Application Note

Food

Addressing Challenges in LC/MS/MS Analysis of Highly Polar Pesticide Residue in Rice

Using the Agilent 1260 Infinity III Prime LC system with the Agilent 6475 triple quadrupole LC/MS as per the FSSAI, eCFR, and EU regulations in cereal matrices

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Abstract

This application note describes a robust, sensitive, and regulatory-compliant workflow for the analysis of polar pesticides in rice using the Agilent 1260 Infinity III Prime LC system coupled with the Agilent 6475 triple quadrupole LC/MS. Quick Polar Pesticides Method (QuPPe-PO version 12.3) was employed for sample preparation and the usage of dynamic MRM (dMRM)-based method was evaluated for key polar analytes—ethephon, fosetyl-Al , glufosinate ammonium, glyphosate, and their metabolites—that are specifically regulated by guidelines across various countries. Matrix-based (pre-spiked) calibration with LOQ of 0.5 to 5 µg/kg with excellent linearity (R^2 > 0.995) across all analytes, and recovery study were performed in rice in accordance with SANTE guidelines[6] while making use of internal standards.

Introduction

India has been the world's largest exporter of rice since 2012 and rice constitutes more than 35% of India's total food grain export volume.[1] The Food Safety and Standards Authority of India (FSSAI)[2], the Electronic Code of Federal Regulations (eCFR)[3], and the European Commission (EURL)[4] have set stringent maximum residue limits (MRLs) for pesticide residues in rice.

Basmati and non-basmati varieties of rice exported from India are subject to heightened scrutiny under domestic and export residue-monitoring programs, making reliable, low-level quantitation of polar pesticides a business-critical analytical capability for Indian testing laboratories and exporters.

Pesticides such as glyphosate, glufosinate, ethephon, and fosetyl-Al, and their metabolites—HEPA, MPPA, NAG, and AMPA—are among the most challenging analytes to assess by conventional reversed-phase chromatography due to their high polarity, poor retention, and susceptibility to matrix interference (Figure 2). Additionally, the various regulations and diversity of matrices make the analysis more challenging.

This study was carried out to address these challenges using the Agilent 6475 triple quadrupole LC/MS, benchmarked directly against the FSSAI, eCFR, and EURL requirements, by using the Quick Polar Pesticides Method (QuPPe-PO)[5] and without using traditional FMOC-Cl-based derivatization methodology.

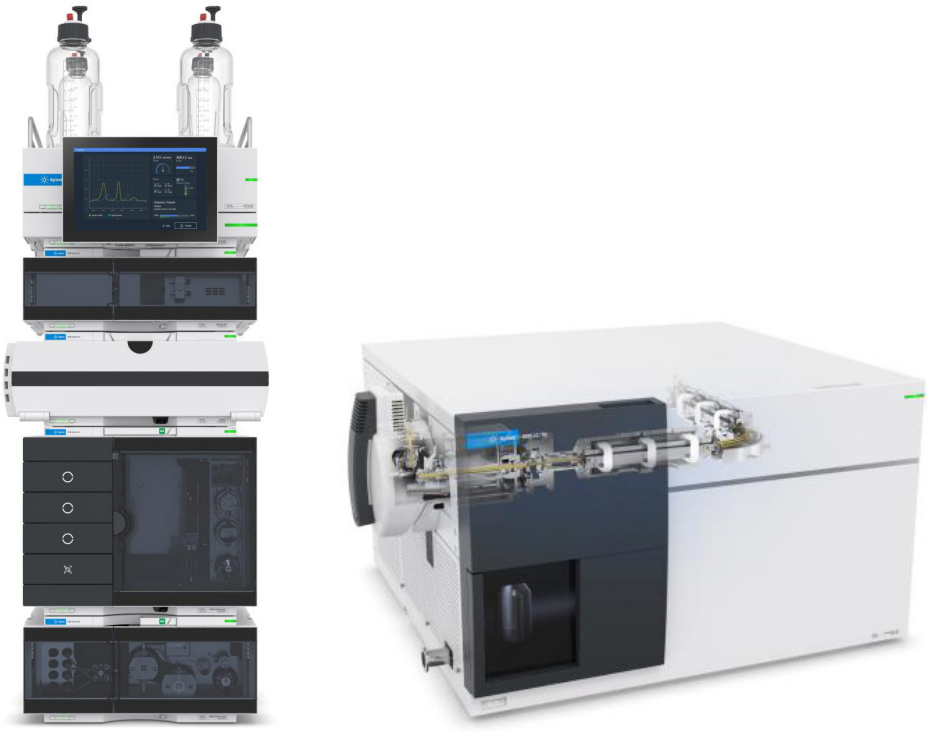


Figure 1. Agilent 1260 Infinity III Prime LC system with the Agilent 6475 triple quadrupole LC/MS.

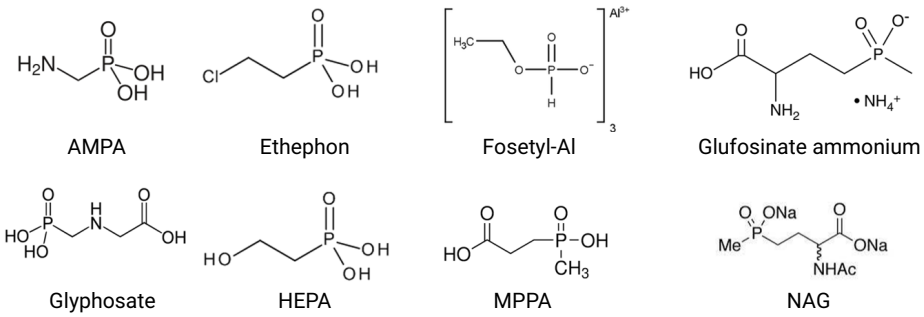


Figure 2. Major polar pesticides and their structures.

Experimental

Instrumentation

- LC System: Agilent 1260 Infinity III Prime LC system
- MS System: Agilent 6475 triple quadrupole LC/MS with AJS source

Software

Agilent MassHunter Workstation software

Lab equipment and consumables

- Multitube shaker
- Water, Solstice Advanced Materials, Inc. (catalog number LC365)
- Methanol, Solstice Advanced Materials, Inc. (catalog number 34966)
- Formic acid, Solstice Advanced Materials, Inc. (catalog number 56302)
- EDTA disodium salt, dihydrate (CAS number 6381-92-6)
- Deep freezer (−20 °C or −80 °C)
- Cryogenic centrifuge (≤ −10 °C with 10,000 g/rcf for 50 mL rotor)
- Polypropylene vial
- Capillary PEEK/ST 0.17 × 500 mm RLO/RLO, Agilent Technologies (p/n G5667-81005)

Chemicals

Individual pesticide reference standards from Toronto Research Chemicals (TRC) and HPC Standards GmbH were procured from Molecules Analytical Lab Solutions (Hyderabad, India). The corresponding CAS numbers of the purchased standards are seen in Table 1.

Preparation of stock and dilutions

Individual standard stock solutions were prepared at a concentration of 1,000 ppm in water. A mixed working standard of 100 ppm was subsequently prepared by appropriately diluting and combining stock solutions with water (see Table 2).

Table 1. Name of pesticides with their Chemical Abstract Service (CAS) numbers as used in the analysis.

Analyte	CAS No.	Abbreviation
Aminomethylphosphonic Acid	1066-51-9	AMPA
Ethephon	16672-87-0	Ethephon
Fosetyl Aluminium	39148-24-8	Fosetyl
Glufosinate Ammonium	77182-82-2	Glufosinate
Glyphosate	1071-83-6	Glyphosate
2-Hydroxyethahephosphonic Acid	22987-21-9	HEPA
Methylphosphonicopropionic Acid	1-5090-23-0	MPPA
n-AcetylGlufosinate	133659-60-6	NAG

Table 2. Preparation of dilutions for polar pesticides.

S. No.	Stock Conc. (ppm)	Stock Volume (mL)	Diluent (mL)	Obtained Conc. (ppm)	Obtained Equivalent in 5 g of Rice (ng/g)
1	100	0.1	0.9	10	500
2	10	0.5	0.5	5	250
3	5	0.4	0.6	2	100
4	2	0.4	0.6	0.8	40
5	0.8	0.5	0.5	0.4	20
6	0.4	0.5	0.5	0.2	10
7	0.2	0.5	0.5	0.1	5
8	0.1	0.4	0.6	0.04	2
9	0.04	0.5	0.5	0.02	1
10	0.02	0.5	0.5	0.01	0.05

1260 Infinity III Prime LC system parameters

Table 3. HPLC parameters.

Parameter	Value
Working Diluent	Methanol:water (1:1) with 0.5% formic acid
MultiWash	Water:acetonitrile (90:10) 20 seconds Water:acetonitrile (10:90) 20 Seconds
Injection Volume	10 µL
Column	BEH-DEA (2.1 × 100 mm, 5 µm), at 50 °C

Sample preparation

Sample extraction followed the EURL-SRM QuPPe-PO method, version 12.3, for cereals, pulses, nuts, and oilseeds.[5] The following steps were used:

1. Weigh 5 g (±0.01) of powdered rice sample in 50 mL PP tube.
2. Add 9 mL of ultrapure water and vortex the tube for 30 minutes.
3. Spike 0.25 mL of solvents standard at different concentration levels and 0.05 mL of IS; vortex for 10 minutes.
4. Add 10 mL of acidified methanol (1% formic acid); vortex for 15 minutes for extraction.
5. Add 0.1 mL of formic acid and keep it in standby for 30 minutes after 2 minutes vortex.
6. Add 1 mL of EDTA (10% w/v) and vortex for 15 minutes for chelation step.
7. Keep PP tubes at –20 °C for 120 minutes (or at –80 °C for 30 minutes).
8. Centrifuge chilled PP tubes using 10,000 g(rcf) speed at –10 °C for 10 minutes.
9. Immediately add 2 mL of supernatant to 2 mL of acetonitrile for precipitation.
10. Vortex the sample for 30 seconds, then centrifuge at 5,000 g at 5 °C for 5 minutes.
11. Transfer the supernatant to a 1 mL PP vial for analysis.

Note: Particle size of rice powder was kept at ~ 0.5 mm by making use of sieve and vortex speed was maintained at 800–1,250 rpm throughout. Matrix-based (prespike) calibration was generated using a 5 g sample extracted into 10 mL water and 10 mL methanol, then diluted 1:1 in acetonitrile, resulting in an overall dilution factor of 8.

Table 4. HILIC gradient elution program used for separation of polar pesticides (overnight conditioning of columns is necessary with initial gradient before analysis).

Time (min)	%A (0.9% FA in water)	%B (0.9% FA in acetonitrile)	Flow (mL/min)
0.0	10.0	90.0	0.5
4.0	85.0	15.0	0.5
13.0	85.0	15.0	0.5
13.5	10.0	90.0	0.5
25.0	10.0	90.0	0.5

Table 5. Agilent Jet Stream (AJS ESI) source parameters using the Agilent 6475 triple quadrupole LC/MS.

Parameter	Positive Mode	Negative Mode
Gas Temperature	220 °C	220 °C
Gas Flow	11 L/min	11 L/min
Nebulizer	30 psi	30 psi
Capillary Voltage	3,000 V	3,500 V
Sheath Gas Temperature	300 °C	300 °C
Sheath Gas Flow	11 L/min	11 L/min
Nozzle Voltage	500 V	1,500 V

Table 6. dMRM method details using the Agilent 6475 triple quadrupole LC/MS.

S. No.	Analyte	Precursor	Product	RT (min)	Fragmentor (V)	CE (V)	CAV (V)	Polarity
1	AMPA	110	62.9	3.5	100	22	3	Negative
	AMPA	110	78.9	3.5	100	34	1	Negative
	AMPA	110	80.9	3.5	100	11	2	Negative
	AMPA-IS	112	63	3.5	100	11	3	Negative
2	Ethephon	145	90.9	9.0	95	19	2	Positive
	Ethephon	145	80.9	9.0	95	27	2	Positive
	Ethephon IS	149	81.0	9.0	95	25	2	Positive
3	Fosetyl-Al	111	82.9	12.0	70	8	1	Positive
	Fosetyl-Al	111	65.0	12.0	70	24	3	Positive
	Fosetyl-Al-IS	114	82.0	12.0	70	8	1	Negative
4	Glufosinate	182.0	136.0	4.5	90	11	5	Positive
	Glufosinate	182.0	119.0	4.5	90	19	6	Positive
	Glufosinate-IS	185.0	139.0	4.5	90	10	6	Positive
5	Glyphosate	170.0	88.0	7.3	60	7	1	Positive
	Glyphosate	170.0	60.0	7.3	60	15	7	Positive
	Glyphosate-IS	173.0	62.0	7.3	60	15	7	Positive
6	HEPA	127.0	46.9	7.0	100	76	1	Positive
	HEPA	127.0	90.9	7.0	100	19	4	Positive
	HEPA-IS	131.0	81.0	7.0	100	25	1	Positive
7	MPPA	153.0	78.9	5.0	80	23	3	Positive
	MPPA	153.0	107.0	5.0	80	15	1	Positive
	Glufosinate-IS	185.0	139.0	4.5	90	10	6	Positive
8	NAG	224.2	136.1	6.0	70	20	5	Positive
	NAG	224.2	56.1	6.0	70	44	5	Positive
	NAG-IS	227.0	56.0	6.0	70	50	5	Positive

Results and discussion

The Dynamic Multiple Reaction Monitoring (dMRM) acquisition mode available on the 6475 LC/TQ provided enhanced sensitivity, improved peak shapes, and efficient monitoring of analytes within their predefined retention time windows. As demonstrated in the chromatograms and accompanying data tables, all eight target polar pesticide analytes—AMPA, ethephon, fosetyl-Al , glufosinate, glyphosate, MPPA, HEPA, and NAG—were successfully separated and detected within a single 14-minute HILIC-based chromatographic run. The method enabled effective retention and resolution of highly polar, early-eluting compounds, including AMPA, which is often considered chromatographically challenging. Clean and symmetrical peak shapes were obtained for all analytes in rice matrix samples spiked at 100 µg/kg, demonstrating excellent chromatographic performance. Furthermore, matrix-matched (prespike) calibration established adequate chromatographic resolution and quantitative performance across all analytes, including the challenging compounds AMPA and glyphosate (Figure 3). The chromatographic overlay obtained from multiple calibration levels in the matrix-based calibration further confirmed the robustness, reproducibility, and consistency of the developed method (Figure 4).

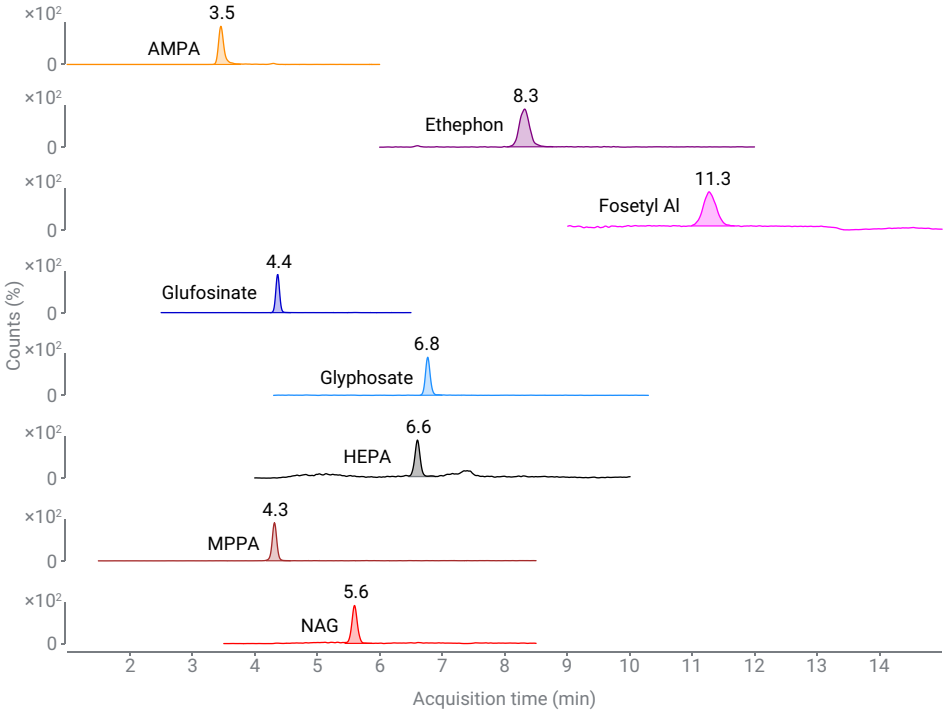


Figure 3. Chromatographic separation and peak shape among polar pesticides in rice matrix.

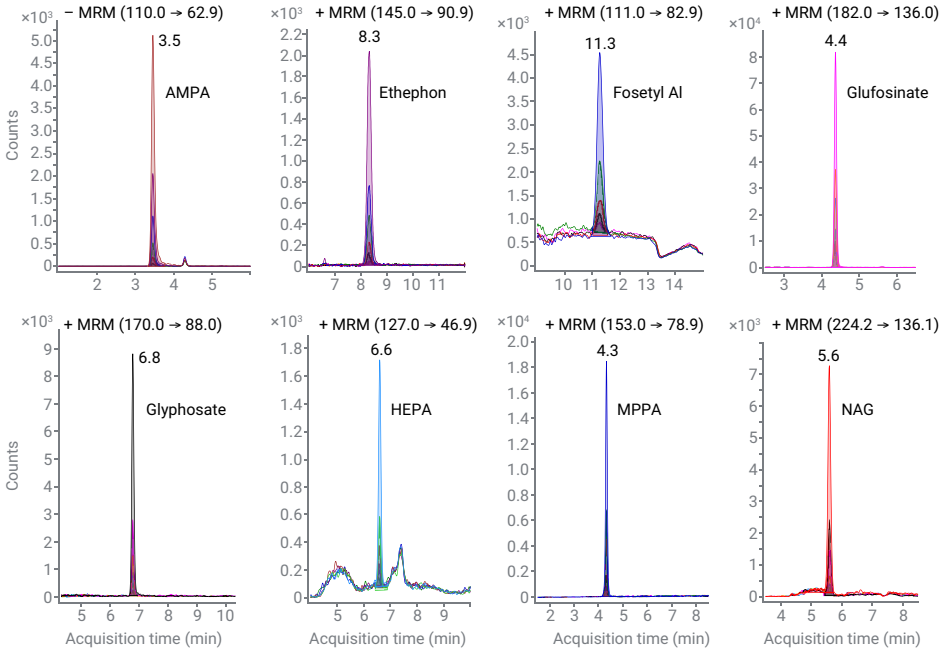


Figure 4. Chromatographic overlay across calibration range in matrix for eight polar pesticides.

Specificity was confirmed by comparing calculated concentrations of each of the pesticides in blank against targeted LOQ level as seen in calibration tables of analytes. No significant interference was observed in the retention times of target analytes in the matrix blank, in accordance with SANTE/11312/2026[6] criterion (response in blank ≤ 30% of the reporting limit or the area response of qualifier/quantifier is not within permissible ± 30% uncertainty).

Matrix-based (prespike) calibration curves were generated for five or more levels across 0.5–100 µg/kg and all analytes exhibited excellent linearity, with R^2 values exceeding 0.995. Recovery experiments were performed by prespiking rice at 10 µg/kg for glufosinate and glyphosate. Spike study for ethephon, MPPA, and NAG was done at 20 µg/kg and for the remaining analytes, at 50 µg/kg. All recoveries were within or close to the acceptable 70–120% range as per method validation parameters and criteria mentioned in SANTE/11312/2026, with the use of isotopically labeled internal standards (Figures 5, 6, 7, 8, 9, 10, 11, and 12).

Sample		AMPA M...	AMPA Results			Qualifier (110.0 -> 80.9...	Qualifier (110.0 -> 78.9...	AMPA-IS...
Name	Type	Exp. Conc.	RT	Calc. Conc.	Accuracy	Ratio	Ratio	RT
Dil	Blank		4.11	1.67				3.33
MB	MatrixBlank		3.48	0.38		28.3	20.5	3.46
L4	Cal	2.0	3.46	1.93	96.4	68.9	96.0	3.46
L5	Cal	5.0	3.45	5.21	104.1	53.5	79.4	3.46
L6	Cal	10.0	3.46	9.91	99.1	43.3	88.1	3.46
L7	Cal	20.0	3.45	20.27	101.4	50.4	95.4	3.46
L8	Cal	40.0	3.45	39.55	98.9	48.3	95.6	3.46
L9	Cal	100.0	3.45	100.14	100.1	49.7	95.9	3.46
Dil	Blank		3.87	0.99				3.45
S50	MatrixSpike		3.47	61.08		63.1	91.5	3.48
S50	MatrixSpike		3.47	54.82		55.3	97.8	3.47
S50	MatrixSpike		3.47	61.82		53.4	93.9	3.48
Dil	DoubleBla...		3.76	236.30				3.44
Sample	Sample		3.35	0.33		170.2		3.48
Sample	Sample		3.73	0.49		49.7	34.4	3.47
Sample	Sample		3.44	0.52		168.8	60.4	3.48

Figure 5. Calibration and recovery at 50 ppb for AMPA.

Sample		Ethephon...	Ethephon Results			Qualifier (145.0 -> 80.9...	Ethephon-...
Name	Type	Exp. Conc.	RT	Calc. Conc.	Accuracy	Ratio	RT
Dil	Blank		7.60	185.79		193.8	7.77
MB	MatrixBlank		8.16	0.00		126.7	8.28
L3	Cal	1.0	8.35	0.97	97.1	64.8	8.28
L4	Cal	2.0	8.36	2.05	102.6	98.2	8.27
L5	Cal	5.0	8.31	4.38	87.7	62.1	8.27
L6	Cal	10.0	8.32	10.59	105.9	70.9	8.27
L7	Cal	20.0	8.31	21.86	109.3	81.4	8.27
L8	Cal	40.0	8.31	39.55	98.9	84.1	8.27
L9	Cal	100.0	8.32	98.60	98.6	82.3	8.27
Dil	Blank		8.22	92.37		57.8	8.23
Sample	Sample		8.01	6.94			8.33
Sample	Sample		8.40	11.57			8.31
Sample	Sample		8.29	5.48		387.4	8.30
S20	MatrixSpike		8.35	23.06		86.4	8.33
S20	MatrixSpike		8.42	24.38		72.7	8.30
S20	MatrixSpike		8.37	20.86		94.4	8.30
Dil	DoubleBla...		7.76	4.39		470.8	8.80

Figure 6. Calibration and recovery at 20 ppb for ethephon.

Sample		Fosetyl M...	Fosetyl Results			Qualifier (111.0 -> 65.0...	Fosetyl-IS...
Name	Type	Exp. Conc.	RT	Calc. Conc.	Accuracy	Ratio	RT
MB	MatrixBlank						11.21
L5	Cal	5.0	11.27	4.82	96.5	13.3	11.21
L6	Cal	10.0	11.27	10.00	100.0	14.4	11.21
L7	Cal	20.0	11.29	19.65	98.3	14.6	11.21
L8	Cal	40.0	11.27	43.16	107.9	13.0	11.21
L9	Cal	100.0	11.27	97.36	97.4	17.4	11.21
Dil	Blank		9.19	4028.36			11.38
Sample	Sample						11.16
Sample	Sample						11.17
Sample	Sample		10.21	7.38			11.18
S50	MatrixSpike		11.19	51.17		16.4	11.16
S50	MatrixSpike		11.21	49.71		12.2	11.17
S50	MatrixSpike		11.21	48.29		12.6	11.16
Dil	Blank		12.21	9621.24			11.22

Figure 7. Calibration and recovery at 50 ppb for fosetyl-Al .

Sample		Glufosina...	Glufosinate Results			Qualifier (182.0 -> 119....	Glufosinat...
Name	Type	Exp. Conc.	RT	Calc. Conc.	Accuracy	Ratio	RT
Dil	Blank		4.39			141.5	
MB	MatrixBlank		4.37	0.00		32.7	4.36
L2	Cal	0.5	4.37	0.50	99.6	28.6	4.36
L3	Cal	1.0	4.37	0.98	97.8	27.3	4.36
L4	Cal	2.0	4.37	1.94	97.1	28.3	4.36
L5	Cal	5.0	4.37	5.16	103.1	25.7	4.36
L6	Cal	10.0	4.37	10.13	101.3	24.9	4.36
L7	Cal	20.0	4.37	20.02	100.1	26.1	4.36
L8	Cal	40.0	4.37	40.77	101.9	25.4	4.36
L9	Cal	100.0	4.37	99.00	99.0	26.0	4.36
Dil	Blank		4.27			220.5	
Sample	Sample		4.42	1.23		28.0	4.40
Sample	Sample		4.42	0.83		27.3	4.41
Sample	Sample		4.42	1.04		29.1	4.40
S10	MatrixSpike		4.42	9.64		32.6	4.40
S10	MatrixSpike		4.42	10.08		30.9	4.40
S10	MatrixSpike		4.42	12.15		29.1	4.40
Dil	DoubleBla...		4.67	3055.30			4.31

Figure 8. Calibration and recovery at 10 ppb for glufosinate.

Sample		Glyphosa...	Glyphosate Results			Qualifier (170.0 -> 60.0...	Glyphosat...
Name	Type	Exp. Conc.	RT	Calc. Conc.	Accuracy	Ratio	RT
Dil	Blank		7.13	71.81			6.86
MB	MatrixBlank		6.74	0.46			6.77
L2	Cal		6.75	0.00	0.0		6.77
L3	Cal	1.0	6.75	1.14	114.5	27.3	6.76
L4	Cal	2.0	6.76	2.10	105.0	34.5	6.76
L5	Cal	5.0	6.77	4.76	95.2	32.5	6.76
L6	Cal	10.0	6.77	8.82	88.2	33.3	6.76
L7	Cal	20.0	6.77	19.85	99.2	33.6	6.76
L8	Cal	40.0	6.77	37.69	94.2	31.0	6.76
L9	Cal	100.0	6.77	103.64	103.6	27.2	6.76
Dil	Blank		6.33	229.84		187.5	6.83
Sample	Sample		6.76	15.50		31.5	6.79
Sample	Sample		6.76	14.57		23.9	6.80
Sample	Sample		6.75	13.03		23.9	6.79
S10	MatrixSpike		6.75	26.62		31.3	6.79
S10	MatrixSpike		6.75	25.01		24.1	6.79
S10	MatrixSpike		6.76	23.23		25.6	6.79
Dil	DoubleBla...		6.91	589.39			6.70

Figure 9. Calibration and recovery at 10 ppb for glyphosate.

Sample		HEPA Me...	HEPA Results			Qualifier (127.0 -> 90.9...	HEPA-IS (...
Name	Type	Exp. Conc.	RT	Calc. Conc.	Accuracy	Ratio	RT
Dil	Blank		6.57	3.72		134.6	7.31
MB	MatrixBlank		6.60	0.36		44.7	6.59
L4	Cal	2.0	6.56	2.30	115.0	86.0	6.58
L5	Cal	5.0	6.60	5.22	104.5	81.2	6.58
L6	Cal	10.0	6.61	8.88	88.8	112.5	6.58
L7	Cal	20.0	6.60	18.12	90.6	96.6	6.59
L8	Cal	40.0	6.60	39.09	97.7	101.1	6.58
L9	Cal	100.0	6.60	103.38	103.4	104.9	6.58
Dil	Blank		6.58	15.73		220.6	7.35
S50	MatrixSpike		6.64	53.97		120.9	6.62
S50	MatrixSpike		6.63	56.61		86.1	6.62
S50	MatrixSpike		6.63	54.88		115.5	6.62
Dil	DoubleBla...		6.72	67.27			7.17
Sample	Sample		6.36	2.18			6.63
Sample	Sample		6.44	1.71		31.7	6.63
Sample	Sample		6.46	12.40		26.0	6.63

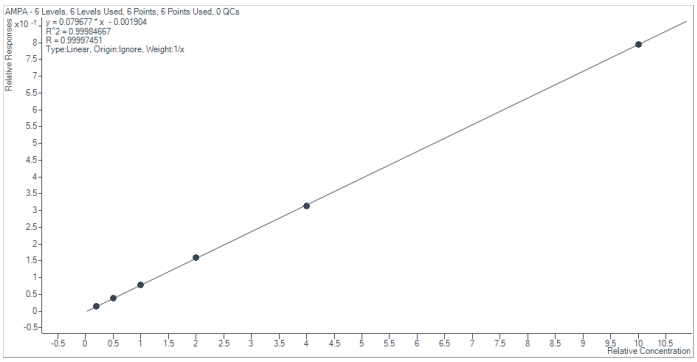
Figure 10. Calibration and recovery at 50 ppb for HEPA.

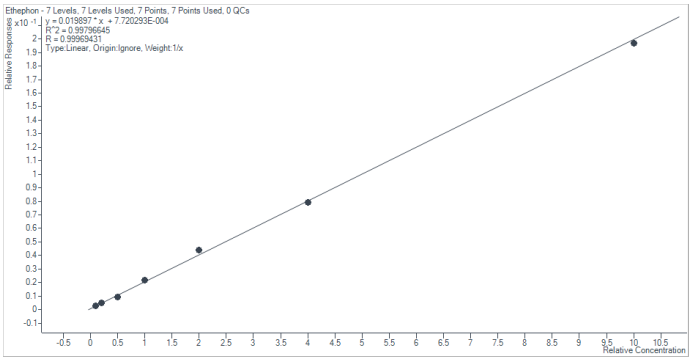
Sample		MPPA M...	MPPA Results			Qualifier (153.0 -> 107....	Glufosinat...
Name	Type	Exp. Conc.	RT	Calc. Conc.	Accuracy	Ratio	RT
Dil	Blank		4.50				
MB	MatrixBlank		4.22	0.27		44.9	4.36
L4	Cal	2.0	4.26	1.92	96.2	41.5	4.36
L5	Cal	5.0	4.28	5.44	108.7	27.8	4.36
L6	Cal	10.0	4.30	10.04	100.4	28.5	4.36
L7	Cal	20.0	4.31	19.19	95.9	33.2	4.36
L8	Cal	40.0	4.31	38.86	97.2	25.2	4.36
L9	Cal	100.0	4.31	101.55	101.6	25.5	4.36
Dil	Blank		4.28				
S50	MatrixSpike		4.31	95.00		36.0	4.40
S50	MatrixSpike		4.31	88.22		28.8	4.40
S50	MatrixSpike		4.32	92.23		28.1	4.40
Dil	Blank		4.02	474.04			4.31
Sample	Sample		4.24	38.98		35.0	4.40
Sample	Sample		4.24	36.63		41.9	4.41
Sample	Sample		4.24	33.52		31.8	4.40
S20	MatrixSpike		4.28	61.63		31.5	4.40
S20	MatrixSpike		4.28	48.88		39.8	4.40
S20	MatrixSpike		4.28	56.62		31.3	4.40

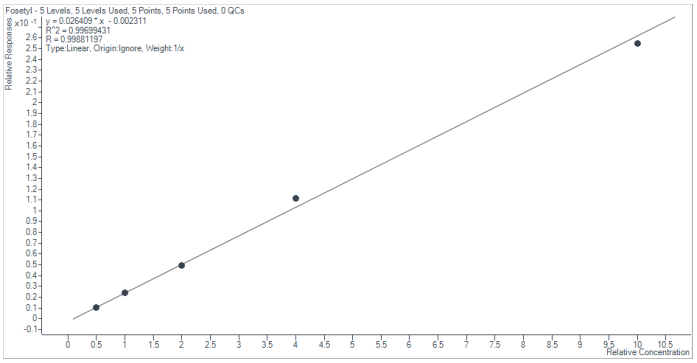
Figure 11. Calibration and recovery at 20 ppb and 50 ppb for MPPA.

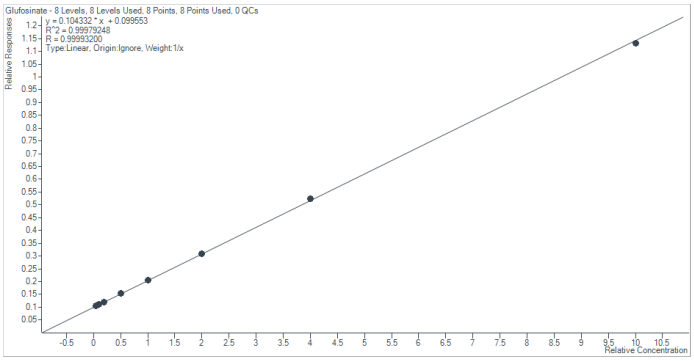
Sample		NAG Met...	NAG Results			Qualifier (224.2 -> 56.1...	NAG-IS (I...
Name	Type	Exp. Conc.	RT	Calc. Conc.	Accuracy	Ratio	RT
Dil	Blank		5.47	100.75			5.67
MB	MatrixBlank		5.59	1.47		3190.9	5.61
L4	Cal		5.82	1.44			5.60
L5	Cal	5.0	5.59	5.28	105.5	200.0	5.60
L6	Cal	10.0	5.59	9.69	96.9	189.3	5.60
L7	Cal	20.0	5.60	19.77	98.8	216.6	5.60
L8	Cal	40.0	5.60	38.99	97.5	218.7	5.60
L9	Cal	100.0	5.60	101.28	101.3	226.0	5.59
Dil	Blank		5.62	85.48			5.66
S50	MatrixSpike		5.65	57.65		201.1	5.65
S50	MatrixSpike		5.65	57.01		217.7	5.66
S50	MatrixSpike		5.65	59.15		194.3	5.65
Dil	Blank		6.03	42.65			6.36
Sample	Sample		5.66	2.12		13714.3	5.66
Sample	Sample		5.80	2.08		283.7	5.66
Sample	Sample		5.69	1.94		57.1	5.66
S20	MatrixSpike		5.68	20.31		214.7	5.66
S20	MatrixSpike		5.66	18.32		235.6	5.66
S20	MatrixSpike		5.64	26.00		200.1	5.65

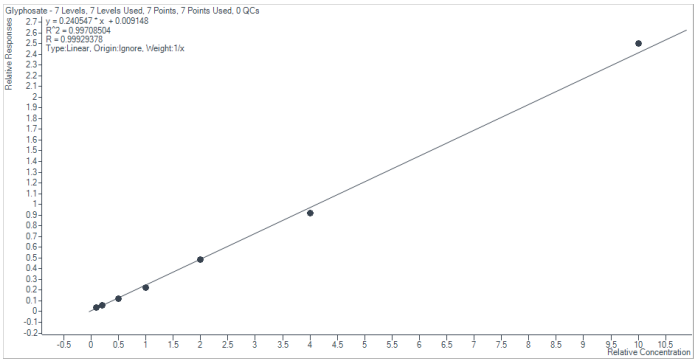
Figure 12. Calibration and recovery at 20 ppb and 50 ppb for NAG.

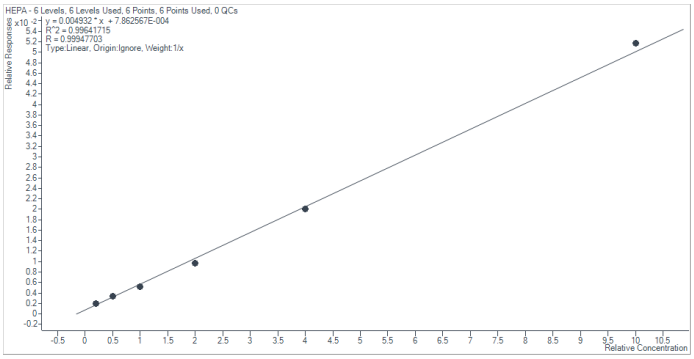


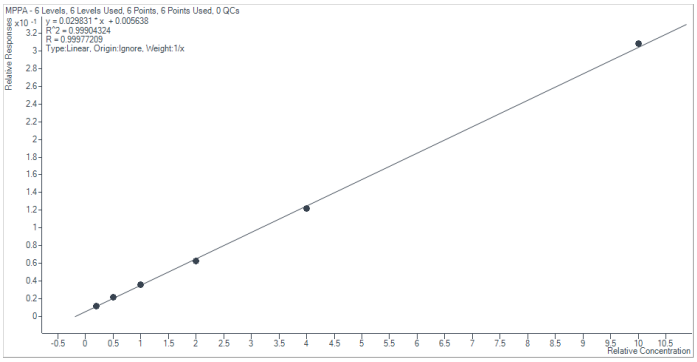












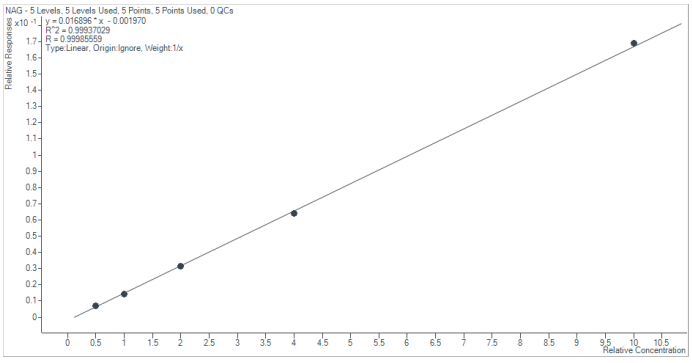


Figure 13. Calibration plot for eight polar pesticides in rice matrix (R^2 values ≥ 0.996).

The method was found to be robust and linear across a wide calibration range. Spike levels are equivalent or lower than the respective MRL value in rice. The method targets ethephon, fosetyl-Al, glufosinate, and glyphosate, along with their key metabolites, as prioritized under the FSSAI, eCFR, and EURL residue-monitoring lists for rice. The targeted MRLs in ppb (µg/kg), obtained calibration, spike levels, and obtained recovery from analysis in triplicate are summarized among various cereal matrices in Table 6.

Table 6. MRL, matrix-based (prespike) calibration range, spike level, and recovery data.

Analyte	FSSAI (µg/kg)	US-CFR (µg/kg)	EURL (µg/kg)	Calibration Range (µg/kg)	Spike Level (µg/kg)	Recovery (Average) (%)
AMPA*	NA	100	NA	2–100	50	118.5
Ethephon	NA	2,000	50	1–100	20	113.8
Fosetyl-Al	NA	NA	NA	5–100	50	99.4
Glufosinate	NA	900	30	0.5–100	10	95.9
Glyphosate	10	100	100	1–100	10	105.8
HEPA	NA	NA	NA	2–100	50	110.3
MPPA**	NA	900	30	2–100	20	96.7
NAG**	NA	900	30	5–100	20	107.7

* AMPA is a metabolite of glyphosate
** MPPA and NAG are metabolites of glufosinate

Conclusion

A sensitive and reliable LC/MS/MS method was developed and verified for the determination of polar pesticide residues in rice using the Agilent 6475 triple quadrupole LC/MS system operating in dynamic Multiple Reaction Monitoring (dMRM) mode. Sample preparation based on the QuPPe-PO method, version 12.3, aligned with EURL-SRM recommendation, enabled efficient extraction of a broad range of highly polar analytes in cereals, such as rice. Matrix-based (prespike) calibration demonstrated excellent linearity over the range of 0.5–100 µg/kg, with correlation coefficients (R^2) exceeding 0.995 for all target compounds.

The methodology confirmed acceptable performance with respect to selectivity, linearity, ion-ratio compliance, and reproducibility. Recovery experiments conducted at concentration levels below the applicable FSSAI, CFR, and EURL maximum residue limits demonstrated satisfactory method accuracy, with recoveries generally falling within the acceptance range of 70–120%. The application of isotopically labeled internal standards further improved quantitative performance and compensated for matrix-related effects.

The results demonstrate that the combination of QuPPe-PO sample preparation method with dMRM-based LC/MS/MS analysis on the Agilent 6475 LC/TQ provides a robust approach for routine monitoring of polar pesticide residues in rice. The method is suitable for regulatory residue testing and fulfills the validation and verification requirements outlined in SANTE/11312/2026, supporting its implementation in food safety and compliance laboratories.

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