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Residue dynamics of fluopyram and trifloxystrobin in grapes: Consumer safety insights from Saudi agriculture

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Summary This study examines the dissipation and residue behavior of fluopyram, trifloxystrobin, and their metabolites (fluopyram-benzamide and trifloxystrobin acid, CGA 321113) in grapes grown in Al-Qassim, Saudi Arabia. The fungicide Luna Sensation® 500 SC (250 g/L fluopyram, 250 g/L trifloxystrobin) was applied at the recommended rate (100–150 g a.i./ha). A multi-residue LC-MS/MS method was developed and validated according to SANTE 11312/2021 guidelines, demonstrating high linearity ($R^2 > 0.999$), low detection limits (0.00021–0.00094 mg/kg), and recoveries of 84.8–94.8% with RSDs < 20%. Dissipation studies revealed first-order kinetics, with fluopyram exhibiting faster degradation ($t_{1/2}$ = 2.23–3.02 days) than trifloxystrobin ($t_{1/2}$ = 3.46–4.04 days). CGA 321113 dissipated to below detectable levels within 3 days, while fluopyram-benzamide remained undetected. Terminal residues were below the maximum residue limits (MRLs) of 3 mg/kg (trifloxystrobin) and 2 mg/kg (fluopyram), with pre-harvest intervals of 6.29 and 2.13 days, respectively. Chronic dietary risk assessments confirmed negligible health risks and provided valuable insights for effective pest management and the safe use of pesticides in Saudi grape cultivation.

Additional keywords: consumer risk assessment, dissipation, field trial, LC–MS/MS, residue definition, risk assessment, terminal residues

Introduction

Grapevine (*Vitis vinifera* L.) is widely cultivated for the sweet, juicy flavor and diverse applications of its fruit. Beyond their culinary appeal, they offer significant health benefits, rich in vitamins C, B1, and B6, as well as antioxidants and phytonutrients protecting against diseases such as cancer, heart disease, and diabetes (Zhou *et al.,* 2022). In 2021, Saudi Arabia produced over 106,400 tons of grapes from approximately 3,925 hectares, making it one of the largest grape producers in the Middle East (Taylor, 2005).

However, due to their high carbohydrate content, grapes are highly susceptible to pests and insects (Conde *et al.,* 2007). Pesticides play a crucial role in protecting crops from diseases and minimizing economic losses (Heshmati *et al.,* 2020), with approximately 7% of agricultural pesticides used in viticulture (Zengin and Karaca, 2018). Several factors, including dosage, formulation, application method, frequency, climate conditions, grape variety, growth dilution, and degradation processes such as photochemical breakdown, influence pesticide dissipation. While pesticides offer essential benefits in agriculture and viticulture, their potential risks must also be taken into consideration (Fantke *et al*., 2012).

Fluopyram (ISO common name; IUPAC: *N-{2-[3-chloro-5-(trifluoromethyl)-2-pyridyl] ethyl}-α,α,α-trifluoro-o-toluamide*; Fig. 1a) is a pyridylethylamide fungicide with broad-spectrum activity (EFSA 2023). It effectively controls pathogens such as *Botrytis* spp., *Sclerotinia* spp., and *Monilia* spp., as well as powdery mildew and leaf spot diseases in various crops (Kandel *et al.,* 2018). Fluopyram functions by inhibiting succinate dehydrogenase (SDH), also known as complex II in the mitochondrial respiratory chain, a key

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component of the tricarboxylic acid cycle (Abad-Fuentes *et al.,* 2015).

Trifloxystrobin (ISO common name; IUPAC: *methyl (2E)-(methoxyimino)(2-{[({(1E)-1-[3-(trifluoromethyl)phenyl]ethylidene}amino)oxy]methyl}phenyl)acetate;* Fig. 1b) is another broad-spectrum fungicide belonging to a class of fluorine-containing compounds (EFSA, 2025). Derived synthetically from naturally occurring strobilurin found in wood-decaying fungi, trifloxystrobin is widely used to combat diseases such as powdery mildew, downy mildew, and anthracnose in fruits, vegetables, and cereals (Ziegler *et al.,* 2003). It disrupts mitochondrial respiration by inhibiting electron transfer in the electron transport chain (Bartlett *et al.,* 2002).

Assessing the metabolism of these fungicides is critical when evaluating their residues. Fluopyram-benzamide (Fig. 1c) and trifloxystrobin acid (CGA 321113) (Fig. 1d) are the primary metabolites of fluopyram and trifloxystrobin, respectively. Research suggests that these metabolites may pose greater environmental and health risks than their parent compounds due to their mobility and persistence (Bartlett *et al.,* 2002; Wei *et al.*, 2016). Therefore, it is essential to quantify fluopyram, trifloxystrobin, and their metabolites to ensure consumer safety (Sharma *et al*., 2022; Cui *et al.,* 2023).

Luna Sensation® 500 SC is a suspension concentrate containing 250 g/L trifloxystrobin and 250 g/L fluopyram. This systemic, preventive, and curative fungicide controls a range of pathogenic fungi, including powdery mildew and botrytis rot in fruits, as well as sclerotinia and monilia diseases in various vegetables and crops (Bayer Crop Science 2026).

Extensive research has employed liquid and gas chromatography to analyze the dissipation behavior of trifloxystrobin and fluopyram residues in various crops, both individually and in combination with other analytes (Jyot *et al.,* 2010; Mohapatra *et al.,* 2010; Sahoo *et al.,* 2012; Patyal *et al.,* 2013; Dong and Hu 2014; Paramasivam *et al.,* 2017; Katna *et al.,* 2018; Sharma *et al.,* 2019; Ren *et al.,* 2023). Investigating the dissipation pattern of a ready-to-use trifloxystrobin and fluopyram formulation in grapevines holds significant practical value. However, data on their behavior in grapevines remains limited, despite similar studies on onions (Sharma *et al.,* 2022) and chili peppers (Mandal *et*

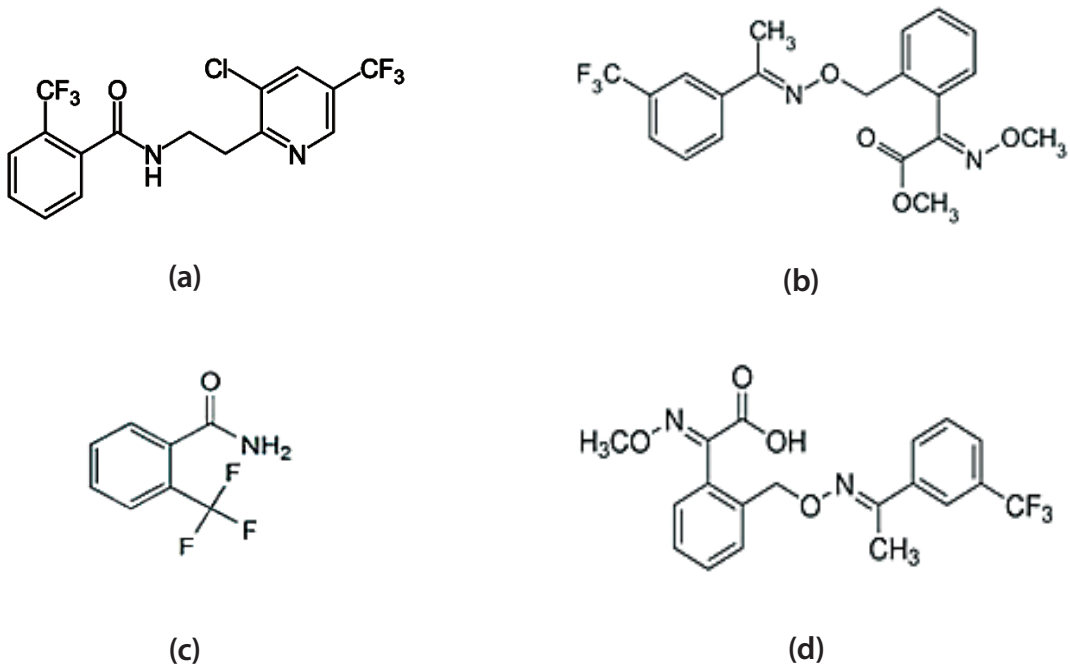

Figure 1. Chemical structure of a) fluopyram, b) trifloxystrobin, c) fluopyram-benzamide and d) CGA 321113 (trifloxystrobin acid).

al., 2023; Parmar *et al.,* 2023).

In this study, the persistence, dissipation kinetics, terminal residues, and consumer risk estimation of a trifloxystrobin and fluopyram formulation (Luna Sensation® 500 SC) under Saudi Arabian climatic and cultivation conditions were assessed. Although Luna Sensation® 500 SC is a registered product and has undergone regulatory evaluation as part of the authorization process, residue behavior can vary with crop, climate, and agricultural practices. Therefore, generating residue and dissipation data under local Saudi field conditions supports evidence-based risk management and helps confirm compliance with relevant maximum residue limits (MRLs) and pre-harvest intervals.

Materials and Methods

Chemicals and Reagents

Certified reference standard of trifloxystrobin (99.4% purity) was obtained from Chem Service Inc. (West Chester, PA, USA). Certified reference standard of fluopyram (98.7% purity), fluopyram-benzamide (99.6% purity), and CGA 321113 (98.68% purity) were sourced from Dr. Ehrenstorfer GmbH (Augsburg, Germany). HPLC-grade methanol, acetonitrile, LC-MS-grade formic acid, and ammonium formate were obtained from Fisher Scientific Ltd. (Loughborough, UK). Sodium chloride, trisodium citrate, disodium hydrogen citrate sesquihydrate and anhydrous magnesium sulfate were purchased from Chem-Lab NV (Zedelgem, Belgium). A ceramic homogenizer was purchased from Chrom Tech, Inc. (Copure®, Apple Valley, MN, USA). Bulk sorbents, including primary secondary amine (PSA) and Octadecylsilane (C18), were obtained from Macherey-Nagel (Chromabond, Düren, Germany). Ultrapure water was produced using an Evoqua Ultra Clear system (Evoqua Water Technologies LLC, Günzburg, Germany). The commercial formulation Luna Sensation® 500 SC (Bayer Crop Science Ltd.) was acquired locally and contains 25% fluopyram and 25% trifloxystrobin.

Standard solution preparation

Individual pesticide standard stock solution of each of the four tested analytes (1000 mg/L each) were prepared in pure acetonitrile. An intermediate standard mixture of acetonitrile was prepared at 100 mg/L by mixing the individual stock solutions. The working standard mixture of the four analytes (10 mg/L) was prepared from intermediate solutions at serial concentrations of 0.001-0.5 mg/L for the construction of solvent-based or matrix-matched calibration curves, respectively, by diluting with acetonitrile or the final sample extracts. All standard solutions were stored in a freezer at -20°C.

Field Experiments

The open-field trials were conducted in Buraidah City, Al-Qassim Region, Saudi Arabia, in August 2024. The cultivated table grape area was divided into 12 plots: four plots were treated with Luna Sensation® 50 SC at the low recommended application rate of 400 mL/ha (equivalent to 100 g a.i./ha of fluopyram and 100 g a.i./ha of trifloxystrobin), and four plots were treated at the high recommended application rate of 600 mL/ha (equivalent to 150 g a.i./ha of each active ingredient). The remaining four plots were maintained as an untreated buffer zone between treated areas to minimize spray drift. The formulation was diluted with tap water to a spray volume of 1000 L/ha and applied using a knapsack sprayer calibrated before application. Spraying was conducted by walking along the vine rows and applying the spray uniformly to the canopy and grape clusters in each plot to achieve consistent coverage.

Approximately 2 kg of grapes were collected from each treated plot at 0 (2 h), 1, 3, 7, 10, 14, and 20 days after application to assess dissipation and to determine half-lives ($t_1/_2$) and pre-harvest intervals (PHIs).

Terminal residues

Terminal residues were assessed in a

separate set of plots (independent of the dissipation study). For the terminal residue study, each application rate (low and high) was applied twice, with a 7-day interval between the first and second applications. Grape samples (~2 kg per plot) were collected at 3 and 7 days after the second (final) application to determine terminal residues under a repeated-use pattern. Applications and sampling were conducted at a late production stage close to commercial maturity to reflect residue-relevant pre-harvest conditions.

During the field trial, temperatures ranged from 29 to 45°C, with a mean relative humidity of 13–18%. Samples were transported under cold conditions, frozen overnight, homogenized, thoroughly mixed, divided into sub-samples, and stored at –20°C until analysis.

LC-MS/MS

The LC-MS/MS analysis was conducted using a Dionex Ultimate 3000 RS UHPLC system (Thermo Fisher Scientific, Austin, TX, USA) coupled with a TSQ Altis triple quadrupole mass spectrometer (Thermo Fisher Scientific, Austin, TX, USA). Chromatographic separation was achieved on an Accucore RP-MS C18 column (100 x 2.1 mm, 2.6 µm film thickness) (Thermo Fisher Scientific, Ireland), maintained at 40°C. The mobile phase comprised (A) water with 0.1% formic acid (v/v) and (B) methanol with 0.1% formic acid (v/v). The gradient elution program began with 10% B, held for 1 minute, and increased to 90% B over 5 minutes, where it was held for 2 minutes. At 8.1 minutes, the mobile phase returned to 10% B and was maintained for 6 minutes, resulting in a total run time of 14 minutes.

The analytes were detected in multiple reaction monitoring (MRM) mode (Table 1), optimized with positive electrospray ionization (ESI+) at a capillary voltage of 3.8 kV. The sheath and auxiliary gas were set to 40 and 10 Arb, respectively, with the vaporizer and ion transfer tube temperatures set to 350°C and 275°C, respectively. High-purity nitrogen was used as a nebulizer gas. Data acquisition and processing were carried out using Trace Finder software (version 4.1).

Extraction and cleanup

Extraction was based on the citrate-buffered QuEChERS approach, followed by dispersive-SPE cleanup using $MgSO_4$, PSA, and C18 to reduce co-extractives from the grape matrix. A 10-g sample of frozen, homogenized berries was weighed into a 50-mL centrifuge tube. For extraction, 10 mL of acetonitrile was added, followed by vigorous vortexing for 2 minutes with a ceramic homogenizer. A salt mixture containing 4 g of magnesium sulfate, 1 g of trisodium citrate, 0.5 g of disodium hydrogen citrate sesquihydrate, and 1 g of sodium chloride was added, then vortexed for 1 minute and centrifuged at 5000 rpm for 5 minutes. Two milliliters of the acetonitrile supernatant were trans-

Table 1. LC-MS / MS Parameters.

Pesticide	t_R (min)	Precursor ion (m/z)	Product ion (m/z)	Collision energy (v)	RF lens (v)
Trifloxystrobin	8.61	409.1	145	44	57
			<u>186</u>	18	57
CGA 321113	8.04	395.1	<u>145</u>	41	50
			186	18	50
Fluopyram	7.68	396.9	<u>208</u>	22	75
			173	29	75
Fluopyram-benzamide	5.76	190.1	<u>170</u>	12	30
			130	22	30

The underlined ions used as quantifier ion.

ferred to a 15 mL tube containing 300 mg of magnesium sulfate, 50 mg of PSA, and 50 mg of C18, vortexed for 30 seconds, and centrifuged again at 5000 rpm for 5 minutes. Finally, 1 mL of the cleaned extract was filtered through a 0.22 µm nylon syringe filter (Whatman, USA) into an LC-MS/MS vial for analysis.

Method validation

The method was validated according to SANTE guidelines (European Commission, 2021). Selectivity was evaluated by analyzing six replicates of blank grape berry samples alongside six replicates of grape berry samples spiked with the target analytes at the method's limit of quantification (LOQ). Chromatographic data were examined to confirm the absence of peaks at the retention times of the target analytes in the blank samples, ensuring no interference from matrix components. Linearity was evaluated using matrix-matched calibration curves prepared at eight concentration levels covering 0.001–0.2 mg/L in the final cleaned extract. The matrix effect (ME) was assessed by comparing the slope of the matrix-matched calibration curve with that of the calibration curve constructed in pure acetonitrile. Detection sensitivity was evaluated using the limit of detection (LOD), defined as the lowest concentration detectable at a signal-to-noise ratio (S/N) of 3. The LOQ was defined as the lowest concentration that could be quantified with acceptable accuracy (70%–120%) and precision (RSD < 20%). Recovery experiments were performed at 0.01–3 mg/kg to assess accuracy across a broad range, while LOQs were confirmed separately at 0.0025 mg/kg (trifloxystrobin, fluopyram) and 0.005 mg/kg (CGA 321113, fluopyram-benzamide) based on S/N ≥ 10 and acceptable accuracy/precision. Accuracy and precision were assessed by fortifying blank grape samples at 0.01, 0.1, 1, and 3 mg/kg (n = 6 per level). Precision at the LOQ was evaluated as intra-day repeatability (n = 6, RSD_r) and inter-day reproducibility (n = 18 across three days, RSD_R). All final residue concentrations are reported as mg/kg (fresh weight) of grape berries.

Calculations

Residual concentrations: The residual concentrations were calculated according to the residue definition for risk assessment, in particular total trifloxystrobin and total fluopyram were expressed as the sum of the parent compound and its specific metabolite, using equations 1 and 2, where a conversion factor to parent compound of 1.04 and 2.1 for trifloxystrobin and fluopyram respectively was used by dividing the molecular weight of the parent compound by the molecular weight of its metabolite:

Total residue of trifloxystrobin = Trifloxystrobin residue + [1.04 × CGA 321113 residue] (Eq. 1)

Total residue of fluopyram= Fluopyram residue + [2.1 × Fluopyram-benzamide residue] (Eq. 2)

Dissipation rates: The dissipation rates of trifloxystrobin and fluopyram were calculated using the first-order kinetics equation (Eq. 3). The half-lives ($t_{1/2}$) were calculated using equation 4 (Hoskins, 1961). The PHI or the safe waiting period was calculated using equation 5 (Hingmire *et al.,* 2015; Abdallah *et al.,* 2023).

$$C_t = C_0 e^{-kt} \quad \text{(Eq. 3)}$$

$$t_{1/2} = \frac{ln2}{k} \quad \text{(Eq. 4)}$$

$$PHI = \frac{lnc_0 - lnMRL}{k} \quad \text{(Eq. 5)}$$

where C_t is the concentration of total trifloxystrobin and fluopyram residues at time t, C_0 is the initial concentration, and *k* is the dissipation rate ($days^{-1}$), *kt* denotes the product of *k* and time *t* (days) in the exponential term of the first-order dissipation equation.

Chronic dietary risk assessment: The long-term dietary risk of total trifloxystrobin and fluopyram was evaluated by determining

the hazard quotient (HQ) using equations 6 and 7 (EFSA 2018; Malhat and Abdallah, 2019) as follows:

$$NEDI = \sum (STMR_i \times F_i) \qquad \text{(Eq. 6)}$$

$$\%HQ_c = \frac{NEDI}{ADI \times bw} \times 100 \qquad \text{(Eq. 7)}$$

where NEDI (mg/kg bw/day) represents the national estimated daily intake, STMR (mg/kg) is the median total residue of the supervised trials, and F_i (kg/day) is the mean daily consumption of fresh table grapes used for chronic exposure assessment (F_i = 0.0168 kg/day) (FAO/WHO, 2014). bw (kg) represents the mean adult body weight (60 kg), and ADI is expressed as mg/kg bw/day. The calculation used STMR values consistent with the residue definition for risk assessment (i.e., total trifloxystrobin and total fluopyram, where total residues represent the parent compound plus relevant metabolites using the conversion factors described in Eqs 1–2). The chronic dietary risk assessment was conducted for adult consumers, and the referenced consumption value was used as an input to evaluate consumer safety under Saudi conditions.

Results and discussion

Method Validation

Selectivity and linearity: The selectivity assessment confirmed the absence of interfering peaks at the retention times of the target analytes in the blank grape berry samples. Spiked samples showed clear, well-resolved peaks corresponding to the analytes, demonstrating the method's ability to accurately differentiate and quantify the target compounds in the grape berry matrix.

The linearity of the LC-MS/MS method was assessed by constructing a matrix-matched calibration curve for each analyte in the final cleaned extract, as described in the proposed procedure. The curves were constructed using eight concentration levels, ranging from 0.001 to 0.2 mg/kg (equivalent to mg/L). Satisfactory linearity was obtained for trifloxystrobin and fluopyram in the range of 0.001-0.2 mg/kg with coefficients of determination (R^2) of higher than 0.9996, and 0.0025-0.2 mg/kg and 0.005-0.2 mg/kg for CGA 321113 and fluopyram-benzamide with coefficients of determination (R^2) of 0.9994 and 0.9995, respectively (Table 2). Samples containing concentrations above the upper limit of the calibration curve are diluted with a blank extract to bring them within the calibration curve's range.

Matrix effects: The signal enhancement or suppression percentage was investigated using the formula ME% = Sm/Ss × 100, where Sm is the slope of the matrix-matched calibration curve, and Ss is the slope of the in-solvent calibration curve. To minimize instrument drift, solvent- and matrix-matched calibration standards were injected in the

Table 2. Validation results.

	Trifloxystrobin	CGA 321113	Fluopyram	Fluopyram-benzamide
Linearity (mg/kg)	0.001-0.2	0.0025-0.2	0.001-0.2	0.005-0.2
Correlation coefficient (r)	0.9996	0.9994	0.9997	0.9995
LOD (mg/kg)	0.00021	0.00055	0.00027	0.00094
LOQ (mg/kg)	0.0025	0.005	0.0025	0.005
RSD_r[a]	10.71	11.33	8.94	11.58
RSD_R[b]	15.42	16.81	14.24	16.25
Matrix effect (%)	-8.16	-6.25	-4.73	+11.14

[a] RSD_r: the relative standard deviation (intra-day repeatability, n=6).
[b] RSD_R: the relative standard deviation (inter-days repeatability, n=18).

same batch using a bracketing sequence and QC checks; therefore, slope differences were attributed to matrix effects rather than signal instability. For all test analytes, a weak matrix effect was observed, with signal suppression noted in trifloxystrobin (-8.16%), CGA 321113 (-6.25%), and fluopyram (-4.73%). In contrast, the fluopyram-benzamide signal was enhanced by 11.14% (Table 2). Ferrer *et al.* (2011) reported a weak matrix effect when values were within ±20% (Ferrer *et al.,* 2011). To achieve more accurate, precise results, matrix-matched calibration curves were used to quantify trifloxystrobin, fluopyram, and their metabolites in the tested samples, thereby compensating for matrix effects (MEs).

LODs and LOQs: The confirmed limits of quantification (LOQs) were 0.0025 mg/kg for trifloxystrobin and fluopyram, with recovery rates of 81.3 ± 9% and 79.8 ± 6%, respectively, and 0.005 mg/kg for CGA 321113 and fluopyram-benzamide, with recovery rates of 83.2 ± 7% and 78.5 ± 7%, respectively. The limits of detection (LODs) were 0.00021 mg/kg for trifloxystrobin and 0.00055 mg/kg for CGA 321113, while fluopyram and fluopyram-benzamide had LODs of 0.00027 mg/kg and 0.00094 mg/kg, respectively. These LOQs were well below the established MRLs of 2 mg/kg for total trifloxystrobin and 3 mg/kg for total fluopyram, highlighting the high sensitivity of the analytical method described. Representative chromatograms for all tested analytes at these LOD and LOQ levels are provided in Figures S1 and S2.

Recovery and precision: The recovery study was conducted using four spiking levels: 0.01, 0.1, 1 and 3 mg/kg. These levels included concentrations lower than, equal to, or higher than the MRL of the tested analytes. For each spiking level, six replicates were analyzed. The recoveries ranged from 90.8 to 94.5% and 84.8-89.7% for trifloxystrobin and its metabolite CGA 321113, respectively, and from 90.7 to 94.8% and 83.3-86.2% for fluopyram and its metabolite fluopyram-benzamide, respectively (Table 3). The relative standard deviations (RSDs) ranged from 2.6% to 8.4%, which satisfied the requirements of the SANTE guideline of 70-120% and RSD of <20%. Method precision was estimated at the LOQ level for each analyte, relative standard deviation (RSD) in terms of intra-day repeatability (RSD_r), which were 10.71, 11.33, 8.94, and 11.58%, and inter-day repeatability (RSD_R) that were 15.42, 16.81, 14.24, and 16.25%, for trifloxystrobin, CGA 321113, fluopyram, and fluopyram-benzamide, respectively (Table 2), which are within the satisfactory limit of < 20%, indicating consistency of the proposed method.

The present study's validation criteria data satisfied the SANTE method validation guideline. It is considered appropriate for quantifying the residue of trifloxystrobin and fluopyram, as well as their metabolites, in grape berries.

Dissipation patterns of trifloxystrobin and fluopyram in grape berries

Dissipation of trifloxystrobin and its metabolite CGA 321113: The dissipation patterns of trifloxystrobin and its metabolite CGA 321113 in grapes are summarized in Table 4.

Table 3. Average recoveries ± RSD (%) of the tested analytes in grape berries.

Spiking level (mg/kg)	Average recoveries (%)			
	Trifloxystrobin	CGA 321113	Fluopyram	Fluopyram-benzamide
0.01	91.4±6.2	87.4±8.4	90.7±3.3	84.8±8.1
0.1	94.5±4.8	85.2±6.5	92.4±5.4	86.2±3.7
1	93.3±2.6	89.7±3.1	94.8±7.8	83.3±4.4
3	90.8±4.1	84.8±4.9	93.1±6.8	85.1±7.1

RSD refers to the relative standard deviation of replicate measurements (n = 6).

Initial deposits of trifloxystrobin (including its metabolite CGA 321113) were 0.97 mg/kg and 1.08 mg/kg for the low (100 g a.i./ha) and high (150 g a.i./ha) application rates, respectively. The dissipation followed first-order kinetics, as demonstrated in the semi-logarithmic dissipation curves (Fig. 2).

By the third day after application, trifloxystrobin residues had dissipated by 49% for the low application rate and 46.46% for the high application rate. By the 10th day, dissipation reached 89.96% and 83.25%, respectively, and by the 20th day, residues decreased by 97.72% and 96.50%, resulting in final residues of 0.022 mg/kg and 0.038 mg/kg, respectively. The half-lives ($t_1/_2$) for trifloxystrobin were calculated to be 3.46 days and 4.04 days for the low and high application rate, with dissipation rate constants (k) of 0.2005 and 0.1714 days^{-1} (Table 4).

The dissipation behavior of trifloxystrobin can be attributed to its lower water solubility (0.16 mg/L at 20°C) and relatively low vapor pressure (1.2×10^{-3} mPa), which likely reduces its rate of volatilization and leaching. The observed hydrolysis of trifloxystrobin, leading to the formation of its metabolite CGA 321113, contributed to overall dissipation. The metabolite CGA 321113 exhibited a rapid decline, with its highest concentration observed at one day post-application and subsequently falling below the detection limit (BDL) by the third day. By the 20th day, metabolite levels were below the quantification limit of both application rates (LOQ).

In previous studies, Mohapatra *et al.* (2010) reported longer half-lives for tri-

Table 4. Dissipation kinetics of total trifloxystrobin and total fluopyram in table grape berries.

	Total trifloxystrobin (mg/kg)		Total Fluopyram (mg/kg)	
	100 g a.i/ha^{-1}	150 g a.i/ha	100 g a.i/ha	150 g a.i/ha
Slope (C_0) (mg/kg)	0.8496	0.9985	1032.3	1215.4
Intercept (k)(day^{-1})	0.2005	0.1714	0.3104	0.2295
R^2	0.9692	0.9902	0.9802	0.9871
$t_{0.5}$ (days)	3.46	4.04	2.23	3.02
EU-MRL (mg/kg)	3	3	2	2
PHI (days)	6.29	6.42	2.13	2.17

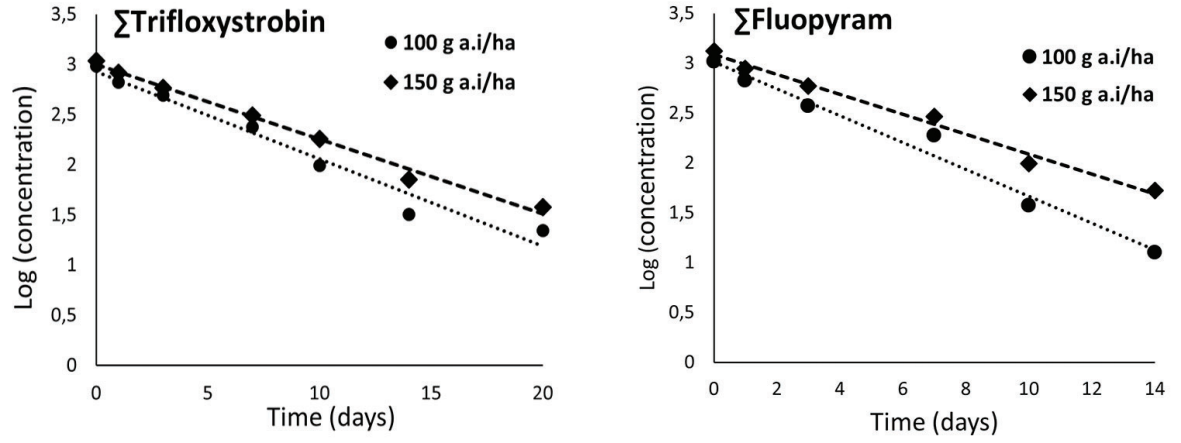

Figure 2. Semi-logarithmic dissipation curves of total trifloxystrobin and fluopyram across high-low doses of 100 and 150 g a.i./ha.

floxystrobin when combined with tebuconazole (Nativo 75 WG), with half-lives of 8.3 to 8.6 days in grapes (Mohapatra *et al.,* 2010). In contrast, shorter half-lives were observed in crops such as chili (1.58–1.81 days) (Sahoo *et al.,* 2012), gherkin (2.9–3.7 days) (Mohapatra, 2015), and tomatoes (1.08–2.13 days) (Sharma *et al.,* 2019). These differences suggest that the dissipation rate of trifloxystrobin depends on crop type and environmental conditions. The primary degradation pathway involves hydrolysis, producing the metabolite CGA 321113 (Banerjee *et al.,* 2006), which has been shown to dissipate rapidly in matrices such as tea (Paramasivam *et al.,* 2017).

Dissipation of fluopyram and its metabolite fluopyram-benzamide: The dissipation kinetics of fluopyram and its metabolite fluopyram-benzamide in grape berries are presented in Table 4. Initial fluopyram deposits were 1.04 mg/kg and 1.32 mg/kg for the low (100 g a.i./ha) and high (150 g a.i./ha) application rates, respectively. The dissipation of fluopyram followed first-order kinetics, as shown by the linear decline in concentration over time on a semi-logarithmic scale (Fig. 2). By the third day, fluopyram residues had dissipated by 64.08% and 55.23% for the low and high application rates, respectively. By the 10th day, dissipation reached 96.38% and 92.55%, with residues falling below detection limits by day 20.

Fluopyram exhibited shorter half-lives than trifloxystrobin, with half-lives of 2.23 and 3.02 days at the low and high application rates, respectively, and dissipation rate constants (k) of 0.3104 and 0.2295 days^{-1}. The faster dissipation of fluopyram can be attributed to its higher water solubility (16 mg/L at 20°C) and greater vapor pressure (2.4×10^{-3} mPa), which enhance volatility and hydrolysis under hot, dry conditions. The absence of detectable levels of fluopyram-benzamide throughout the study suggests that it undergoes rapid degradation or is not formed in grape tissues. Fluopyram's half-life vary across different crops, with values ranging from 4.04 to 5.18 days in pomegranates (Patil *et al.,* 2018), 6.48 to 6.60 days in watermelons (Dong and Hu, 2014), and 3.8 to 4.4 days in peppers, tomatoes, and cucumbers (Wei *et al.,* 2016). Patel *et al.* (2016) reported longer half-lives of 8.85 to 9.12 days in onions (Patel *et al.* 2016), highlighting the influence of crop type and environmental conditions on residue dissipation.

The distinct dissipation rates of trifloxystrobin and fluopyram can be attributed to their physicochemical properties. Trifloxystrobin's lower solubility and vapor pressure result in slower dissipation, whereas fluopyram's higher solubility and vapor pressure enhance its degradation, particularly under high-temperature and low-humidity conditions. This was evident from the study results, which showed that residues of both fungicides declined below the relevant EU MRLs. For trifloxystrobin, the EU MRL for table grapes is 3 mg/kg (EU-MRL database). For fluopyram, EU legislation distinguishes between table grapes (2 mg/kg) and wine grapes (1.5 mg/kg) (EU-MRL database); since the present study was conducted on table grapes, compliance was assessed against the 2 mg/kg MRL. The calculated pre-harvest intervals (PHIs) were 6.29 days (trifloxystrobin) and 2.13 days (fluopyram). The extended ripening period in grapes, combined with the environmental conditions in the Al-Qassim region of Saudi Arabia—high temperatures (29 to 45°C) and low humidity (13% to 18%)—likely contributed to the rapid dissipation observed The findings confirm that both fungicides, when applied at recommended rates, are safe for use in grape cultivation in the Al-Qassim region, as they meet food safety standards well before harvest.

Terminal Residues of Trifloxystrobin and Fluopyram

Terminal residue results are presented for the parent compounds (trifloxystrobin and fluopyram) (Table 5). The metabolites (CGA 321113 and fluopyram-benzamide) were below the LOQ in samples collected; therefore, total residues (parent + metabolites) were effectively equivalent to parent

residues under the conditions of this study. At a lower application rate of 100 g a.i/ha, after two applications with a short 3-day harvest interval, trifloxystrobin residues were 0.663 mg/kg, and fluopyram residues were 0.55 mg/kg. Extending the harvest interval to 7 days resulted in a significant reduction in residues to 0.38 mg/kg for trifloxystrobin and 0.21 mg/kg for fluopyram, demonstrating effective dissipation over time Increasing the application rate to 150 g a.i/ha raised residues up to 0.74 mg/kg for trifloxystrobin and 0.67 mg/kg for fluopyram with the same 3-day interval. However, a longer 7-day interval effectively reduced residues, underscoring the benefit of extended pre-harvest periods in promoting dissipation.

All recorded residue levels were well below the established MRLs of 3 mg/kg for trifloxystrobin and 2 mg/kg for fluopyram, indicating compliance with safety guidelines. The calculated PHIs were approximately 6.29 days for trifloxystrobin, 2.13 days for fluopyram at the lower application rate, and 6.42 days and 2.17 days at the higher application rate. Given the combined use of these fungicides, a 7-day PHI is recommended to ensure compliance and safeguard consumer health.

Chronic dietary risk assessment

The dietary risk analysis, using National Estimated Daily Intake (NEDI) and Hazard Quotient (HQ) metrics (Table 6), confirmed a low consumer risk, even with higher application rates. For the 100 g a.i/ha application rate with two applications and a 3-day harvest interval, NEDI values were 1.40E-04 mg/kg bw for trifloxystrobin and 1.12E-04 mg/kg bw for fluopyram. The corresponding HQ values were 0.14% and 0.93%, which dropped to 0.06% and 0.47% with a 7-day interval.

Table 5. Terminal residues of trifloxystrobin and fluopyram in grapevine.

Dosages (g a.i/ha)	Number of applications	Harvest interval (days)	Mean residues (mg/kg)	
			Trifloxystrobin	Fluopyram
100	2	3	0.6633	0.5517
		7	0.3782	0.2113
150	2	3	0.7426	0.6738
		7	0.4572	0.2324

Mean residues are reported for the parent compounds; metabolites were below the LOQ in terminal samples; therefore, total residues were effectively equivalent to parent residues under these conditions.

Table 6. Supervised trials median residues (STMR), national estimated daily intake (NEDI, mg/kg bw/day), and hazard quotient (HQ, %) values of trifloxystrobin and fluopyram in grape berries.

Dosages (g a.i/ha)	Number of applications	Harvest interval (days)	STMR (mg/kg)		NEDI (mg/kg bw/day)		HQ (%)	
			Trifloxystrobin	Fluopyram	Trifloxystrobin	Fluopyram	Trifloxystrobin	Fluopyram
100	2	3	0.524	0.418	1.40E-04	1.12E-04	0.140	0.934
		7	0.228	0.208	6.13E-05	5.59E-05	0.061	0.466
150	2	3	0.613	0.513	1.65E-04	1.38E-04	0.165	1.147
		7	0.294	0.267	7.89E-05	7.15E-05	0.079	0.596

STMR values correspond to total residues according to the risk-assessment residue definition; metabolites were <LOQ in terminal samples.

At the higher 150 g a.i/ha application rate, NEDI values rose slightly to 1.65E-04 mg/kg bw for trifloxystrobin and 1.38E-04 mg/kg bw for fluopyram after the second application with a 3-day interval. HQ values of 0.165% and 1.147% were observed for trifloxystrobin and fluopyram, respectively. These figures are significantly below the 100% threshold, demonstrating that residues, even with intensive use, remain at safe levels for chronic exposure.

The extended 7-day PHI for the combination of trifloxystrobin and fluopyram ensures that residue levels drop to safe levels, minimizing dietary risk. This adjustment is crucial, particularly in grape cultivation in Saudi Arabia, as it aligns with both national and international MRLs. The findings also support the safe use of the 50% SC formulation (containing 25% each of Trifloxystrobin and Fluopyram) in integrated pest management. The data provide a reliable foundation for establishing national MRLs, which support sustainable agricultural practices and ensure consumer safety.

Conclusion

In the current study the efficient dissipation of fluopyram and trifloxystrobin, along with their primary metabolites, in grape berries grown under the hot, dry conditions of the Al-Qassim region, Saudi Arabia was investigated. The developed and validated LC-MS/MS method proved adequate for the simultaneous determination of fungicides and their metabolites, providing reliable results for routine residue monitoring. High temperatures and low humidity contributed to the rapid dissipation of fluopyram, with rates reaching levels well below established MRLs before harvest.

The study confirmed that the PHIs of 6.29 days for trifloxystrobin and 2.13 days for fluopyram are adequate as to ensure consumer safety.. The results, therefore, highlighted the excellent suitability of the Luna Sensation 500 SC formulation for safe, practical application in integrated pest management in grape cultivation. These studies provide the information needed to establish national MRLs, which will contribute to the development of sustainable agriculture in compliance with food safety regulations in Saudi Arabia.

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Authorship contribution

Saleh Alhewairini: Review & editing, Project administration, Funding acquisition. Osama Abdallah and Ayman F. Omar: Conceptualization, Writing – original draft, Validation, Resources, Methodology, Investigation. Khaled Al-Jamhan and Fahad Almulhim: Sample collection, Formal analysis, Data curation, Software.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Data availability

Data will be made available on request.

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Συμπεριφορά υπολειμμάτων φυτοπροστατευτικών ουσιών fluopyram και trifloxystrobin και εκτίμηση διατροφικής επικινδυνότητας σε σταφύλια στη Σαουδική Αραβία

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Περίληψη Η παρούσα μελέτη διερευνά τη συμπεριφορά των υπολειμμάτων των δραστικών ουσιών fluopyram και trifloxystrobin σε σταφύλια που καλλιεργήθηκαν στην περιοχή Al-Qassim της Σαουδικής Αραβίας. Οι δραστικές ουσίες εφαρμόστηκαν με ψεκασμό φυλλώματος, μέσω σκευάσματος τύπου SC, στην εγκεκριμένη συνιστώμενη δόση (100–150 g δ.ο./ha). Για τον προσδιορισμό των υπολειμμάτων αναπτύχθηκε και επικυρώθηκε αναλυτική μέθοδος LC-MS/MS σύμφωνα με τις ευρωπαϊκές κατευθυντήριες οδηγίες, παρουσιάζοντας υψηλή γραμμικότητα ($R^2 > 0,999$), χαμηλά όρια ανίχνευσης (0,00021–0,00094 mg/kg) και ικανοποιητικές ανακτήσεις (84,8–94,8%) με σχετικές τυπικές αποκλίσεις (RSD) < 20%. Οι δοκιμές αποδόμησης έδειξαν κινητική πρώτης τάξης, με το fluopyram να αποδομείται ταχύτερα ($t_{1/2}$ = 2,23–3,02 ημέρες) σε σύγκριση με το trifloxystrobin ($t_{1/2}$= 3,46–4,04 ημέρες). Ο μεταβολίτης CGA 321113 μειώθηκε σε επίπεδα κάτω από το όριο ανίχνευσης εντός 3 ημερών από τον ψεκασμό, ενώ ο fluopyram-benzamide δεν ανιχνεύθηκε. Τα τελικά υπολείμματα βρέθηκαν κάτω από τα ανώτατα επιτρεπτά όρια (MRLs) των 3 mg/kg για το trifloxystrobin και των 2 mg/kg για το fluopyram, με τελευταία επέμβαση πριν την συγκμιδή (PHI) 6,29 και 2,13 ημερών, αντίστοιχα. Τα αποτελέσματα καταδεικνύουν αμελητέο κίνδυνο χρόνιας διατροφικής έκθεσης και τεκμηριώνουν την ασφαλή χρήση των εν λόγω φυτοπροστατευτικών ουσιών στην αμπελοκαλλιέργεια της Σαουδικής Αραβίας.

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Supplementary material

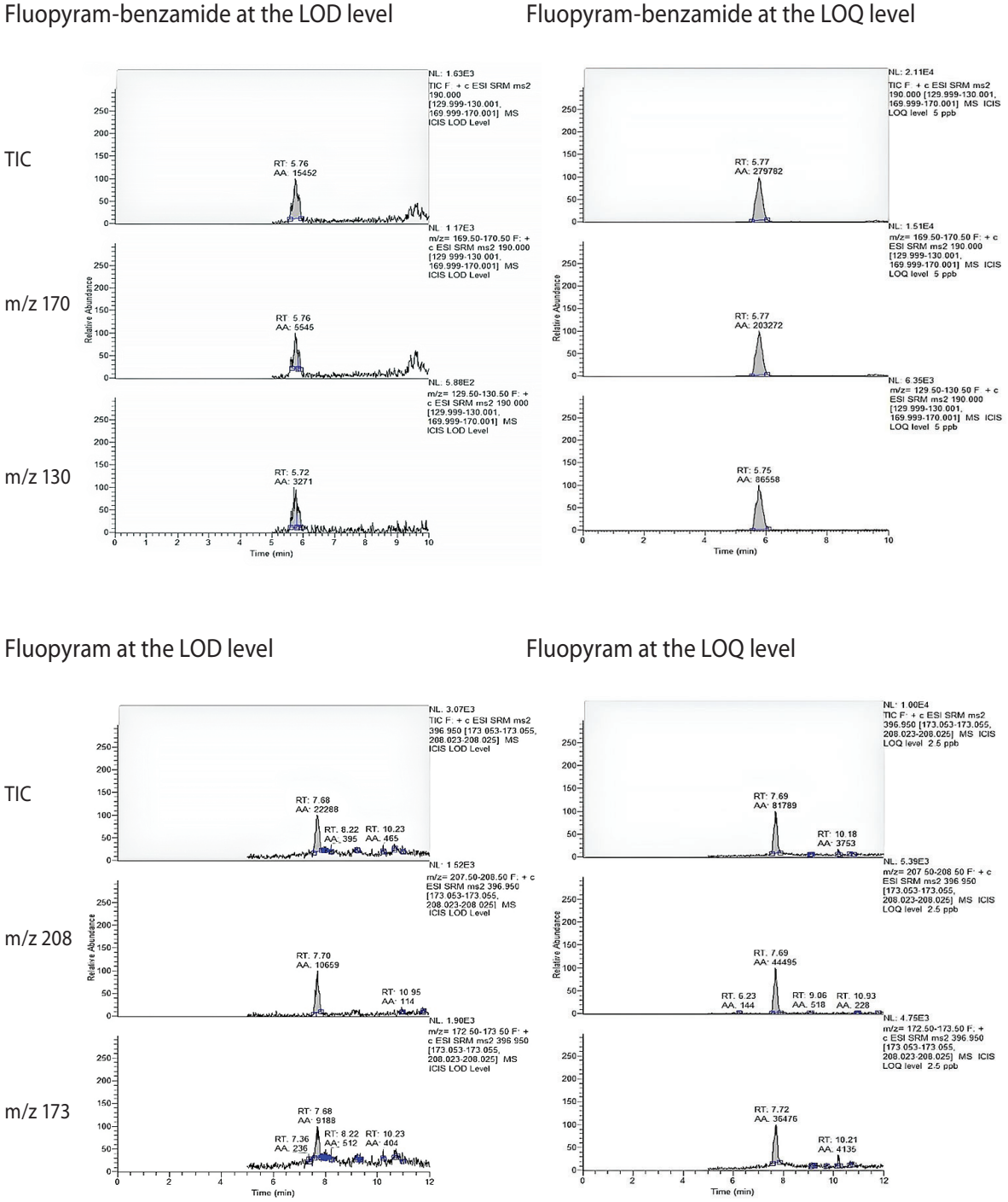

Figure S1. Representative LC-MS/MS chromatograms of Fluopyram and Fluopyram-benzamide at the Limit of Detection (LOD) and Limit of Quantitation (LOQ).

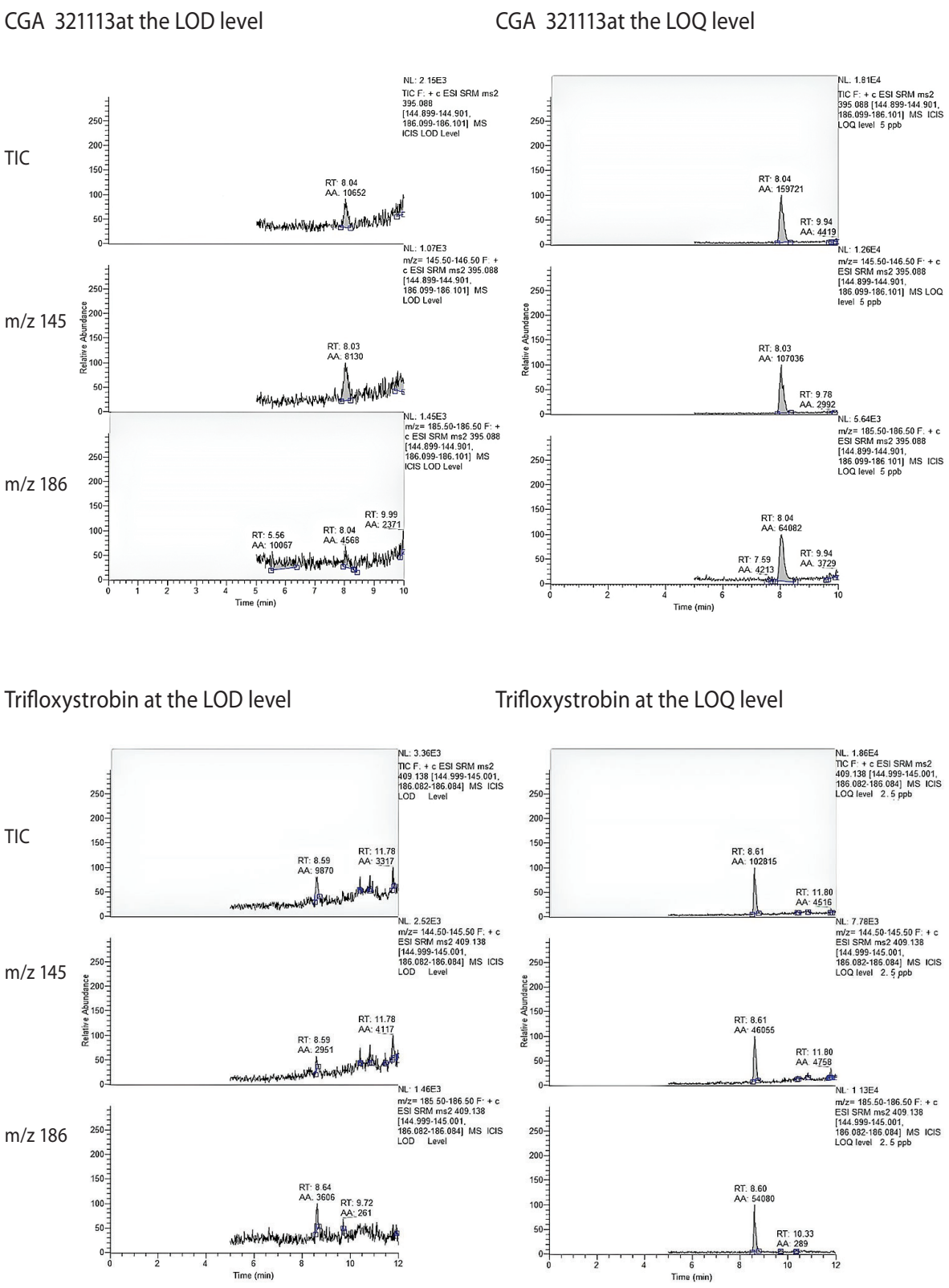

Figure S2. Representative LC-MS/MS chromatograms of CGA 31113 and trifloxystrobin at the Limit of Detection (LOD) and Limit of Quantitation (LOQ).