0.01	1.00	0.10	SWSTD 2
19.99 (SS3)	0.01	1.00	0.20	SWSTD 3
49.98 (SS4)	0.01	1.00	0.50	SWSTD 4
79.97 (SS5)	0.01	1.00	0.80	SWSTD 5
99.96 (SS6)	0.01	1.00	1.00	SWSTD 6

The reference standard working solutions **SWSTD 1 to SWSTD 6** were injected onto the LC-MS-MS accordance with **section 2.7.12** and peak area was plotted against concentration (µg/L). The correlation coefficient (r), slope (b) and intercept (a) were calculated.

2.7.6   Linear Dynamic Range (LDR) for Ground Water

Working Solution (µg/L)	Solution Taken (mL)	Final volume (mL) in ground water	Obtained Concentration (µg/L)	Sample Identification
4.00 (SS1)	0.01	1.00	0.04	GWSTD 1
10.00 (SS2)	0.01	1.00	0.10	GWSTD 2
19.99 (SS3)	0.01	1.00	0.20	GWSTD 3
49.98 (SS4)	0.01	1.00	0.50	GWSTD 4
79.97 (SS5)	0.01	1.00	0.80	GWSTD 5
99.96 (SS6)	0.01	1.00	1.00	GWSTD 6

The reference standard working solutions **GWSTD 1 to GWSTD 6** were injected onto the LC-MS-MS accordance with **section 2.7.12** and peak area was plotted against concentration (µg/L). The correlation coefficient (r), slope (b) and intercept (a) were calculated.

2.7.7   Linear Dynamic Range (LDR) for Drinking Water

Working Solution (µg/L)	Solution Taken (mL)	Final volume (mL) in Drinking water	Obtained Concentration (µg/L)	Sample Identification
4.00 (SS1)	0.01	1.00	0.04	DWSTD 1
10.00 (SS2)	0.01	1.00	0.10	DWSTD 2
19.99 (SS3)	0.01	1.00	0.20	DWSTD 3
49.98 (SS4)	0.01	1.00	0.50	DWSTD 4
79.97 (SS5)	0.01	1.00	0.80	DWSTD 5
99.96 (SS6)	0.01	1.00	1.00	DWSTD 6

The reference standard working solutions **DWSTD 1 to DWSTD 6** were injected onto the LC-MS-MS accordance with **section 2.7.12** and peak area was plotted against concentration (µg/L). The correlation coefficient (r), slope (b) and intercept (a) were calculated.

2.7.8   **Working solution for Precision (% RSD) and Accuracy (% Recovery) at LOQ and 10 x LOQ**

Fortific-ation level	Concentration of Test Item working Solution (µg/L)	Volume (mL) taken	Final volume (mL) in Acetonitrile	Concentration Obtained (µg/L)	Identification of Working Solution
LOQ	4996.00 (TIB)	0.01	10	5.00	LOQSS1
10 LOQ		0.10	10	49.96	LOQSS2

2.7.9   **Precision (% RSD) and Accuracy (% Recovery) at LOQ and 10 x LOQ for Surface Water**

Fortific-ation level	Replic-ation	Concentration of Test Item working Solution (µg/L)	Volume (mL) taken	Final volume (mL) surface water	Concentration Obtained (µg/L)	Identification of Working Solution
LOQ	R1		0.01	1.00	0.05	SWLOQ1
	R2		0.01	1.00	0.05	SWLOQ2
	R3	5.00 (LOQSS1)	0.01	1.00	0.05	SWLOQ3
	R4		0.01	1.00	0.05	SWLOQ4
	R5		0.01	1.00	0.05	SWLOQ5
10 X LOQ	R1		0.01	1.00	0.50	SW 10 LOQ1
	R2		0.01	1.00	0.50	SW 10 LOQ2
	R3	49.96 (LOQSS2)	0.01	1.00	0.50	SW 10 LOQ3
	R4		0.01	1.00	0.50	SW 10 LOQ4
	R5		0.01	1.00	0.50	SW 10 LOQ5

\# The above prepared sample solutions along with surface water were injected onto LC-MS-MS accordance with **section 2.7.12.**

2.7.10   **Precision (% RSD) and Accuracy (% Recovery) at LOQ and 10 x LOQ for Ground Water**

Fortific-ation level	Replic-ation	Concentration of Test Item working Solution (µg/L)	Volume (mL) taken	Final volume (mL) ground water	Concentration Obtained (µg/L)	Identification of Working Solution
LOQ	R1		0.01	1.00	0.05	GWLOQ1
	R2		0.01	1.00	0.05	GWLOQ2
	R3	5.00 (LOQSS1)	0.01	1.00	0.05	GWLOQ3
	R4		0.01	1.00	0.05	GWLOQ4
	R5		0.01	1.00	0.05	GWLOQ5
10 X LOQ	R1		0.01	1.00	0.50	GW 10 LOQ1
	R2		0.01	1.00	0.50	GW 10 LOQ2
	R3	49.96 (LOQSS2)	0.01	1.00	0.50	GW 10 LOQ3
	R4		0.01	1.00	0.50	GW 10 LOQ4
	R5		0.01	1.00	0.50	GW 10 LOQ5

\# The above prepared sample solutions along with ground water were injected onto LC-MS-MS accordance with **section 2.7.12.**

2.7.11   **Precision (% RSD) and Accuracy (% Recovery) at LOQ and 10 x LOQ for Drinking Water**

Fortific-ation level	Replic-ation	Concentration of Test Item working Solution (μg/L)	Volume (mL) taken	Final volume (mL) Drinking water	Concentration Obtained (μg/L)	Identification of Working Solution
LOQ	R1	5.00 (LOQSS1)	0.01	1.00	0.05	DWLOQ1
	R2		0.01	1.00	0.05	DWLOQ2
	R3		0.01	1.00	0.05	DWLOQ3
	R4		0.01	1.00	0.05	DWLOQ4
	R5		0.01	1.00	0.05	DWLOQ5
10 X LOQ	R1	49.96 (LOQSS2)	0.01	1.00	0.50	DW 10 LOQ1
	R2		0.01	1.00	0.50	DW 10 LOQ2
	R3		0.01	1.00	0.50	DW 10 LOQ3
	R4		0.01	1.00	0.50	DW 10 LOQ4
	R5		0.01	1.00	0.50	DW 10 LOQ5

The above prepared sample solutions along with drinking water were injected onto LC-MS-MS accordance with **section 2.7.12.**

2.7.12   **Instrumental Parameters**

System	:	AB MDS Sciex API 4000 Q trap mass spectrometer coupled with Nexera X2 HPLC system
Mobile Phase	:	Acetonitrile (60): 0.1 % Formic Acid in water (40) v/v
Column	:	Waters X-Bridge C-18, (150 X 4.6 mm, 3.5 μm particle size)
Injection volume	:	10 μL
Flow Rate	:	0.8 mL/minute
Data handling system	:	Analyst (Version 1.5.2)

MS Parameters

(a) Source Parameters

Curtain Gas	:	10 psi
Collision Gas	:	Medium
Ion source voltage	:	5500 mV
Temperature	:	550°C
Gas-1	:	50 psi
Gas-2	:	60 psi

(b) Compound Parameters

Declustering potential	:	87 mV
Entrance potential	:	6 mV
Collision Energy	:	23 mV
Collision cell Exit Potential	:	15 mV
Ionisation	:	Positive Mode

(c) MRM (Q1/Q3)

Bensulfuron methyl	:	Q1 Mass: 411.1, Q3 Mass: 149.2 (Quantification)
		Q1 Mass: 411.1, Q3 Mass: 182.3 (Confirmation)

1.   **GENERAL INFORMATION**

1.1   **Study Director**

Arun Raithatha, M.Sc.

Deputy Study Director

Moinuddin, M. Pharm.

1.2   **Test Facility Management**

Dr. Nadeem Ahmad Khan

1.3   **Study Schedule**

Study Initiation Date	:	August 04, 2014
Experiment Start Date	:	August 06, 2014
Experiment Completion	:	Latest by August 2014
Draft Report Submission	:	Latest by August 2014
Final Report Submission	:	Within two weeks from the date of receipt of comments on the final draft report from the Sponsor.

1.4   **Study Plan and Amendment (if any) Distribution**

a. Original copy in Archive and Study Sponsor; b. Photocopy to Study Director and QAU

2.   **INTRODUCTION**

2.1   **Objective**

The objective of this study is to validate the analytical method for determination of Bensulfuron methyl concentration in surface water, ground water and drinking water.

2.2   **Guidance Documents**

European Commission 2000: Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4), and Annex III (part A, Section 5) of Directive 91/414; SANCO/3029/99 rev. 4 of 11 July 2000. "Directorate General Health and Consumer Protection".
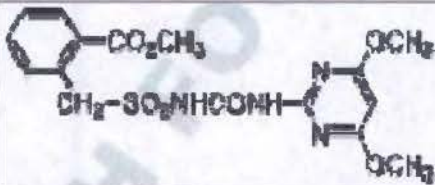
European Commission 2010: EU guidance document on analytical methods for the determination of residues (Post-registration monitoring and control); SANCO/825/00 rev. 8.1, 2010.

U.S. EPA, 1996: OCSPP (OPPTS) 860.1340 "Residue Analytical Method" (EPA 712-C-96-174). The United States Environmental Protection Agency, Residue Chemistry Test Guidelines (August 1996).

2.3 Test Item

The Test Item Data Sheet has been completed by the Sponsor. The representative sample of the test item will be retained for archive purpose. Any residual test item will be disposed of at JRF after the expiry date unless otherwise instructed by the Sponsor. The details relating to the test item (as furnished by the study Sponsor) are as follows:

Test Item Name	Bensulfuron methyl technical
IUPAC Name	methyl α-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl)-o-toluate
C.A. Name	methyl 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)-amino]carbonyl] amino] sulfonyl]methyl]benzoate
CAS Number	83055–99–6
Molecular Formula	$C_{16}H_{18}N_4O_7S$
Molecular Weight	410.4
Molecular Structure	
Batch/Lot Number	AUG12SHF31
Analysed Purity	98.93% (Information taken from JRF GLP CoA)
Manufactured by	DuPont, China
Supplied to JRF by	RiceCo LLC, USA
Date of Manufacture	August 2012
Date of Expiry	January 2016
Appearance	White colored powder
Other Characteristics (pH, Solubility, Specific gravity)	pH: 6.4 – 6.9, Solubility: 67 mg/L (in water at pH-7 and 25 °C)
Test Item Characterisation under GLP	Yes
Storage Condition (at JRF)	As per the instruction received from the Sponsor on storage of the test item, the test item will be stored: Storage Temperature: Room Temperature Storage Container : In original container as supplied by the Sponsor Storage Location : Test Item Control Office, JRF
JRF Test Item Code	BMT222

Source: IUPAC Name, CA Name, CAS Number, Molecular weight and Molecular structure were obtained from e-Pesticide Manual, fifteenth edition, Version 5.1 (2010).

2.4   Reference Standard

Details of the reference standard are mentioned below:

Reference Standard	Bensulfuron methyl
IUPAC Name	methyl α-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl)-o-toluate
C.A. Name	methyl 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)-amino]carbonyl]amino] sulfonyl]methyl]benzoate
Molecular Weight	410.41
Molecular Formula	$C_{16}H_{18}N_4O_7S$
CAS N°	83055–99–6
Lot N°	20503
Certification Date	September 18, 2012
Expiry Date	September 17, 2016
Purity	98.0%
Source	Dr. Enhenstrofer , Germany
Storage Condition	Refrigerator
JRF Entry Number	PC-2978

2.5   Reference Standard Retention

The reference standard will be retained at the Department of Chemistry until the declared expiry or re-assay date.

3.   GOOD LABORATORY PRACTICE (GLP)

3.1   GLP Compliance

This study will be conducted in compliance with the OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17, N° 1, Environment Directorate, Organisation for Economic Cooperation and Development, Paris (1998) and all subsequent OECD consensus documents.

3.2   Standard Operating Procedures (SOPs)

Unless otherwise specified all procedures mentioned in the study plan are subject to detailed Standard Operating Procedures of Jai Research Foundation.

3.3   Amendment to Study Plan

This study plan may be subject to amendment. Amendment to study plan, whether initiated by the Study Director or Sponsor will be generated, authorized by the Study Director and sent to the Sponsor for approval.

In the event that circumstances dictate immediate action, the nature of these circumstances will be communicated to the Sponsor as soon as practicable (by telephone, facsimile transmission or e-mail) and confirmed as soon as possible by way of formal study plan amendment.

3.4   Deviation from the Study Plan

Any deviation from the study plan will be documented in study file and reported in the study report.

3.5   Quality Assurance

This study plan has been verified by JRF Quality Assurance Unit (QAU) and documented (Number 63811). The QAU of JRF will inspect the critical phase(s) of the study by study based inspection and/or process based inspection. The raw data, draft and final reports will be audited to ensure that the final report accurately reflect the raw data. The audit/inspection reports   will be provided to the Study Director and the Test Facility Management. The date of audits/inspections and reporting of findings to the Study Director and the Test Facility Management will be incorporated in the study report.

4.   EXPERIMENTAL PROCEDURE

4.1   Test System

A] Surface Water

Sampling Location   :   Daman Ganga River, Near N. H. No. 8, Near: Jai Research
                              Foundation, Vapi, Gujarat, India.

B] Ground Water

Source   :   Jai Research Foundation, Vapi, Gujarat, India.

C] Drinking Water

Source   :   Market (will be purchased from commercial source)

The test systems with information of following pysico-chemical properties will be used. The complete details of the source and characterization results will be reported in the report.

(i)   pH

(ii)   Dissolve organic carbon

(iii)   Hardness

(iv)   Conductivity

4.2 Equipment/Instruments

pH meter, conductivity meter, cyclo mixer, analytical balance, variable micro pipette and LC-MS- MS .

4.3 Solvents and Chemicals

Acetonitrile, formic acid, milli-Q water and other chemicals and solvents required for analysis.

4.4 Validation of the Method

The LC-MS-MS analytical method for the determination of Bensulfuron methyl technical active ingredient concentrations in surface water, drinking water and ground water will be validated. The validation will cover the aspects viz., (i) specificity, (ii) linear dynamic range (LDR), (iii) limit of detection (LOD), (iv) limit of quantitation (LOQ), (v) precision (%, RSD) and accuracy (%, Recovery) at LOQ and 10 x LOQ level.

4.4.1 Preparation of Bensulfuron methyl Stock Solutions

A known quantity of Bensulfuron methyl technical will be weighed in a volumetric flask of known capacity, dissolved in few mL acetonitrile and the volume will be made upto the mark with acetonitrile.

4.4.2 Specificity

The solvent (used for solution preparation), mobile phase, reference standard solution and test item solution will be injected onto LC-MS-MS using selected parameters. The interference (if any), in the determination of active ingredient in test item will be reported. Interference should not exceed 30% peak area of the target active ingredient at LOQ level.

4.4.3 Limit of Detection (LOD)

The minimum quantity of Bensulfuron methyl, which could be detected by the LC-MS-MS with signal to noise ratio of $3 \pm 0.5: 1$, will be considered as limit of detection (LOD).

4.4.4 Limit of Quantification (LOQ)

The minimum quantity of Bensulfuron methyl fortified in the test media, which could be quantified by the LC-MS-MS with signal to noise ratio $\geq 10:1$, will be considered as limit of quantification (LOQ).

4.4.5 Linear Dynamic Range

Six different concentrations of Bensulfuron methyl reference standard will be prepared in diluent and injected onto LC-MS-MS. The Standard 1 to 6 will be injected in singlet. The linear calibration curve will be established by plotting the mean peak area against concentration (μg/L). The correlation coefficient ($r > 0.99$), intercept (a) and slope (b) will be calculated. The regression equation will be established.

4.4.6 Precision (% RSD) and Accuracy (% Recovery) for Surface Water, Drinking Water and Ground Water

Precision and accuracy of the method will be reported at each fortification level i.e., at LOQ and 10 X LOQ along with two sets of control. Recovery of the Bensulfuron methyl will be determined by fortification of working stock solution to the surface, drinking and ground water. Five determinations will be made at each fortification level. The Bensulfuron methyl active ingredient concentration in each replicate will be injected; the mean of active ingredient concentration, SD and % RSD will be calculated and reported.

The Bensulfuron methyl active ingredient concentration in surface, drinking and ground water will be calculated by linear $1/x^2$ using the following formula:

$$Y = bX + a$$

$$\text{Bensulfuron methyl Concentration } (\mu g/L) = \frac{Y - a}{b} \times D$$

Where,

Y	=	Peak area of the sample
a	=	Constant
b	=	Slope of the curve
D	=	Dilution factor (if applicable)

Accuracy/recovery values should fall within 70 to 110%. If the majority of recoveries do not fall within the expected range the reason will be identified and corrections will be made to the method. If necessary the validation will be repeated.

$$\% \text{ Recovery (Accuracy)} = \frac{\text{Recovered Concentration}}{\text{Fortified Concentration}} \times 100$$

The % RSD will be calculated at each fortification level. Precision will be defined by the relative standard deviation (% RSD). The precision (% RSD) will be calculated using the formula provided as follows:

$$\text{Precision (\% RSD)} = \frac{\text{Standard deviation}}{\text{Mean Recovered Concentration}} \times 100$$

4.5   Instrumental Parameters

System	:	AB MDS Sciex API 4000 Q trap mass spectrometer coupled with Nexera X2 HPLC system
Mobile Phase	:	Acetonitrile (60): 0.1 % Formic Acid in water (40) v/v
Column	:	Waters X-Bridge C-18, (150 X 4.6 mm, 3.5 μm)
Injection volume	:	10 μL
Flow Rate	:	0.8 mL/minute
Data handling system	:	Analyst (Version 1.5.2)

4.5.1   MS Parameters

(a) Source Parameters

Curtain Gas	:	10 psi
Collision Gas	:	Medium
Ion source voltage	:	5500 mV
Temperature	:	550°C
Gas-1	:	50 psi
Gas-2	:	60 psi

(b) Compound Parameters

Declustering potential	:	87 mV
Entrance potential	:	6 mV
Collision Energy	:	23 mV
Collision cell Exit Potential	:	15 mV
Ionisation	:	Positive Mode

(c) MRM (Q1/Q3)

(Bensulfuron methyl)	:	Q1 Mass: 411.1, Q3 Mass: 149.2