**BUREAU OF INDIAN STANDARDS**

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| *भारतीय मानक मसौदा***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 MULTI-RESIDUE PESTICIDES 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, synthetic pyrethroids, neonicotinoids etc. In addition, other pesticides classes like triazines, ureic, amides, nitro compounds, plant growth regulators, benzimidazoles, phthalimides, bipyridyl compounds, dithiocarbamate compounds and analogues of copper or mercury etc. are also being used in agriculture for control of various pests and diseases. Because of their widespread use in agricultural and other practices, their residues are detected in various food and feed matrices including milk and milk products through direct or as indirect source necessitating their residual analysis for regulatory compliance.

The indicative list of pesticides required to be tested in milk and milk products in India are specified in FSSR, 2011 regulation. For a complete analysis of the listed pesticides of milk and milk products, laboratories require multiple extraction and analysis protocols due to the diverse physicochemical nature of these listed pesticides. Among the regulated pesticides, a major class of pesticides (>85%) is being analyzed following QuEChERS based multi-residue extraction, d-SPE cleanup and analysis by LC-MS/MS and GC-MS/MS. The rest of the analytes are analyzed by specific single residue methods. This Indian standard document details the procedure to be followed for extraction, cleanup and analysis of multi-residue pesticides from milk and milk products following QuEChERS extraction by acetonitrile solvent, d-SPE cleanup and GC-MS/MS or LC-MS/MS analysis. For extraction of multi-residue pesticides from milk and milk products, appropriate commercial QuEChERS kits having similar composition of salts described in this standard can also be used after required performance verification.

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 extraction and quantification of multi-residue pesticides from milk and milk products following QuEChERS extraction and analysis by liquid and gas chromatography coupled with mass spectrometry.

**2 PRINCIPLE**

In general, QuEChERS based multi-residue analysis of pesticide residues involves solvent to solvent extraction with acetonitrile of a pre-weighed samples in the presence of chemical sorbents/salts followed by clean-up of the organic phase with dispersive cleanup sorbets such as C18, Primary Secondary Amine (PSA), MgSO4 etc*.* and reconstitution in suitable solvents prior to instrumental analysis. This standard covers the extraction of multi-residue pesticides amenable to QuEChERS extraction with acetonitrile from milk and milk products and their detection using liquid and gas chromatography coupled with mass spectrometer.

**3 REQUIREMENTS**

**3.1 Apparatus/Instruments**

1. Freezer capable of -20 °C;
2. Sample homogenizer/mixer;
3. Second stage sample mixture;
4. Analytical Balance – Readable to 0.20g, semi microbalance;
5. Centrifuge (≥10000 rpm);
6. Nitrogen evaporator with temperature controlled water bath;
7. Oven;
8. 50 ml and 15 ml PP centrifuge tubes and tube shaker;
9. Micro centrifuge tubes;
10. Glass centrifuge tubes – 15 ml;
11. Filter paper;
12. Nylon syringe filter – 0.2 μm;
13. Micro centrifuge tubes (with 150 mg MgSO4 & 50 mg PSA per 2ml);
14. Variable volume pipettes capable of accurately delivering 20 μL – 5000μL;
15. Plastic syringe – 5 ml;
16. Glass auto-sampler vials & caps – 2 ml;
17. Glass volumetric flasks – Class A;
18. Graduated cylinders – Class A;
19. PTFE vials and bottles/flasks;
20. Tube mixture; and
21. Conical glass stopper flasks/bottle.

 **3.2** **Materials and Reagents**

1. Certified reference standards (ISO/IEC 17034:2016 certified);
2. Magnesium and sodium sulfate, anhydrous;
3. Sodium acetate;
4. Acetic acid;
5. Hydrochloric acid (HCL);
6. Ethyl acetate LCMS grade;
7. Acetonitrile, LCMS grade;
8. Methanol (MeOH), LCMS grade;
9. Ammonium formate, LCMS grade;
10. Formic acid, LCMS grade;
11. Disodium dihydrogen phosphate buffers;
12. Deionized distilled HPLC grade Water;
13. Diluent (1: 1 MeOH: Water);

**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 CALIBRATION STANDARD PREPARATION**

**5.1.** **Solvent Based:** Using working standard (WS) mixture of appropriate concentration, solvent based calibration standards in the range of 0.001 μg/ml – 0.2 μg/ml are prepared in methanol and water for LC-MS and ethyl acetate for GC-MS. For LC-MS, the ratio of methanol and water is maintained to be 1:1 in all the calibration standards to maintain uniform solubility of analytes and avoid possible effects on peak shapes.

NOTE –

1. Solvent based linearity can be used for quantification, only when suitable Isotopically labelled internal standards are being used and added to samples prior to extraction.
2. The calibration range may vary based on the instrument sensitivity and sample dilution.

**Table 1 Preparation of Calibration Standards (Solvent-based) for LC-MS**

 **(Exemplary only)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **SI. No.** | **WS Concentration****(µg/mL)** | **Volume of WS taken****(µL)** | **Methanol (µL)** |  **Water or 0.1%formic acid solution****(µL)** | **Calibration Standard Concentration (µg/mL)** |
|  | 1.0 | 200 | 300 | 500 | 0.20 |
|  | 1.0  | 100 | 400 | 500 | 0.10 |
|  | 1.0 | 50 | 450 | 500 | 0.05 |
|  | 0.1 | 100 | 400 | 500 | 0.01 |
|  | 0.1 | 50 | 450 | 500 | 0.005 |
|  | 0.1  | 20 | 480 | 500 | 0.002 |
| 7 | 0.1 | 10 | 490 | 500 | 0.001 |

**Table 2 Preparation of Calibration Standards (Solvent-based) for GC-MS**

 **(Exemplary only)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **SI. No.** | **WS Concentration****(µg/mL)** | **Volume of WS taken****(µL)** | **Ethyl Acetate (µL)** | **Calibration Standard Concentration (µg/mL)** |
|  | 1.0 | 200 | 800 | 0.20 |
|  | 1.0  | 100 | 900 | 0.10 |
|  | 1.0 | 50 | 950 | 0.05 |
|  | 0.1 | 100 | 900 | 0.01 |
|  | 0.1 | 50 | 950 | 0.005 |
|  | 0.1  | 20 | 980 | 0.002 |
| 7 | 0.1 | 10 | 990 | 0.001 |

**5.2** Procedural Calibration**:** Procedural Calibration Standards (pre-extraction spiked matrix calibration standards) – A set of control matrix samples are spiked at desired concentration levels (e.g. 0.010 μg/g – 0.5 μg/g) and extracted as per the protocol. The final extracts are injected to establish procedural calibration standard.

NOTE –

1. Appropriate range of spiking for procedural calibration standards shall be selected based on sample dilution and desired limit of quantification (LOQ) to be achieved.

**5.3** In absence of control matrix, standard addition technique can be used for quantification. In this, sample extracts (after initial judgment of concentration present in sample) are spiked at various concentrations using at least three times higher concentrations. From the y-intercept and slope of calibration equation, concentration of analyte in the sample is calculated.

**6 SAMPLE PREPARATION AND METHOD OF ANALYSIS**

**6.1** **Multi-residue extraction of pesticides: GC-MS/MS and LC-MS/MS**

Take weight of 10.00 g ± 0.01 g of homogenized sample for products containing approximately less than 30 % total solids (liquid milk, *Dahi*, buttermilk, and milk) or 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) or 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 centrifuge tube and shake the tubes 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 MgSO4 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 the extract into a clean-up tube containing 150 mg MgSO4, 50 mg PSA and 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 and analyze by GC-MS/MS.

For LC-MS/MS amenable pesticides, transfer 2 ml of supernatant into a clean-up tube containing 150 mg MgSO4, 50 mg PSA and 50 mg C-18. 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 and analyze by GC-MS/MS.

**WORK FLOW DIAGRAM for EXTRACTION PROCEDURE**

Weigh 10.00 g ± 0.01 g of homogenized sample (5 g ± 0.01 g for products containing approximately more than 30 percent total solids, 2 g ± 0.01 g 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 

 Shake tubes 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 MgSO4 and 1.5 g of sodium acetate

Shake tubes for a minute and centrifuge at 4000 rpm for 10 min



 GC MS/MS LC MS/MS



















1. **INSTRUMENTAL PARAMET**
2. **6.**
3. **INSTRUMENT PARAMETERS- GC-MS/MS**

**6.1 Indicative GC Oven and MS Conditions**

**6.1.1** *Oven Temperature Program*

1. 60 ºC for 1 min;
2. 40 ºC per min to 170 ºC;
3. 10 ºC per min to 310 ºC hold for 3 min; and
4. Run time: 20.75 min.

**6.1.2** *GC Injection Conditions*

1. *Liner*: 2 mm id;
2. *Injection volume*: 1 µl (Syringe size 10 µl);
3. *Injection mode*: Split less;
4. Inlet temperature: 280 ºC; and
5. Septum purge: 3 ml/min.

**6.1.3** *GC Column and Flow Conditions*

1. Column: DB5MS/ RX5/HP5 or equivalent column (dimension 15 m x 250 µm x 0.25 µm)
2. *Carrier gas* – Helium; Flow rate: 3 ml/min.

**6.1.4** *Indicative**MS Conditions*

1. MS source: 70eV;
2. Source temperature: 280 ºC;
3. Quadruple temperature: 150 ºC;
4. Transfer line temperature: 280 º C;
5. Helium quench gas: 2.25 ml/min; and
6. N2 collision gas: 1.5 ml/min.

**Table -2 MRM Parameters for GC-MS/MS Analysis (For Reference Only)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sl. No.** | **Name of Pesticide** | **RT** | **MRM****Transitions** | **CE (Ev)** |
|  | 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 |
|  | Chlorothalonil | 8.42 | 265.9 >230.9(q1) | 20 |
| 265.9 >168 (q2) | 30 |
| 196.9 > 169 (Q) | 15 |
|  | Chlorpyriphos | 9.85 | 198.9 >171 (q1) | 15 |
| 313.8 >257.8(q2) | 15 |
|  | Cypermethrin (sum ofisomers) | 16.62 | 181.2 > 152.1(Q) | 25 |
| 181 > 152.1 (q)165.1 > 91.1 (q) | 2515 |
|  | Deltamethrin | 18.20 | 252.9 > 93 (Q)181 > 152.1 (q1)250.7 > 172 (q2) | 15255 |
|  | Dichlorvos | 4.65 | 184.9 > 93 (Q)144.9 > 109 (q)109 > 79 (q) | 10105 |
|  | Etofenprox | 16.89 | 163 > 107.1 (Q)163 > 135.1 (q1)107 > 77 (q2) | 201015 |
|  | Fenproprathrin | 14.12 | 181.1 > 152.1(Q)125 > 55.1 (q1)207.9 > 181 (q2) | 25105 |
|  | Fenvalerate (Sum of isomers) | 17.46 (I)17.66 (II) | 167 > 125.1 (Q)167 > 88.9 (q1)224.9 > 119 (q2) | 54015 |
|  | Fipronil | 10.46 | 366.8 > 212.8(Q)254.9 > 228 (q1)350.8 > 54.8(q2) | 251515 |
|  | Phorate | 7.5 | 260 > 75 (Q)128.9 > 65 (q)121 > 65 (q) | 51510 |
|  | Pirimiphos Methyl | 9.50 | 290 > 125 (Q)232.9 > 151 (q1)232.9 > 125 (q2) | 2055 |
|  | Aldrin | 10.02 | 262.9 >192.9 (Q)262.9 > 90.9(q1)264.9 > 92.9(q2) | 353535 |
|  | Lindane | 8.17 | 181 > 145 (Q)216.9 > 182 (q1)218.9 > 83.1(q2) | 1555 |
|  | Chlordane (Sum of isomers) | 11.11(cis) 11.35(tran) | 271.8 > 236.9(Q)374.8 > 65.8(q1)372.8 > 65.9(q2) | 151525 |
|  | Chlorfenvinphos | 10.60 | 266.9 > 159 (Q)266.9 > 81 (q1)294.9 > 66.9(q2) | 20305 |
|  | Beta-Cyfluthrin | 16.43 | 162.9 > 127 (Q)198.9 > 70.1(q1)206 > 150 (q2) | 52540 |
|  | Lambda -Cyhalothrin | 14.89 | 181.1 > 152.1(Q)181.1 > 77 (q1)208.1 >181.1(q2) | 304510 |
|  | Dieldrin | 11.83 | 262.9 > 193 (Q)262.9 > 191 (q1)277 > 241 (q2) | 35355 |
|  | Alpha-Endosulfan | 11.35 | 194.9 > 125 (Q)194.9 > 160 (q1)194.9 > 159 (q2) | 2055 |
|  | Beta-Endosulfan | 12.40 | 206.9 > 172 (Q)194.9 >158.9(q1)194.9 >124.9(q2) | 151025 |
|  | Fenthion | 9.92 | 278 > 109 (Q)124.9 > 79 (q1)124.9 > 47 (q2) | 15510 |
|  | Pirimiphos Ethyl | 10.18 | 318.1 > 166.1(Q)318.1 > 182 (q1)152.1 > 84 (q2) | 101010 |

NOTE – The MRM transition provided is indicative only. Ion (m/z) selection and mass parameters may vary depending on the instrument used and optimization may be performed for better stability and sensitivity of ions.

**7 INSTRUMENTAL PARAMETERS – LC-MS/MS**

**7.1 Indicative Instrument Conditions**

**7.1.1** *Instrument Settings for* LC-MS/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

|  |  |  |  |
| --- | --- | --- | --- |
| **UHPLC****gradient** | **Time****(Min.)** | **Mobile Phase** **A (%)** | **Mobile Phase****B (%)** |
| 0 | 98 | 2 |
| 0.5 | 98 | 2 |
| 15 | 2 | 98 |
| 17 | 2 | 98 |
| 17.5 | 98 | 2 |
| 22 | 98 | 2 |

**7.2 Indicative MS Conditions**

1. Mode : ESI +ve, Capillary (kV) : 1.00
2. Source offset (V) : 80.0, Source temperature (°C) : 150
3. Desolvation Temperature (°C) : 500
4. Cone Gas Flow (L/h) : 150, Desolvation Gas flow (ml/Min)
5. 1000 Collision Gas Flow (Bar) : 0.15

Table -3 MRM Parameters for LC-MS/MS Analysis (For Reference Only)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sl. No.** |  **Name of Pesticide** | **RT** | **MRM****Transitions** | **Cone(V)** | **CE(eV)** |
|  | Acephate | 1.54 | 183.9 > 142.8(Q)183.9 > 124.9(q) | 2020 | 1012 |
|  | Methamidophos | 1.22 | 141.9 > 124.8(Q)141.9 > 93.9(q) | 3030 | 1412 |
|  | Acetamiprid | 6.02 | 223.0 > 126.0(Q)223.0 > 56.1(q) | 3030 | 2015 |
|  | Azoxystrobin | 11.60 | 404.1 > 372.0(Q)404.1 > 328.9(q) | 1515 | 830 |
|  | Benomyl | 8.70 | 291.0 > 192.0(Q)291.0 > 160.0(q) | 2222 | 1628 |
|  | Carbendazim | 3.40 | 192.1 > 160.1(Q)192.1 > 132.1(q) | 1010 | 1530 |
|  | Bitertanol | 13.90 | 338.1 > 98.9 (Q)338.1 > 70.1(q) | 3030 | 168 |
|  | Buprofezin | 14.84 | 306.1 > 201.0(Q)306.1 > 57.4(q) | 3131 | 1220 |
|  | Carbaryl | 9.13 | 202.1 > 145.1(Q)202.1 > 127.1(q) | 2525 | 1025 |
|  | Carbofuran | 8.58 | 222.1 > 165.1(Q)222.1 > 123.0(q) | 55 | 1020 |
|  | 3-hydroxy carbofuran | 5.75 | 238.0 > 163.0(Q)238.0 > 107.0(q) | 3434 | 1616 |
|  | Chlorantraniliprole | 11.01 | 484.0 > 453.0(Q)484.0 > 286.0(q) | 1818 | 1712 |
|  | Chlothianidin | 5.04 | 250.0 > 169.0(Q)250. 0 > 132.0(q) | 2525 | 1015 |
|  | Difenoconazole | 14.33 | 406.1 > 337.2(Q)406.1 > 250.9(q) | 3535 | 1225 |
|  | Dimethoate | 5.40 | 230.0 > 198.8 (Q)230.0 > 124.8(q) | 2020 | 1022 |
|  | Dinotefuran | 2.53 | 203.2 > 129.1(Q)203.2 > 114.1(q) | 1010 | 1015 |
|  | Edifenphos | 13.50 | 311.0 > 111.0(Q)311.0 > 109.0(q) | 2323 | 2632 |
|  | Emamectin Benzoate | 15.68 | 886.6 > 158.0(Q)886.6 > 82.0(q) | 4545 | 3735 |
|  | Ethion | 15.22 | 385.0 > 199.0(Q)385.0 > 142.9(q) | 3030 | 1025 |
|  | Flubendiamide | 13.33 | 683.3 > 408.2(Q)683.3 > 274.2(q) | 55 | 516 |
|  | Flusilazole | 13.02 | 316.0 > 247.0(Q)316.0 > 165.0(q) | 55 | 2025 |
|  | Imidacloprid | 5.14 | 256.1 > 209.0(Q)256.1 > 174.9(q) | 2525 | 1220 |
|  | Indoxacarb | 14.40 | 528.1 > 217.9(Q)528.1 > 202.9(q) | 3030 | 2540 |
|  | Kresoxim Methyl | 13.21 | 314.2 > 131.0(Q)314.2 > 115.9 q | 3030 | 2512 |
|  | Methomyl | 3.58 | 162.9 > 105.9(Q)162.9 > 88.0(q) | 1515 | 1010 |
|  | Metolachlor | 12.83 | 284.1 > 252.1(Q)284.1 > 176.1(q) | 1717 | 1525 |
|  | Monocrotophos | 4.31 | 224.1 > 127.1(Q)224.1 > 98.0(q) | 2626 | 1512 |
|  | Oxydemeton-Methyl | 5.41 | 263.0 > 169.0(Q)263.0 > 120.9(q) | 2020 | 1314 |
|  | Penconazole | 13.32 | 284.0 > 159.0(Q)284.0 > 70.1(q) | 1515 | 2515 |
|  | Phenthoate | 13.12 | 321.0 > 135.0(Q)321.0 > 79.1(q) | 99 | 2040 |
|  | Phorate sulphones | 9.90 | 293.2 > 171.2(Q)293.2 > 97.1(q) | 2020 | 1010 |
|  | Phorate sulphoxides | 9.79 | 277.0 > 143.0(Q)277.0 > 96.9 (q) | 2424 | 2032 |
|  | Propiconazole | 13.67 | 342.1 > 158.9(Q)342.1 > 69.1(q) | 3535 | 2030 |
|  | Pyraclostrobin | 13.85 | 388.1 > 193.9(Q)388.1 > 163.0(q) | 2525 | 1225 |
|  | Tebuconazole | 13.35 | 308.2 > 125.1(Q)308.2 > 70.1(q) | 2020 | 4024 |
|  | Thiacloprid | 6.88 | 253.0 > 125.8(Q)253.0 > 90.0(q) | 3535 | 2040 |
|  | Thiamethoxam | 3.88 | 292.0 > 211.2(Q)292.0 > 132.0(q) | 2525 | 1020 |
|  | Thiophanate methyl | 8.56 | 343.0 > 151.0(Q)343.0 > 93.0(q) | 2828 | 2240 |
|  | Trichlorfon | 5.23 | 257.0 > 109.0(Q)257.0 > 79.0(q) | 2828 | 1830 |
|  | Triadimefon | 12.14 | 294.1 > 196.9(Q)294.1 > 69.1(q) | 3030 | 1620 |

NOTE –The MRM Transition provided is indicative only. Ion (m/z) selection, source and mass parameters may vary depending on the instrument used and optimization may be performed for better stability and sensitivity of ions.

**8. SEQUENCE OF INJECTION**

**8.1** Inject one blank as well as a standard mixture to ensure that the system is ready for the sample analysis.

**8.2** Inject the solvent blank before and after standards to check the system free from carry over.

**8.3** Inject mixture of solvent standards / matrix / procedural standards at least 5 levels including LOQ level.

**8.4** Inject reagent blank and quality control sample.

**8.5** Inject samples.

**8.6** If there are more than ten samples in a batch, after every 10 sample inject one reagent blank and one calibration standard to check carry over and overall performance of the analytical instrument.

**9.    IDENTIFICATION/CONFIRMATION, CALCULATIONS AND REPORTING OF RESULTS**

**9.1 Identification/Confirmation and Quantitation:**

**9.1.1** Check the acquired data for standards as well as samples

**9.1.2** After data processing, check the two transition per analyte are present at same (expected) retention time. Then calculate the ion ratio for two transitions. The ion ratio values should match within 30 percent deviation of the reference standard ion ratio. Once confirm this identification criterion then start the quantification.

**9.1.3** For quantitation, check the retention times (± 0.1 min) and response of calibration standards is proportionally increased with respect to concentration. Prepare a quantitation method using an optimum level of concentration. By applying the quantitation method, prepare the calibration curve for the standards by using the linear equation with 1/x weighting factor.

**9.1.4** Check linearity providing correlation coefficient >0.99 and residuals within ± 20 percent for all the target analytes.

**9.2 Reporting of the Results**

**9.2.1** Results should be reported in mg kg-1

**9.2.2** Convert the residue concentration to their parent component and then add the residue values where the residue definition is expressed as sum of residues.

**9.2.3** Appropriate dilution factor associated with the sample preparation should be applied in final residue calculation/ quantification.

**9.2.4** Apply a suitable conversion factor for the analytes as per the residue definitions given in the regulatory guidelines wherever applicable.

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