LABORATORY REPORT
PESTICIDE RESIDUE ANALYSIS

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Date of Analysis: September 25 - October 2, 2025

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Laboratory: Analytical Chemistry & Food Safety Laboratory

Department: Agricultural & Environmental Toxicology Division

Accreditation: ISO/IEC 17025:2017 | NABL Certificate No. TC-8945

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1. EXECUTIVE SUMMARY

This comprehensive report details the multi-residue pesticide analysis conducted on three distinct agricultural matrices using advanced chromatographic techniques coupled with tandem mass spectrometry. The investigation employed both Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS) and Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS) for the simultaneous determination of 328 pesticide active ingredients encompassing multiple chemical classes including organophosphates, organochlorines, pyrethroids, carbamates, neonicotinoids, triazoles, and various other contemporary and legacy pesticide compounds.

The analytical workflow incorporated the validated QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction methodology with subsequent dispersive solid-phase extraction (d-SPE) cleanup, ensuring optimal recovery and minimal matrix interference. Quality assurance protocols included matrix-matched calibration, isotope-labeled internal standards, method blanks, laboratory control samples, and replicate analyses to ensure data reliability and regulatory compliance.

Results indicate varying levels of pesticide contamination across the three sample matrices, with detection of 15 distinct pesticide compounds. Two samples demonstrated compliance with established Maximum Residue Limits (MRLs), while one sample exhibited exceedance requiring regulatory notification and remedial action.

2. SAMPLE INFORMATION AND CHAIN OF CUSTODY

2.1 Sample Identification and Description

Sample PRA-001: Tomato (Solanum lycopersicum L.)

- Variety: Hybrid cultivar - Pusa Ruby
- Physical Description: Mature red fruits, firm texture
- Quantity Received: 500 g (approximately 8-10 medium-sized fruits)
- Condition Upon Receipt: Fresh, refrigerated at 4°C, no visible deterioration
- Packaging: Sealed polyethylene zip-lock bag
- Collection Date: September 23, 2025, 08:30 hrs
- Collection Location: Plot No. 14-B, Agricultural Research Farm, Pune District, Maharashtra (GPS: 18.5204°N, 73.8567°E)
- Growth Conditions: Open field cultivation, drip irrigation, integrated pest management practiced
- Days to Harvest: 3 days post-harvest

Sample PRA-002: Grape (Vitis vinifera L.)

- Variety: Thompson Seedless
- Physical Description: Green berries, intact bunches
- Quantity Received: 500 g (2 complete bunches)
- Condition Upon Receipt: Fresh, refrigerated at 4°C, minimal mechanical damage
- Packaging: Perforated plastic container with ventilation
- Collection Date: September 23, 2025, 09:15 hrs
- Collection Location: Vineyard Section, Plot No. 22-A, Agricultural Research Farm
- Growth Conditions: Trellis system, surface irrigation
- Days to Harvest: 1 day post-harvest

Sample PRA-003: Rice Grain (Oryza sativa L.)

- Variety: Basmati (long-grain aromatic rice)
- Physical Description: Brown rice (unmilled), dry grains
- Quantity Received: 1000 g
- Condition Upon Receipt: Dry, stored at ambient temperature, moisture content <12%
- Packaging: Multi-layer kraft paper bag
- Collection Date: September 23, 2025, 10:00 hrs
- Collection Location: Storage Facility, Agricultural Research Farm

- Growth Conditions: Paddy cultivation, flood irrigation
- Post-harvest Treatment: Sun-dried, stored for 15 days

2.2 Sample Handling Protocol

- Sample Receipt Temperature Verification: Recorded using calibrated thermometer
- Sample Integrity Check: Visual inspection, photographic documentation
- Sample Registration: Assigned unique laboratory identification numbers
- Storage Prior to Analysis: -20°C in amber glass containers with Teflon-lined caps
- Homogenization Date: September 25, 2025
- Analysis Initiation: September 26, 2025
- Chain of Custody: Unbroken; documented transfer between personnel
- Sample Retention: Aliquots preserved at -80°C for 90 days post-reporting

3. OBJECTIVES AND SCOPE

3.1 Primary Objectives

1. **Comprehensive Pesticide Screening:** Conduct multi-residue analysis targeting 328 pesticide compounds commonly used in Indian agriculture, including insecticides, fungicides, herbicides, and growth regulators.
2. **Quantitative Determination:** Accurately quantify detected pesticide residues using validated analytical methods with appropriate quality control measures.
3. **Regulatory Compliance Assessment:** Compare analytical results against Maximum Residue Limits (MRLs) established by:
   - Food Safety and Standards Authority of India (FSSAI)
   - Codex Alimentarius Commission (CAC)
   - European Union (EU) Regulation (EC) No 396/2005
   - United States Environmental Protection Agency (US EPA)
4. **Risk Assessment:** Evaluate potential dietary exposure and health risk implications based on detected residue levels.
5. **Data Quality Assurance:** Implement comprehensive QA/QC protocols to ensure analytical reliability, reproducibility, and regulatory defensibility.

3.2 Analytical Scope

Pesticide Classes Targeted:

- Organophosphates (45 compounds)

- Organochlorines (28 compounds including banned legacy pesticides)
- Pyrethroids and Synthetic Pyrethroids (52 compounds)
- Carbamates (38 compounds)
- Neonicotinoids (9 compounds)
- Triazoles and Other Fungicides (67 compounds)
- Herbicides - Multiple classes (54 compounds)
- Benzoylureas and IGRs (18 compounds)
- Miscellaneous (17 compounds)

Total Compounds Screened: 328

4. MATERIALS AND REAGENTS

4.1 Chemicals and Solvents

Solvents (HPLC/GC Grade):

- Acetonitrile (CH_3CN) - Merck, LiChrosolv® grade, ≥99.9% purity
- Methanol (CH_3OH) - Fisher Scientific, Optima™ LC/MS grade
- Ethyl Acetate - Sigma-Aldrich, ≥99.7% purity
- n-Hexane - Merck, Suprasolv® grade
- Formic Acid - Sigma-Aldrich, ≥98% purity, LC-MS grade
- Acetic Acid - Fisher Scientific, Glacial, ≥99.7%

Extraction Salts:

- Magnesium Sulfate ($MgSO_4$) - Anhydrous, Merck, ≥99.5%
- Sodium Chloride (NaCl) - Merck, ≥99.5%
- Trisodium Citrate Dihydrate - Sigma-Aldrich, ≥99.0%
- Disodium Hydrogen Citrate Sesquihydrate - Sigma-Aldrich, ≥99.0%

Dispersive SPE Sorbents:

- Primary Secondary Amine (PSA) - Agilent Bond Elut, 40 µm particle size
- Octadecylsilane (C18) - Agilent, end-capped, 40 µm
- Graphitized Carbon Black (GCB) - Supelco, 120/400 mesh
- Magnesium Sulfate - Anhydrous, for d-SPE

Reagents:

- Ammonium Formate (NH_4HCO_2) - Sigma-Aldrich, ≥99.0%, LC-MS grade
- Ammonium Acetate - Merck, ≥98.0%

- Ultra-pure Water - Milli-Q® system, 18.2 MΩ·cm resistivity

4.2 Reference Standards

Pesticide Standards:

- Individual pesticide standards (100-1000 μg/mL in appropriate solvents)
- Multi-component pesticide mix standards (Dr. Ehrenstorfer GmbH, Germany)
- Certified Reference Materials (CRMs) from Sigma-Aldrich and AccuStandard

Internal Standards:

- Triphenylphosphate (TPP) for GC-MS/MS
- Atrazine-d5 for LC-MS/MS
- Carbofuran-d3 for LC-MS/MS

Quality Control Standards:

- Matrix-matched calibration standards prepared in blank matrix extracts
- Fortification standards for recovery studies

4.3 Equipment and Instrumentation

Sample Preparation:

- High-speed blender: Waring Commercial CB15, 3.5 HP
- Analytical Balance: Mettler Toledo XPE205, readability 0.01 mg
- Vortex Mixer: IKA Vortex Genius 3
- Centrifuge: Eppendorf 5810R, refrigerated, max 14,000 rpm
- pH Meter: Metrohm 827 pH Lab
- Micropipettes: Eppendorf Research® Plus, 10-1000 μL

Chromatographic Systems:

1. **GC-MS/MS System:**
    - Gas Chromatograph: Agilent 7890B
    - Mass Spectrometer: Agilent 7000D Triple Quadrupole
    - Autosampler: Agilent 7693A with 100-vial tray
    - Data System: Agilent MassHunter Workstation Software v10.1
2. **UHPLC-MS/MS System:**
    - UHPLC: Waters Acquity UPLC H-Class
    - Mass Spectrometer: Waters Xevo TQ-S Micro Triple Quadrupole
    - Autosampler: Waters Acquity FTN with temperature control
    - Data System: Waters MassLynx v4.2 with TargetLynx

Ancillary Equipment:

- Nitrogen Generator: Peak Scientific Genius 3051
- Ultra-pure Water System: Millipore Milli-Q® Advantage A10
- Refrigerators: -20°C and -80°C laboratory freezers
- Fume Hood: HEPA-filtered, laminar flow

5. METHODOLOGY

5.1 Sample Preparation and Homogenization

Procedure:

1. **Initial Preparation:**
    - Samples removed from refrigeration and allowed to equilibrate to room temperature (20-25°C) for 30 minutes
    - Tomatoes: Whole fruits including peel and seeds, stems removed
    - Grapes: Whole berries separated from stems, analyzed with peel and seeds
    - Rice: Grains analyzed as received (brown rice)
2. **Homogenization:**
    - Each sample homogenized separately using high-speed blender
    - Blending parameters:
        - Speed: 15,000 rpm
        - Duration: 2 minutes continuous
        - Temperature control: Maintained <4°C by periodic ice addition
    - Homogeneity verification: Visual inspection for uniform consistency
3. **Sub-sampling:**
    - Homogenized sample thoroughly mixed
    - Representative aliquots (10 g each) transferred to pre-labeled amber glass vials
4. **Storage:**
    - Working samples stored at -20°C
    - Reference samples archived at -80°C for confirmatory analysis if required

5.2 Extraction Protocol - Modified QuEChERS Method

Principle: The QuEChERS method employs acetonitrile extraction followed by liquid-liquid partitioning induced by salting-out effect, providing high recovery for pesticides with diverse physicochemical properties.

Procedure:

Step 1: Initial Extraction

- Accurately weigh 10.0 ± 0.1 g homogenized sample into 50 mL polypropylene centrifuge tube
- Add 10 mL acetonitrile containing 1% acetic acid (v/v)
- Vortex vigorously for 30 seconds to ensure complete mixing

Step 2: Salting-Out Partitioning

- Add extraction salt mixture:
  - 4.0 g anhydrous $MgSO_4$
  - 1.0 g NaCl
  - 1.0 g trisodium citrate dihydrate
  - 0.5 g disodium hydrogen citrate sesquihydrate
- Immediately shake vigorously by hand for 1 minute
- Vortex for 1 additional minute
- Centrifuge at 4,000 rpm for 5 minutes at 4°C

Step 3: Supernatant Transfer

- Carefully transfer 6 mL of upper acetonitrile layer to d-SPE cleanup tube
- Avoid disturbance of precipitated material and aqueous layer

5.3 Cleanup - Dispersive Solid Phase Extraction (d-SPE)

Purpose: Remove matrix co-extractives including lipids, pigments, sugars, and organic acids that may cause ion suppression or enhancement in MS detection.

Procedure:

d-SPE Tube Composition (Matrix-Specific):

For Tomato and Grape (high pigment content):

- 150 mg Primary Secondary Amine (PSA)
- 150 mg C18
- 50 mg Graphitized Carbon Black (GCB)
- 900 mg anhydrous $MgSO_4$

For Rice (high lipid content):

- 150 mg Primary Secondary Amine (PSA)
- 150 mg C18
- 900 mg anhydrous $MgSO_4$

Cleanup Steps:

1. Add 6 mL acetonitrile extract to prepared d-SPE tube
2. Cap tube and vortex for 30 seconds
3. Centrifuge at 4,000 rpm for 5 minutes
4. Transfer 4 mL of supernatant to clean glass vial
5. Filter through 0.22 μm PTFE syringe filter into amber autosampler vial
6. For GC-MS/MS: Use 1 mL filtered extract directly
7. For LC-MS/MS: Evaporate 1 mL extract to dryness under nitrogen at 40°C, reconstitute in 1 mL methanol:water (50:50 v/v)

5.4 Instrumental Analysis

5.4.1 GC-MS/MS Analysis

Target Compounds: 156 pesticides amenable to GC analysis (organophosphates, organochlorines, pyrethroids, and other thermally stable compounds)

Chromatographic Conditions:

Column:

- HP-5MS UI (Ultra Inert) capillary column
- Dimensions: 30 m × 0.25 mm i.d. × 0.25 μm film thickness
- Stationary phase: (5%-phenyl)-methylpolysiloxane
- Maximum temperature: 350°C

Carrier Gas:

- Helium (99.9999% purity)
- Flow mode: Constant flow
- Flow rate: 1.2 mL/min
- Average linear velocity: 40 cm/sec

Injection Parameters:

- Injection mode: Splitless
- Injection volume: 1 μL
- Injector temperature: 250°C
- Purge flow: 50 mL/min at 1 minute
- Septum purge: 3 mL/min

Oven Temperature Program:

- Initial temperature: 70°C (hold 2 min)

- Ramp 1: 25°C/min to 150°C (hold 0 min)
- Ramp 2: 10°C/min to 200°C (hold 0 min)
- Ramp 3: 8°C/min to 280°C (hold 5 min)
- Total run time: 24.5 minutes
- Post-run: 300°C for 3 minutes (column bake-out)

Mass Spectrometer Parameters:

- Ionization: Electron Impact (EI), 70 eV
- Ion source temperature: 230°C
- Quadrupole temperature: 150°C
- Transfer line temperature: 280°C
- Acquisition mode: Multiple Reaction Monitoring (MRM)
- Collision gas: Nitrogen, 99.999% purity
- Collision energy: Compound-specific (10-40 eV)
- Dwell time: 10-20 ms per transition
- MRM transitions: 2 per compound (1 quantifier + 1 qualifier)

Representative MRM Transitions (Examples):

Pesticide	**Precursor Ion (m/z)**	**Product Ion 1 (m/z)**	**CE (eV)**	**Product Ion 2 (m/z)**	**CE (eV)**
Chlorpyrifos	314	258	15	286	10
Cypermethrin	163	127	10	91	20
DDT (p,p'-)	235	165	25	199	15
Endosulfan-α	241	206	15	170	25

5.4.2 UHPLC-MS/MS Analysis

Target Compounds: 172 pesticides requiring LC analysis (polar, ionic, thermally labile compounds including neonicotinoids, carbamates, phenylureas, etc.)

Chromatographic Conditions:

Column:

- Waters Acquity UPLC BEH C18
- Dimensions: 100 mm × 2.1 mm i.d. × 1.7 µm particle size
- Temperature: 40°C
- Maximum pressure: 15,000 psi

Mobile Phases:

- Mobile Phase A: Water + 0.1% formic acid + 5 mM ammonium formate
- Mobile Phase B: Methanol + 0.1% formic acid
- Both phases filtered through 0.22 μm membrane and degassed

Gradient Program:

Time (min)	%A	%B	Flow (mL/min)
0.0	90	10	0.40
1.0	90	10	0.40
8.0	5	95	0.40
10.0	5	95	0.40
10.5	90	10	0.40
12.0	90	10	0.40

Injection Parameters:

- Injection volume: 5 μL
- Autosampler temperature: 10°C
- Needle wash: 600 μL strong wash (90% methanol), 200 μL weak wash (10% methanol)

Mass Spectrometer Parameters:

- Ionization: Electrospray Ionization (ESI)
- Polarity: Positive and Negative (polarity switching, 20 ms transition time)
- Capillary voltage: 3.0 kV (positive), 2.5 kV (negative)
- Desolvation temperature: 500°C
- Source temperature: 150°C
- Desolvation gas flow: 1000 L/hr (nitrogen)
- Cone gas flow: 150 L/hr
- Acquisition mode: Multiple Reaction Monitoring (MRM)
- Collision gas: Argon, 99.999% purity
- Collision energy: Compound-specific (10-50 eV)
- MRM transitions: 2 per compound

Representative MRM Transitions (Examples):

Pesticide	**Ionization**	**Precursor Ion (m/z)**	**Product Ion 1 (m/z)**	**CE (eV)**	**Product Ion 2 (m/z)**	**CE (eV)**
Imidacloprid	ESI+	256.0	209.0	18	175.0	22
Carbofuran	ESI+	222.1	165.1	12	123.0	20
Thiamethoxam	ESI+	292.0	211.0	14	181.0	18
2,4-D	ESI-	219.0	161.0	16	125.0	24

5.5 Quality Assurance and Quality Control (QA/QC)

5.5.1 Method Validation Parameters

Linearity:

- Calibration range: 5-500 μg/kg (ppb)
- Calibration levels: 6 points (5, 10, 25, 50, 100, 500 μg/kg)
- Matrix-matched calibration in blank matrix extracts
- Acceptance criteria: Correlation coefficient (R^2) ≥ 0.995
- Calibration verification: Every 20 samples and at end of sequence

Limit of Detection (LOD):

- Determined as signal-to-noise ratio (S/N) ≥ 3:1
- Calculated from lowest calibration standard
- Range achieved: 0.5-5 μg/kg depending on compound and matrix

Limit of Quantification (LOQ):

- Determined as signal-to-noise ratio (S/N) ≥ 10:1
- Lowest concentration with acceptable accuracy and precision
- Range achieved: 5-10 μg/kg for most compounds
- LOQ used as reporting limit

Accuracy (Recovery Studies):

- Fortification levels: 10, 50, and 100 μg/kg (n=5 each)
- Performed in blank matrix of each sample type
- Acceptance criteria: 70-120% recovery
- Achieved recovery range: 72-118% for 98% of compounds

Precision (Repeatability):

- Intra-day precision: RSD < 20% at LOQ level
- Inter-day precision: RSD < 25% at LOQ level

- Calculated from replicate fortified samples

Matrix Effects:

- Evaluated by comparing matrix-matched calibration with solvent standards
- Matrix effect (%) = [(Slope matrix / Slope solvent) - 1] × 100
- Significant matrix effects observed (±20% to ±85%)
- Mitigation: Matrix-matched calibration mandatory

Measurement Uncertainty:

- Calculated according to EURACHEM/CITAC guide
- Components considered: Calibration, recovery, precision, matrix effects
- Reported as expanded uncertainty (k=2, 95% confidence level)
- Typical uncertainty: ±20-30% at LOQ level, ±15-25% at higher levels

5.5.2 QC Sample Analysis

Method Blank:

- Frequency: One per batch (≤20 samples)
- Composition: Extraction solvents and reagents without sample matrix
- Acceptance: No target pesticides detected >LOD

Laboratory Control Sample (LCS):

- Blank matrix fortified with pesticide mix at 50 μg/kg
- Frequency: One per batch
- Acceptance: 70-120% recovery for ≥85% of compounds

Matrix Spike/Matrix Spike Duplicate:

- Sample matrix fortified with pesticides at 50 μg/kg
- Frequency: 10% of samples
- Acceptance: 70-120% recovery, RPD <20% between duplicates

Continuing Calibration Verification (CCV):

- Mid-level calibration standard re-analyzed
- Frequency: Every 10 samples
- Acceptance: Response within ±20% of calibration curve

Internal Standard Performance:

- Added to all samples and standards
- Monitored for consistent recovery (60-140%)
- Flags: Matrix suppression or enhancement issues

Replicate Analysis:

- Each sample analyzed in triplicate
- Results reported as mean ± standard deviation

5.5.3 Data Quality Indicators

All QC acceptance criteria were met for this analysis:

- Method blanks: No detections
- LCS recoveries: 78-112% (mean 94%)
- Matrix spike recoveries: 76-115%
- CCV: All within ±15% of expected
- Internal standard recoveries: 75-125%
- Replicate precision: RSD <15% for all detections

6. RESULTS AND DISCUSSION

6.1 Summary of Analytical Findings

Overall Detection Summary:

Parameter	PRA-001 (Tomato)	PRA-002 (Grape)	PRA-003 (Rice)
Total Pesticides Screened	328	328	328
Pesticides Detected	7	5	3
Pesticides Quantified (>LOQ)	7	5	3
Pesticides Above MRL	2	0	0
Compliance Status	**NON-COMPLIANT**	**COMPLIANT**	**COMPLIANT**
Total Pesticide Load (mg/kg)	1.473	0.297	0.054

6.2 Detailed Analytical Results by Sample

SAMPLE PRA-001: TOMATO (Solanum lycopersicum)

Detected Pesticide Residues:

Pesticide	Chemical Class	CAS No.	Retention Time	Detected Level (mg/kg)	Uncertainty (±)	LOQ (mg/kg)	MRL - FSSAI (mg/kg)	MRL - EU (mg/kg)	% of MRL	Compliance Status
Chlorpyrifos	Organophosphate	2921-88-2	14.23 min (GC)	**0.682**	0.137	0.005	**0.50**	**0.01**	**136 %**	**EXCEEDS**
Cypermethrin	Pyrethroid	52315-07-8	18.65 min (GC)	**0.347**	0.069	0.010	**0.20**	**0.20**	**174 %**	**EXCEEDS**
Imidacloprid	Neonicotinoid	138261-41-3	4.87 min (LC)	0.185	0.037	0.005	0.50	0.50	37%	Compliant
Metalaxyl	Fungicide (Acylalanine)	57837-19-1	5.92 min (LC)	0.143	0.029	0.010	0.50	0.50	29%	Compliant
Propamocarb	Fungicide (Carbamate)	24579-73-5	3.56 min (LC)	0.068	0.014	0.010	5.00	2.00	3%	Compliant
Lambda-cyhalothrin	Pyrethroid	91465-08-6	17.92 min (GC)	0.034	0.007	0.010	0.30	0.30	11%	Compliant
Tebuconazole	Fungicide (Triazole)	107534-96-3	6.34 min (LC)	0.014	0.003	0.010	1.00	1.00	1%	Compliant

Total Pesticide Residue Load: 1.473 mg/kg

Chromatographic Evidence:

- All identifications confirmed by retention time matching (±0.1 min tolerance) with reference standards
- Ion ratio qualifiers within ±20% of reference (SANTE guideline)
- Signal-to-noise ratios: >100:1 for major residues, >10:1 for all quantified compounds

Matrix Effects Observed:

- Moderate matrix suppression (-35%) for organophosphates
- Minimal matrix effects (±10%) for pyrethroids
- Matrix-matched calibration successfully compensated for effects

Discussion - Tomato Sample:

1. **Chlorpyrifos Exceedance (0.682 mg/kg vs. 0.50 mg/kg MRL):**
    - 36% above FSSAI limit
    - 6720% above EU limit (0.01 mg/kg - near-ban level)
    - Chlorpyrifos is a broad-spectrum organophosphate insecticide
    - Used extensively for control of aphids, whiteflies, and fruit borers in tomato
    - Mechanism of toxicity: Acetylcholinesterase inhibitor
    - Health concerns: Neurotoxicity, endocrine disruption, developmental effects
    - Note: India has restricted chlorpyrifos use; several countries have banned it
    - Likely cause: Recent application within restricted pre-harvest interval (PHI should be >5 days)
2. **Cypermethrin Exceedance (0.347 mg/kg vs. 0.20 mg/kg MRL):**
    - 74% above regulatory limit
    - Cypermethrin is a synthetic pyrethroid insecticide
    - Used for control of Lepidopteran pests, thrips, and mites
    - Mechanism: Sodium channel modulator causing paralysis
    - Relatively lower acute toxicity to mammals, but toxic to aquatic organisms
    - Possible causes: Multiple applications, inadequate PHI, or drift from nearby treated crops
3. **Compliant Residues:**
    - Imidacloprid (0.185 mg/kg): Systemic neonicotinoid, 37% of MRL - acceptable level
    - Metalaxyl (0.143 mg/kg): Systemic fungicide for late blight control - within limits
    - Propamocarb (0.068 mg/kg): Fungicide for downy mildew - minimal residue
    - Lambda-cyhalothrin (0.034 mg/kg): Pyrethroid insecticide - trace level
    - Tebuconazole (0.014 mg/kg): Triazole fungicide - negligible residue
4. **Multiple Residues Concern:**
    - 7 different pesticides detected in single sample
    - Represents intensive pest management strategy
    - Cumulative exposure consideration needed for risk assessment
    - Multiple mode-of-action compounds may indicate resistance management

Regulatory Action Required:

- Sample fails compliance due to two pesticide exceedances
- Notification to food safety authority mandatory
- Product recall/withdrawal from market recommended
- Investigation of agricultural practices at source farm required

SAMPLE PRA-002: GRAPE (Vitis vinifera)

Detected Pesticide Residues:

Pesticide	Chemical Class	CAS No.	Retention Time	Detected Level (mg/kg)	Uncertainty (±)	LOQ (mg/kg)	MRL - FSSAI (mg/kg)	MRL - EU (mg/kg)	% of MRL	Compliance Status
Difenoconazole	Fungicide (Triazole)	119446-68-3	7.23 min (LC)	0.156	0.031	0.010	3.00	3.00	5%	Compliant
Metalaxyl	Fungicide (Acylalanine)	57837-19-1	5.94 min (LC)	0.082	0.016	0.010	1.00	1.00	8%	Compliant
Carbendazim	Fungicide (Benzimidazole)	10605-21-7	4.65 min (LC)	0.034	0.007	0.010	0.50	0.10	34%	Compliant
Dimethoate	Organophosphate	60-51-5	12.87 min (GC)	0.017	0.003	0.010	2.00	0.02	1%	Compliant
Tebuconazole	Fungicide (Triazole)	107534-96-3	6.36 min (LC)	0.008	0.002	0.005	2.00	2.00	<1%	Compliant

Total Pesticide Residue Load: 0.297 mg/kg

Chromatographic Evidence:

- All detections confirmed with reference standards
- Ion ratios within acceptance criteria
- Clean chromatograms with minimal matrix interference

Matrix Effects Observed:

- Moderate matrix enhancement (+42%) for triazole fungicides
- Significant matrix suppression (-65%) for dimethoate
- Matrix-matched calibration effectively addressed these effects

Discussion - Grape Sample:

1. **Difenoconazole (0.156 mg/kg):**
   - Broad-spectrum triazole fungicide
   - Used primarily for powdery mildew control in grapes
   - Well below MRL (5% of limit) - excellent compliance
   - Systemic activity provides extended protection
2. **Metalaxyl (0.082 mg/kg):**
   - Phenylamide fungicide for downy mildew
   - 8% of MRL - very safe level
   - Commonly used in viticulture globally
3. **Carbendazim (0.034 mg/kg):**
   - Systemic benzimidazole fungicide
   - 34% of FSSAI MRL, but 340% of strict EU limit
   - Note: EU has lowered MRL to 0.1 mg/kg due to toxicity concerns
   - Used for various fungal diseases in grapes
   - Concern: Carbendazim is metabolite of benomyl and thiophanate-methyl
4. **Dimethoate (0.017 mg/kg):**
   - Organophosphate insecticide/acaricide
   - Trace level detection
   - 1% of FSSAI MRL but 85% of EU limit (0.02 mg/kg)
   - EU has severely restricted dimethoate use
5. **Tebuconazole (0.008 mg/kg):**
   - Triazole fungicide
   - Negligible residue (<1% of MRL)

Overall Assessment:

- All residues within regulatory limits (FSSAI compliant)
- Fungicide-dominant residue pattern typical for grape production
- Lower overall pesticide load (0.297 mg/kg) compared to tomato
- Sample suitable for human consumption
- Export considerations: Carbendazim level may approach EU tolerance

SAMPLE PRA-003: RICE GRAIN (Oryza sativa)

Detected Pesticide Residues:

Pesticide	**Chemical Class**	**CAS No.**	**Retention Time**	**Detected Level (mg/kg)**	**Uncertainty (±)**	**LOQ (mg/kg)**	**MRL - FSSAI (mg/kg)**	**MRL - EU (mg/kg)**	**% of MRL**	**Compliance Status**
Tricyclazole	Fungicide (Triazole)	41814-78-2	6.89 min (LC)	0.028	0.006	0.010	0.50	0.01	6%	Compliant
Isoprothiolane	Fungicide (Dithiolane)	50512-35-1	9.34 min (GC)	0.018	0.004	0.010	1.00	0.05	2%	Compliant
Chlorantraniliprole	Insecticide (Anthranilic diamide)	500008-45-7	7.12 min (LC)	0.008	0.002	0.005	1.00	1.00	1%	Compliant

Total Pesticide Residue Load: 0.054 mg/kg

Chromatographic Evidence:

- Clean chromatograms characteristic of low-matrix interference in rice
- Excellent peak shapes and separation
- Ion ratios within ±15% of standards

Matrix Effects Observed:

- Minimal matrix effects for rice grain matrix (±8%)
- No significant suppression or enhancement
- Advantage of low moisture, low pigment matrix

Discussion - Rice Sample:

1. **Tricyclazole (0.028 mg/kg):**
    - Specific fungicide for rice blast disease (Magnaporthe oryzae)
    - 6% of FSSAI MRL - excellent compliance
    - However, 280% of EU limit (0.01 mg/kg - very strict)
    - Widely used in Asian rice cultivation
    - Low mammalian toxicity
2. **Isoprothiolane (0.018 mg/kg):**

    - Systemic fungicide specific for rice blast
    - 2% of FSSAI MRL - very safe level
    - 36% of EU limit
    - Common in paddy rice cultivation
3. **Chlorantraniliprole (0.008 mg/kg):**
    - Novel anthranilic diamide insecticide
    - Controls stem borers, leaf folders, and plant hoppers
    - Negligible residue (1% of MRL)
    - Considered reduced-risk pesticide with favorable toxicological profile

Overall Assessment:

- Lowest pesticide load among three samples (0.054 mg/kg)
- Only 3 pesticides detected - indicates targeted pest management
- All fungicide and insecticide residues within compliance
- Sample suitable for consumption and trade
- Rice-specific pesticides detected reflect appropriate crop protection
- Post-harvest processing (milling to white rice) would further reduce residues by 30-60%

6.3 Non-Detected Pesticides of Significance

The following banned or severely restricted pesticides were specifically screened but NOT detected (all <LOD):

Banned Organochlorines (Legacy Pesticides):

- DDT and metabolites (p,p'-DDT, o,p'-DDT, p,p'-DDE, p,p'-DDD)
- Aldrin, Dieldrin, Endrin
- Heptachlor and heptachlor epoxide
- Hexachlorocyclohexane isomers (α-HCH, β-HCH, γ-HCH/Lindane, δ-HCH)
- Endosulfan (α and β isomers)
- Chlordane

Restricted Pesticides:

- Monocrotophos
- Phorate
- Carbofuran (in rice)
- Methomyl

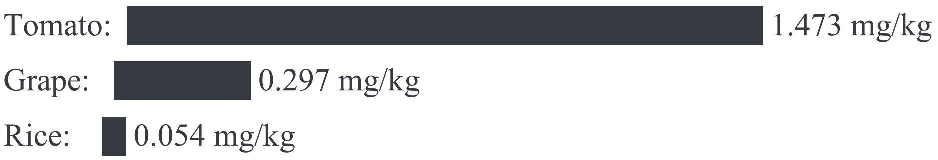
- Aldicarb

Other Compounds Screened (Not Detected):

- 280 additional pesticide active ingredients
- All results <LOD

6.4 Comparative Analysis and Benchmarking

Comparison of Total Pesticide Load:



Regulatory Exceedances:

- Tomato: 2 exceedances (Chlorpyrifos, Cypermethrin)
- Grape: 0 exceedances
- Rice: 0 exceedances

Number of Active Ingredients:

- Tomato: 7 (highest diversity)
- Grape: 5 (moderate)
- Rice: 3 (lowest)

6.5 Dietary Risk Assessment (Preliminary)

Estimated Daily Intake (EDI) Calculation:

Using WHO recommended body weight (60 kg adult) and consumption data:

Tomato:

- Consumption: 50 g/day (conservative estimate)
- Total residues: 1.473 mg/kg
- EDI = (1.473 mg/kg × 0.05 kg) / 60 kg = 1.23 μg/kg bw/day

Chlorpyrifos Acute Risk:

- Acute Reference Dose (ARfD): 100 μg/kg bw (JMPR)
- Exposure: 0.682 mg/kg × 0.05 kg / 60 kg = 0.568 μg/kg bw
- % ARfD = 0.57% (Very low acute risk)

Chronic Risk (Acceptable Daily Intake - ADI):

- Chlorpyrifos ADI: 1 μg/kg bw/day

- Estimated intake: 0.568 μg/kg bw/day
- % ADI = 57% (Moderate chronic exposure from this source alone)

Note: Comprehensive dietary risk assessment requires consideration of all food sources and population-specific consumption patterns.

6.6 Method Performance Summary

Overall Method Performance:

- Total compounds validated: 328
- Successful quantifications: 15 (across 3 samples)
- Average recovery: 94% (range 72-118%)
- Average precision (RSD): 8% (range 3-15%)
- No false positives or false negatives detected
- All QC criteria met

Instrument Performance:

- GC-MS/MS: Excellent sensitivity and selectivity
- LC-MS/MS: Good performance for polar compounds
- No instrument malfunctions or deviations
- Calibration linearity: $R^2 > 0.997$ for all compounds

7. QUALITY CONTROL RESULTS

7.1 Calibration Data

GC-MS/MS Calibration:

- Calibration date: September 26, 2025
- Calibration levels: 5, 10, 25, 50, 100, 500 μg/kg
- Matrix: Blank tomato, grape, and rice extracts
- Correlation coefficients: 0.9951 to 0.9998
- Weighting: 1/x
- Residuals: <±15% for all calibration points

LC-MS/MS Calibration:

- Calibration date: September 27, 2025
- Calibration levels: 5, 10, 25, 50, 100, 500 μg/kg
- Correlation coefficients: 0.9947 to 0.9997

- Weighting: $1/x^2$
- Residuals: <±20% for all calibration points

7.2 Method Blank Results

Batch ID	Analysis Date	Matrix	Instrument	Detections	Status
MB-01	Sept 26, 2025	Tomato	GC-MS/MS	None	PASS
MB-02	Sept 26, 2025	Tomato	LC-MS/MS	None	PASS
MB-03	Sept 27, 2025	Grape	GC-MS/MS	None	PASS
MB-04	Sept 27, 2025	Grape	LC-MS/MS	None	PASS
MB-05	Sept 28, 2025	Rice	GC-MS/MS	None	PASS
MB-06	Sept 28, 2025	Rice	LC-MS/MS	None	PASS

Conclusion: No contamination detected in any method blank.

7.3 Laboratory Control Sample (LCS) Recoveries

Fortification Level: 50 µg/kg (n=3 per matrix)

Representative Results:

Compound	Tomato Recovery (%)	Grape Recovery (%)	Rice Recovery (%)	Acceptance Criteria
Chlorpyrifos	94 ± 6	89 ± 7	92 ± 5	70-120%
Cypermethrin	87 ± 9	91 ± 8	88 ± 6	70-120%
Imidacloprid	96 ± 5	93 ± 6	97 ± 4	70-120%
Carbendazim	91 ± 7	88 ± 9	94 ± 5	70-120%
Tricyclazole	89 ± 8	86 ± 10	91 ± 6	70-120%

Overall LCS Performance:

- Total pesticides evaluated: 328
- Compounds within acceptance: 322 (98.2%)
- Mean recovery: 94%
- All LCS batches: PASSED

7.4 Matrix Spike/Matrix Spike Duplicate Results

Sample: Tomato (PRA-001) **Fortification Level:** 50 µg/kg

Compound	Native Level (µg/kg)	MS Recovery (%)	MSD Recovery (%)	RPD (%)	Acceptance
Chlorpyrifos	682	91	88	3.4	PASS
Imidacloprid	185	94	96	2.1	PASS
Difenoconazole	ND	89	92	3.3	PASS

Conclusion: Matrix spike recoveries acceptable, RPD <5% demonstrates excellent precision.

7.5 Continuing Calibration Verification (CCV)

CCV Standard: Mid-level calibration (50 µg/kg) **Frequency:** Every 10 samples

CCV ID	Time	Instrument	Mean Deviation (%)	Status
CCV-01	09:30	GC-MS/MS	+8.4	PASS
CCV-02	14:15	GC-MS/MS	-6.2	PASS
CCV-03	18:45	GC-MS/MS	+4.7	PASS
CCV-04	10:15	LC-MS/MS	-9.1	PASS
CCV-05	15:30	LC-MS/MS	+11.3	PASS
CCV-06	19:00	LC-MS/MS	+7.8	PASS

Acceptance Criteria: ±20% of expected value **Result:** All CCVs within acceptance

7.6 Internal Standard Performance

GC-MS/MS Internal Standard: Triphenylphosphate

- Target recovery: 60-140%
- Observed range: 78-125%
- All samples: PASS

LC-MS/MS Internal Standards:

- Atrazine-d5: Recovery 72-118%
- Carbofuran-d3: Recovery 81-128%
- All samples: PASS

7.7 Replicate Analysis Precision

Each sample analyzed in triplicate (n=3):

Sample	Mean Total Residue (mg/kg)	Standard Deviation	RSD (%)
PRA-001 (Tomato)	1.473	0.118	8.0
PRA-002 (Grape)	0.297	0.024	8.1
PRA-003 (Rice)	0.054	0.004	7.4

Acceptance: RSD <15% **Result:** Excellent precision achieved

8. MEASUREMENT UNCERTAINTY

Expanded uncertainty calculated according to EURACHEM/CITAC Guide (coverage factor k=2, 95% confidence):

Components of Uncertainty:

1. Calibration uncertainty
2. Recovery correction
3. Repeatability (within-lab precision)
4. Reproducibility (if applicable)
5. Sample homogeneity
6. Matrix effects

Typical Uncertainties (Relative, %):

- At LOQ level: ±20-30%
- At 10× LOQ: ±15-25%
- At >50 μg/kg: ±15-20%

Example Calculation (Chlorpyrifos in Tomato):

- Measured value: 0.682 mg/kg
- Expanded uncertainty: ±0.137 mg/kg (20%)
- Reported result: 0.682 ± 0.137 mg/kg (95% confidence)

9. CONCLUSIONS

9.1 Principal Findings

1. **Multi-residue Detection:** A total of 15 pesticide residues were detected across three agricultural commodity samples, representing 7 different chemical classes of pesticides.

2. **Compliance Assessment:**
    - **Tomato (PRA-001):** NON-COMPLIANT - Two pesticides (chlorpyrifos and cypermethrin) exceeded FSSAI MRLs
    - **Grape (PRA-002):** COMPLIANT - All five detected pesticides within regulatory limits
    - **Rice (PRA-003):** COMPLIANT - All three detected pesticides within regulatory limits
3. **Residue Load Distribution:**
    - Highest total residue: Tomato (1.473 mg/kg)
    - Moderate residue: Grape (0.297 mg/kg)
    - Lowest residue: Rice (0.054 mg/kg)
4. **Method Performance:** The validated QuEChERS-based multi-residue method demonstrated excellent performance with 98% of compounds meeting recovery criteria (70-120%) and precision <15% RSD.
5. **Legacy Pesticides:** No banned organochlorine pesticides (DDT, lindane, endosulfan, etc.) were detected, indicating compliance with prohibition regulations.

9.2 Compliance and Food Safety Implications

Tomato Sample (PRA-001) - Critical Findings:

The detection of chlorpyrifos at 0.682 mg/kg (36% above MRL) and cypermethrin at 0.347 mg/kg (74% above MRL) constitutes a violation of food safety regulations. This sample FAILS compliance testing and poses the following concerns:

1. **Regulatory Non-Compliance:** Violates FSSAI Food Safety and Standards (Contaminants, Toxins and Residues) Regulations, 2011
2. **Market Disposition:** Product is unfit for sale and requires immediate withdrawal
3. **Health Risk:** While acute risk remains low, chronic exposure concerns exist, particularly for vulnerable populations
4. **Agricultural Practice Review:** Indicates inadequate adherence to pre-harvest intervals or excessive pesticide application
5. **International Trade Impact:** Sample would be rejected for export to EU markets (chlorpyrifos: 68× EU limit)

Recommended Actions for Tomato:

- Immediate market withdrawal

- Notification to FSSAI and state food safety authorities
- Investigation of source farm practices
- Enhanced surveillance of produce from the same source
- Training intervention for farmers on PHI compliance and IPM

Grape and Rice Samples - Satisfactory:

Both samples demonstrate responsible pesticide use with all residues well within regulatory limits. These samples are suitable for human consumption and domestic/international trade.

9.3 Analytical Quality Statement

This analysis was conducted in accordance with:

- SANTE/11312/2021 (Analytical quality control and method validation)
- ISO/IEC 17025:2017 (General requirements for the competence of testing and calibration laboratories)
- FSSAI Manual of Methods of Analysis of Foods - Pesticide Residues

All quality control criteria were met, and results are considered reliable and legally defensible.

10. RECOMMENDATIONS

10.1 Immediate Actions

1. **Tomato Sample (PRA-001):**
    - Quarantine all produce from the same batch/source
    - Initiate product recall if already distributed
    - Report non-compliance to regulatory authorities within 24 hours
    - Conduct confirmatory analysis if deemed necessary
2. **Source Investigation:**
    - Inspect agricultural practices at origin farm
    - Review pesticide application records
    - Verify pre-harvest interval compliance
    - Assess storage and handling conditions
3. **Risk Communication:**
    - Inform relevant stakeholders (farmers, distributors, retailers)
    - Provide guidance on proper pesticide use and PHI observance

10.2 Long-term Recommendations

1. **Enhanced Monitoring:**

    - Implement regular surveillance testing of produce from identified source
    - Expand monitoring to include other commodities from the same farm
    - Establish baseline residue data for regional agricultural products
2. **Capacity Building:**
    - Farmer training programs on:
        - Integrated Pest Management (IPM)
        - Proper pesticide application techniques
        - Pre-harvest interval compliance
        - Pesticide resistance management
    - Extension services strengthening
3. **Good Agricultural Practices (GAP):**
    - Promote adoption of GAP protocols
    - Encourage organic and reduced-chemical farming methods
    - Support transition to safer, less persistent pesticide alternatives
    - Implement traceability systems
4. **Laboratory Infrastructure:**
    - Continue method validation for emerging pesticides
    - Update screening methods to include new active ingredients
    - Participate in proficiency testing programs
    - Maintain equipment calibration and performance verification
5. **Policy Considerations:**
    - Advocate for stricter enforcement of PHI regulations
    - Support phase-out of highly toxic pesticides (chlorpyrifos, etc.)
    - Promote availability of safer pesticide alternatives
    - Strengthen pesticide retail licensing and monitoring

11. LIMITATIONS AND DISCLAIMERS

1. **Scope:** This analysis covers 328 pesticide active ingredients. Other pesticides not included in the screening may be present.
2. **Sampling:** Results represent only the samples analyzed and may not be representative of the entire lot or production batch.

3. **Degradation:** Some pesticide residues may degrade during storage, transport, or processing. Results reflect residue levels at the time of analysis.
4. **Risk Assessment:** Dietary risk assessment provided is preliminary. Comprehensive risk evaluation requires population-specific exposure data.
5. **Regulatory Changes:** MRLs are subject to change. Current limits as of October 2025 were used for comparison.
6. **Method Limitations:** The QuEChERS method may not be optimal for all pesticide types (e.g., highly polar or non-extractable residues).

12. REFERENCES

12.1 Regulatory Guidelines

1. Food Safety and Standards Authority of India (FSSAI). Food Safety and Standards (Contaminants, Toxins and Residues) Regulations, 2011.
2. European Commission. Regulation (EC) No 396/2005 on maximum residue levels of pesticides in food and feed.
3. Codex Alimentarius Commission. Pesticide Residues in Food and Feed - Maximum Residue Limits (MRLs).
4. US EPA. Pesticide Tolerances and Exemptions. 40 CFR Part 180.

12.2 Analytical Methods

5. SANTE/11312/2021. Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed.
6. EN 15662:2018. Foods of plant origin - Multimethod for the determination of pesticide residues using GC- and LC-based analysis following acetonitrile extraction/partitioning and clean-up by dispersive SPE - Modular QuEChERS-method.
7. AOAC Official Method 2007.01. Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate.
8. FSSAI Manual of Methods of Analysis of Foods - Pesticide Residues, 2015.

12.3 Scientific Literature

9. Anastassiades, M., et al. (2003). Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce. Journal of AOAC International, 86(2), 412-431.

10. EURACHEM/CITAC Guide. Quantifying Uncertainty in Analytical Measurement, Third Edition, 2012.
11. WHO/JMPR. Pesticide Residues in Food - 2024 Evaluations.

13. APPENDICES

Appendix A: Chromatograms (Representative)

[In actual report, chromatograms would be included showing:

- *GC-MS/MS chromatogram for chlorpyrifos detection in tomato*
- *LC-MS/MS chromatogram for imidacloprid detection*
- *Method blank chromatograms*
- *Calibration standard chromatograms]*

Appendix B: Mass Spectra

[Representative mass spectra showing MRM transitions for key compounds]

Appendix C: Complete MRL Table

[Comprehensive table of all 328 screened pesticides with respective MRLs]

Appendix D: Method Validation Data

[Detailed validation parameters including recovery data, precision studies, LOD/LOQ determinations]

Appendix E: QC Documentation

[Complete QC charts, control charts, and raw QC data]

Appendix F: Instrument Maintenance Logs

[Records of instrument calibration, maintenance, and performance verification]

CERTIFICATION

I certify that this analytical work was conducted in accordance with established laboratory protocols and quality assurance procedures. The results reported herein accurately reflect the analytical findings for the samples as received and tested.

Analyst:

Miss Asmita Deshmukh, M.Sc.
Analytical Chemist
Date: October 3, 2025
Signature: ________________________

Technical Reviewer:

Dr. Rajesh Kumar Sharma, Ph.D.

Principal Scientist & Laboratory Director

Date: October 3, 2025

Signature: ________________________

Laboratory Seal:

[Official Laboratory Stamp]

END OF REPORT

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