FARELABS

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Report

On

Effect of 4Well Alkaline Drops on the Removal of Pesticide Residues in Water

Submitted To

Darju9 Enterprises Pvt. Ltd.

41/35, West Punjabi Bagh,

New Delhi-110026

FARE Labs Pvt. Ltd.

L-17/3, DLF Phase II, MG Road, IFFCO Chowk, Gurgaon

Haryana-122002, India

Tel: 0124-4034205, 4223207-08

Fax: 0124-4036038

E-mail: farelabs@farelabs.com, Website: www.farelabs.com

Project Title	:	**Effect of 4Well Alkaline Drops on the Removal of Pesticide Residues in Water**
Project Study held at	:	FARE Labs Pvt. Ltd. L-17/3, DLF Phase II, MG Road, Gurgaon, Haryana
Principal Investigator	:	**Dr. Meenakshi Tripathi** Quality Manager, FARE Labs Pvt. Ltd.
Project Execution Team	:	**Dr. Konda Reddy Kunduru** Scientist C **Ms. Nidhi** Scientist C **Mr. Mithilesh Kumar** Scientist B **Mr. Ashutosh Tiwari** Scientist B **Ms. Astha Kumari** Scientist B
Project Duration	:	10/08/2023 to 15/08/2023

ACKNOWLEDGEMENT

FARE Labs Pvt. Ltd. takes this opportunity to express the thanks to Darju9 Enterprises Pvt. Ltd. for the project on "Effect of 4Well Alkaline Drops on the Removal of Pesticide Residues in Water".

Mr. D. Mathur
Director

Dr. Meenakshi Tripathi
Quality Manager

TEST REPORT

Issued to: Darju9 Enterprises Pvt. Ltd.
41/35, West Punjabi Bagh,
New Delhi-110026

J.O. No.: FL/W/SL/10082023-002
Report Date: 15-08-2023
Sample Receipt Date: 10-08-2023
Account Manager: BD Team 1
Credit Manager: Gulab Singh

Customer Provided Information: #
Nature/Name of the Sample : **4 Well Alkaline Drops**

Laboratory Provided Information:
Sample Quantity & Packaging : 30ml x 1, Glass Bottle
Date of Performance of Test : 10th – 15th August, 2023
Method of Sampling : Sample is provided by Darju9 Enterprises Pvt. Ltd., through Courier.

Analysis Report

S. No.	Parameters	Test Results
1.	Effect of 4Well Alkaline Drops on the Removal of Pesticide Residues in Water	Report Attached

Reviewed by

Authorized Signatory
Dr. Konda Reddy Kunduru
Scientist-C

1. INTRODUCTION

This report summarizes the work on the "Effect of 4Well Alkaline Drops on the Removal of Pesticide Residues in Water".

2. OBJECTIVES

The objective of the study was as under:

- To test the 4Well Alkaline Drops effect on the removal of pesticide residues in water sample

3. DETAILS OF EXPERIMENTS

3.1. EQUIPMENTS

- Gas Chromatography -Tandem Mass Spectrometer - Agilent 7000C Series , 7890B GC-MS/MS (or) equivalent and Agilent 6460 & 1260 Series LC-MS/MS Conditions -Multi Residue Method
- Nitrogen Concentrator Low Volume , temperature controlled
- Micropipettes 10-100 µL capacity and 100-1000 µL capacity, calibrated
- Ultrasonic Bath, temperature controlled
- Water bath, temperature controlled
- Mechanical Shaker , Horizontal
- Vortex Mixer

3.2. CHEMICALS AND GLASSWARES

- Volumetric Flasks - 10ml and 100 ml
- Eppendorf tube 2 ml
- Auto sampler vials
- Milli - Q Water
- Sodium Chloride, Merck
- Anhydrous Sodium Sulfate, AR Grade
- Methanol, HPLC Grade
- Ethyl acetate, HPLC Grade
- Certified Reference Material
- Separating Funnel-2 Litre
- Dichloromethane- HPLC Grade
- Hexane -HPLC Grade
- Acetonitrile- (J.T. Baker)
- Filter Paper-0.2 µm

3.3. PREPARATION OF STANDARDS

3.3.1. Preparation of Pesticide Standard Stock Solution (1000 mg/L)

Weighed equivalent to 10mg (±0.1 mg) of standard into a 10mL volumetric flask and dissolved in Acetonitrile and Made into the volume with the same. Labelled the name of the standard, concentration and preparation and expiry date and stored the solution in a refrigerator at 4-8 °C. Prepared the stock standards individually.

3.4. EXTRACTION PROCEDURE

3.4.1. Method for Multi-Residues Extraction

- 150 mL of water sample was taken in a separating funnel (500 mL) and added 8 gm of sodium chloride, and shake well until sodium chloride dissolved in it. (The purpose of adding NaCl was to avoid emulsion during solvent extraction).

- Followed by added of 25 ml Dichloromethane through measuring Cylinder into separating funnel and mixed well for 20 minutes and kept it aside to stand for 5 minute for the separation of the organic layer.
- The bottom separated organic layer was collected in round Bottom Flask. (Note: no water droplets should not enter the organic layer).
- Dried the organic layer by adding small amount of Na_2SO_4 in to round bottom flask and mixed by shaking the flask for 2 minutes
    - (Na_2SO_4 activated with acetone and dry it in oven for 1 hour at 140 °C and cool in Desiccator and place in air tight container).
- Then transferred the organic layer in to another Round Bottom Flask.
- Add 25 ml Dichloromethane with measuring cylinder in same separatory
- Funnel and repeat the above mentioned procedure twice.
- Collected all the organic dichloromethane layer and evaporated using Nitrogen Concentrator at 35°C until complete to dryness.
- To the round bottom flask, added of 5 ml hexane and evaporated until complete to dryness.
- Reconstituted with 1 ml of ethyl acetate and filter through 0.20 Micron Nylon filter membrane and inject on GCMS/MS
- Reconstituted with 1 ml of 50:50 water: ACN and filtered through 0.20 Micron Nylon filter membrane and inject on LCMS/MS.

3.5. EQUIPMENT METHODS

3.5.1. Agilent 7000C & 7890B GCMSMS Conditions for Multi Residues Method

Equipment	Gas Chromatograph- Mass Spectrometer (GCMSMS)		
Make & Model	AGILENT- 7000C; 7890B		
Column	HP5-MS (30 m X 250 mm x 0.25 µm)		
Gas Chromatography Conditions			
Injection Volume	1 µl		
Oven Program			
	Temp (°C / min)	Temp (°C)	Hold Time (Min.)
		70	2
	25	150	0
	3	200	0
	8	280	10
Injector temperature program	Temp (°C / min)	Temp((°C)	Hold Time (Min.)
Total Flow	25 ml/min -	280	-
Purge Flow to split vent	30 ml/min		
Carrier Gas	Helium		
Carrier gas flow rate	2.66 ml/min		
Maximum Temperature	325 °C		
Run time	41.86 min		
MSMS Conditions			
Acquisition Mode		MRM	
Collision Gas		Nitrogen	
Ion Source Temperature		280 °C	

3.5.2. Agilent 6460 & 1260 Series LC-MSMS Conditions -Multi Residue Method

Equipment	Liquid Chromatography-Tandem Mass Spectrometer (LCMSMS)		
Make & Model	AGILENT 6460 –MSMS, 1260 Infinity –LC		
Column	Zorbax RRHD-C18,1.8 µm, 3.0 X 100 mm, Pressure-600 bar		
Liquid Chromatography Conditions			
Mobile Phase A	5mM Ammonium Formate and 0.01% Formic acid in water		
Mobile Phase B	Acetonitrile (0.1%)		
Flow Rate	0.5ml/minute		
Injection Volume	2.0 µl/min		
Column Temperature	40°C		
Mode	Gradient		
	Time (Min.)	A %	B %
	0	90	10
	1	90	10
	3	10	90
	8	10	90
	9	90	10
	15	90	10
MSMS Conditions			
Mode	ESI Positive, Negative		
Gas temperature (°C)	300		
Gas flow (l/min)	5		
Nebulizer (psi)	45		
Sheath Gas Temp.	250		
Sheath Gas Flow	5		
Capillary voltage	3000		
Delta EMV	500		

3.6. CALCULATIONS

Reference Material – Salt & Purity Correction Formula:

$$X = \frac{(Molecular\ weight\ of\ Compound\ with\ salt - \text{Salt Mass})}{Molecular\ weight\ of\ Compound\ with\ salt} * Reference\ Material\ Weight * Purity$$

Purity = x/100 where x = percentage of compound

Final concentration in solution Y (mg/L) = X *1000/V where V is the makeup volume.

3.7. BATCH SEQUENCE AND QUALITY CONTROL ACCEPTANCE CRITERIA

S. No.	QC Point	Criteria	Run
1	Solvent Blank	Analyte Free	1
2	Calibration Standard-5 Levels	$R^2 \geq 0.9900$	each 1
3	Matrix Blank	Analyte Free (or) <30% LOD	1
4	QC Recovery @ LOQ	70% to 120% Refer Validation data (or) as prescribed in the standard	1
5	Reagent Blank	Analyte Free	1

3.8. Laboratory Quality Control Check

- Spike concentrations at MRL level for any pesticides to check the recovery.
- Suitable concentration at or around LOQ level was taken for spike and recovery for the analyte having LOQ>0.01ug/L.
- Calculate the recovery and compare against the standard method validation study.

CALCULATIONS

$$Conc, mg/ltr. = \frac{(Concentration\ From\ calibration\ X\ Volume\ make\ up\ )}{Volume\ of\ sample(ml)}$$

Results with Recovery Correction: Calculated concentration X Recovery factor

$$Conc, mg/ltr = \frac{(\text{Sample Area K Standard Con.(ppmK Volume make up)})}{\text{Standard Area K Volume of sample(ml)}}$$

3.9. Preparation Test Water Sample

- The milli-Q water (Resistivity at 25 °C, 18.2 MΩ.cm) from our facility was used to prepare the pesticide residues spiked (100 µg/L) water sample.
- The above prepared pesticide residues spiked sample was used throughout the study

4. RESULTS

The water sample without the addition of 4Well alkaline drops and with the addition of 2 drops and 4 drops of 4Well alkaline drops were extracted (Figure 1) for the pesticide residues in to organic solvent and injected as per the requirement of the analysis in GC-MS/MS and LC-MS/MS. The analysis results are included in the Table 1 & Table 2.

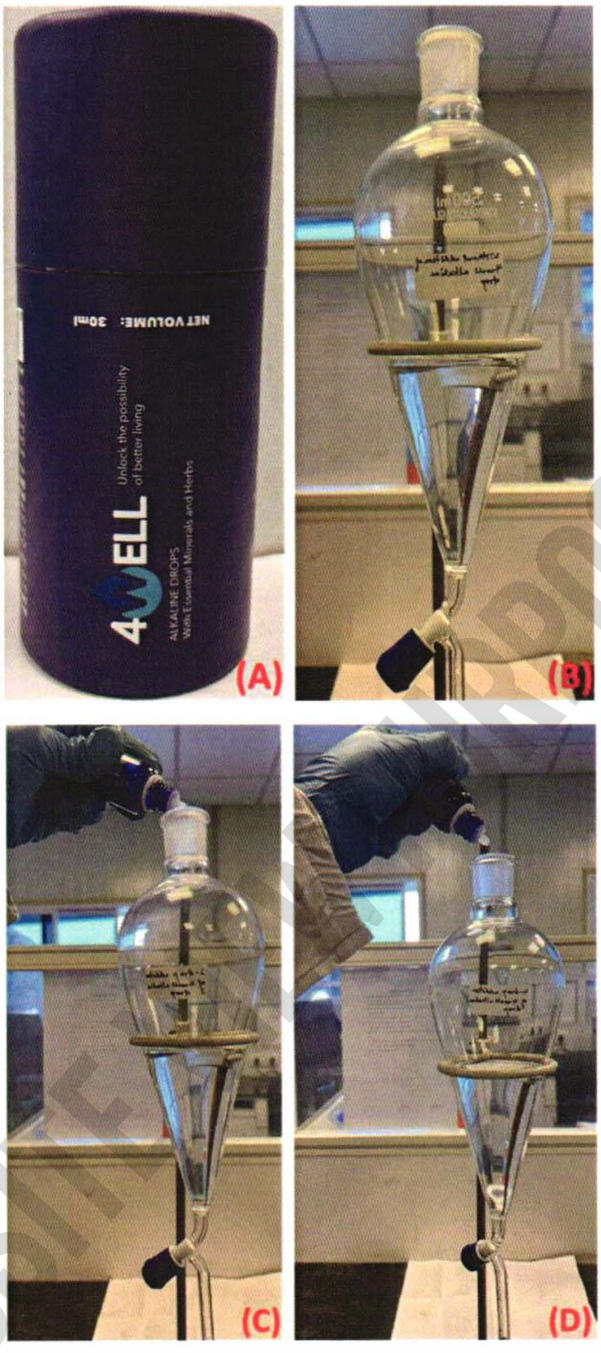

Figure 1: (A) Picture of 4Well Alkaline Drops sample as received, (B) Extraction of pesticide residues present in the water without the addition of 4Well Alkaline Drops, (C) Extraction of pesticide residues present in the water with the addition of 4 drops of 4Well Alkaline Drops, (D) Extraction of pesticide residues present in the water with the addition of 4 drops of 4Well Alkaline Drops.

Table 2: Pesticide residues concentrations in water without the addition and the addition of 4 drops of 4 well alkaline drop and the % variations in the reduction of the pesticide residues in water.

S. No.	Parameter-Pesticide Residues	Unit	Without the addition of 4Well Alkaline Drops (µg/l)	With the addition of 4 drops of 4Well Alkaline Drops (µg/l)	% Variation w.r.t analysis without addition of 4Well Alkaline Drops (4drops)
1	2,4-Dichlorophenoxyacetic acid	µg/L	108.65	97.33	10.41
2	Alachlor	µg/L	103.55	88.32	14.70
3	Aldrin	µg/L	90.35	85.36	5.52
4	Alpha HCH	µg/L	94.32	65.23	30.84
5	Atrazine	µg/L	98.65	94.78	3.92
6	Beta HCH	µg/L	112.57	88.01	21.81
7	Butachlor	µg/L	102.36	50.37	50.79
8	Chlorpyriphos	µg/L	98.56	79.34	19.50
9	Delta HCH	µg/L	99.75	80.64	19.15
10	Dieldrin	µg/L	79.68	75.94	4.69
11	Endosulfan-alpha	µg/L	103.98	96.57	7.126
12	Endosulfan-beta	µg/L	97.15	94.44	2.78
13	Endosulfan-sulphate	µg/L	99.98	97.32	2.66
14	Ethion	µg/L	94.37	55.62	41.06
15	Gamma - HCH (Lindane)	µg/L	108.94	89.57	17.78
16	Isoproturon	µg/L	93.42	84.35	9.70
17	Malathion	µg/L	111.28	93.34	16.12
18	Methyl parathion	µg/L	92.74	84.33	9.06
19	Monocrotophos	µg/L	104.36	95.87	8.13
20	Phorate	µg/L	93.46	84.35	9.74
21	2,4-DDT	µg/L	103.28	91.54	11.36
22	4,4-DDT	µg/L	104.65	94.65	9.55
23	2,4-DDE	µg/L	110.64	92.75	16.16
24	4,4-DDE	µg/L	101.56	90.23	11.15
25	2,4-DDD	µg/L	109.75	93.68	14.64
26	4,4-DDD	µg/L	99.75	92.47	7.29
27	Azinphos methyl	µg/L	102.64	91.37	10.98
28	Captan	µg/L	97.44	93.55	3.99
29	Dimethoate	µg/L	107.64	96.57	10.28
30	Disulfoton	µg/L	96.45	84.35	12.54

CONCLUSIONS

- The reduction in the pesticide residues concentration was studied with and without the addition of 4Well Alkaline Drops and found that the addition of 4 drops of 4Well Alkaline Drops was effective.

REFERENCES

AOAC 990.06, 21st Edition 2019, Chapter -10

ABBREVIATIONS

ABBREVIATIONS	TERMS
SOP	Standard Operating Procedure
GC-MS/MS	Gas Chromatography Mass Spectrometry /Mass Spectrometry
LC-MS/MS	Liquid Chromatography Mass Spectrometry /Mass Spectrometry
MRM	Multiple Reaction Monitoring
LOD	Limit of Detection
LOQ	Limit of Quantification