JAI RESEARCH FOUNDATION

Original Amended Final Report I (2 of 2)

STUDY TITLE

INDEPENDENT LABORATORY VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF RESIDUES OF CAPTAN IN DRINKING WATER AND SURFACE WATER BY GC-MS

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

GUIDELINES: SANTE/2020/12830, Rev.1 (February 24, 2021)
OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation

STUDY DIRECTOR/REPORT AUTHOR: PURUSHOTTAM TRIVEDI

STUDY COMPLETION: NOVEMBER 02, 2021

AMENDED REPORT SUBMISSION: JULY 29, 2025

SPONSORS

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C/O MAKHTESHIM AGAN OF NORTH AMERICA, INC (dba ADAMA)
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DEPARTMENT OF CHEMISTRY
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TEST FACILITY STUDY NUMBER: 228-2-13-29211
UPL STUDY NUMBER: UPL/2021/0595
ADAMA REFERENCE NUMBER: 000109249

1. INTRODUCTION

1.1 Study Objective

The objective of this study was to independently validate the analytical method for determination of residue of Captan in drinking water and surface water by GC-MS analysis as part of ILV. The methodology to validate was as per Study entitled "Validation of an analytical method for the determination of Captan in drinking water and surface water", Study code of testing facility: 20041453/01-RVW, Performed at GAB Biotechnologie GmbH & GAB Analytik GmbH Eutin Str. The study was conducted in compliance with the OECD Principles of GLP (1998).

1.2 Study Guidelines

The present study was conducted according to:

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

SANTE/2020/12830: Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes, Rev.1 (February 24, 2021).

OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation. January 2012.

1.3 Test Facility and Study Period

This study was performed at the Department of Chemistry, Jai Research Foundation, Valvada – 396 105 Dist. Valsad, Gujarat, India.

Study Initiation	:	September 29, 2021
Experiment Start	:	October 01, 2021
Experiment Completion	:	October 26, 2021
Study Completion	:	November 02, 2021
Amended Report Submission	*:*	*July 29, 2025*

1.4 Personnel Involved in the Study

Study Director	:	Purushottam Trivedi, Ph.D.
Deputy Study Director	:	A.H. Patel, M.Sc.

1.5 Archives

All original raw data including any storage medium for electronically recorded data, documentation, chromatograms, the signed study plan, the *study plan amendments,* the draft report, *draft amended report I,* original final report 1 of 2, *original amended final report I (1 of 2),* and representative sample of the test item 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 dispose of the material.

2. EXPERIMENTAL PROCEDURE

2.1 Test Item

Details of Test Item provided by the Sponsor are:

Test Item	Captan
IUPAC Name	3a,4,7,7a-tetrahydro-2-[(trichloromethylthio)]-1H-isoindole-1,3(2H)-dione
Molecular Weight	300.58 g/moL
CAS N°	133-06-2
Lot N°	625-054-00
Analysis Date	September 17, 2021
Expiry Date	September 17, 2023
Appearance	White Solid
Analysed Purity (Provided by Sponsor)	100 % (Refer Certificate of Analysis in APPENDIX 3)
Supplied to JRF by	ADAMA Makhteshim Ltd
Storage Condition (at JRF)	As per the instruction received from the Sponsor on storage of the test item, was stored as follows: Storage Temperature : Room temperature (15 to 25 °C) Storage Condition : Stored in a well-closed container Protection from light Storage Container : In an original container as supplied by the Sponsor Storage Location : Test Item Control Office, JRF

2.2 Equipment/Instruments

Sr. N°	Instrument/Apparatus	Model	Make / Supplier
1	GC-MS	7890B/5977A MSD	Agilent Technologies
2	Balance	GR-202	Adair and Dutt
3	Refrigerator	Eon	Godrej
4	Sonicator	UCB 70D	Spectra lab instrument Pvt. Ltd.
5	Nitrogen Evaporator	Caterpillar	Takahe Analytical Instruments
6	Spinix Vortex Shaker	Spinix	Tarsons
7	SPE Manifold	-	Waters
8	Magnetic Stirrer	MS-H-Pro^{+}	DLAB Instrument Pvt. Ltd.
9	Micropipette	-	Eppendorf

2.3 Solvents and Chemicals

Sr. N°	Name	Grade	Source
1	Acetone	HPLC	Advent
2	n-Hexane	Milli-Q	Rankem
3	Acetic Acid	HPLC	Finar
4	Citral	HPLC	Sigma Aldrich
5	Bond Elute PPL Cartridge	-	Agilent Technologies

2.4 Test System

Drinking water samples was collected from the JRF Golghar, JRF Vapi, Valsad Gujarat, India on date October 01, 2021 and transported to laboratory. Surface water samples was collected from the Daman Ganga River, Vapi, and Valsad Gujarat, India on date October 01, 2021, and transported to laboratory. Same samples were sent for physical chemical parameter determination at Pollucon Lab Surat, Gujarat, India (non-GLP). For the analysis JRF does not claim the GLP compliance.

Parameter	Drinking Water	Surface Water	Method
Appearance	Clear Transparent Liquid	Yellowish Turbid Liquid	By Visual Inspection
pH (at 20 °C)	7.14	7.48	IS 3025 (Part 11) 2019
Specific Electric Conductivity (at 20 °C, µS/cm)	639	175	IS 3025 (Part 14) 2019
Total Hardness (mmol/L)	3.54	0.79	IS 3025 (Part 21) 2019
Spectral absorption Coefficient (at 254 nm, m^{-1})	3.341	3.435	-
Dissolved content (mg/L)	8.06	6.97	APHA (23rd Edition 2017) 5310 B

2.5 Independent Laboratory Validation of an Analytical Method

The analytical method for the determination of concentration of captan in drinking water and surface water was validated by analyses of test item. The validation covered the following aspects namely (i) Matrix Effect, (ii) Calibration (Linearity), (iii) Limit of Detection (LOD), (iv) Limit of Quantification (LOQ), (v) Recovery and Repeatability (%, RSD), (vi) Selectivity/Specificity and (vii) Standard solution stability, extract stability and confirmation of analyte.

2.5.1 Matrix Effect

2.5.1.1 Preparation of Stock Solution

Weight (mg) of Test Item	Purity (%)	Final Volume (mL)	Volume Made up with	Obtained Concentration (µg/L)	Identification of Test Item Stock Solution
10.01	100	10	Acetone	1001000	A
Identification of Test Item Solution	**Solution Taken (mL)**	**Final Volume (mL)**	**Volume Made up with**	**Obtained Concentration (µg/L)**	**Identification of Test Item Working Solution**
A	1.0	10	Acetone	100100	A1
A1	0.15	10	Acetone	1501.50	A2
A2	0.33	1.0	n-Hexane	495.50	MMDSW01
A2	0.33	1.0	Blank Extract of DW*	495.50	MMDW02
A2	0.33	1.0	Blank Extract of SW*	495.50	MMSW01
A1	0.05	10	Acetone	500.50	A3
A3	0.10	1.0	n-Hexane	50.05	MMDSW02
A3	0.10	1.0	Blank Extract of DW*	50.05	MMDW03
A3	0.10	1.0	Blank Extract of SW*	50.05	MMSW02

Note: DW = Drinking Water, SW = Surface Water, MMSW = Matrix Match Surface Water, MMDW = Matrix Match Drinking Water and MMDSW = Matrix Match Drinking Surface Water (prepared in solvent)

*Blank Extract of DW and Blank Extract of SW = A volume of 500 ml of blank drinking water and blank surface water were taken into glass bottle and extracted as described in section 2.5.1.2 from step 2.

The solutions MMDSW02, MMDW03, MMSW02, MMDSW01, MMDW02, MMSW01 were injected in triplicates onto the GC-MS in accordance with **section 2.5.8** along with the blank solvent (n-Hexane), blank extract of drinking water (B-DW) and blank extract of surface water (B-SW).

2.5.1.2 Extraction Procedure

1. A volume of 500 mL of water sample (drinking water and surface water- blank or fortified) were taken into glass bottles separately.
2. A volume of 0.2 mL of acetic acid was added. The sample was homogenized for half a minute using a magnetic stirrer.
3. SPE cartridges were attached to a vacuum manifold using PTFE connectors and conditioned with each 3 mL of acetone, followed by 2 x 3 mL demineralized water.

4. The water samples were attached to a vacuum manifold using PTFE connector and passed through the SPE cartridges at about 1-2 drops per second.
5. After sampling, the cartridges were dried by sucking air through them for 5 minutes using the vacuum manifold.
6. 2 mL of volumetric flasks was placed under the cartridges and residue of captan compound was eluted with 2.5 mL of acetone.
7. The solvent was evaporated to dryness using a gentle nitrogen stream and was reconstituted in 1 mL of n-hexane.
8. A volume of 10 µL citral were added into each sample. The samples were injected on GC/MS.

2.5.2 Selectivity/Specificity

2.5.2.1 Preparation of Working Solutions

Identification of Solution and Concentration (µg/L)	Solution Taken (mL)	Final Volume (mL)	Volume Made up with	Obtained Concentration (µg/L)	Identification of Working Solution
A2 - 1501.50	0.1	1.0	n-Hexane	150.15	A4
A4 -150.15	0.1	1.0	Blank Extract of DW*	15.02	MDWL01
A4 – 150.15	0.1	1.0	Blank Extract of SW*	15.02	MSWL01
A1 – 100100	0.1	10.0	Acetone	1001.00	FS01
A1 - 100100	0.1	10.0	Acetone	1001.00	FS02

*Blank Extract of DW and Blank Extract of SW = A volume of 500 ml of blank drinking water and blank surface water were taken into glass bottle and extracted as described in section 2.5.1.2 from step of 2.

For fortified samples at LOQ level, 500 mL of blank drinking water and surface water sample transferred into 500 mL reagent bottle and added a volume of 0.05 mL of FS01 and FS02 respectively and then extracted as per section 2.5.1.2. from step 2. Identification of the sample was DW-LOQ1 and SW-LOQ1.

The solutions MDWL01, MSWL01, DW-LOQ1 and SW-LOQ1 were injected onto the GC-MS in accordance with **section 2.5.8** along with the blank solvent (n-Hexane), blank extract of drinking water (B-DW) and blank extract of surface water (B-SW).

2.5.3 Calibration (Linearity)

Calibration level covered a range linearity from 30% of the LOQ and 20% above the highest level. The calibration range were 15.02 to 750.75 µg/L (Equivalent to 0.03 µg/L to 1.5 µg/L in water after Solid Phase Extraction) (Refer Amendment No. 2)

2.5.3.1 Preparation of Working Solutions

Identification of Test Item Solutions and Conc. (µg/L)	Solution Taken (mL)	Final Volume (mL)	Volume Made up with	Obtained Concentration (µg/L)*	Identification of Test Item Working Solutions
A - 1001000	0.10	10	Acetone	10010.0	K01
	0.15	10		15015.0	K02
K01 - 10010	0.75	5	n-Hexane	1501.5	K03
	0.50	5		1001.0	K04
K02 - 15015	0.25	5		750.75	L06
K01 - 10010	0.25	5		500.50	L05
	0.125	5		250.25	L04
K04 - 1001	0.50	5		100.10	L03
L05 - 500.50	0.50	5		50.05	L02
K03 - 1501.5	0.05	5		15.02	L01

*(15.02 µg/L to 750.75 µg/L, Equivalent to 0.03 µg/L to 1.5 µg/L in water after Solid Phase Extraction)

The working solutions **L01, L02, L03, L04, L05, and L06,** were injected onto the GC-MS in accordance with **section 2.5.8** and peak area was plotted against concentration. The slope (b) and intercept (a) were calculated. The sample acquisition and quantification were performed by Mass hunter Quantitative Analysis Software.

2.5.4 Recovery and Repeatability (% RSD)

2.5.4.1 Preparation of Test Item Working Solutions in Drinking Water

Identification of Test Item Solution and Concentration (µg/L)	Solution Taken (mL)	Final Volume (mL)	Volume Made up with	Obtained Concentration (µg/L)	Identification of Test Item Working Solution
A1 - 100100	1.0	10	Acetone	10010.0	FS03
FS03 - 10010	1.0	10		1001.0	FS04

For control samples, 500 mL of blank drinking water and surface water sample were taken into glass bottle and extracted as described in section 2.5.1.2 from step 2. Identification for control drinking water sample were DW-C I and DW-C II and for control surface water were SW-C I and SW-C II. For fortified samples, 500 mL of blank drinking water and surface water sample transferred into 500 mL reagent bottle and solution fotified according to the table below and then extracted as per section 2.5.1.2.

Test Specimen	Level of Fortification (µg/L)	Replication	Concentration of fortification solution (µg/L)	Volume of fortification solution (mL)	Sample Volume (mL)	Sample ID
Drinking Water	LOQ (0.1)	DQR1	FS04 – 1001.0	0.05	500	DW-LOQ01
		DQR2		0.05	500	DW-LOQ02
		DQR3		0.05	500	DW-LOQ03
		DQR4		0.05	500	DW-LOQ04
		DQR5		0.05	500	DW-LOQ05
	10 x LOQ (1.0)	DHR1	FS03 – 10010.0	0.05	500	DW-10LOQ01
		DHR2		0.05	500	DW-10LOQ02
		DHR3		0.05	500	DW-10LOQ03
		DHR4		0.05	500	DW-10LOQ04
		DHR5		0.05	500	DW-10LOQ05
Surface Water	LOQ (0.1)	QR1	FS04 – 1001.0	0.05	500	SW-LOQ01
		QR2		0.05	500	SW-LOQ02
		QR3		0.05	500	SW-LOQ03
		QR4		0.05	500	SW-LOQ04
		QR5		0.05	500	SW-LOQ05
	10 x LOQ (1.0)	HR1	FS03 – 10010.0	0.05	500	SW-10LOQ01
		HR2		0.05	500	SW-10LOQ02
		HR3		0.05	500	SW-10LOQ03
		HR4		0.05	500	SW-10LOQ04
		HR5		0.05	500	SW-10LOQ05

The solutions of each matrix for captan at LOQ [DW-LOQ01 to DW-LOQ05], [SW-LOQ01 to SW-LOQ05] and 10 x LOQ level [DW-10LOQ01 to DW-10LOQ05], [SW-10LOQ01 to SW-10LOQ05] along with two control extract samples [DW-C I and DW-C II, SW-C I and SW-C II] were injected onto GC-MS in accordance with the parameters described in **section 2.5.8**

2.5.5 Extract Stability

For extract stability in drinking water and surface water at LOQ and 10 x LOQ level, 500 mL of drinking and surface water sample were taken into three-three separate 500 mL reagent bottles and fortification solution added according to the table below and then extracted as per section 2.5.1.2.

Test Specimen	Level of Fortification (µg/L)	Replication	Concentration of fortification solution (µg/L)	Volume of fortification solution (mL)	Sample Volume (mL)	Sample ID
Drinking Water	LOQ (0.1)	EX DW Q1	FS04 – 1001.0	0.05	500	EXDQ1
		EX DW Q2		0.05	500	EXDQ2
		EX DW Q3		0.05	500	EXDQ3
	10 x LOQ (1.0)	EX DW 10-Q1	FS03 – 10010.0	0.05	500	EXD10Q1
		EX DW 10-Q2		0.05	500	EXD10Q2
		EX DW 10-Q3		0.05	500	EXD10Q3
Surface Water	LOQ (0.1)	EX SW Q1	FS04 – 1001.0	0.05	500	EXSQ1
		EX SW Q2		0.05	500	EXSQ2
		EX SW Q3		0.05	500	EXSQ3
	10 x LOQ (1.0)	EX SW 10-Q1	FS03 – 10010.0	0.05	500	EXS10Q1
		EX SW 10-Q2		0.05	500	EXS10Q2
		EX SW 10-Q3		0.05	500	EXS10Q3

For control samples 500 mL of blank drinking water and surface water sample were taken into glass bottle and extracted as described in section 2.5.1.2 from step 2. Identification of control drinking water sample were EX-CI DW and for control surface water were WX-CI SW.

Extract solutions of drinking and surface water at LOQ and 10 x LOQ level were prepared in triplicates on dated October 08, 2021, and stored in refrigerator. The same samples were injected on to GC-MS against fresh linearity as described in section 2.5.3 on dated October 26, 2021 (18 Day) in accordance with **section 2.5.8**. All solutions were stored in a refrigerator when not in use.

The solutions of each matrix for captan at LOQ [EXDQ1 to EXDQ3], [EXSQ1 to EXSQ3] and 10 x LOQ level [EXD10Q1 to EXD10Q3], [EXS10Q1 to EXS10Q3] along with control extract samples [EX-CI DW and WX-CI SW] were injected onto GC-MS in accordance with the parameters described in **section 2.5.8.**

2.5.6 Standard Stability

2.5.6.1 Preparation of Standard Working Solutions

Weight (mg) of Test Item	Purity (%)	Final Volume (mL)	Volume Made up with	Obtained Concentration (µg/L)	Identification of Test Item Stock Solution
10.43	100	10	Acetone	1043000	N
Dilution of Working solution					
Identification of Test Item Solution and Concentration (µg/L)	**Solution Taken (mL)**	**Final Volume (mL)**	**Volume Made up with**	**Obtained Concentration (µg/L)**	**Identification of Working Solution**
A (1001000)	0.15	10.0	Acetone	15015.00	AN01
AN01 (15015.00)	0.25	5.0	n-Hexane	750.75	SSS-OLD
N (1043000)	0.15	10.0	Acetone	15645.00	N02
N02 (15645)	0.25	5	n-Hexane	782.25	SSS-NEW

Standard Solutions SS-OLD and SS-NEW were injected onto GC-MS by making five injections of each sample on dated 26.10.2021 in accordance with **section 2.5.8**.

2.5.7 Calculation

The intercept (a) and slope (b) were calculated, and the regression equation was established to calculate the concentration of standard and the concentration of analyte. The sample acquisition and quantification was performed by Mass hunter Quantitative Analysis Software.

Matrix effects was calculated using following equation:

$$\text{Matrix Effect \%} = \frac{\text{Peak area of matrix match standard} - \text{Peak area of standard in solvent}}{\text{Peak area of standard in solvent}} \times 100$$

The regression residual “di” was calculated using following formula.

$di = Yi – Yyi$

where Yi is the measured value i;

Yyi is the estimated value which corresponds to yi and is derived from the calibration function.

$$\text{Active Ingredient concentration, (µg/L)} = \frac{Y - a}{b} \times D$$

Where,

- Y = Peak area of the sample
- a = Intercept
- b = Slope of the line
- D = Dilution factor

The repeatability (% RSD) was calculated using the following formula:

$$\text{Repeatability (\% RSD)} = \frac{\text{Standard Deviation}}{\text{Mean (\%) Recovery}} \times 100$$

The % Recovery was calculated using the following formula:

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

The following equation was used to calculate the residue R in samples

$R = C \times V_{End} / V$

Where:

R: Residue found, in µg/L

C: Captan Concentration in Final Extract

V_{End}: Final volume of Extract

V: Sample Volume

2.5.8 Instrumental Parameters

Instrument	:	GC-MS [Agilent Technologies 7890B Mass Hunter 5977-A-MSD]
Column	:	HP 5 MS [30 m x 0.25 mm x 0.25 µm film thickness]
Carrier gas	:	Helium, 1 mL/min
Injection	:	2 µL pulsed Splitless at 200 °C, 150 kPa for 1 min.
Temperature program	:	90 °C, 20 °C/min to l30°C, 10 °C/min to 195 °C, 5 °C/min to 205 °C, 30 °C/min to 310 °C, hold 5 min
Temperatures	:	Transfer line: 280 °C Ionizer: 230 °C (EI+, 70 eV) Quadrupole: 150 °C
SIM masses	:	Quantifier Ion = 264 m/z Qualifier Ion I = 266 m/z Quantifier Ion II = 114 m/z
Retention Time	:	Captan – 11.8 minutes (approximately)

2.5.9 Communication with Method Developer

Primary method validation and independent laboratory validation were carried out at different locations, by different study personnel, and using different instrumentation, equipment, and stocks of chemicals. No direct communication with the method developer or individuals previously familiar with the method was required to perform the analysis. The method was sufficiently detailed and robust to allow independent execution without additional guidance.

4. CONCLUSION

From the results of the independent laboratory method validation, it is concluded that the method is sensitive, precise and accurate for the determination of residue of captan in drinking water and surface water with an LOQ of 0.1 µg/L. Stock solutions of captan was stable for at least 26 days at refrigerated condition. Drinking water and surface water extract solutions were stable for at least 18 days at refrigerator condition. The results of validation criteria are within the guideline SANTE/2020/12830, Rev.1 (February 24, 2021) and OCSPP 850.6100 specified limit. *Primary method validation and independent laboratory validation were carried out at different locations, by different study personnel, and using different instrumentation, equipment, and stocks of chemicals. No direct communication with the method developer or individuals previously familiar with the method was required to perform the analysis. The method was sufficiently detailed and robust to allow independent execution without additional guidance.*

5. REFERENCES

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

JRF/PC/SOP-184, 2021: Validation of Analytical Method for Determination of Active Ingredient Concentration and Stability of Test Item in Matrix.

JRF/PC/SOP-4176: 2021 “Analytical Method and Independent Laboratory Validation for the Determination of Residue of Compound in Matrix”.

OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation. January 2012.

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SANTE/2020/12830, 2021: Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes Rev.1 (February 24, 2021).

Thom, Marcel., 2005 Validation of an Analytical Method for the Determination of Captan in Drinking Water and Surface Water Study code of testing facility: 20041453/01-RVW April 27, 2005, Study Report of Calliope S.A.S. Route d Artix BP80 64150 Noguères France.