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जीसी-एमएस/एमएस और एलसी एमएस/एमएस द्वारा दूध और दूध उत्पादों (एमएमपी) के कीटनाशकों के अवशेषों का निर्धारण – परीक्षण पद्धति

Draft Indian Standard

DETERMINATION OF PESTICIDE RESIDUES IN MILK AND MILK PRODUCTS BY GC-MS/MS AND LC MS/MS – METHOD OF TEST

ICS 65.100.10

Pesticide Residues Analysis Sectional Committee, FAD 27	Last Date of Comments – 21 April 2024

FOREWORD

The substances intended for preventing, destroying, and repelling any 'pest' are known as pesticides. Several hundred pesticides of different chemical nature are currently used for agricultural purposes all over the world. Pesticides are mainly divided into classes, like organochlorine, organophosphorous, organocarbamates, and pyrethroides. In addition, other pesticides classes are triazines, ureic, amides, nitro compounds, plant growth regulators, benzimidazoles, phtalamides, bipyridyl compounds, ethylene bromide, sulfur containing dithiocarbamate compounds and analogues of copper or mercury. Because of their widespread use in agricultural and other practices, they are detected in various food and feed matrices.

The indicative list of pesticides required to be tested in milk and milk products are given in Annex A. For a complete analysis of the listed pesticides of milk and milkproducts laboratory requires performing six different methods due to the different physicochemical nature of these listed pesticides. As showed in table 1, out of the total of 54 pesticides 48 pesticides can be analyzed following QuEChERS based multi-residue extraction and detection by LC-MS/MS MR-1 (Multi-Residue) and GC-MS/MS MR-1 respectively. Pesticides of plant growth category *i.e.* 2, 4-Dichlorophenoxy Acetic Acid and Methyl Chlorophenoxy Acetic Acid (MCPA) can be analyzed using LC-MS/MS MR- 2 protocol. Glufosinate Ammonium, Paraquat dichloride (Determined as Paraquat cations), triacontanol, and Dithiocarbamates (Mancozeb and Metiram as CS2) can be tested using four different single residue analysis protocols using LC-MS/MS SR-1 (Single Residue), LC-MS/MS SR-2, GC-MS/MS SR-3, and GC-MS SR-4 respectively. The details of these extraction protocols and their respective instrumental parameters and MRM methods are given under the sample preparation and method of analysis section of this document. For extraction of pesticide residues from milk and milk products, appropriatecommercial QuEChERS kits having similar composition can be used. However, in such cases lab is required to do verification of the kit.

For the purpose of deciding whether a particular requirement of the standard is complied with, the

final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 2022 ‘Rules for rounding off numerical values (*second revision*)’. The number of significant places retained in the rounded-off value should be the same as that of the specified value in this standard

1 SCOPE

This standard prescribes the multi-residue method for quantification of pesticide residues in milk and milk products.

2 PRINCIPLE

In general, QuEChERS based multi-residue analysis of pesticide residues involves solvents-solvent extraction of pre-weighed samples in the presence of chemical sorbents/salts followed by clean-up separated organic phase with C_{18}, Primary Secondary Amine (PSA), $MgSO_4$ etc. and reconstitution in suitable solvents for instrumental analysis. Few chemically different classes of pesticides require to perform methods with different principles for their extraction which involves steps like saponification, derivatisation, digestion of analyte to release CS_2 and extraction using a specific solvents or cartridge at specific *p*H, temperature, and flow conditions. This standard covers the extraction of a variety of pesticide residues from milk and milk products and their detection using liquid and gas chromatography coupled with Mass spectrometer.

3 REQUIREMENTS

3.1 Apparatus/Instruments

a) Freezer capable of -20 °C;
b) Sample homogenizer/mixer;
c) Second stage sample mixture;
d) Analytical Balance – Readable to 0.20g, semi microbalance;
e) Centrifuge (Thermo scientific sorvall legend xtr or equivalent);
f) Nitrogen evaporator apparatus with heated water bath;
g) Oven;
h) 50 ml and 15 ml PP centrifuge tubes and tube shaker;
j) Micro entrifuge tubes;
k) Glass centrifuge tubes – 15 ml;
m) 1000 mg C18 SPE Columns/cartridge;
n) Filter paper;
p) Nylon syringe filter – 0.2 µm;
q) Micro centrifuge tubes (150 mg $MgSO_4$ & 50 mg PSA per 2ml);
r) Variable volume pipettes capable of accurately delivering 20 µL – 5000µL;
s) Plastic syringe – 5 ml;
t) Glass auto-sampler vials & caps – 2 ml;
u) Glass volumetric flasks – Class A;
v) Graduated cylinders – Class A;
w) PTFE vials and bottles/flasks;
y) Tube mixture; and
z) Conical glass stopper flasks/bottle.

3.2 Materials and Reagents

a) Certified reference standards;
b) Magnesium and sodium sulfate, anhydrous;
c) Sodium chloride and ethylenediaminetetraacetic acid (EDTA);
d) Sodium acetate;
e) Acetic acid;
f) Ethanol;
g) Sodium hydroxide (NaOH);
h) Heptane;
j) Iso-Octane;
k) Hydrochloric acid (HCL);
m) Ethyl acetate LCMS grade;
n) Acetonitrile, LCMS grade;
p) Methanol (MeOH), LCMS grade;
q) Ammonium formate, LCMS grade;
r) Formic acid, LCMS grade;
s) Disodium dihydrogen phosphate buffers;
t) Deionized distilled HPLC grade Water;
u) Diluent (1: 1 MeOH: Water);
v) Toluene;
w) Hexane;
y) N, O-Bis-trimethylsilyl-trifluoroacetamide (BSTFA); and
z) Tin (II) chloride.

4 PREPARATION OF REAGENTS / STANDARDS

Stock standards of pesticides were prepared from analytical grade Certified Reference Standards (CRS) of purity preferably more than 95 percent. An amount of about 10 mg of pure CRS were accurately weighed, dissolved and diluted to 10 ml with a suitable solvent (toluene, methanol, acetonitrile, etc.) for better solubility. All the standards were labelled for a minimum requirement of identification (name, date of preparation, date of expiry, concentration) and stored at -20 ºC in a dark (amber) color bottle that prevents any loss of solvent and entry of water. Stock standards need to be checked for solubility, there shall not be any visible solid precipitates, if required standards were sonicated for dissolution especially where solubility at a lower temperature is limited. Calculate the concentration of stock standard considering its purity and salts, if any. Intermediate standards of 10 mg/l in a group were prepared by pipetting required volume of stock standard and dissolving it in a 10 ml volumetric flask with a solvent used in stock standards preparation. A mixture of working standards of 1 mg/l from a mixture of all intermediate standards is prepared by dissolving the required volume of all intermediate standards in a volumetric flask of 10 ml with a solvent used in intermediate standards preparation.

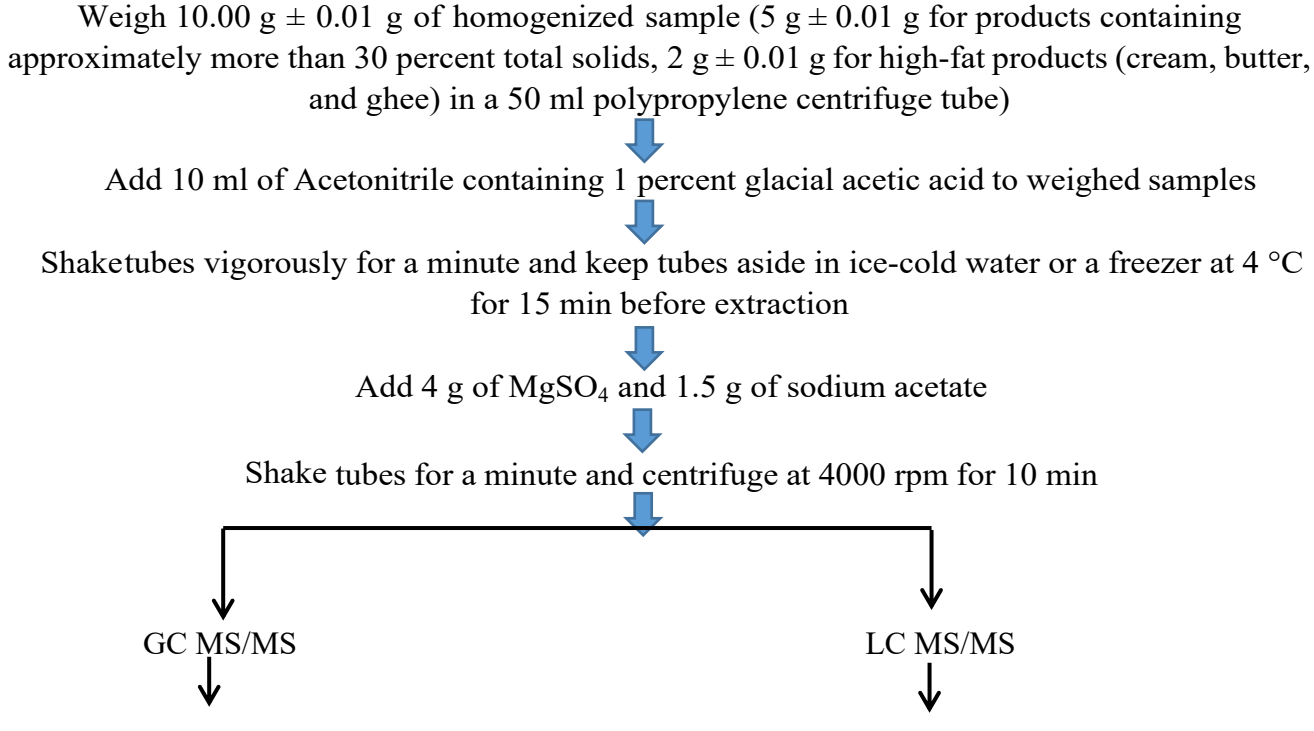
5 SAMPLE PREPARATION AND METHOD OF ANALYSIS

5.1 Multiresidue Analysis of Pesticides (GC-MS/MS MR-1 and LC-MS/MS MR-1)

Take weight of 10.00 g ± 0.01 g of homogenized sample for products containingapproximately less than 30 % total solids (liquid milk, *Dahi*, buttermilk, and milk), 5 g ± 0.01 g of homogenized sample for products containing approximately more than 30 % total solids (ice-cream, milk powder, paneer, cheese, *khoa*, and other traditional Indian dairy products) and 2 g ± 0.01 g of homogenized sample for high-fat products (cream, butter, and ghee) in a 50 ml polypropylene centrifuge tube. Add 10 ml of Acetonitrile containing 1 percent glacial acetic acid to weighed samples in 50 ml centrifuge tube and shaketubes vigorously for a minute and keep tubes aside in ice-cold water or a freezer at 4 °C for 15 min before extraction. Add 4 g of $MgSO_4$ and 1.5 g of sodium acetate and shake tubes for a minute and centrifuge at 4000 rpm for 10 min. For GC-MS/MS amenable pesticides, take 2 ml of supernatant into a 20 ml glass tube, dry the sample by using a nitrogen evaporator at 40 °C. Reconstitute with 2 ml of ethyl acetate and vortex for 30 secs for cream, butter, and ghee samples reconstitute with 1 ml ethyl acetate. Transfer extract into a clean-up tube containing 150 mg $MgSO_4$ 50 mg PSA,50 mg C18. Vortex the clean-up tubes for 2 min and centrifuge at 4000 rpm for 10 min.

Transfer the cleaned extract into a 2 ml auto-sampler GC vial through a 0.2 µm syringe filter. For LC-MS/MS amenable pesticides, transfer 2 ml of supernatant into a clean-up tube containing 150 mg $MgSO_4$ 50 mg PSA, 50 mg C18. Vortex the cleanup tubes and centrifuge at 4000 rpm for 10 min. Take 1 ml of supernatant into a 20 ml glass tube, dry the sample by using a nitrogen evaporator at 40 °C and reconstitute it with mobile phase A: B (80: 20). Transfer the cleaned extract into a 2 ml auto-sampler vial through a 0.2 µm syringe filter.

EXTRACTION PROCEDURE



Take 2 ml of supernatant into a 20 ml glass tube

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Drythe sample by using a nitrogen evaporator at 40 °C

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Reconstitute with 2 ml of ethyl acetate and vortex for 30 seconds for cream, butter, and ghee samples reconstitute with 1 ml ethyl acetate.

↓

Transfer extract into a clean-up tube containing 150 mg $MgSO_4$ 50 mg PSA,50 mg C18.

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Vortex the clean-up tubes for 2 mins and centrifuge at 4000 rpm for 10 mins.

↓

Transfer the cleaned extract into a 2 ml auto-sampler GC vial through a 0.2 µm syringe filter.

Transfer 2 ml of supernatant into a clean-up tube containing 150 mg $MgSO_4$ 50 mg PSA, 50 mg C18.

↓

Vortex the clean-up tubes and centrifuge at 4000 rpm for 10 min.

↓

Transfer 2 ml of supernatant into a clean-up tube containing 150 mg $MgSO_4$, 50 mg PSA, and 50 mg C18.

↓

Transfer 2 ml of supernatant into test tubes and dry the sample by using a nitrogen evaporator at 40 °C.

↓

Reconstitute it with 1ml of mobile phase A: B (80: 20).

↓

Transfer the cleaned extract into a 2 ml auto-sampler vial through a 0.2 µm syringe filter.

6 INSTRUMENTAL PARAMETERS - GC-MS/MS MR-1 MULTIRESIDUE METHOD

6.1 Instrument Conditions (Agilent 7010B or Equivalent)/GC Oven Conditions

6.1.1 *Oven Temperature Program*

a) 60 ºC for 1 min;
b) 40 ºC per min to 170 ºC;
c) 10 ºC per min to 310 ºC hold for 3 min; and
d) *Run time* – 20.75 min.

6.1.2 *GC Injection Conditions*

a) *Liner* – 2 mm id;
b) *Injection volume* – 1 µl (Syringe size 10 µl);
c) *Injection mode* – Split less;
d) Inlet temperature – 280 ºC; and
e) Septum purge – 3 ml/min.

6.1.3 *GC Column Flow Conditions*

a) *Carrier gas* – Helium; and
b) DB5MS or equivalent 15 m x 250 µm x 0.25 µm or equivalent, Column 1 and 2 of 15 m each connected through union - Column 1 flow – 1.197 ml/min and Column 2 flow – 1.397 ml/min.

6.1.4 *MS Conditions*

a) *MS source* – 70eV;
b) *Source temperature* – 280 ºC;
c) *Quadruple temperature* – 150 ºC;
d) *Transfer line temperature* – 280 º C;
e) *Helium quench gas* – 2.25 ml/min; and
f) *N2 collision gas* – 1.5 ml/min.

Table -2 MRM Transition (Accepted)

Multiresidue analysis of multiclass pesticides (GC-MS/MS MR1)				
Sl. No.	**Name of Pesticide**	**RT**	**MRM Transitions**	**CE (Ev)**
1.	Bifenthrin	13.94	181.2>165.2(Q1)	25
			181.2 >166.2(q1)	10
			166.2 >165.2(q2)	20
			265.9 >133 (Q1)	45
2.	Chlorothalonil	8.42	265.9 >230.9(q1)	20
			265.9 >168 (q2)	30
			196.9 > 169 (Q)	15
3.	Chlorpyriphos	9.85	198.9 >171 (q1)	15
			313.8 >257.8(q2)	15
4.	Cypermethrin (sum of isomers)	16.62	181.2 > 152.1(Q)	25
			181 > 152.1 (q)	25
			165.1 > 91.1 (q)	15
5.	Deltamethrin	18.20	252.9 > 93 (Q)	15
			181 > 152.1 (q1)	25
			250.7 > 172 (q2)	5
6.	Dichlorvos	4.65	184.9 > 93 (Q)	10
			144.9 > 109 (q)	10
			109 > 79 (q)	5
7.	Etofenprox	16.89	163 > 107.1 (Q)	20
			163 > 135.1 (q1)	10
			107 > 77 (q2)	15
8.	Fenproprathrin	14.12	181.1 > 152.1(Q)	25
			125 > 55.1 (q1)	10
			207.9 > 181 (q2)	5
9.	Fenvalerate (Sum ofisomers)	17.46 (I)	167 > 125.1 (Q)	5

		17.66 (II)	167 > 88.9 (q1) 224.9 > 119 (q2)	40 15
10.	Fipronil	10.46	366.8 > 212.8(Q) 254.9 > 228 (q1) 350.8 > 54.8(q2)	25 15 15
11.	Phorate	7.5	260 > 75 (Q) 128.9 > 65 (q) 121 > 65 (q)	5 15 10
12.	Pirimiphos Methyl	9.50	290 > 125 (Q) 232.9 > 151 (q1) 232.9 > 125 (q2)	20 5 5
13.	Aldrin	10.02	262.9 >192.9 (Q) 262.9 > 90.9(q1) 264.9 > 92.9(q2)	35 35 35
14.	Lindane	8.17	181 > 145 (Q) 216.9 > 182 (q1) 218.9 > 83.1(q2)	15 5 5
15.	Chlordane (Sum ofisomers)	11.11(cis) 11.35(tran)	271.8 > 236.9(Q) 374.8 > 65.8(q1) 372.8 > 65.9(q2)	15 15 25
16.	Chlorfenvinphos	10.60	266.9 > 159 (Q) 266.9 > 81 (q1) 294.9 > 66.9(q2)	20 30 5
17.	Beta-Cyfluthrin	16.43	162.9 > 127 (Q) 198.9 > 70.1(q1) 206 > 150 (q2)	5 25 40
18.	Lambda -Cyhalothrin	14.89	181.1 > 152.1(Q) 181.1 > 77 (q1) 208.1 >181.1(q2)	30 45 10
19.	Dieldrin	11.83	262.9 > 193 (Q) 262.9 > 191 (q1) 277 > 241 (q2)	35 35 5
20.	Alpha-Endosulfan	11.35	194.9 > 125 (Q) 194.9 > 160 (q1) 194.9 > 159 (q2)	20 5 5
21.	Beta-Endosulfan	12.40	206.9 > 172 (Q) 194.9 >158.9(q1) 194.9 >124.9(q2)	15 10 25
22.	Fenthion	9.92	278 > 109 (Q) 124.9 > 79 (q1) 124.9 > 47 (q2)	15 5 10
23.	Pirimiphos Ethyl	10.18	318.1 > 166.1(Q) 318.1 > 182 (q1) 152.1 > 84 (q2)	10 10 10

7 INSTRUMENTAL PARAMETERS – LC-MS/MS MR-1 MULTIRESIDUE METHOD

7.1 Instrument Conditions (Waters Xevo TQS or Equivalent)

7.1.1 *Instrument Settings for* LCMS/MS

7.1.1.1 *Mobile phase A* – 0.1 percent formic acid and 5mM ammonium formate in water: methanol 90:10

7.1.1.2 *Mobile phase B* – 0.1 percent formic acid and 5mM ammonium formate in methanol: water -90:10

7.1.1.3 *Flow rate* – 0.4 ml/min, Injection Volume – 5 µL

7.1.1.4 *Column temperature* – 40 ºC, Column: BEH C18 1.7 µm, 2.1 X 100 mm or equivalent

7.1.1.5 *Run time* – 22 minutes

	Time (Min.)	Mobile Phase A (%)	Mobile Phase B (%)
UPLC gradient	0	98	2
	0.5	98	2
	15	2	98
	17	2	98
	17.5	98	2
	22	98	2

7.2 MS Conditions

a) *Mode* – ESI $^{+ve}$, Capillary (kV) – 1.00
b) *Source offset* (V) – 80.0, Source temperature (˚C) – 150
c) *Desolvation Temperature* (˚C) – 500
d) *Cone Gas Flow* (L/h) – 150, Desolvation Gas flow (ml/Min)
e) *1000Collision Gas Flow* (Bar) – 0.15

Table -3 MRM Transition

Sl. No.	Name of Pesticide	RT	MRM Transitions	Cone(V)	CE(eV)
1.	Acephate	1.54	183.9 > 142.8(Q) 183.9 > 124.9(q)	20 20	10 12
2.	Methamidophos	1.22	141.9 > 124.8(Q) 141.9 > 93.9(q)	30 30	14 12
3.	Acetamiprid	6.02	223.0 > 126.0(Q) 223.0 > 56.1(q)	30 30	20 15
4.	Azoxystrobin	11.60	404.1 > 372.0(Q) 404.1 > 328.9(q)	15 15	8 30
5.	Benomyl	8.70	291.0 > 192.0(Q)	22	16

			291.0 > 160.0(q)	22	28
6.	Carbendazim	3.40	192.1 > 160.1(Q) 192.1 > 132.1(q)	10 10	15 30
7.	Bitertanol	13.90	338.1 > 98.9 (Q) 338.1 > 70.1(q)	30 30	16 8
8.	Buprofezin	14.84	306.1 > 201.0(Q) 306.1 > 57.4(q)	31 31	12 20
9.	Carbaryl	9.13	202.1 > 145.1(Q) 202.1 > 127.1(q)	25 25	10 25
10.	Carbofuran	8.58	222.1 > 165.1(Q) 222.1 > 123.0(q)	5 5	10 20
11.	3-hydroxy carbofuran	5.75	238.0 > 163.0(Q) 238.0 > 107.0(q)	34 34	16 16
12.	Chlorantraniliprole	11.01	484.0 > 453.0(Q) 484.0 > 286.0(q)	18 18	17 12
13.	Chlothianidin	5.04	250.0 > 169.0(Q) 250. 0 > 132.0(q)	25 25	10 15
14.	Difenoconazole	14.33	406.1 > 337.2(Q) 406.1 > 250.9(q)	35 35	12 25
15.	Dimethoate	5.40	230.0 > 198.8 (Q) 230.0 > 124.8(q)	20 20	10 22
16.	Dinotefuran	2.53	203.2 > 129.1(Q) 203.2 > 114.1(q)	10 10	10 15
17.	Edifenphos	13.50	311.0 > 111.0(Q) 311.0 > 109.0(q)	23 23	26 32
18.	Emamectin Benzoate	15.68	886.6 > 158.0(Q) 886.6 > 82.0(q)	45 45	37 35
19.	Ethion	15.22	385.0 > 199.0(Q) 385.0 > 142.9(q)	30 30	10 25
20.	Flubendiamide	13.33	683.3 > 408.2(Q) 683.3 > 274.2(q)	5 5	5 16
21.	Flusilazole	13.02	316.0 > 247.0(Q) 316.0 > 165.0(q)	5 5	20 25
22.	Imidacloprid	5.14	256.1 > 209.0(Q) 256.1 > 174.9(q)	25 25	12 20
23.	Indoxacarb	14.40	528.1 > 217.9(Q) 528.1 > 202.9(q)	30 30	25 40
24.	Kresoxim Methyl	13.21	314.2 > 131.0(Q) 314.2 > 115.9 q	30 30	25 12
25.	Methomyl	3.58	162.9 > 105.9(Q) 162.9 > 88.0(q)	15 15	10 10
26.	Metolachlor	12.83	284.1 > 252.1(Q) 284.1 > 176.1(q)	17 17	15 25

27.	Monocrotophos	4.31	224.1 > 127.1(Q) 224.1 > 98.0(q)	26 26	15 12
28.	Oxydemeton-Methyl	5.41	263.0 > 169.0(Q) 263.0 > 120.9(q)	20 20	13 14
29.	Penconazole	13.32	284.0 > 159.0(Q) 284.0 > 70.1(q)	15 15	25 15
30.	Phenthoate	13.12	321.0 > 135.0(Q) 321.0 > 79.1(q)	9 9	20 40
31.	Phorate sulphones	9.90	293.2 > 171.2(Q) 293.2 > 97.1(q)	20 20	10 10
32.	Phorate sulphoxides	9.79	277.0 > 143.0(Q) 277.0 > 96.9 (q)	24 24	20 32
33.	Propiconazole	13.67	342.1 > 158.9(Q) 342.1 > 69.1(q)	35 35	20 30
34.	Pyraclostrobin	13.85	388.1 > 193.9(Q) 388.1 > 163.0(q)	25 25	12 25
35.	Tebuconazole	13.35	308.2 > 125.1(Q) 308.2 > 70.1(q)	20 20	40 24
36.	Thiacloprid	6.88	253.0 > 125.8(Q) 253.0 > 90.0(q)	35 35	20 40
37.	Thiamethoxam	3.88	292.0 > 211.2(Q) 292.0 > 132.0(q)	25 25	10 20
38.	Thiophanate-Methyl	8.56	343.0 > 151.0(Q) 343.0 > 93.0(q)	28 28	22 40
39.	Trichlorfon	5.23	257.0 > 109.0(Q) 257.0 > 79.0(q)	28 28	18 30
40.	Triadimefon	12.14	294.1 > 196.9(Q) 294.1 > 69.1(q)	30 30	16 20

8 MULTIRESIDUE ANALYSIS OF 2, 4D & MCPA - PGR (LC-MS/MS MR-2)

Take a weight of 10.00 g ± 0.01 g of homogenized sample for products containing approximately less than 30 percent total solids (liquid milk, *Dahi*, buttermilk, and milk), 5 g ± 0.01 g of homogenized sample for products containing approximately more than 30 percent total solids (ice-cream, milk powder, paneer, cheese, *khoa*, and other traditional Indian dairy products) and 2 g ± 0.01 g of homogenized sample for high-fat products (cream, butter and ghee) in a 50 ml polypropylene centrifuge tube. Add 10 ml of Acetonitrile containing 1 percent acetic acid to weighed samples in 50 ml centrifuge tube and shake tubes vigorously for a minute and keep tubes aside for 15 min. Add 4gm $MgSO_4$ and 1 g sodium chloride shake tubes for a minute and centrifuge at 4000 rpm for 10 min. Transfer 6 ml raw extract into a 15 ml centrifuge tube and to these tubes add 1.5 g magnesium sulfate and 0.5 g sodium chloride. Vortex tubes for a minute and centrifuge at 4000 rpm for 10 min and dilute 1 ml supernatant with 1 ml water (1:1 dilution). Shake shortly to mix and filter through syringe filter into an LC-MS/MS vial. Instrumental

Parameter – LC-MS/MS MR-2 (PGR) Multiresidue method 2 Instrument conditions (Waters Xevo TQS or equivalent)

EXTRACTION PROCEDURE

Take a weight of 10.00 g ± 0.01 g of homogenized (5 g ± 0.01 g of homogenized sample for products containing approximately more than 30 % total solids) and 2 g ± 0.01 g of homogenized sample for high-fat products (cream, butterand ghee) in a 50 ml polypropylene centrifuge tube.

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Add 10 ml of Acetonitrile containing 1 percent acetic acid to weighed samples in 50 ml centrifuge tube and shake tubes vigorously for a minute and keep tubes aside for 15 min.

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Add 4gm $MgSO_4$ and 1 g sodium chloride shake tubes for a minute and centrifuge at 4000 rpm for 10 min.

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Transfer 6 ml raw extract into a 15 ml centrifuge tube and to these tubes add 1.5 g magnesium sulfate and 0.5 g sodium chloride.

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Vortex tubes for a minute and centrifuge at 4000 rpm for 10 min and dilute 1 ml supernatant with 1 ml water (1:1 dilution).

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Shake shortly to mix and filter through syringe filter into an LC-MS/MS vial.

8.1 Instrument Settings for LCMS/MS

a) Mobile phase A – 0.1 percent acetic acid in water;
b) Mobile phase B – 0.1 % acetic acid in acetonitrile;
c) Flow rate – 0.4 ml/min;
d) Column: BEH or equivalent C18 1.7 µm, 2.1 X 100 mm;
e) Column temperature – 40 ºC;
f) Injection volume – 5 Ml; and
g) Run time – 11 minutes.

	Time (Min.)	**Mobile Phase A (%)**	**MobilePhase B (%)**
UPLC Gradient	0.0	90	10
	0.5	90	10
	4.0	10	90
	4.5	10	90
	5.0	90	10
	8.0	90	10

8.2 MS Conditions

a) Mode – ESI;

b) Capillary (kV) – 2;
c) Cone (V) – 20;
d) Source offset (V) – 80;
e) Source temperature (°C) – 150;
f) Desolvation temperature (°C) – 550;
g) Cone gas flow (L/h) – 150;
h) Desolvation gas flow (ml/Min) – 1000;
j) Collision gas flow (ml/Min) – 0.15; and
k) Nebuliser gas flow (Bar) – 7.

Table -4 MRM Transition Multiresidue analysis of PGR pesticides (LC-MS/MS MR2)

Sl. No.	Name of Pesticide	RT	MRM Transitions	Cone(V)	CE (eV)
1.	2,4-D	3.46	218.9 > 125.0(Q) 218.9 > 160.9 (q)	20 20	25 15
2.	MCPA	3.46	199 > 140.9(Q) 199 > 154.9(q)	30 30	18 18

~~**9 CALCULATION**~~

9. IDENTIFICATION/CONFIRMATION, CALCULATIONS AND REPORTING OF RESULTS

9.1 Identification/Confirmation

9.1.1 Review the chromatograms to verify that the analyte peaks are within the retention time windows and that the peaks are integrated correctly.
9.1.2 Calculate the ion ratio of the response.
9.1.3 The relative intensities or ratios of selective ions, expressed as a ratio relative to the most intense ion that are used for identification, should match with the reference ion ratio. The reference ion ratio is the average obtained from solvent standards measured in the same sequence and under the same conditions as the samples.

9.2 Calculations

9.2.1 Generate a linear curve fit to each analyte in standard curve using normalized response to concentration (µg/kg or ppb).
9.2.2 Mass lynx and Mass hunter software provided in the instrument can be used for auto Quantitation by using linear regressio:

$$(y = mx + b)$$

where

y = peak area/height;

x = analyte concentration in µg/kg;

m = slope of curve; and

b = Intercept of y.

9.3 Reporting of the Results

9.3.1 Report results in parts per million (ppm).

ANNEX A

(foreword)

INDICATIVE LIST OF PESTICIDES TO BE ANALYSED

Sl. No.	Name of the Insecticide
1.	2,4-Dichlorophenoxy Acetic Acid
2.	Acephate (expressed as mixture of Methamidophos and acephate).
3.	Acetamiprid
4.	Azoxystrobin
5.	Sum of benomyl and carbendazim expressed as carbendazim
6.	Bifenthrin
7.	Bitertanol
8.	Buprofezin
9.	Carbaryl
10.	Carbendazim
11.	Carbofuran (sum of carbofuran and 3-hydroxy carbofuran expressed as carbofuran)
12.	Chlorantraniliprole
13.	Chlorothalonil
14.	Chlorpyriphos
15.	Chlothianidin (Chlothianidin and its metabolites)
16.	Cypermethrin (sum of isomers) (Fat soluble residue)
17.	Deltamethrin (Decamethrin)
18.	Dichlorvos (DDVP) (content of di- chloroacetaldehyde (D.C.A.) be reported where possible)
19.	Difenoconazole
20.	Dimethoate
21.	Dinotefuran
22.	Dithiocarbamates Mancozeb and Metiram as CS2
23.	Edifenphos
24.	Emamectin Benzoate
25.	Ethion (Residues to be determined as ethion and its oxygen analogue and expressed as ethion)
26.	Ethofenprox (Etofenprox)
27.	Fenpropathrin
28.	Fenvalerate (Fat soluble residue)
29.	Fipronil
30.	Flubendiamide
31.	Flusilazole

32.	Glufosinate Ammonium
33.	Imidacloprid
34.	Indoxacarb
35.	Kresoxim Methyl
36.	Methomyl
37.	Methyl Chlorophenoxy Acetic Acid (MCPA)
38.	Metolachlor
39.	Monocrotophos
40.	Oxydemeton-Methyl
41.	Paraquat dichloride (Determined as Paraquatcations)
42.	Penconazole
43.	Phenthoate
44.	Phorate (sum of Phorate, its oxygen analogue and their sulphoxides and sulphones)
45.	Pirimiphos-methyl
46.	Propiconazole
47.	Pyraclostrobin
48.	Tebuconazole
49.	Thiacloprid
50.	Thiamethoxam
51.	Thiophanate-Methyl
52.	Trichlorfon
53.	Triacontanol
54.	Triadimefon