Multi-residue pesticide analysis in chicken: LC-MS/MS method development and validation

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ABSTRACT

The presence of pesticide residue in meat has been of great public health significance. In order to address the existing gap in the literature, a rapid and sensitive method was standardised and validated to detect and quantify multi-residue pesticides in animal origin food. Thus, a highly sensitive method based on modified QuEChERS (quick, easy, cheap, effective, rugged and safe) extraction coupled with Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) analysis has been standardised and validated to determine simultaneously 20 selected pesticide residues in chicken. The developed method is satisfactory in terms of accuracy (relative recoveries range between 73.26 to 116%) and sensitivity (limits of detection in the range of 4.41 to 5.86 μg/kg). The method accuracy and precision (RSD≤20%) complied with performance criteria of the SANTE/11312/2021 analytical quality control procedure. The application of validated methodology to chicken samples collected from retail shops and farms of Mumbai, Palghar, Thane, Satara and Pune districts of Maharashtra revealed the presence of trace levels of carbendazim at concentrations below the Maximum Residue Limit (MRL). The study of pesticide multi residues should be further explored along with regular monitoring and surveillance to ensure food safety and public health.

Keywords: Chicken, LC-MS/MS, Pesticides, Quechers, Validation

India, with its agrarian economy, relies substantially on animal husbandry and poultry as key income sources, meeting the nation's growing food and protein demands (Chatterjee and Raj Kumar 2015). The prevalent use of pesticides to control vector-borne diseases in poultry has led to significant public health concerns. Amongst the Indian states, Maharashtra reported to be the highest user of pesticides (GOI, 2021). Poultry are often indirectly exposed to pesticides through contaminated feed and water (Kumar et al. 2013). Prolonged low-dose exposure in both poultry and humans has been linked to a range of toxic effects, from skin irritation and nausea to chronic impacts, including cancer, asthma, immunosuppression and hormonal disruptions (Khilare et al. 2016). The ingestion of pesticides through baits, granules, treated seeds and sprays poses direct risks, affects growth rates and liver health of poultry (Khandia et al. 2020). The consumption of pesticide-contaminated meat has non-carcinogenic health risks, especially for children (Tongo and Ezemonye 2015). Traditionally, organochlorine pesticides were predominant, but their environmental persistence has led to a shift toward organophosphates, carbamates and pyrethroids (Mitra et al. 2011). Currently, India ranks fourth in global pesticide

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production, with Indian pesticide industry valued at approximately ₹260 billion in 2024 and is expected to expand to around ₹440.1 billion by 2033 growing at a compound annual growth rate (CAGR) of 5.72% throughout during the period of 2025 to 2033 (IMARC 2024). The majority of pesticide use is attributed to insecticides, followed by herbicides, fungicides and bactericides. Chlorpyrifos remains one of the most widely used insecticides (Nayak and Solanki 2021). The classification and effects of selected pesticides in the study are enlisted in Table 1.

Addressing pesticide residues in food requires rigorous monitoring and analysis to comply with MRL set by International Organizations. Traditional analysis techniques have low sensitivity and specificity in complex biological matrices like chicken. The QuEChERS extraction approach combined with LC–MS/MS (Anastassiades *et al.* 2003, Schneider *et al.* 2015), offers improved accuracy and sensitivity for detecting pesticide residues across diverse pesticide classes (Vogeser and Parhofer 2007, Hajrulai-Musliu *et al.* 2021). Hence, the rapid and sensitive LC-MS/MS method was developed and validated for multiresidue analysis in chicken.

MATERIALS AND METHODS

Sample preparation and storage: A total of 120 samples (60 from Mumbai markets and 60 from farms in Thane, Satara, and Pune districts) were collected in sterile

Table 1. Classification and effect of selected pesticides

Group of pesticides	Type of action	Public health effects		
	Orga	nophosphate		
Profenofos	Insecticide and Acaricide	Neurotoxic, chronic neurological impairment		
Acephate		Respiratory distress, neurotoxicity		
Chlorpyriphos		Developmental neurotoxicity (children)		
Phenthoate	Insecticide	Neurotoxic effects (Tremors)		
Phorate Sulfone		Acute and chronic neurotoxic		
Phorate Sulfoxide		Acute and enronic neurotoxic		
	In	secticide		
Imidacloprid		Neurotoxic, viz. cognition and motor functions.		
Thiamethoxam	Insecticide	Developmental neurotoxicity (children)		
Clothianidin		Neurological impairment and teratogenic		
Carbendazim		Teratogenic		
Difenoconazole		Hepatotoxicity and thyroid toxicity with chronic exposure		
Penconazole	Fungicide	Endocrine disruption and hepatotoxicity		
Triadimefon		Teratogenicity		
Edifenphos		Hepatotoxicity		
Indoxacarb	Insecticide	Neurotoxicity		
Flusilazole	Fungicide	Teratogenicity		
Chlorantraniliprole	Insecticide	Neurotoxicity		
	Co	arbamate		
Carbofuran	Insecticide	Cardiotoxicity and neurotoxicity		
Thiodicarb	Insecticide and Molluscicide	Respiratory distress, neurotoxicity		
	P_{Σ}	yrethroid		
Fenpropathrin	Insecticide and Acaricide	Neurotoxicity		

polyethylene bags, labelled and transported to the lab on ice, stored at -20°C and thawed at room temperature before analysis.

Chemicals and standards: LCMS grade Acetonitrile (MeCN), methanol and water were obtained from Honeywell international Inc. (Germany) whereas NaOAc (anhydrous sodium acetate), MgSO₄ (magnesium sulphate), PSA (Primary Secondary Amine), Formic acid (LC-MS LiChropur) and Ammonium Formate were procured from Merck India Pvt. Ltd. All 20 pesticide standards (% purity) viz. acephate (99.6%), carbendazim (98.7%), chlorpyrifos (98.5%), clothianidin (99.9%), edifenfos (99.4%), flusilazole (99.4%), indoxacarb (95%), penconazole (99%), phorate sulphone (95%), phorate sulfoxide (98.9%), profenofos (97.2%), thiamethoxam (99.3%), triadimefon (99%), phenthoate (98.3%), difenoconazole (95.5%), chlorantraniliprole (96.6%), carbofuran (99.8%), fenpropathrin (99.2%), imidacloprid (99.7%), methomyl (98%) and thiodicarb (98.1%) were purchased from Sigma Aldrich.

Preparation of standard solution: Individual 1 mg/mL stock solution for all 20 selected standards were prepared in acetonitrile, with calibration standards at 5, 10, 20, 50, 100, and 150 μ g/kg.

Sample extraction protocol: Extraction was carried using method reported by Hajrulai-Musliu *et al.* (2021) with slight modifications. Chicken samples were homogenised prior to use and $10 \text{ gm} \pm 0.1 \text{ gm}$ was uniformly spiked with the pesticide standards and incubated for 10 min before

subjected to extraction. Addition of acidified acetonitrile (10 mL) as extraction solvent, followed by vigorous shaking for 1 min on a vortex mixer, followed by end to end shaking for 25 min and then incubated at -20°C for 5 min. Subsequently, MgSO₄ (4 gm) and NaOAc (1.5 gm) were added to the sample, shaken thoroughly and centrifuged for 5 min at 7000 rpm at 4°C. The resultant supernatant (5 mL) was transferred to 15 mL centrifuge tube for clean-up by addition of MgSO₄ (750 mg) and PSA (150 mg) and vortex for 2 min. The tubes were centrifuged at 10000 rpm at 4°C for 5 min. The supernatant (1 mL) was diluted with water (1 mL) and filtered through the 0.22 µm syringe filter and analysis was performed by LC-MS/MS.

Liquid chromatography parameters: LC-MS/MS analysis was conducted on an Ultra-High Performance Liquid Chromatography System (Waters ACQUITY UPLC H-class) coupled with a triple quadrupole mass spectrometer (Waters XEVO TQ-S micro) and controlled by Mass Lynx software, version 4.2. Chromatographic separation was achieved on an ACQUITY UPLC BEH C18 column (2.1 \times 50 mm, 1.7 μ m) at 45°C with a 5 μ L injection volume, and the auto-sampler was set at 10°C. The mobile phase included Eluent A (water) and Eluent B (methanol) with 5 mM ammonium formate and 0.2% formic acid. Gradient elution: 90-10% B (0 min); 10-90% B (0.0-15.0 min). Data was processed with Target Lynx.

MS/MS parameters: The mass spectrometer (Waters XEVO TQ-S micro) operated in positive ion mode (ESI+), with auto-optimization of MS parameters, viz. MRM

(Multiple Reaction Monitoring) transitions, CV (cone voltage) and CE (collision energy) via the IntelliStart tool with desolvation temperature of 600°C, cone gas (50 L/h), source temperature (150°C), desolvation gas flow (1000 L/h) and a capillary voltage (1.5 kV), achieving optimal peak shapes and signal intensity. Selected compounds had symmetric peak and the maximum signal intensity with these values. Nitrogen (\geq 99% purity), generated using a nitrogen generator (Peak Scientific, Billerica, MA, USA), was used as ESI source nebulizer.

Method validation: The method was validated per SANTE/11312/2021 guidelines, evaluating linearity, accuracy, precision, LOD (limit of detection) and LOQ (limit of quantification). Linearity was assessed using a six-point calibration curve (5–150 μ g/Kg) on the basis of matrix-match calibration. The LOQ was set at 10 μ g/Kg. Recoveries and precisions were tested by spiking chicken meat at 10, 50, and 100 μ g/Kg (n=6) for 20 selected pesticides. Each pesticide was analysed with two MRM transitions and retention time tolerance (t_R) of \pm 0.1 min for residue identification.

RESULTS AND DISCUSSION

Optimization of MS/MS parameters: A multi-residue method was developed for the simultaneous detection of twenty pesticides in a single run. To optimize analyte response, individual standards (1 μ g/mL) were directly infused into the MS/MS detector. For each analyte, two transitions with the maximum response were selected: highest intensity transition as the quantifier ion and the other as the qualifier ion. Table 2 enlists the optimized MS/MS parameters. Dwell time was set to achieve 10.23 points per peak, ensuring good peak shape and an adequate signal-to-noise ratio (S/N).

Optimization of LC parameters: Optimizing LC parameters is crucial for achieving high sensitivity, good resolution and effective ionization with minimal interference. Various mobile phases were tested, including combinations of formic acid (0.1%) and ammonium salts (10 mM Ammonium Acetate and 5mM Ammonium Acetate) in water and methanol. The best results, with optimal resolution and peak shape were obtained using 5 mM Ammonium Formate with 0.2% formic acid in both water (aqueous phase) and methanol (organic phase). Xie et al. (2015) indicated that adding formic acid improves analyte ionization in ESI+ mode. The gradient program is enlisted above, with chromatograms shown in Figure 1a and 1b.

Optimization of sample extraction: Optimizing multiclass, multi-residue pesticide extraction from chicken meat is a challenge due to matrix complexity and varied pesticide characteristics. Acidified acetonitrile (MeCN) was selected as the extraction solvent (Choi et al. 2015), over MeCN:EtOAc acid (49.5:49.5:1) (Hajrulai-Musliu et al. 2021), as it provided a cleaner extract, improving detection in ESI mode and enhanced extraction efficiency for polar compounds, viz. acephate as compared to ethyl acetate (Mastovska and Lehotay, 2004). Since chicken

Table 2. Optimized Mass Spectrometric Parameters for selected pesticide standards

pesticide standards						
Name of pesticide	Parent ion	Product ion	Cone	Collision		
	(m/z)	(m/z)	(V)	(V)		
Acephate	183.9043	125.0034	10	18		
песрпас	103.7073	142.9536	10	10		
Carbendazim	192.9043	131.9851	42	30		
Caroenaaziiii	172.7013	159.9734	42	16		
Chlorpyrifos	349.9000	97.0000	27	32		
emorpymos	317.7000	198.0000	27	20		
Clothianidin	249.9129	131.8565	14	14		
	,,,,,,	168.8915	14	12		
Edifenphos	310.9932	110.9486	38	20		
		282.9198	38	12		
Flusilazole	316.1323	165.0310	5	26		
11001102010	010.1020	247.0118	5	18		
Indoxacarb	528.0388	149.9277	2	22		
	220.0200	248.9419	2	16		
Penconazole	284.1601	70.0049	38	14		
1 0110 0111112010	201001	158.9215	38	26		
Phorate sulphone	293.0346	142.9642	10	10		
-		230.9311	10	8		
Phorate	277.0397	96.8865	2	32		
sulphoxide	27710057	198.9218	2	8		
Profenofos	372.9032	302.7830	44	18		
		344.7946	44	12		
Thiamethoxam	292.0512	132.0000	22	22		
1111411114111	2,2.0012	211.2000	22	12		
Triadimefon	296.1407	69.0248	14	20		
	_, ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	198.9793	14	14		
Phenthoate	320.9987	78.9879	8	40		
1 110111110 1110	520.5507	246.9397	8	10		
Difenoconazole	405.9689	110.9478	78	58		
2110110 00111112010	.00.,00,	250.9085	78	24		
Chlorantraniliprole	481.8750	111.9231	50	74		
Ститантиниргого	101.0750	283.8715	50	12		
Carbofuran	222.1371	122.9968	2	20		
Curoorum	222.13/1	165.0444	2	10		
		97.0000	24	34		
Fenpropathrin	350.1000	125.0000	24	14		
Imidacloprid	256.1000	175.1000	25	20		
1		209.1000	25	15		
Thiodicarb	355.0081	88.1000	17	16		
		108.1000	17	16		
Methomyl	163.0000	88.0000	10	1010		
		106.0000	10			

contains around 70% water, additional water was omitted to prevent interference with analyte partitioning into acetonitrile (Cutillas and Fernández-Alba 2021). End-to-end shaking for 25 min improved the recovery (Hajrulai-Musliu *et al.* 2021). The sample was subjected to 5 min incubation at -20°C before adding MgSO₄ and NaOAc minimized heat-induced pesticide degradation. MgSO₄ and PSA were used for cleanup to remove fatty acids and lipids (Chung and Chan 2010), and the MeCN extract was diluted with water (1:1) for analysis.

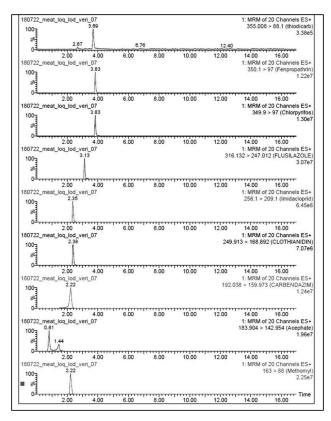


Fig. 1a. Chromatograms of 12 of 20 selected pesticide standards spiked in blank chicken meat at 100µg/Kg concentration.

Validation of method

Linearity: Linearity was assessed at six concentration levels for pesticide residues. The solvent linearity demonstrated a good correlation within the reported concentration range, with a regression coefficient (r^2) of ≥ 0.99 for all analytes. Retention times for analytes in solvent and matrix-matched standards was in accordance with the SANTE/11312/2021 guidelines. A matrix-matched calibration curve was used for quantification, to mitigate matrix effects (Cortese *et al.* 2020). Table 3 shows the retention times, linear ranges, and r^2 values for each analyte. These findings align with studies by Lee *et al.* (2022) and Kang *et al.* (2020), who reported similar linearity for insecticides (difenoconazole and flusilazole) and other pesticides (chlorpyrifos, phenthoate, profenofos, triadimefon, edifenphos and penconazole), respectively.

Limit of Detection and Limit of Quantification: The LOD and LOQ were determined at signal-to-noise ratios of 3 and 10, respectively (Weng et al. 2020). In current study, LOD values ranged from 4.41 to 5.86 μg/kg, and LOQ values ranged from 9.91 to 11.03 μg/kg, aligning with Wei et al. (2015), who reported LODs of 0.5 to 5 μg/kg. Pang et al. (2009) reported higher LOQs for thiamethoxam (40 μg/kg), clothianidin (24 μg/kg), phorate sulfoxide (200 μg/kg) and fenpropathrin (40 μg/kg). Thiodicarb was quantified as methomyl, as it rapidly degraded to methomyl upon fortification into livestock products, consistent with findings by Rahman et al. (2017). The LOD and LOQ for methomyl were 5.41 and 11.0 μg/kg, respectively, with

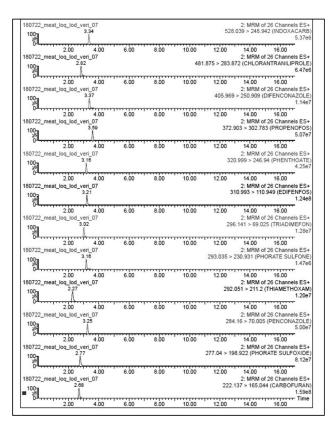


Fig. 1b. Chromatograms of 8 of 20 selected pesticide standards spiked in blank chicken meat at 100µg/Kg concentration.

LOQ below the FSSAI MRL (20 μg/kg) (2020). Although much lower LOQ was reported by Rahman *et al.* (2017) using LC-FLD and Wu *et al.* (2013) using LC-MS/MS.

Recovery and precision: Recovery and precision were evaluated using three different concentrations spiked in chicken samples, with each spike assessed in six replicates. Intra-day recovery and precision were determined by analyzing samples on the same day, while inter-day recovery and precision were assessed over three consecutive days, also in six replicates. The results of the present study are presented in Table 4.

Average recoveries for all 20 selected pesticides were statistically evaluated. Intra-day recoveries ranged from 73.26% to 116.72% with RSD values of 0.77% to 13.07%. Inter-day average recoveries were between 85.93% and 110.79%, with RSD values from 2.23% to 19.39%. Lichtmannegger *et al.* (2015) reported recoveries of 93-100% in pork using GC-MS/MS, consistent with this study. Lee *et al.* (2022) reported recoveries of 75-81% for 32 pesticides in pork, beef and chicken, also aligning with these results. Rani *et al.* (2021) reported recovery of imidacloprid in biological matrices at 88-98% using LC-MS/MS.

Robustness: Robustness was assessed by varying extraction parameters (shaking time) and instrumental parameters (injection volume and column temperature). The shaking time was reduced to 20 min, yielding recoveries of 76.71 to 115% with RSD of 1.32 to 9.74%. Injection volumes of 3 μL and 7 μL resulted in recoveries

Table 3. Retention time, LOD, LOQ, Regression Coefficient and linear range of selected pesticides

			Parameter		
Name of Pesticide	RT (min)	LOD μg/kg	LOQ μg/kg	\mathbf{r}^2	Linear range μg/kg
Acephate	0.81 ± 0.01	4.90 ± 0.18	9.95 ± 0.14	0.998	
Carbendazim	2.21 ± 0.01	4.71 ± 0.35	9.91 ± 0.16	0.995	
Chlorpyrifos	3.83 ± 0.00	4.58 ± 0.23	10.25 ± 0.16	0.997	
Clothianidin	2.35 ± 0.01	5.83 ± 0.62	10.58 ± 0.13	0.992	
Edifenphos	3.21 ± 0.00	4.68 ± 0.20	10.56 ± 0.10	0.995	
Flusilazole	3.13 ± 0.00	4.70 ± 0.24	11.00 ± 0.16	0.992	
Indoxacarb	3.34 ± 0.01	5.73 ± 0.22	10.43 ± 0.23	0.998	
Penconazole	3.25 ± 0.00	4.73 ± 0.11	10.78 ± 0.17	0.996	
Phorate sulphone	3.17 ± 0.01	4.98 ± 0.45	10.51 ± 0.50	0.991	
Phorate sulphoxide	2.77 ± 0.00	4.98 ± 0.11	10.61 ± 0.17	0.995	
Profenofos	3.59 ± 0.01	4.86 ± 0.22	10.70 ± 0.22	0.998	5-150
Thiamethoxam	2.25 ± 0.00	4.96 ± 0.38	10.21 ± 0.20	0.990	
Triadimefon	3.02 ± 0.01	5.30 ± 0.28	10.56 ± 0.27	0.991	
Phenthoate	3.16 ± 0.00	4.41 ± 0.25	10.55 ± 0.66	0.995	
Difenoconazole	3.38 ± 0.01	5.00 ± 0.14	10.80 ± 0.26	0.996	
Chlorantraniliprole	2.82 ± 0.00	5.08 ± 0.15	10.80 ± 0.25	0.994	
Carbofuran	2.68 ± 0.00	5.25 ± 0.22	10.41 ± 0.08	0.991	
Fenpropathrin	3.83 ± 0.01	4.61 ± 0.15	10.35 ± 0.15	0.998	
Imidacloprid	2.35 ± 0.00	5.86 ± 0.64	10.31 ± 0.22	0.991	
Thiodicarb	3.69 ± 0.01	-	-	-	
Methomyl	2.22 ± 0.01	5.41 ± 0.93	11.00 ± 0.20	0.995	

RT=retention time, Results are expressed in \pm SD, n=6.

Table 4. Inter and intra-day RSD and recoveries of 20 selected pesticides spiked in chicken meat

Name of pesticide	Spiked concentration	Intra- day study		Inter- day study	
	- (μg/Kg)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
	10	76.48	11.20	86.86	17.32
Acephate	50	102.97	2.37	99.56	3.08
	100	73.26	2.56	93.21	18.54
	10	90.77	7.09	92.56	9.68
Carbendazim	50	105.42	2.28	101.43	4.15
	100	102.72	7.48	99.74	3.05
	10	101.41	2.36	92.64	8.57
Chlorpyrifos	50	99.07	2.11	99.72	3.76
1.5	100	109.00	1.98	101.08	8.39
	10	103.23	8.23	110.79	6.06
Clothianidin	50	115.63	3.70	101.31	12.33
	100	107.35	11.34	96.47	9.79
Edifenphos	10	104.90	1.09	96.83	8.01
	50	105.28	2.19	102.08	4.73
	100	105.30	4.10	102.87	5.71
Flusilazole	10	97.41	4.89	95.16	19.39
	50	103.40	4.13	98.55	5.87
	100	95.49	13.92	104.23	7.37
Indoxacarb	10	96.92	2.56	88.09	11.64
	50	104.10	3.45	99.50	5.64
	100	98.38	2.00	95.66	6.21

(Table 4 continued ...)

Table 4. Concluded ...

Name of pesticide	Spiked concentration	Intra- day study		Inter- day study	
	(μg/Kg)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Penconazole	10	105.40	1.72	93.5	12.94
	50	102.74	1.81	102.78	3.88
	100	105.58	2.30	102.54	5.78
Phorate sulphone	10	107.72	6.41	99.6	18.23
	50	117.18	4.94	106.33	6.41
	100	104.66	10.82	106.88	2.23
	10	100.91	0.77	90.6	9.31
Phorate sulphoxide	50	104.40	4.12	99.84	3.84
	100	98.85	2.54	99.70	2.78
	10	108.38	4.37	95.5	8.91
Profenofos	50	103.97	4.71	102.55	2.74
	100	104.69	3.65	98.78	4.99
	10	104.39	7.04	97.03	6.26
Thiamethoxam	50	116.72	3.88	102.7	8.73
	100	104.79	8.51	95.51	6.60
	10	92.76	3.54	95.13	10.20
Triadimefon	50	102.74	2.15	101.54	4.65
	100	99.57	4.73	98.62	4.60
	10	97.25	3.73	93.3	17.47
Phenthoate	50	105.23	5.50	102.36	3.36
	100	97.20	7.82	105.77	7.19
	10	96.25	2.02	88.06	8.73
Difenoconazole	50	101.02	2.35	100.65	4.07
	100	100.59	1.43	98.32	5.26
	10	98.58	1.37	85.93	12.77
Chlorantraniliprole	50	103.30	3.56	100.27	3.22
	100	95.89	6.81	95.59	3.29
	10	98.58	1.37	95.56	9.39
Carbofuran	50	106.28	3.34	102.51	3.76
	100	100.79	2.99	100.70	2.22
	10	100.08	2.79	92.93	8.15
Fenpropathrin	50	99.90	2.98	100.77	2.86
	100	110.33	2.11	102.55	7.73
Imidacloprid	10	107.22	8.29	103.43	11.13
	50	115.10	3.44	96.77	16.82
	100	107.00	9.65	93.27	13.26
Thiodicarb/ Methomyl	10	113.04	7.54	108.03	5.16
	50	90.86	13.07	99.06	6.68
	100	89.93	8.27	93.53	5.55

RSD=Relative Standard Deviation; n=6

of 86.65 to 106.36% (RSD 2.64% to 12.77%) and 83.16 to 110.33% (RSD 2.29 to 9.92%), respectively. Column temperature studies at 40°C and 50°C produced recoveries of 92.94 to 112.50% (RSD 2.59 to 10.32%) and 97.08% to 108.68% (RSD 2.62 to 11.09%), respectively. Retention time of all 20 pesticide standards were within the ± 0.1 min tolerance limit.

Method application to field samples: A total of 120 samples were analyzed, from local and farms to assess the method's applicability for routine pesticide residue analysis. Trace amounts of carbendazim were detected in one local market sample from Mumbai region at 11.2 μg/

kg, below the FSSAI (2020) MRL. In contrast, Osaili *et al.* (2023) reported that 83% of chicken samples exceeded the prescribed MRL for carbamates. All pesticide residues in farm samples were below detection limits, and none exceeded MRLs (FSSAI 2020, EU 2010, CODEX 2020). These findings align with Lee *et al.* (2022), who reported difenoconazole or flusilazole was not detected in chicken samples using GC-MS/MS, and Gomez-Perez *et al.* (2014), who reported absence of acephate in chicken samples analyzed using UHPLC coupled with Orbitrap Mass Spectrometry. Oliveira *et al.* (2018) also reported absence of carbofuran, profenofos and triadimefon in beef samples

using LC-MS and QuEChERS extraction. However, Anagnostopoulos *et al.* (2015) reported two meat samples positive for chlorpyrifos when analyzed by LC-MS/MS.

A multi-residue method was developed for the simultaneous detection and quantification of 20 selected pesticides using Liquid Chromatography-Tandem Mass Spectrometry and its applicability was tested on 120 chicken samples. The findings indicated no risk associated with chicken consumption in the Mumbai and studied areas. Continuous surveillance and monitoring using innovative screening methods for detection of pesticide residues in animal-origin food are essential to ensure food safety and safeguard public health.

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