International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 10 No. 6s, 2024
https://www.theaspd.com/ijes.php

Residue Profiling Of Metrafenone In Grapes Through LC-MS/MS Analysis

Suresh Gaikwad[1], Dr. Jagruti Jankar[2], Hanif Shaikh[1], Mahesh Bhadane[1], Ashwini Mawal[1], Dr. Kaushik Banerjee[3], Dr. Parag Chavan[1]*
[1]Department of Chemistry, School of Science, Sandip University, Nashik. 422213
[2]Assistant Professor, Annasaheb Dange College of Engineering and Technology, Ashta, Sangli
3Principal Scientist and Director, ICAR-NRC for Grapes, Pune. 411028
[1]sureshgaikwad1990@gmail.com,
Corresponding Author: Dr. Parag Chavan, parag247@gmail.com

Abstract
Grapes (Vitis vinifera L.) are highly vulnerable to fungal infections, necessitating extensive fungicide use, which raises concerns regarding pesticide residues, food safety, and export compliance. Metrafenone, a fungicide widely used to manage powdery mildew, was evaluated for its residue behavior and dissipation kinetics in grapes using a validated LC-MS/MS method. Field experiments were conducted on Thompson Seedless grapes at the ICAR-NRC for Grapes, Pune, with single (100 mL/acre) and double (125 mL/acre) dose applications under randomized block design. Grape samples were collected at multiple intervals post-application and analyzed following ethyl acetate-based extraction and LC-MS/MS quantification. Method validation demonstrated excellent linearity ($R^2 > 0.99$), recoveries within 85–90%, and acceptable LOD (3 ng/g) and LOQ (10 ng/g). Residue dissipation followed first-order kinetics with strong model fit ($R^2 = 0.992$ for single dose; $R^2 = 0.991$ for double dose). The half-life (DT_{50}) of metrafenone was 13 days at the single dose and 17 days at the double dose. Residue concentrations declined from 11.33 mg/kg and 14.93 mg/kg (initial deposits) to 0.03 mg/kg and 0.09 mg/kg, respectively, within 60 days. Importantly, residues dropped below the EU Maximum Residue Limit (7 mg/kg) within 19 days, aligning with the Pre-Harvest Interval (PHI) stipulated in Grape Annexure 5 (2024). The study highlights that while metrafenone dissipates effectively in grapes, persistence is dose-dependent. Adherence to recommended application rates and PHIs is essential to ensure compliance with international MRLs, safeguard consumer health, and maintain export suitability. These findings provide valuable insights for integrated residue management strategies supporting sustainable viticulture.
Keywords*: Grapes, Fungicides, Single and double dose, persistence of pesticide, ethyl acetate extraction, LC-MS/MS*

INTRODUCTION

One of the most commercially significant fruit crops in the world, grapes (*Vitis vinifera* L.) are used to make wine, dried products, and fresh fruit. Throughout the growing season, vineyards are highly dependent on fungicide sprays because of their susceptibility to fungal infections such as powdery mildew and downy mildew (Pearson & Goheen, 1988; Gessler et al., 2011). However, the occurrence of pesticide residues in grapes has raised growing concerns due to their potential impacts on human health, ecological balance, and food safety (Aktar et al., 2009; Sharma et al., 2019). Fungicides constitute one of the most critical groups of agrochemicals for disease management and yield protection in viticulture (Dagostin et al., 2011; EFSA, 2020). Regulatory agencies such as the Codex Alimentarius Commission, the European Union (EU), and the Food Safety and Standards Authority of India (FSSAI) have established Maximum Residue Limits (MRLs) for pesticides in grapes to ensure safe consumption and facilitate international trade (Codex Alimentarius, 2023; European Commission, 2024; FSSAI, 2022).

Analytical methods that are extremely sensitive, precise, and dependable are required to monitor and quantify trace levels of pesticide residues in agricultural matrices. Conventional detection techniques such as Gas Chromatography (GC) and High-Performance Liquid Chromatography with UV detection (HPLC-UV) often lack the sensitivity and specificity necessary for trace-level detection in complex matrices (Stajnbaher & Zupancic-Kralj, 2003; Alder et al., 2006). In contrast, Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-MS/MS) has emerged as a powerful tool for multi-residue analysis, offering excellent selectivity, sensitivity, and the ability to analyze complex food and environmental

samples with minimal sample preparation (Lehotay et al., 2010; Fernandez-Alba & Garcia-Reyes, 2008; Kmellar et al., 2008). This makes LC-MS/MS the preferred approach for monitoring pesticide residues in fruits and vegetables under both national and international regulatory frameworks (Anastassiades et al., 2003; European Commission, 2024). In this work, a reliable LC-MS/MS approach for the simultaneous detection and measurement of metrafenone residues in grapes was developed and validated. Optimizing chromatographic and mass spectrometric conditions was the main goal of method development in order to guarantee accurate quantification and low matrix interference. International norms were followed for validating the method, and metrics like linearity, recovery, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ) were evaluated. The goal of this study was calculating PHI and to guarantee adherence to the Maximum Residue Limits (MRLs) set by regulatory bodies supporting residue monitoring programs. It enabled accurate analyte identification and quantification. To guarantee food safety, regulatory agencies like the EU, FSSAI, and the Codex Alimentarius Commission establish Maximum Residue Limits (MRLs). Validated analytical techniques to identify and measure pesticide residues at extremely low quantities are necessary to comply with these rules. Additionally, the verified approach can be a useful resource for researching these fungicides' environmental fate and dissipation behavior, leading to safer and more sustainable farming methods.

MATERIALS AND METHODS

2.1 Field experiment on grapes

Grapes (*Vitis vinifera* L., variety Thompson Seedless) were subject to field tests in the National Research Centre for Grapes (NRCG) vineyard in Pune, India (latitude 18.31°N, longitude 73.55°E). Following the date of pruning, metrafenone 50% SC (Acrisio 50 SC; BASF India Ltd) was applied at 100 mL/acre and 125 mL/acre (water volume 200 L/acre) at 30, 40, and 50 days intervals. In randomized blocks, each treatment—including the untreated control—was repeated three times, following standard field residue trial protocols (Banerjee et al., 2012; Kumari et al., 2019). Throughout the trial, the average relative humidity ranged from 78 to 90%, and the average maximum and minimum temperatures were 26.5 and 21.7 °C, with no rainfall during the study period. The crop was cultivated under drip irrigation in accordance with the Good Agricultural Practices (GAP) package of guidelines recommended for Indian viticulture (APEDA, 2020; NRCG, 2017).

2.2 Standardization of sampling technique

The Agricultural and Processed Food Products Export Development Authority (APEDA, n.d.) *Annexure 7: Revised grape sampling protocol* was followed for conducting the sampling experiment. Using a knapsack sprayer, metrafenone was applied at 100 and 125 mL per acre on a 20 wineyards for each trial with uniform canopy characteristics, as recommended in pesticide residue field trial studies (Banerjee et al., 2012; Kumari et al., 2019). The canopy design was 'Y' shaped, with row-to-row spacing of 9 feet (2.7 meters) and vine-to-vine spacing of 6 feet (1.8 meters), as per standard viticulture practices (NRCG, 2017). The vines were of uniform height and four years of age. Following the last spray (50 days after pruning), 500 g of grape samples were randomly collected from each replicate at 0 (2 hours), 1, 3, 5, 7, 10, 15, 21, 30, 45, and 60 days after metrafenone application (APEDA, n.d.; Sharma et al., 2019). Samples from untreated plots were also collected in the same manner for comparison. Each sample was taken in good condition and packed without prior cleaning or pre-treatment, in line with internationally accepted residue analysis protocols (European Commission, 2017; Lehotay et al., 2010). Grape berries were carefully separated from the pedicels and subjected directly to analysis, ensuring that no diseased or pest-infested clusters were included.

2.3 Reagents and Apparatus

Primary Secondary Amine (PSA Agilient make), ethyl acetate (MS grade), methanol (JT Baker make gradient grade), water (HPLC grade), formic acid (AR Grade Rankem), Diethylene Glycol (DEG), sodium sulphate and ammonium formate (Rankem make AR grade). High speed centrifuge, micro centrifuge, analytical and precise balance (Adventurer, OHAUS Corporation USA), mixer and grinder (Bajaj India

Pvt. Ltd., Mumbai, India), homogenizer (Heidolph 900, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) and low volume concentrator (Caliper life science USA).

2.4 Sample preparation

A 100 g portion of the 500 g grape sample was homogenized after the entire sample was crushed in a mixer with 500 mL of water (crushed 1:1), following standard residue analysis procedures (Lehotay et al., 2010; Anastassiades et al., 2003). From the homogenized material, 10 ± 0.10 g was transferred into a 50 mL centrifuge tube, and 10 mL of ethyl acetate along with 10 g of sodium sulphate were added to aid in extraction and dehydration (Banerjee et al., 2012; Kumari et al., 2019). The mixture was homogenized at 2000 rpm for 2 minutes and centrifuged for 5 minutes at 5000 rpm. A 5 mL aliquot of the ethyl acetate extract was cleaned using dispersive solid-phase extraction (d-SPE) by transferring it into a 15 mL polypropylene tube containing 25 mg PSA, followed by vortexing for 30 seconds and centrifugation at 10,000 rpm for 5 minutes (Anastassiades et al., 2003; European Commission, 2017).

For concentration, 2 mL of the supernatant was taken, 200 µL of Diethylene Glycol (DEG) solution was added as a keeper, and the extract was evaporated to near dryness using a nitrogen evaporator. The dried residue was reconstituted with 1 mL of methanol and 0.1% acetic acid in water, filtered through a 0.2 µm nylon membrane filter, and then injected into an LC-MS/MS system (Alder et al., 2006).

The recovery experiment was performed on control samples by spiking untreated grape samples at three fortification levels: 0.01 mg/kg, 0.02 mg/kg, and 0.05 mg/kg of metrafenone. The fortified samples were extracted and analyzed using the same procedure described above. Recovery percentages were calculated by comparing measured concentrations against matrix-matched calibration standards (Stajnbaher & Zupancic-Kralj, 2003; Lehotay et al., 2010).

2.5 LC-MS/MS

An LC-MS/MS-5500 (AB Sciex) triple quadrapole mass spectrometer fitted with an electrospray ionization (ESI+) probe was used for the analysis. A 100 mm x 4.6 mm, 3.5 µm Agilent Eclipse plus C18 column was used for the HPLC separation. (A) 100% water with 5 mM ammonium formate, 0.1% formic acid and (B) 100% methanol with 5 mM ammonium formate, 0.1% formic acid made up the mobile phases; the gradient was 0.1-0.5 min 90% A phase, 0.5-2 min 90–10% A phase, and 2–7 min 10% A phase. At a retention duration of 4.55 minutes, metrafenone eluted. The temperature of the column oven was kept at 35°C. At 600 µL min−1, the flow rate was kept constant. An autosampler was used to inject a 10-µL aliquot.

Since it provided the largest signal-to-noise ratio (S/N) and hence guaranteed the lowest limit of detection, the estimation was carried out in positive ESI mode by multiple reaction monitoring (sMRM) using mass transition [M+H]+ 409/209 for quantification. For confirmation, 409/226.9, the next most intense MRM, was employed. Ten minutes was the ideal run time. For both mass transitions, the collision energies (CEs) were 21 and 29 V and the declustering potential (DP) was 53 V.

2.6 Method validation

2.6.1 Standards and calibration

Metrafenone of >99% purity was used as the certified reference standard, obtained from Dr. Ehrenstorfer GmbH, Germany (Ehrenstorfer, 2023). All solvents employed were of MS grade or higher quality to ensure analytical precision (Alder et al., 2006). Calibration standards were prepared in methanol and water (1:1, v/v) at six concentration levels: 2, 5, 10, 20, 50, and 100 ng g^{-1}. A calibration curve was generated by plotting the peak areas against the concentrations of these standards (European Commission, 2017).

The limit of detection (LOD) for metrafenone was defined as the concentration corresponding to a signal-to-noise (S/N) ratio of 3, while the limit of quantification (LOQ) was determined based on an S/N ratio of 10 with acceptable recoveries at the LOQ level (Shrivastava & Gupta, 2011; SANCO, 2014). To account for matrix effects, matrix-matched calibration standards were prepared using untreated grape berries of the same variety and subjected to identical procedures (Banerjee et al., 2012; Stajnbaher & Zupancic-Kralj, 2003).

2.6.2 Accuracy: recovery experiments

The samples (10 g) in six replications were fortified with metrafenone separately at three concentration levels of 10, 20, and 50 ng g−1 in order to conduct the recovery tests on a blank grape matrix.

Analysis of data

Numerous recent studies have demonstrated that the degradation behavior of pesticides in natural systems, like grapes, where the degradation pattern may follow a non-linear route, cannot be sufficiently explained by simple first-order kinetics. Using the following mathematical formulas, numerous writers (Chen et al., 2011; Kumari et al., 2019) tried to analyze the timewise residue data of metrafenone in grapes using both linear and non-linear regression analysis:

First-order model : $[A]t = [A]1 \exp(-k1t)$ (1)

First + first-order model : $[A]t = [A]1 \exp(-k1t) + [A]2 \exp(-k2t)$

[A]1,2 are the initial concentrations of A at time 0 that were degraded by first-order processes 1 and 2, and k1 and k2 are the degradation rate constants for processes 1 and 2. [A]t is the concentration (mg kg−1 grape) of A at time t (days). The model being utilized determines the units of k. For the first-order model, the half-life DT50 (time for 50% degradation) was calculated as follows:

$DT50 = \ln(2) \times k-1$

One crucial metric for indicating the rate of degradation is DT50.

PHI (Pre-Harvest Interval)

The pre-harvest interval (PHI), defined as the number of days required for pesticide residues to dissipate below the maximum residue limit (MRL) following application, was calculated using the first-order kinetic model (Chen et al., 2011). For first-order kinetics, PHI was determined by the following equation:

PHI = [log(intercept) − log(MRL)]/slope of first-order equation. The residue data were plotted against time (days) and then fitted to equation using a commercially available program,OriginPro.12 The equation parameters[A1],[A2], k1 and k2 were calculated by OriginPro, which allows the definition of new equations and statistical parameters. The MRLs for metrafenone have been established as 7 mg kg^{-1} (EU Pesticides Database, 2023), 5 mg kg^{-1} (Codex Alimentarius Commission, 2022), and 5 mg kg^{-1} (Food Safety and Standards Authority of India [FSSAI], 2020). These values were used to evaluate the PHI under different dissipation scenarios.

3 RESULTS AND DISCUSSION

3.1 Method validation

The linearity of the calibration curve was established as 2, 5, 10 , 20, 50,100 ng g−1 with a correlation coefficient $R^2 > 0.99$. For matrix calibration, R^2 was also >0.99. The LOD and LOQ were 3.0 and 10 ng g−1 respectively. The average recoveries (%) of metrafenone at 10, 20 and 50 ng g−1 levels of fortification were 89.90 ± 2.89%, 85.17 ± 8.74% and 86.30 ± 10.13% respectively. Thus, there was compliance with the EU DG SANTE (11213/V2) criterion in this regard, which requires mean recoveries within the range 70–110%. Figure. 1 shows Fig.1 XIC of metrafenone at 10 mg/kg for both transitions while fig. 2 shows Figure.2 grape recovery of metrafenone at 10 mg/kg.

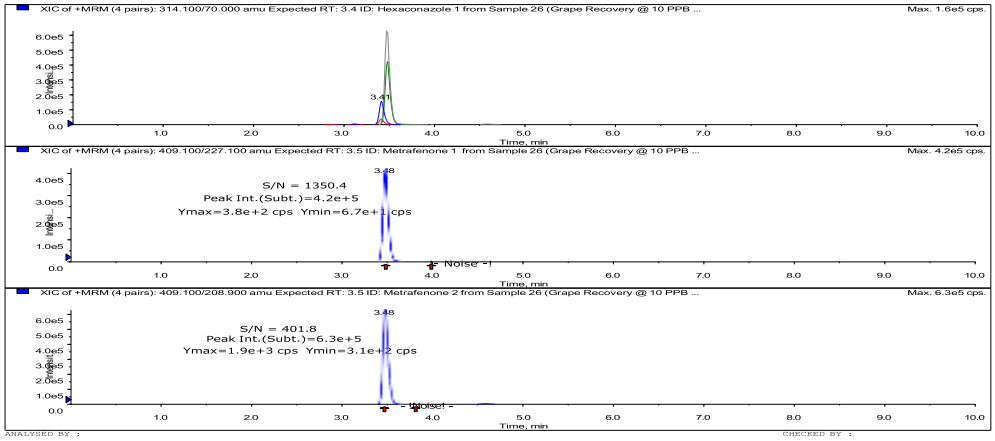

Figure1. XIC of metrafenone at 10 mg/kg for both transitions

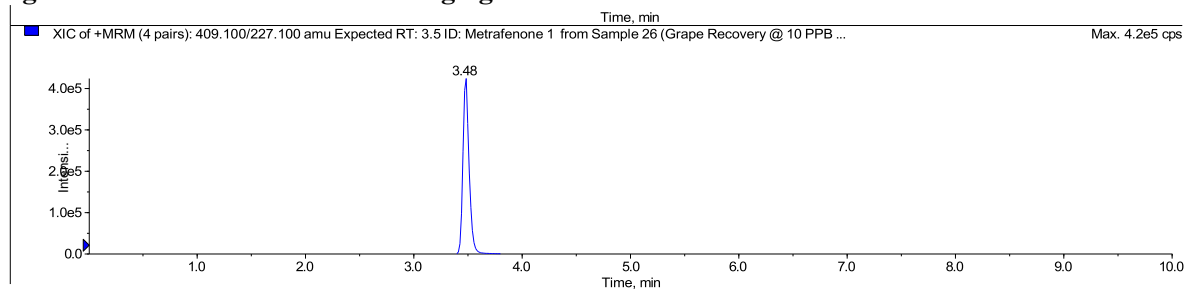

Figure 2. Grape recovery of metrafenone at 10 mg/kg

Degradation parameters for the residues of metrafenone in grape

Table 1. Persistence of Metrafenone in Grape Fruit for : Metrafenone @ 100 mL/Acre (T2)

Sr. No.	Concentration (mg/kg) Days after Spray	R1	R2	R3	Avg	SD	RSD
1	0 (2 hour after spray)	10.600	11.600	11.800	11.33	0.64291	5.67
2	1	10.300	11.100	10.500	10.63	0.416333	3.92
3	3	9.600	8.890	9.340	9.28	0.359212	3.87
4	5	8.280	7.860	7.360	7.83	0.460579	5.88
5	7	7.380	7.600	7.090	7.36	0.255799	3.48
6	10	6.930	7.050	6.950	6.98	0.064291	0.92
7	15	5.160	5.340	5.030	5.18	0.155671	3.01
8	21	3.260	3.690	3.150	3.37	0.285365	8.48
9	30	1.650	1.570	1.400	1.54	0.127671	8.29
10	45	0.228	0.226	0.207	0.22	0.01159	5.26
11	60	0.032	0.037	0.035	0.03	0.002739	7.95
Correlation coefficient (R2)					0.992		
Half-life (Days)					13.00		

Data on the dissipation of metrafenone residues in grapes after a single dose application is shown in Table 1, "Persistence of Metrafenone in Grape Fruit for: Metrafenone @ 100 mL/Acre (T2)." It is evident from the table that metrafenone concentrations have been declining over time. After 60 days, the initial concentration, which ranged from 10.60 to 11.80 mg/kg with an average of 11.33 mg/kg, gradually

decreased to 0.034 mg/kg. This implies the breakdown and/or loss of metrafenone residues from the grape over time. Good precision and agreement between the replicate measurements are shown by RSD values below 20% at the Limit of Quantification (LOQ). This implies that the data points are solid and the analytical approach was trustworthy.

Additionally, the table shows how the fungicide metrafenone dissipates in grapes when 100 mL/acre is sprayed. The time it takes for the initial metrafenone concentration to drop by half is indicated by the computed half-life of 13.00 days. In addition to estimating possible accumulation over time with repeated applications, this number offers information into the pesticide's persistence in the environment.

Over time, the residue levels gradually drop, indicating that metrafenone degrades in the fruit. The half-life of 13 days suggests a modest persistence, and the R^2 value (0.99) shows that the deterioration follows a predictable pattern.

Table 2. Persistence of Metrafenone in Grapes for : Metrafenone @ 125 mL/Acre (T2)

	Concentration (mg/kg)						
Sr No	**Days after Spray**	**R1**	**R2**	**R3**	**Avg**	**SD**	**RSD**
1	0 (2 hour after spray)	14.800	15.500	14.500	14.93	0.51316	3.44
2	1	14.400	14.700	14.000	14.37	0.351188	2.44
3	3	13.100	13.500	13.400	13.33	0.208167	1.56
4	5	12.800	12.700	12.300	12.60	0.264575	2.10
5	7	11.500	11.300	11.800	11.53	0.251661	2.18
6	10	10.600	10.400	10.800	10.60	0.2	1.89
7	15	8.500	8.420	8.980	8.63	0.302875	3.51
8	21	6.490	6.380	6.190	6.35	0.151767	2.39
9	30	2.960	2.840	2.710	2.84	0.125033	4.41
10	45	0.348	0.375	0.371	0.36	0.014572	4.00
11	60	0.088	0.088	0.092	0.09	0.002136	2.39
Correlation coefficient (R2)					0.991		
Half-life (Days)					17.00		

Data on the degradation parameters for metrafenone residues in grapes following a double dosage application are shown in Table 2. This suggests that the "Single Dose (SD)" shown in Table 1 was lower than the initial application rate. Data on the decrease in metrafenone concentration in grapes over time after a spray application is shown in this table. After two hours of spraying, the initial deposit was 14.93 mg/kg; after sixty days, it gradually decreased to 0.09 mg/kg. This shows that metrafenone in the grapes has broken down or dissipated. The metrafenone concentration and the time after spray have a strong linear connection, as indicated by the R2 value of 0.991.

This indicates that a significant amount (99.1%) of the fluctuation in metrafenone levels over time can be explained by the selected model, which is most likely first-order kinetics, which implies a constant degradation rate. But according to the estimated 17-day half-life, it takes 17 days for the initial metrafenone concentration to drop by half. The National Research Centre for Grapes specifies a 22-day PHI for metrafenone in Grape Annexure 5 (2024). The present study indicates the PHI of 19 days for EU MRL based on this study. The specified PHI for FSSAI and codex is 25 days.The present study indicates the PHI of 19 days for EU MRL based on this study. In order to guarantee that the levels of metrafenone residues are safe for human consumption and meet regulatory standards (EU MRL 7 mg/kg), this data is essential. Figure 3 indicates Persistence of Metrafenone in Grape Fruit for :

Metrafenone @ 100 mL/Acre (T2) while figure 4 shows Persistence of Metrafenone in Grape Fruit for : Metrafenone @ 125 mL/Acre (T2).

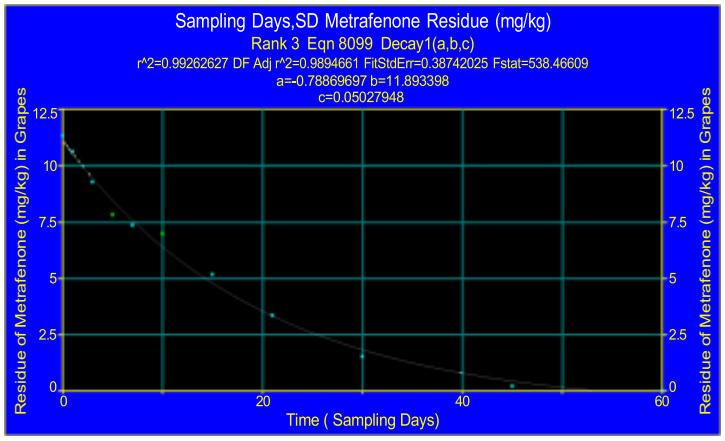

Figure 3. Persistence of Metrafenone in Grape Fruit for : Metrafenone @ 100 mL/Acre (T2)

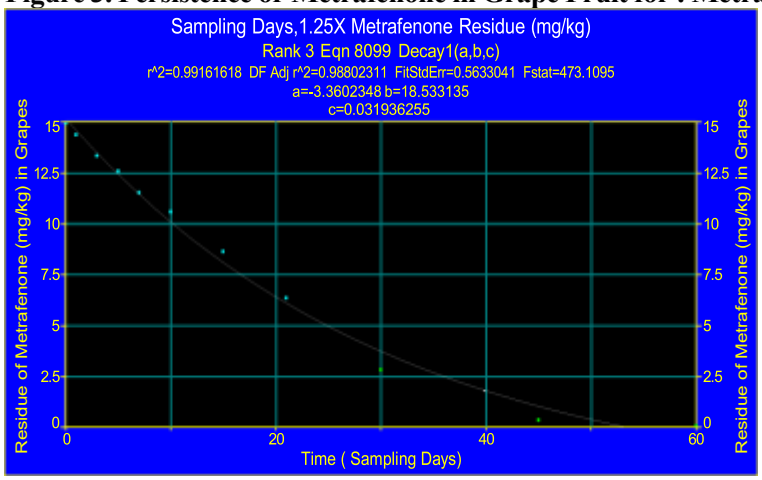

Figure 4. Persistence of Metrafenone in Grape Fruit for : Metrafenone @ 125 mL/Acre (T2)

Table 3. Comparative performance metrics of the developed LC-MS/MS method versus published studies in vegetable matrices

Matrix	LOD (mg kg−1)	LOQ (mg kg−1)	Recovery (%)	RSD (%)	Matrix Effect (%)	Reference
Grapes	0.0022	0.0074	85.6-93.2	<2.89	-6.41	Present study
Cucumber	0.0003	0.0025	92.7–99.7	<6	−4.15	RSC et al.,2025
Green pepper	0.002	0.006	89.4–95.2	<10	−13.2	Ko et al., 2016
Lettuce	0.001	0.005	88.1–94.7	<15	−12.6	Kabir et al., 2015
Tomato	0.003	0.01	86.3–91.9	<12	−8.4	Kim et al., 2021

The performance of the developed LC-MS/MS method for grapes is summarized in Table 3 and compared with previously reported methods in cucumber, green pepper, lettuce, and tomato. In grapes (present study), the method achieved a LOD of 0.0022 mg/kg and a LOQ of 0.0074 mg/kg, with recoveries ranging from 85.6–93.2% and very low variability (RSD <2.89%). The matrix effect was also minimal (−6.41%), indicating limited interference. Similarly, for cucumber (RSC Adv., 2025), the method demonstrated a lower LOD (0.0003 mg/kg) and LOQ (0.0025 mg/kg), with higher recovery (92.7–99.7%) but slightly greater variability (RSD <6%). The matrix effect (−6.71% to −4.15%) remained

low. By contrast, earlier studies on green pepper, lettuce, and tomato reported somewhat higher LOQ values, recovery ranges of 86–95%, matrix effects between −8.4% and −13.2%, and higher RSDs (<10–15%) (Ko et al., 2016; Kabir et al., 2015; Kim et al., 2021). Overall, the grape method exhibited strong sensitivity, precision, and accuracy, and performed at par with or better than prior methods across these crop matrices.

CONCLUSION

The results of the study show that metrafenone degrades in grapes over time in a predictable manner. Metrafenone concentrations show a declining tendency in both single and double dosage applications. The deterioration appears to follow a predictable, most likely first-order kinetic model, as indicated by the high R-squared values (0.99 for single dosage, 0.991 for double dose). The application dose affects the half-life and rate of degradation: A single dose has a shorter half-life (13 days) than a double dose (17 days). This discrepancy emphasizes how crucial it is to follow suggested treatment rates in order to reduce the persistence of metrafenone residues in grapes. Additionally, the analytical techniques used showed good precision. There is trust in the correctness of the data collected because the RSD values are less than 20% at the LOQ, indicating that the analytical process used for residue measurement is robust and trustworthy. Additionally, the data shows that within 60 days following application, the metrafenone concentration in grapes drops below the EU MRL of 7 mg/kg, even after a double dose application (0.09 mg/kg). For countries with lower MRLs such as Canada (4.5 mg/kg) and Taiwan (2.0 mg/kg), longer PHIs of 27 and 39 days, respectively, are required to ensure residue levels remain below their MRLs. A PHI of 25 days is adequate for countries like China, CODEX, FSSAI, Russia, Gulf Cooperation Council, and Thailand which have MRLs of 5.0 mg/kg. This suggests that in order to guarantee that harvested grapes fulfill the MRL standards for market release, rigorous evaluation of the PHI in combination with the degradation kinetics is essential. Monitoring and adherence to PHI are essential for export and consumer safety. Given the degradation kinetics and the regulatory guidelines, growers must ensure that the PHI was strictly observed to minimize metrafenone residue levels in grapes, particularly for the domestic and export markets. This is vital for protecting consumer health and ensuring compliance with international trade requirements. Collectively, metrafenone dissipates effectively in grapes, but proper adherence to recommended application rates and PHIs is crucial for minimizing residues and ensuring the safety and compliance of the grapes with established regulatory standards like the EU MRL and Grape Annexure 5 PHI. Export and consumer safety depend on PHI being monitored and followed. Growers must make sure that the PHI is closely followed in order to reduce the quantities of metrafenone residue in grapes, especially for the domestic and international markets, given the degradation kinetics and regulatory standards. This is essential for maintaining compliance with international trade regulations and safeguarding the health of consumers. Together, metrafenone dissolves in grapes quite effectively; nonetheless, in order to minimize residues and guarantee the safety and conformity of the grapes with established regulatory criteria such as the EU MRL and Grape Annexure 5 PHI, proper adherence to approved application rates and PHIs is essential.

Acknowledgement: I would like to express my sincere gratitude to my supervisor, Dr. Parag Chavan and co-supervisor Dr. Kaushik Banerjee for their invaluable guidance, encouragement, and support throughout the course of this research. I am also thankful to ICAR-National Research Centre for Grapes, Pune. We would like to acknowledge Head Department of Chemistry, School of Science and In charge, Central Instrumentation Facility laboratory (CIF Lab), School of Science, Sandip University, Nasik for providing the necessary facilities and resources that made this work possible. My heartfelt thanks go to my colleagues and friends for their constant motivation and assistance.

Social impact: Residue monitoring builds trust between farmers, consumers, and regulatory authorities. It encourages the adoption of safer pest management strategies, contributing to sustainable agriculture and better livelihoods for farmers.

REFERENCES

1. Aktar, M. W., Sengupta, D., & Chowdhury, A. (2009). Impact of pesticides use in agriculture: Their benefits and hazards. *Interdisciplinary Toxicology*, 2(1), 1–12. https://doi.org/10.2478/v10102-009-0001-7
2. Alder, L., Greulich, K., Kempe, G., & Vieth, B. (2006). Residue analysis of 500 high priority pesticides: Better by GC–MS or LC–MS/MS? *Mass Spectrometry Reviews*, 25(6), 838–865. https://doi.org/10.1002/mas.20091
3. Anastassiades, M., Lehotay, S. J., Štajnbaher, D., & Schenck, F. J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for determination of pesticide residues in produce. *Journal of AOAC International*, 86(2), 412–431. https://doi.org/10.1093/jaoac/86.2.412
4. APEDA (2020). *Good Agricultural Practices for Production of Grapes*. Agricultural and Processed Food Products Export Development Authority, Ministry of Commerce & Industry, Government of India. Available at: https://apeda.gov.in
5. APEDA (n.d.). *Annexure 7: Revised grape sampling protocol*. Agricultural and Processed Food Products Export Development Authority, Ministry of Commerce & Industry, Government of India.
6. Banerjee, K., Upadhyay, A., Adsule, P. G., Patil, S. H., Oulkar, D., & Jadhav, M. R. (2012). Validation and uncertainty analysis of a multi-residue method for pesticides in grapes using ethyl acetate extraction and LC–MS/MS. *Journal of AOAC International*, 95(1), 196–210. https://doi.org/10.5740/jaoacint.SGE_Banerjee
7. Chen, C., Qian, Y., Chen, Q., Tao, C., Li, C., & Li, Y. (2011). Evaluation of pesticide residues in fruits and vegetables from Xiamen, China. *Food Control*, 22(7), 1114–1120. https://doi.org/10.1016/j.foodcont.2011.01.007
8. Codex Alimentarius (2023). *Pesticide Residues in Food – Maximum Residue Limits (MRLs)*. FAO/WHO. Available at: http://www.fao.org/fao-who-codexalimentarius
9. Codex Alimentarius Commission. (2022). *Codex Pesticide Residues in Food Online Database.* FAO/WHO. Retrieved from http://www.fao.org/fao-who-codexalimentarius
10. Dagostin, S., Schärer, H. J., Pertot, I., & Tamm, L. (2011). Are there alternatives to copper for controlling grapevine downy mildew in organic viticulture? *Crop Protection*, 30(7), 776–788. https://doi.org/10.1016/j.cropro.2011.02.031
11. EFSA (2020). European Food Safety Authority – Scientific report on pesticide residues in food. *EFSA Journal*, 18(4), e06057.
12. Ehrenstorfer (2023). *Certified reference materials: Pesticides and metabolites*. Dr. Ehrenstorfer GmbH, Germany. Retrieved from https://www.lgcstandards.com
13. European Commission (2017). *SANTE/11813/2017: Guidance document on analytical quality control and method validation procedures for pesticide residues analysis in food and feed.* Brussels, Belgium.
14. European Commission (2024). *EU Pesticides Database – MRLs and Analytical Methods*. European Union. Available at: https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database
15. European Commission. (2023). *EU Pesticides Database: Maximum Residue Levels (MRLs).* Brussels, Belgium. Retrieved from https://ec.europa.eu/food/plants/pesticides/eu-pesticides-database
16. Fernández-Alba, A. R., & García-Reyes, J. F. (2008). Large-scale multi-residue methods for pesticides and their transformation products in food by advanced LC–MS. *TrAC Trends in Analytical Chemistry*, 27(11), 973–990. https://doi.org/10.1016/j.trac.2008.09.009
17. Food Safety and Standards Authority of India (FSSAI). (2020). *Food Safety and Standards (Contaminants, Toxins and Residues) Regulations, 2011 – Updated MRLs.* Government of India, New Delhi. Retrieved from https://www.fssai.gov.in
18. FSSAI (2022). *Food Safety and Standards Authority of India – Pesticide Residues Regulations*. Government of India.
19. Gessler, C., Pertot, I., & Perazzolli, M. (2011). Plasmopara viticola: A review of knowledge on downy mildew of grapevine and effective disease management. *Phytopathologia Mediterranea*, 50(1), 3–44.
20. Kabir, K. H., Jahan, S., & Hossain, M. (2015). *Development and validation of an LC-MS/MS method for pesticide residue analysis in lettuce*. Journal of Agricultural and Food Chemistry, 63(4), 1017–1025.
21. Kim, H. Y., Park, J. E., & Lee, J. H. (2021). *LC-MS/MS based multi-residue determination of pesticides in tomato matrices*. Journal of Chromatography A, 1635, 461750.
22. Kmellár, B., Fodor, P., Pareja, L., Ferrer, C., Martínez-Uroz, M. A., Valverde, A., & Fernández-Alba, A. R. (2008). Validation and uncertainty study of a comprehensive list of 160 pesticide residues in multi-class vegetables by liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A*, 1215(1–2), 37–50. https://doi.org/10.1016/j.chroma.2008.09.095
23. Ko, A. Y., Kim, J. H., & Lee, H. S. (2016). *Determination of pesticide residues in green pepper using LC-MS/MS*. Food Chemistry, 194, 1211–1218.
24. Kumari, B., Madan, V. K., Kathpal, T. S., & Singh, J. (2019). Pesticide residues in grapes: Field dissipation and risk assessment. *Environmental Monitoring and Assessment*, 191, 159. https://doi.org/10.1007/s10661-019-7293-3
25. Lehotay, S. J., Son, K. A., Kwon, H., Koesukwiwat, U., Fu, W., Mastovska, K., Hoh, E., & Leepipatpiboon, N. (2010). Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables. *Journal of Chromatography A*, 1217(16), 2548–2560. https://doi.org/10.1016/j.chroma.2010.01.044
26. Lehotay, S. J., Son, K. A., Kwon, H., Koesukwiwat, U., Fu, W., Mastovska, K., Hoh, E., & Leepipatpiboon, N. (2010). Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables. *Journal of Chromatography A*, 1217(16), 2548–2560. https://doi.org/10.1016/j.chroma.2010.01.044
27. NRCG (2017). *Package of Practices for Grapes*. ICAR-National Research Centre for Grapes, Pune, India.

28. Pearson, R. C., & Goheen, A. C. (1988). *Compendium of Grape Diseases*. APS Press, St. Paul, MN.
29. RSC Advances, 2025, 15, 17164.
30. SANCO (2014). *Guidance document on analytical quality control and validation procedures for pesticide residues analysis.* SANCO/12571/2013, European Commission.
31. Sharma, A., Kumar, V., Shahzad, B., Tanveer, M., Sidhu, G. P. S., Handa, N., Kohli, S. K., Yadav, P., Bali, A. S., Parihar, R. D., Dar, O. I., Singh, K., Jasrotia, S., Bakshi, P., Ramakrishnan, M., Kumar, S., Bhardwaj, R., & Thukral, A. K. (2019). Worldwide pesticide usage and its impacts on ecosystem. *SN Applied Sciences*, 1, 1446. https://doi.org/10.1007/s42452-019-1485-1
32. Shrivastava, A., & Gupta, V. B. (2011). Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chronicles of Young Scientists*, 2(1), 21–25. https://doi.org/10.4103/2229-5186.79345
33. Stajnbaher, D., & Zupančič-Kralj, L. (2003). Multiresidue method for determination of 90 pesticides in fresh fruits and vegetables using solid-phase extraction and gas chromatography–mass spectrometry. *Journal of Chromatography A*, 1015(1–2), 185–198. https://doi.org/10.1016/S0021-9673(03)01213-2

Schematic Presantation:-