

SUMMARY

STUDY TYPE : Independent Laboratory Validation; OCSPP 860.1340, SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1.

TEST ITEM : Propanil [Purity: 99.0 %] and 3,4-Dichloroaniline [Purity: 99.3 %]

CITATION : Gopal Upadhyay. Independent Laboratory Validation of an Analytical Method for the Determination of Propanil and 3,4-Dichloroaniline in Surface and Drinking water by LC-MS/MS Analysis, Jai Research Foundation, India. Laboratory report number: 228-2-14-19546; May 21, 2018.

SPONSOR : Propanil Task Force II, Washington D.C., USA.

EXECUTIVE SUMMARY: Analytical methods for the analysis of the test/reference substances propanil and its metabolite 3,4-dichloroaniline (3,4-DCA) in surface and drinking water have been independently validated at 0.1 µg/L (LOQ) and 1.0 µg/L (10X LOQ).

Propanil and 3,4-dichloroaniline (3,4-DCA) were analyzed by liquid chromatography with tandem mass spectrometry with electrospray ionization in positive polarity mode [LC-MS/MS; ESI (+)]. Propanil and 3,4-DCA were quantitated using a single point calibration of a mixture of the corresponding matrix matched standards. The standard solutions were analyzed several times to bracket the samples. The concentration in the samples was calculated by comparing peak areas of propanil and 3,4-DCA of the analyzed samples to the specific analytical reference standards.. The amount of propanil was determined for the quantitation ion m/z 218.1>162.1 and for the confirmation ion 218.1>127.2; the amount of 3,4-DCA was determined for the quantitation ion m/z 162.1>127.1 and for the confirmation ion m/z 162.1>74.0.

No specific interference was observed in specificity experiment performed at JRF India.

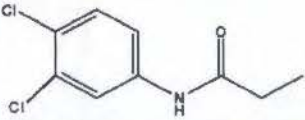
The recovery data at the LOQ and 10X LOQ levels meet the acceptance criteria for validation of the analytical methods. This study fulfils registration requirements as outlined in EPA guidelines OCSPP 860.1340.

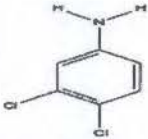
The validation covered the aspects namely: (i) specificity, (ii) linearity, (iii) precision (% RSD) and (iv) accuracy (% recovery).

2. EXPERIMENTAL PROCEDURE

2.1 Reference Standards

Reference Standards of propanil and 3,4-dichloroaniline were used as the test item.

Reference Standard	Propanil
IUPAC Name	3',4'-dichloropropanilide
CAS N°	709-98-8
Molecular Weight	218.08 g/mol
Molecular Structure	
Lot N°	40316
Expiry Date	March 25, 2020
Purity	99.0 % (Refer Certificate of Analysis in APPENDIX 4)
Source	Dr. Ehrenstorfer
Storage Condition	Refrigerator
JRF Entry Number	PC-3785

Reference Standard	3,4-Dichloroaniline
IUPAC Name	3,4-Dichloroaniline
CAS N°	95-76-1
Molecular Formula	C ₆ H ₅ Cl ₂ N
Molecular Weight	162.02 g/mol
Molecular Structure	
Batch N°	13509KQV
Reassay Date	September 1, 2018
Analysed Concentration under GLP by JRF America	99.3 % (Refer Certificate of Analysis in APPENDIX 4)
Source	Sigma Aldrich
Storage Condition	Ambient
JRF Entry Number	PC-4247

Source of IUPAC Name, CAS N°, Molecular Structure, Molecular weight and Molecular Formula: Certificate of Analysis and JRF America, Inc., Study Number: AU-2017-40.

2.2 Equipments/Instruments

Sr. N ^o	Instrument/Apparatus	Model	Make / Supplier
1	Mass Spectrometer	API 6500 Q trap	AB Sciex
2	HPLC	Nexera X2	Shimadzu
3	Analytical Balance	GR 202	Adair Dutt
4	Refrigerator	Eon	Godrej
5	Micropipette	-	eppendorf

2.3 Solvents and Chemicals

Sr. N ^o	Name	Grade	Source
1	Acetonitrile	LC-MS	Honey well
2	Methanol	LC-MS	Honey well
3	Acetic Acid	LC-MS	Merck
4	Water	LC-MS	Merck
5	Distilled water	Type-II	Merck

2.4 Outline of Method

The Propanil and 3,4-Dichloroaniline concentrations in surface water and drinking water were determined by using an LC-MS-MS method.

2.5 Test System

A) Surface Water

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

B) Drinking Water

Sampling Location : Jai Research Foundation, Vapi, Gujarat, India.

Note: The test system characterisation was done at JRF India via JRF RES-2-14-20143. Refer Certificate of Characterisation of Surface and Drinking Water (refer APPENDIX 3)

2.6 Independent Laboratory Validation of an Analytical Method for Quantity of Propanil and 3,4-Dichloroaniline in Surface and Drinking Waters by LC-MS/MS

The analytical method for the determination of quantity of Propanil and 3,4-Dichloroaniline in surface water and drinking waters was validated. The validation covered the aspects viz., specificity, linearity, precision (% RSD) and accuracy (% Recovery) at LOQ and 10 x LOQ level.

2.6.1 Preparation of Propanil and 3,4-Dichloroaniline Reference Standards Stock Solutions

Name of Reference Standards	Purity (%)	Weight of Reference Standards (mg)	Capacity of Volumetric Flask (mL)	Volume Made up with	Concentrations Obtained (µg/L)	Stock Solutions Identification
Propanil	99.0	10.72	10	Acetonitrile	1061280.000	RS-A
3,4-Dichloroaniline	99.3	10.65	10	Methanol	1057545.000	RS-B

2.6.1.1 Preparation of Propanil and 3,4-Dichloroaniline Reference Standards Working Solutions

Volume Taken (mL)		Final Volume (mL)	Volume Made up with	Obtained Concentrations ($\mu\text{g/L}$)		Identification of Reference Standards Stock Solutions
				A	B	
0.08 (RS-A)	0.08 (RS-B)	10	Diluent	8490.240	8460.360	AB
0.04 (AB)		25	Diluent	13.584	13.537	AB-1
2.88 (AB-1)		5	Diluent	7.824	7.797	AB-2
0.1 (AB-2)		1.0	Surface water	0.782	0.780	SW-AB
0.1 (AB-2)		1.0	Drinking water	0.782	0.780	DW-AB

2.6.1.2 Preparation of Blank Working Solutions in Surface Water and Drinking Water

Volume Taken (mL)	Final Volume (mL)	Volume Made up with	Obtained Concentrations ($\mu\text{g/L}$)	Identification of Reference Standards Stock Solutions
2.88 (Diluent)	5.0	Surface water	-	BF
0.100 (BF)	1.0	Surface water	-	SW-BF
0.100 (BF)	1.0	Drinking water	-	DW-BF

Note: Diluent is Methanol (80%) and water (20%) v/v. 'A' - Propanil and 'B' - 3,4-Dichloroaniline.

The solvent (acetonitrile, methanol), blank working solutions (SW-BF and DW-BF), reference standards working solutions (SW-AB and DW-AB) and blank media (surface water and drinking water) were injected onto LC-MS/MS in accordance with section 2.6.6.

2.6.2 Linearity

2.6.2.1 Preparation of Propanil and 3,4-Dichloroaniline Reference Standards Linearity Spiking Solutions

Identification of Reference Standards Working Solution ($\mu\text{g/L}$)			Solution Taken (mL)	Final Volume (mL)	Obtained Concentrations ($\mu\text{g/L}$)		Identification of Reference Standards Working Solutions
A	ID	B			A	B	
13.584	AB-1	13.537	2.88	5	7.824	7.797	SS-6
			1.44	5	3.912	3.899	SS-5
			0.72	5	1.956	1.949	SS-4
			0.36	5	0.978	0.975	SS-3
			0.18	5	0.489	0.487	SS-2
			0.09	5	0.245	0.244	SS-1

The above reference standards linearity spiking solutions (SS-1, SS-2, SS-3, SS-4, SS-5 and SS-6) were prepared in diluent.

2.6.2.2 Preparation of Propanil and 3,4-Dichloroaniline Reference Standards Working Solutions in Surface water

Identification of Reference Standards Working Solutions ($\mu\text{g/L}$)			Solution Taken (mL)	Final Volume (mL)	Obtained Concentrations ($\mu\text{g/L}$)		Identification of Reference Standards Working Solutions
A	ID	B			A	B	
0.245	SS-1	0.244	0.1	1	0.025	0.024	SW-L1
0.489	SS-2	0.487	0.1	1	0.049	0.049	SW-L2
0.978	SS-3	0.975	0.1	1	0.098	0.098	SW-L3
1.956	SS-4	1.949	0.1	1	0.196	0.195	SW-L4
3.912	SS-5	3.899	0.1	1	0.391	0.390	SW-L5
7.824	SS-6	7.797	0.1	1	0.782	0.780	SW-L6

The above reference standards working solutions were prepared in surface water.

2.6.2.3 Preparation of Propanil and 3,4-Dichloroaniline Reference Standards Working Solutions in Drinking water

Identification of Reference Standards Working Solutions ($\mu\text{g/L}$)			Solution Taken (mL)	Final Volume (mL)	Obtained Concentrations ($\mu\text{g/L}$)		Identification of Reference Standards Working Solutions
A	ID	B			A	B	
0.245	SS-1	0.244	0.1	1	0.025	0.024	DW-L1
0.489	SS-2	0.487	0.1	1	0.049	0.049	DW-L2
0.978	SS-3	0.975	0.1	1	0.098	0.098	DW-L3
1.956	SS-4	1.949	0.1	1	0.196	0.195	DW-L4
3.912	SS-5	3.899	0.1	1	0.391	0.390	DW-L5
7.824	SS-6	7.797	0.1	1	0.782	0.780	DW-L6

The above reference standards working solutions were prepared in drinking water.

The reference standards working solutions SW-L1, SW-L2, SW-L3, SW-L4, SW-L5, SW-L6 and DW-L1, DW-L2, DW-L3, DW-L4, DW-L5, DW-L6 for surface water and drinking water were injected onto the LC-MS-MS accordance with section 2.6.6 and peak area was plotted against concentration ($\mu\text{g/L}$). The correlation coefficient (r), slope (b) and intercept (a) were calculated.

2.6.3 Precision (% RSD) and Accuracy (% Recovery)

2.6.3.1 Preparation of Propanil and 3,4-Dichloroaniline Reference Standards Working Solutions at LOQ and 10 x LOQ in Surface Water

Fortification Level ($\mu\text{g/L}$)	Replication	Concentration of Reference Standards Working Solutions ($\mu\text{g/L}$)	Volume Taken (mL)	Final Volume (mL)	Sample ID
LOQ A - (0.100) B - (0.099)	R1	SS-3 A - (0.978) B - (0.975)	0.102	1	SW-Q1
	R2		0.102	1	SW-Q2
	R3		0.102	1	SW-Q3
	R4		0.102	1	SW-Q4
	R5		0.102	1	SW-Q5
	R6		0.102	1	SW-Q6
	R7		0.102	1	SW-Q7

Note: 'A' - Propanil and 'B' - 3,4-Dichloroaniline.

10 X LOQ A - (1.005) B - (1.002)	R1	AB-1 A - (13.584) B - (13.537)	0.074	1	SW-10Q1
	R2		0.074	1	SW-10Q2
	R3		0.074	1	SW-10Q3
	R4		0.074	1	SW-10Q4
	R5		0.074	1	SW-10Q5

All the above solutions were prepared in drinking water. The solutions at LOQ and 10 x LOQ level along with two control samples (surface water) were injected onto LC-MS/MS in accordance with the parameters described in section 2.6.6.

2.6.3.2 Preparation of Propanil and 3,4-Dichloroaniline Reference Standards Working Solutions at LOQ and 10 x LOQ in Drinking Water

Fortification Level (µg/L)	Replication	Concentration of Reference Standards Working Solutions (µg/L)	Volume Taken (mL)	Final Volume (mL)	Sample ID
LOQ A - (0.100) B - (0.099)	R1	SS-3 A - (0.978) B - (0.975)	0.102	1	DW-Q1
	R2		0.102	1	DW-Q2
	R3		0.102	1	DW-Q3
	R4		0.102	1	DW-Q4
	R5		0.102	1	DW-Q5
	R6		0.102	1	DW-Q6
	R7		0.102	1	DW-Q7
10 X LOQ A - (1.005) B - (1.002)	R1	AB-1 A - (13.584) B - (13.537)	0.074	1	DW-10Q1
	R2		0.074	1	DW-10Q2
	R3		0.074	1	DW-10Q3
	R4		0.074	1	DW-10Q4
	R5		0.074	1	DW-10Q5

Note: 'A' - Propanil and 'B' - 3,4-Dichloroaniline.

The above solutions (SW-10Q1 to SW-10Q5) and (DW-10Q1 to DW-10Q5) were further diluted as mentioned in the section 2.6.4.

All the above solutions were prepared in drinking water. The solutions at LOQ and 10 x LOQ level (diluted) along with two control samples (drinking water) were injected onto LC-MS/MS in accordance with the parameters described in section 2.6.6.

2.6.4 Dilution of 10 x LOQ Solutions for Surface and Drinking Waters

A volume of 0.1 mL from each 10 x LOQ sample was transferred to an eppendorf containing 0.9 mL of surface water or drinking water, vortexed and injected onto LC-MS/MS in accordance with the parameters described in section 2.6.6. The dilution factor for 10 x LOQ Solutions was 10.

2.6.5 Typical Calculation

Example for Propanil (Quantitation) in Surface water

$$(\%) \text{ Recovery} = \frac{\text{Calculated Concentration}}{\text{Nominal Concentration}} \times 100 = \frac{0.095}{0.100} \times 100 = 95.00$$

The quantity of Propanil and 3,4-Dichloroaniline in media and regression equation was established by analyst® software version 1.6.2.

2.6.6 Instrumental Parameters

Instrument : LC-MS/MS [API 4000 Mass spectrometer coupled with Nexera X2 HPLC System]
 Column : Luna 3 μ m 100 A° (100 x 2.0 mm)
 Flow Rate : 500 μ L/min
 Injection Volume : 20 μ L
 Oven Temperature : Ambient
 Detector : Sciex 6500
 Run Time : 12 minutes
 Mobile phase : A) 0.1% Acetic acid in LC-MS grade water
 B) 0.1% Acetic acid in LC-MS grade methanol

Gradient Program:

Time (min)	A%	B%	Flow (μ L/min)
0.00	80	20	500
2.00	80	20	500
5.00	10	90	500
9.00	10	90	500
10.00	80	20	500
12.00	80	20	500
12.01	80	20	500

Acquisition Ions and Compound Dependent Parameters:

Analyte	Mass Transition (m/z)	Dwell (msec)	DP (V)	CE (V)	CXP (V)
Propanil (Quantitation)	218.1/162.1	150	40	23	12
Propanil (Confirmatory)	218.1/127.2	150	40	35	12
3,4-Dichloroaniline (Quantitation)	162.1/127.1	150	40	27	12
3,4-Dichloroaniline (Confirmatory)	162.1/74.0	150	40	65	7

Typical MS/MS Voltage Conditions:

Ionization Mode	ESI
Scan Type	MRM
Polarity	Positive
Resolution – Q1	Unit
Resolution – Q3	Unit
Curtain Gas (N ₂)	10
GS1	50
GS2	60
CAD gas (N ₂)	High
Ion Spray (V)	5500
Temperature (°C)	400
EP	10

APPENDIX 2 (Continued)

1. GENERAL INFORMATION

1.1 Study Director

Gopal Upadhyay, M.Sc.

Deputy Study Director

Basheer Shaik, M.Sc.

1.2 Test Facility Management

Dr. Nadeem Ahmad Khan

1.3 Study Schedule

Study Initiation Date	:	April 26, 2018
Experiment Start Date	:	April 28, 2018
Experiment Completion	:	Latest by May 2018
Draft Report Submission	:	Latest by May 2018
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 perform independent laboratory validation of the analytical method for determination of Propanil and 3, 4-Dichloroaniline in surface and drinking waters by LC-MS/MS following the methods used in JRF America study no. AU-2017-40.

2.2 Regulatory Guidelines

Commission Regulation (EU) No 283/2013 setting out the data requirement for active substances, in accordance with Regulation (EC) No 1107/2009.

SANCO/825/00 rev. 8.1 (November 16, 2010), Guidance Document on Pesticide Residue Analytical Methods (Post-registration Requirements for Annex II and Annex III).

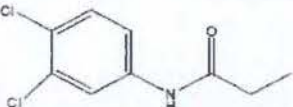
U.S. EPA OCSPP 860.1340 "Residue Analytical Method" August 1996.

U.S. EPA, 2012: OCSPP 850.6100 "Environmental Chemistry Methods and Associated Independent Laboratory Validation" January 2012.

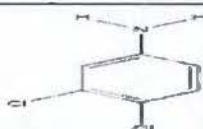
APPENDIX 2 (Continued)

2.3 Test Item

Reference Standard of Propanil and 3, 4-Dichloroaniline will be used as the test item.

Reference Standard	Propanil
IUPAC Name	3',4'-dichloropropionanilide
CAS N°	709-98-8
Molecular Weight	218.08 g/mol
Molecular Structure	
Lot N°	40316
Expiry Date	March 25, 2020
Purity	99.0 %
Source	Dr.Ehrenstorfer
Storage Condition	Refrigerator
JRF Entry Number	PC-3785

Source of IUPAC Name, Source of CAS N°, Molecular Structure and Molecular weight: Certificate of Analysis, e-Pesticide Manual, 15th Edition, 2010.

Reference Standard	3, 4-Dichloroaniline
IUPAC Name	3, 4-Dichloroaniline
CAS N°	95-76-1
Molecular Formula	C ₆ H ₅ Cl ₂ N
Molecular Weight	162.02 g/mol
Molecular Structure	
Batch N°	13509KQV
Reassay Date	September 1, 2018
Purity	99.3%
Source	Sigma Aldrich
Storage Condition	Ambient
JRF Entry Number	PC-4247

Source of IUPAC Name, CAS N°, Molecular Structure, Molecular weight and Molecular Formula: Certificate of Analysis and from e-Pesticide Manual, 15th Edition, 2010.

APPENDIX 2 (Continued)

2.4 Reference Standards Retention

The reference standards will be retained at the Department of Chemistry till 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 Co-operation and Development, Paris (1998) and all subsequent OECD consensus documents.

Good Laboratory Practice standard is considered by the United States Environmental Protection Agency to be compatible with the Environmental Protection Agency (EPA-FIFRA) Title 40 of the US Code of Federal Regulations Part 160, 16 October 1989.

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 Sponsor or the Study Director will be generated, authorized by the Study Director and will be 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 will be confirmed as soon as possible by way of formal study plan amendment.

3.4 Deviation(s)

Any deviation(s) will be approved by the Study Director, documented in the 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 100653). 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.

APPENDIX 2 (Continued)

4. EXPERIMENTAL PROCEDURE

4.1 Equipment/Instruments

LC-MS/MS, Column, Micropipette, Centrifuge, Analytical balance, Vortex mixer, Refrigerator and Sonicator.

4.2 Solvents and Chemicals

Acetonitrile, Water (Distilled and Milli-Q), Acetic acid, Methanol.

4.3 Test System

4.3.1 Water information

Test system is Surface water and Drinking water. Surface water samples will be collected from the local region of Gujarat, India. Drinking water available at JRF will be used for the validation. All the characteristics and source of test media will be incorporated in the raw data and in the final report as well.

4.4 Validation of the Method

The analytical method will be validated for determination of Propanil and 3, 4-Dichloroaniline in surface and drinking waters. The validation will cover the aspects: (i) Specificity, (ii) Linearity, (iii) Accuracy (%Recovery) and Precision (% RSD).

4.4.1 Specificity

The solvent (acetonitrile and methanol), test item (i.e Propanil & 3, 4-Dichloroaniline) solution and blank of surface and drinking waters will be injected onto LC-MS/MS using section 4.7. The interference (if any) in the determination of active ingredient concentration will be reported.

4.4.2 Linearity

At least five different concentrations of Propanil and 3,4-Dichloroaniline reference standard solutions will be prepared and analyzed to empirically determine the linearity of the detector response using $1/x$ as weighting factor. The calibration curve will extend beyond (by at least 20%) the highest and lowest nominal concentration (30% of LOQ, if sensitivity allows of it) of the analyte in the relevant analytical solutions. The correlation coefficient ($r > 0.99$), intercept (a) and slope (b) will be calculated. The regression equation will be established.

4.4.3 Accuracy and Precision

Accuracy and precision of the analytical method will be performed at two fortification level i.e., at LOQ and $10 \times$ LOQ along with two untreated control sample and a reagent blank sample. LOQ & $10 \times$ LOQ will be fortified with Propanil and 3, 4-Dichloroaniline at $0.1 \mu\text{g/L}$ and $1.0 \mu\text{g/L}$ for surface and drinking waters. Seven determinations at LOQ and five determinations at $10 \times$ LOQ fortification level will be made. The active ingredient concentration in each level, mean of active ingredient concentration, SD and % RSD will be calculated and reported.

APPENDIX 2 (Continued)

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Recovery values should fall within 70 to 120%. The RSD of replicate recovery measurements should not exceed the level of 20 % at or above the LOQ, and any interference should be negligible ($\leq 30\%$ of the response found in the sample fortified at LOQ). If the majority of recoveries do not fall within the range the reason will be identified and with the Sponsor's permission an additional attempt will be made with such modifications that are allowed within the method. Should a second attempt fail, the Sponsor will again be notified and an additional attempt will be made.

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

4.4.4 Confirmation of Analyte

The LC-MS/MS analytical method for the determination of Propanil and 3, 4-Dichloroaniline concentrations in surface and drinking waters will be confirmed by additional MRM mass transition.

4.5 Preparation of Propanil and 3, 4-Dichloroaniline Stock Solution

A known quantity of Propanil and 3,4-Dichloroaniline standard will be weighed in a volumetric flask of known capacity, dissolve Propanil using Acetonitrile (LCMS grade) and 3, 4-Dichloroaniline stock in Methanol (LCMS grade) l. Different concentration of standard solutions will be prepared in test system.

4.6 Extraction Procedure

Extraction procedure for sample preparation will be followed as per described method in JRFA Method AU-287R0.

4.7 Instrumental Parameters

Instrument	: LC-MS/MS
Column	: Luna 3 μ m 100 A $^{\circ}$, 100 x 2.0mm,
Flow Rate	: 500 μ L/min
Injection Volume	: 20 μ L
Injector Temperature	: Ambient
Detector	: Sciex 6500
Run Time	: 12 minutes
Mobile phase	: A) 0.1% Acetic acid in LC-MS grade water B) 0.1% Acetic acid in LC-MS grade methanol

APPENDIX 2 (Continued)

Gradient Program:

Time(min)	A%	B%	Flow(μ L/min)
0.00	80	20	500
2.00	80	20	500
5.00	10	90	500
9.00	10	90	500
10.00	80	20	500
12.00	80	20	500
12.01	80	20	500

Acquisition Ions and Compound Dependent Parameters:

Analyte	Mass Transition (m/z)	Dwell (msec)	DP (V)	CE (V)	CXP (V)
Propanil (Quantitation)	218.1/162.1	150.0	40	23	12
Propanil (Confirmatory)	218.1/127.2	150.0	40	35	12
3,4-Dichloroaniline (Quantitation)	162.1/127.1	150.0	40	27	12
3,4-Dichloroaniline (Confirmatory)	162.1/74.0	150.0	40	65	7

Typical MS/MS Voltage Conditions:

Ionization Mode	ESI
Scan Type	MRM
Polarity	Positive
Resolution – Q1	Unit
Resolution – Q3	Unit
Curtain Gas (N ₂)	10
GS1	50
GS2	60
CAD gas (N ₂)	High
Ion Spray (V)	5500
Temperature (°C)	400
EP	10

Note: MS settings as provided above should be used as guidelines only. For optimal results, compound and source optimization should be performed by the analyst. Any such changes will be recorded in the raw data form and in the report.

APPENDIX 2 (Continued)

5. REPORT

Unless otherwise requested by the Sponsor, original final report 2 of 2 will be issued along with one soft copy in PDF. The report will include the following information:

- Description of the reference standard along with certificates of analysis
- Complete details of the experimental procedure
- Summary of observations
- Results
- Mass Chromatograms
- Personnel involved in the study
- The signed study plan and study plan amendment(s) [if any]
- Record of deviation(s) (if any)

6. ARCHIVES

On completion of the study, all original raw data including any storage medium for electronically recorded data, documentation, the signed study plan, any study plan amendment, the draft report and original final report 1 of 2 will be retained in the GLP Archives at Jai Research Foundation for a period of ten years. At the end of this period, the Sponsor's instructions will be sought to either extend the archiving period or return the archived material to the Sponsor or for the material to be disposed of.

7. REFERENCES

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APPENDIX 2 (Continued)

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