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ORIGINAL ARTICLE

High performance liquid chromatography Ultraviolet method for the determination of fludioxonil fungicide residues: Application on rice grains cultivated in Pakistan

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KEYWORDS

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Extraction;
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Abstract An analytical method was developed and validated for the determination of fludioxonil in rice samples. Rice samples for the study were collected from different regions of Pakistan. The method was based on safe and cost-effective extraction of fludioxonil from rice grains using acetone and methanol (1:1), efficient clean-up through homogenous mixture of acidic aluminium (12 g) and activated charcoal (1 g) followed by liquid chromatographic determination with UV detection. Quantification was performed on Prospher Star C18 (5 μm, 25 × 0.46 cm) column maintaining the temperature 40 °C and detector wavelength 212 nm using mobile phase 50:50 v/v methanol-water (pH 3.3) employing flow rate 1.0 mL min^{-1} and 20 μL injection volume. The method showed linearity (0.01–16 mg^{-1}) with correlation coefficient greater than 0.998. The proposed method was precisely validated for rice sample of all regions, showing recoveries higher than 98%. Rice samples collected from Badin, Multan, Hyderabad, Lahore, Jahania and Sarghoda was found to have fludioxonil residues 0.046, 0.045, 0.043, 0.040, 0.024 and 0.016 mg Kg^{-1} respectively, all below the maximum residual limit (MRL) level i.e. 0.05 mg Kg^{-1} whereas samples collected from Khanewal

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and Gularchi showed fludioxonil residue above MRL i.e. 0.065 and 0.058 mg Kg^{-1} respectively. However, fludioxonil residues was not detected in rice sample collected from city Makhdumpur.

1. Introduction

Rice is the important staple sustenance and leading food commodity of developing world, which ranks third on the basis of its consumption. However rice crop is subjected to a number of diseases affecting quality and reducing the crop yield worldwide. Pakistan's chief summer crop is rice. It is cultivated over almost 11% area of total agricultural land distinguishing Pakistan as a top producer of rice commodity. Pakistani rice is famous for its taste and aroma and it is a major source of foreign export earnings. Punjab and Sindh are major rice producing provinces in Pakistan, and accounts for about 88% of total rice production (Pakistan Rice Annual Apr-2015).

Rice commodity is majorly affected by fungi e.g. fusarium species spoiled by penicillium and Aspergillus (Park et al., 2005). Fludioxonil, chemically 4-(2, 2-difluoro-1,3-benzo dioxol-4-yl)-1H-pyrrole-3-carbonitril (Fig. 1) is non-systematic, colorless and odorless phenylpyrrole fungicide. It is strongly effective against fungal pathogens like stem-base browning, snow mold and seeding blight and can be used to inhibit black spot, gray mold and storage mold (Agostini et al., 2006; Errampalli et al., 2006; Li et al., 2014; Ren et al., 2016). It is applied on various fruits, vegetables and cereal crop (Edmunds and Holmes, 2009; Gaurilcikiene et al., 2008; Martinez et al., 2005; Munitz et al., 2013; Zhao et al., 2010). Literature survey reveals that unsafe interaction of fludioxonil can have negative health consequences on applicators and farmers. It's excessive exposure can cause diseases like cancer (Go et al., 2017), it is also toxic for aquatic life (Verdisson et al., 2001). CODEX ALIMENTARIUS standard has defined 0.05 mg kg^{-1} maximum residual limit (MRL) of fludioxonil for cereal grains (Codex Pesticides Residues in Food Online Database, 2018).

The determination of pesticide residues in food commodities is of major importance in relation to public health. Regular monitoring of pesticide residue is one of the necessary steps to achieve adequate level of consumer protection. Asia is on top in the world with highest average pesticide usage i.e. 6.5–60 kg ha^{-1} (Carvalho, 2017). Pakistan is the second largest pesticide consumer country in south Asia (Waheed et al., 2017). Various analytical methods have been reported for the quantitative determination of fludioxonil in variety of fruits, vegetables and food commodities. Residue of fludioxonil was quantitatively analyzed in blueberries (Munitz et al., 2013) and in grape and lettuce by GC-NPD (Marín et al., 2003). Otero et al. developed a gas chromatographic method for its determination in white wine (Otero et al., 2002). F.J. Camino-Sanchez (Camino-Sánchez et al., 2011) and D.Stajnbaher determined different pesticides in vegetables and fruits by solid phase extraction followed by gas chromatography (Štajnbaher and Zupančič-Kralj, 2003). Fludioxonil has been determined in fruits (Lee et al., 2012) and in the fermentative process of must (Vaquero-Fernández et al., 2008) by HPLC-DAD. Mercader et al., applied ELISA technique for fludioxonil determination in fruit juices (Mercader et al., 2014). However, in spite of importance of rice as one of the most common staple foods, very few studies have been conducted on determination of fludioxonil residues in rice (Kecojević et al., 2021; Ko et al., 2015). These studies used more advanced and sophisticated detectors as compared to UV detection. However, R&D laboratories of developing countries such as Pakistan often face financial constraints and operate with limited resources. As a consequence, usually low cost analytical solutions are opted for routine analysis without significantly compromising on quality of analysis. UV-detectors provide advantage of being simple, low cost and easily maintainable. HPLC-UV methods can be reasonable choice in such scenarios. To the best of our knowledge, no liquid chromatographic methods-ultraviolet have been reported for fludioxonil residues in rice samples.

Present study reports development and validation of simple and cost effective liquid-chromatographic method with UV-detection for the determination of fludioxonil residues in rice grains. After appropriate pre-treatment method for its extraction and purification, liquid chromatography with UV-detection was used as analytical technique followed by validation employing FDA guidelines (FDA 2015). Rice samples were collected from nine different regions of two major rice-producing provinces of Pakistan namely Punjab (Khanewal, Multan, Lahore, Jahania, Sarghoda and Makhdumpur) and Sindh (Gularchi, Badin and Hyderabad).

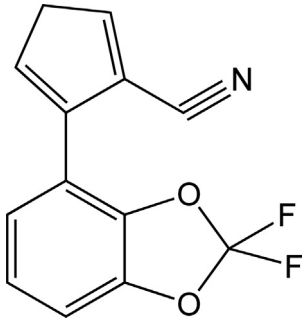

Fig. 1 Representative structural formula of fludioxonil.

2. Experimental

2.1. Chemicals and reagents

Fludioxonil (97% pure) was supplied by Department of Plant Protection. Analytical grade solvents methanol (99%), dichloromethane (98%), acetone (98%), anhydrous sodium sulfate and activated charcoal were purchased from Merck (Darmstadt, Germany). Chemically pure acidic aluminum oxide pH 4.5 ± 0.5, Brockmann activity grade I was purchased from Fluka (Switzerland). Filter membranes with pore size 0.45 μm were purchased from Merck (Darmstadt, Germany).

2.2. Instrumentation

Liquid chromatographic system (Shimadzu Corporation, Japan) equipped with dual LC-20 AT solvent delivery modules connected with DGU-20A3/20A5 on-line degasser, fitted with rheodyne manual injector connected with SPD-20A/20AV UV/VIS detector and Shimadzu CBM-20A communication bus module. Data acquisition was performed on LC solution GPC Chromatographic software (version 1.25). λ_{max} was measured on Shimadzu-1800 double beam UV/vis spectrophotometer. Rotary evaporator (Heidolph G_3 Germany) was used to concentrate the sample.

2.3. Chromatographic conditions

Separation was achieved on Purospher Star, C_{18} (5 µm, 25 × 0. 46 cm) column (Merck, Germany) maintaining the column temperature at 40 °C with optimized parameters including mobile phase methanol: water 50:50 (v/v) with pH adjusted at 3.3 using *o*-phosphoric acid (85%), observing detector response at 212 nm. Fludioxonil was eluted isocratically maintaining flow rate 1.0 mL min^{-1}. Prior to introducing into the system, all the solutions were filtered through 0.45 µm millipore filter followed by degassing on ultrasonic bath (Elma LC-30H model Singen, Germany).

2.4. Calibration curve

Accurately weighted 0.01 g fludioxonil standard was dissolved in 25 mL methanol to obtain 400 mg L^{-1} stock standard solutions. It was prepared once and stored at 4 °C protected from light. Seven calibration standards of fludioxonil within the linearity range 0.01–16 mg L^{-1} were prepared in 25 mL volumetric flask. Calibration standard were prepared fresh daily and filtered through 0.45 µm filter before injecting in to the system.

2.5. Method validation

Validation of method was performed according to Food and Drug Administration (FDA) guidelines in term of accuracy, linearity, precision, specificity, system suitability, limit of detection (LOD) and limit of quantification (LOQ). Efficiency of column performance was evaluated in term of tailing factor, number of theoretical plates and capacity factor. Six different concentration levels were used for calibration study. Linearity and regression characteristics were evaluated by using intercept, slope, correlation coefficient, standard error and standard error estimate. Percent recovery was calculated to determine accuracy of the method. LOD and LOQ were also calculated. For robustness study, minor deliberate changes were introduced in order to check the persistence of method. A robust assay for analysis of fludioxonil was performed and validated. The chromatographic parameters were deliberately varied including mobile phase composition methanol: water 50:50, pH 2.6–3.6, wavelength 212 nm ± 2, flow rate in range of 0.7–1.2 mL min^{-1} and chromatographic response was monitored. In order to ensure the quality of analytical measurement of fludioxonil, study samples were injected into chromatograph before assessing each validation parameter. It was confirmed in terms of instrument performance and analytical method performance. Instrument performance was assessed by using diluent as blank and also by injecting calibration standards as study sample in the range 0.01, 4.0 and 16 µg mL^{-1}), whereas analytical method performance was confirmed by using spiked rice samples (i.e. spiking to the control rice samples).

2.6. Rice sample analysis

2.6.1. Sample collection

Control rice samples (free from fludioxonil) were obtained from crop disease research institute, PARC, Karachi. Nine samples were collected from two provinces of Pakistan, Punjab (Khanewal, Multan, Lahore, Jahania, Sarghoda and Makhdumpur) and Sindh (Gularchi, Badin, Multan, Hyderabad). Fig. 2 presents distribution of rice sampling regions and strategy to obtain representative rice sample for the study. Purposeful sampling was carried out to get the representative samples. Rice field was equally divided into nine parts by sketching imaginary lines. Approximating 50 g of rice samples were randomly collected from each part, homogenously mixed and considered as one composite sample. All the nine composite samples were collected following the same strategy.

2.6.2. Fortification

Accurately weighed 100 g of all rice samples were separately soaked in 50 mL acetone followed by addition of 100 µL of 10 ppm fludioxonil working standard solution and homogenized. The contents were allowed to penetrate in rice grains by keeping in the dark for 24 h. After the grains have been well dried, all the samples were separately pulverized with a mechanical hand grinder to increase the surface area and to ensure better extraction of fludioxonil from rice grain sample.

2.6.3. Extraction

The extraction was accomplished following the procedure described by Uddin et.al (Uddin et al., 2011). Along with fortified samples, a blank was also concurrently processed for extractions. Into a centrifuging tube, accurately weighed 4 g of already fortified and pulverized rice sample was transferred followed by addition of 75 mL mixture of acetone and methanol (1:1) in two times. The contents were vigorously stirred and centrifuged at 2500 rpm for three minutes; supernatant was collected into the conical flask passing through Whatman filter paper supported by filter funnel. Into a separating funnel already containing 200 mL of 2.5% sodium sulfate solution, the filtered rice extract was transferred followed by addition of 25 mL of dichloromethane by rinsing the respective conical flask. The contents were vigorously stirred and then allowed for layer separation. The lower layer containing fludioxonil extract was collected in a conical flask; the procedure was repeated with addition of 25 mL portion of dichloromethane twice in order to get maximum extraction of fludioxonil. Furthermore, the contents were passed through the glass column containing about 25 g of anhydrous sodium sulfate (Na_2SO_4) supported by glass wool. Finally, a 10 mL portion of dichloromethane was further passed to sweep the contents from the column completely. Moisture-free extract was then concentrated to approximately 2 mL on rotary evaporator. Analysis was performed in triplicate for each sample.

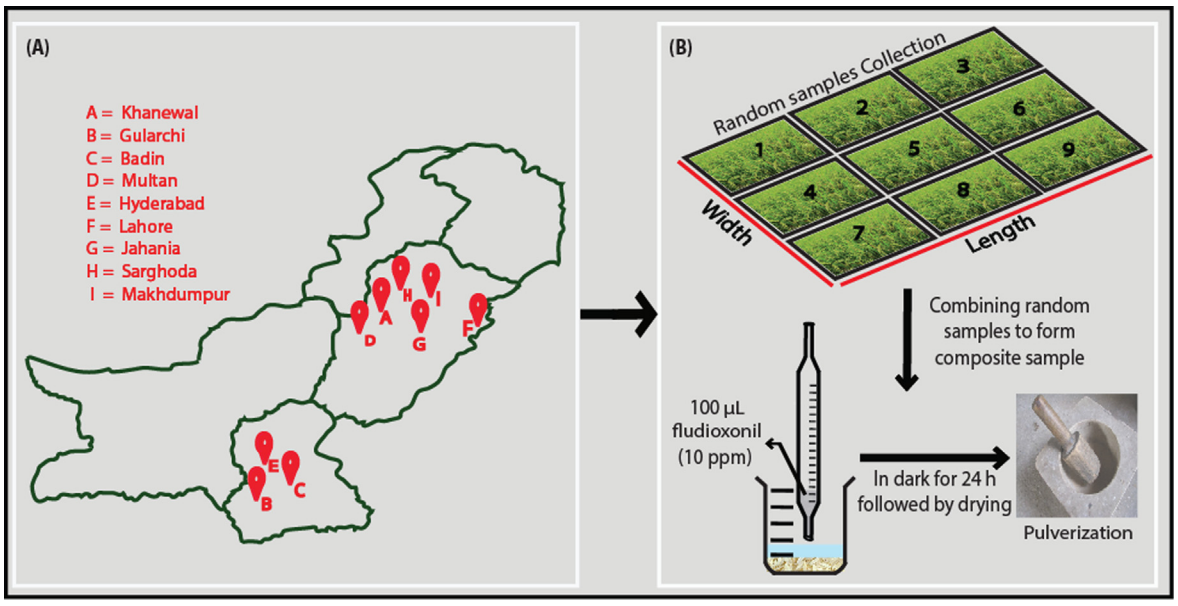

Fig. 2 Rice sampling regions (A) and strategy to obtain representative rice sample (B).

2.6.4. Clean-up

Activated charcoal column was prepared for clean-up of extract. Prior to column preparation, acidic aluminum, charcoal and sodium sulfate were activated at 110 °C for 6 h. The column was packed by placing evenly a wad of cotton wool at the bottom. On the top of the plug, a layer of anhydrous Na_2SO_4 was poured, subsequently, addition of homogenous mixture of acidic aluminum (12 g) and activated charcoal (1 g), finally another layer of anhydrous Na_2SO_4 was transferred at the top of homogenous mixture. The column was then loaded with concentrated extract followed by addition of 120 mL dichloromethane in three small portion.

2.6.5. Preparation of sample for analysis

The eluted extract was then placed on rotary evaporator for complete evaporation of solvent. The dried flask contains the expected residues of fludioxonil which was then dissolved in 2 mL methanol for its quantitative determination. The samples in 2 mL methanol were mostly observed to be opaque. The clear and transparent samples obtained by filtration through 0.45 µm millipore filter paper was injected to the system for chromatographic analysis. Steps for extraction and clean-up were repeated for all the other samples before preparation for chromatographic analysis.

3. Results and discussion

3.1. Method optimization

In order to establish the optimum reliable analytical condition and to obtain the maximum sensitivity for identification and quantification of fludioxonil, numerous parameters were varied to set the best chromatographic condition. Maximum wavelength of fludioxonil was measured on Shimadzu-1800 double beam UV–vis spectrophotometer i.e. 212 nm (Fig. 3). Instrumental parameters including flow rate, composition and ratio of mobile phase and its pH were separately studied

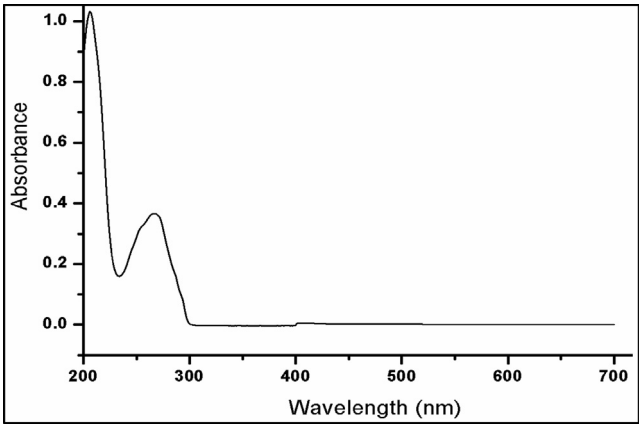

Fig. 3 UV–visible spectra of fludioxonil standard in methanol.

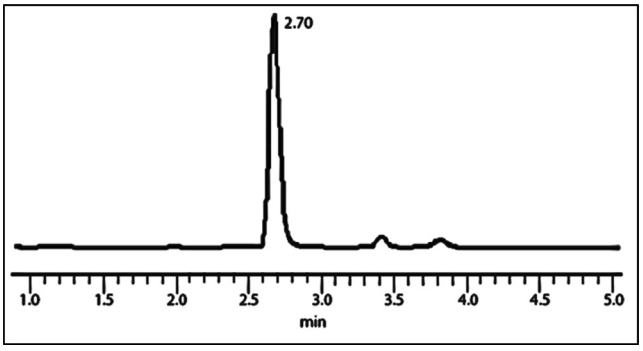

Fig. 4 Representative chromatogram of fludioxonil 400 mg Kg^{-1} in reference standard.

using 10 µg mL^{-1} fludioxonil standard solution prepared in methanol. In order improve the selectivity in reversed-phase high performance liquid chromatography (HPLC), different

Table 1 System suitability parameters for the analysis of fludioxonil.

Parameters	Retention time (min)	Theoretical plates	Tailing factor	Capacity factor	Separation factor
Fludioxonil	2.7	5106	1.616	2.177	1.22

Table 2 Regression characteristics for the analysis of fludioxonil.

Linearity (mg L^{-1})	Slope	Intercept	Correlationcoefficient	LOD (mg L^{-1})	LOQ (mg L^{-1})
0.01–16	35,549	1079.8	0.998	0.0042	0.0126

Table 3 Recovery, repeatability and reproducibility of fludioxonil in fortified rice grain.

Spiked (mg Kg-1)	Found (mg Kg-1)	% Recovery	%RSD*	%RSD**
16.000	15.35	95.96	3.78	4.14
8.000	7.58	94.75	5.97	7.37
4.000	3.68	91.94	7.80	6.51
1.000	0.95	95.17	4.40	5.25
0.100	0.10	98.11	2.13	2.44
0.050	0.05	98.48	1.38	2.84
0.010	0.01	98.44	1.38	1.41

* Intra-day, **Inter-day.

Table 4 Robustness of the proposed method for the detection of fludioxonil.

Parameters	Variation	Tailing factor	Capacity factor	Theoretical plates
Flow rate (mL min^{-1})	0.9	1.184	2.683	4973
	1.0	1.227	2.075	5227
	1.1	1.163	2.483	5307
Wavelength (nm)	210	1.104	2.378	5107
	212	1.027	2.075	5429
	214	0.980	2.889	5094
Mobile phase	48:52	1.173	2.219	5004
	50:50	1.227	2.075	5217
	52:48	1.160	2.096	5332

combination of organic solvents were tried such as acetonitrile: water and methanol:water. It was observed that increasing the percentage of organic modifier caused reduction in retention time i.e. acetonitrile showed less retention time of analyte as compare to methanol. However, because of being carcinogenic nature of acetonitrile, methanol was preferred. Methanol:water in the ratio 80:20, 70:30, 60:40 and 50:50 with pH in the range 2.0–4.0 were tried to avoid retention and selectivity changes. The flow rate of mobile phase was varied between 0.7 mL min^{-1} and 1.2 mL min^{-1}. The best results in terms of short retention time, high resolution and good peak symmetry were obtained with mobile phase ratio 50:50 v/v methanol: water adjusting eluent pH 3.3 at detector wavelength 212 nm and flow rate 1.0 mL min^{-1}. The representative chromatogram of standard fludioxonil is shown in Fig. 4.

3.2. Method validation

In order to establish the appropriateness of method for its future application, developed method was validated according to Food and Drug Administration (FDA) guidelines. Validated parameters include system suitability, linearity, preci-

Table 5 Comparison of the presented method with other methods.

S. No	Food Commodity	Technique	Mobile phase	Linearity	Regression Equation	LOD	LOQ	Ref
1	Cabbage	LC-MS/MS	0.1% HCOOH and 0.4 mM ammonium formate in water: MeOH	10–800 μg kg^{-1}		0.005 mg kg^{-1}	0.01 mg kg^{-1}	(Kecojević et al. 2021)
2	Rice	LC-MS/MS	(Gradient elution)	5–400 μg kg^{-1}		0.005 mg kg^{-1}	0.01 mg kg^{-1}	(Kecojević et al. 2021)
3	Marijuana	LC-MS/MS	0.1% HCOOH in water: ACN (Gradient elution)		y = 53.3x + 59.8	0.08 μg kg^{-1}	0.28 μg kg^{-1}	(Daniel et al., 2019)
4	Cherry	LC-MS/MS	ACN:0.2% acetic acid-5 mM/L ammonium acetate (90:10)	0.005–5 mg kg^{-1}	y = 22,531x + 5196.1	0.005 mg kg^{-1}	0.01 mg kg^{-1}	(Yao et al. 2021)
5	Must	HPLC-DAD	Water:ACN (Gradient elution)	35–20000 μg L^{-1}	y = (83978.3 ± 2445.2) x + (2113.5 ± 24940.0)	30.9 μg L^{-1}	35 μg L^{-1}	(Vaquero-Fernández et al., 2008)
6	Wine	HPLC-DAD		177.7–20000 μg L^{-1}	y = (81817.5 ± 4175.9) x + (−13705.3 ± 42599.4)	173.9 μg L^{-1}	177.7 μg L^{-1}	(Vaquero-Fernández et al., 2008)
7	Lettuce	HPLC-DAD	0.1 %TFA in water: MeOH (Gradient elution)	1.2–9.6 mg kg^{-1}		0.37 mg kg^{-1}	1.24 mg kg^{-1}	(Melo et al. 2012)
8	Strawberries	HPLC-DAD	Water:ACN (Gradient elution)	0.02–5.0 mg kg^{-1}		0.01 mg kg^{-1}	0.020 mg kg^{-1}	(Machado and Dol, 2021)
9	Proposed method	HPLC-UV	MeOH:water (50:50)	0.01–16 μg mL^{-1}	y = 35549x + 1079.8	0.0042 μg mL^{-1}	0.0126 μg mL^{-1}	–

Table 6 Detection of fludioxonil in rice grain samples collected from nine different regions of Pakistan.

Location of rice grain sample	Detection level(mg Kg^{-1})
Khanewal	0.065
Gularchi	0.058
Badin	0.046
Multan	0.045
Hyderabad	0.043
Lahore	0.040
Jahania	0.024
Sarghoda	0.016
Makhdumpur	ND*

*Not detected.

sion, accuracy, limit of detection, limit of quantification and robustness (FDA 2015).

3.2.1. System suitability test

System suitability is an important step of method validation which represents the efficiency of column. It was evaluated by injecting the standard fludioxonil solution into the system six times on each day of analysis. The data obtained for system suitability of the proposed method represented in Table 1 shows capacity factor (k′) 2.177, theoretical plates (N) 5106, tailing factor (T) 1.16 and separation factor (α) 1.22. Number of theoretical plates above 2000 and tailing factor below 2 show good system suitability of the method.

3.2.2. Linearity

Calibration curve was plotted by triplicate analysis of seven different calibration standards of fludioxonil in methanol:water 50:50 (v/v) diluent in the range 0.01 to 16 mg L^{-1}. Regression data showed correlation coefficient 0.998, which lie in the acceptable range, established by FDA guidelines. Linearity and regression data including slope and intercept are represented in Table 2.

3.2.3. Precision

Precision of the method was confirmed by introducing seven calibration standard of fludioxonil within the linearity range 0.01 to 16 mg L^{-1} three times within the same day (intra-day precision) and on two consecutive days (inter-day precision) of method validation. The % relative standard deviation (RSD) values within-day and in between two consecutive days was found to be in the range 1.38–7.80% and 1.41–7.37% respectively fulfilling the acceptance criteria of RSD (Table 3). Therefore, the proposed method encounters the performance requirements and is appropriate for the daily screening of fludioxonil residue in rice sample.

3.2.4. Accuracy

For evaluation of accuracy, control rice samples were spiked with fludioxonil over the linearity range 0.01–16 mg L^{-1}. The recoveries were observed between 91.94 and 98.48% indicating

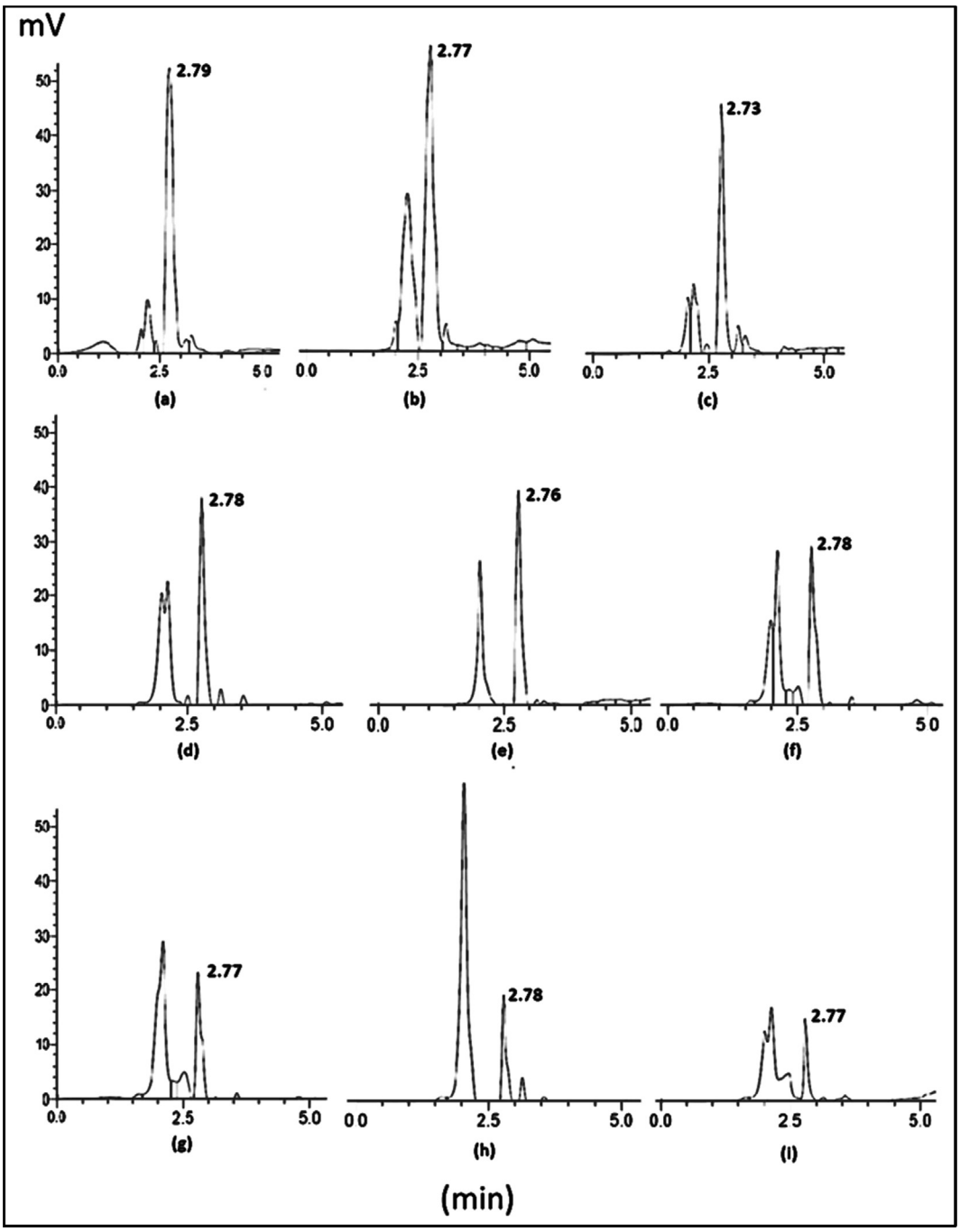

Fig. 5 Chromatograms representing the detection level of fludioxonil in rice samples collected from (a) Khanewal, (b) Gularchi, (c) Badin, (d) Multan, (e) Hyderabad, (f) Lahore, (g) Jahania, (h) Sarghoda, (i) Makhdumpur.

that the method may meet the routine monitoring requirements of fludioxonil (Table 3).

3.2.5. *Limit of detection and quantification*

Detection and quantitation limits of fludioxonil were evaluated based on standard solutions. These were determined in relation to the chromatographic signal higher than three times and ten times to the baseline noise respectively. LOD and LOQ of fludioxonil were found to be 0.0042 and 0.0126 mg L^{-1} demonstrating the sensitivity of proposed method (Table 2).

3.2.6. *Robustness*

The robustness of the proposed method was assessed by evaluating the capability of method to withstand intended variation in the chromatographic parameters of developed analytical method. Parameters including mobile phase composition and pH, wavelength and flow rate were intentionally changed and compatibility of method was assessed. Theoretical plates and tailing factor represented in Table 4 confirms suitability of method for routine analysis.

3.3. Method comparison

Literature survey reveals various liquid chromatographic methods for the determination of fludioxonil residues in different food commodities. Table 5 represents the comparison of reported method with the proposed liquid chromatographic method for fludioxonil determination. Most of these reported methods used acetonitrile based mobile phases. Acetonitrile is considered as problematic solvent due to its negative environmental impact and increasing cost (Deineka et al., 2021; Kannaiah and Sugumaran, 2022). Proposed HPLC-UV method can be considered advantageous as compared to other proposed methods on the basis of lower LOD and LOQ, use of environmentally benign solvent system i.e. MeOH:water (50:50), simple isocratic elution instead of gradient one, wider linearity range and better sensitivity (i.e. slope of the regression equation). Moreover, these merits were achieved using low cost single wavelength UV detector instead of high cost and more sophisticated detectors being used in other reported studies.

3.4. Recovery of fludioxonil

The applicability of the proposed method was assessed by analysing detection level of fludioxonil in rice grains collected from the rice producing fields of Punjab and Sindh. Sample pre-treatment was carried out to minimize co-extractives and interferences during fludioxonil analysis in rice sample. For this purpose, our previously reported optimized method for the extraction of pesticide residue followed by clean-up from rice commodity with lesser matrix influence was applied (Uddin et al., 2011) on all nine rice samples collected from Punjab and Sindh. Analysis was performed by comparing the un-spiked rice samples using spiked one at 1.0 mg Kg^{-1} fortification level. The spiked samples were prepared by transferring known quantity of fludioxonil to 4 g finely ground rice sample separately followed by extraction and purification. The un-spiked rice samples were simultaneously processed along with spiked ones for recovery check. The extraction recovery values were obtained as average of triplicate measurement and compared with maximum residual limit (MRL) i.e. 0.05 mg Kg^{-1}. The fludioxonil residue was detected to be below MRL level i.e. 0.046, 0.043, 0.045 and 0.040 mg Kg^{-1} in Badin, Hyderabad, Multan and Lahore. It was found to be very low in Sargodha and Jahania samples i.e. 0.016 and 0.024 mg Kg^{-1} respectively. The data represents that skilled or trained farmer had monitored the field and rice samples cultivated in the fields of Badin, Hyderabad, Multan and Lahore are safe for consumption. The fludioxonil concentration was high in Khanewal and Gularchi samples i.e. 0.065 and 0.058 mg Kg^{-1} respectively. It represents that untrained farmers had excessively sprayed the pesticide on rice field without considering its potential dangerous effects. Fludioxonil was not detected in rice sample collected from city Makhdumpur. It may be due to human error or it is possible that its quantity is below the detection limit. One possible reason may be that fludioxonil have not been sprayed in the field. The results are represented in Table 6 and comparison of chromatographic response of un-spiked and spiked samples has been depicted in Fig. 5.

4. Conclusion

An inexpensive, simple and efficient LC-UV method for quantitative determination of fludioxonil residues in rice commodity has been reported for the laboratories that don't have access to modern extraction techniques. Developed method has been successfully applied for analysis of rice samples that were collected from Khanewal, Gularchi, Badin, Multan, Hyderabad, Lahore, Jahania, Sarghoda and Makhdumpur region of Punjab and Sindh. Analysis involved extraction of fludioxonil from rice with reduced amount of solvent and less matrix effect. Chromatographic analysis presented the sensitivity and specificity for the fludioxonil determination in rice samples; the method was then validated according to the FDA guidelines 2015, proving suitability of method. Results demonstrated satisfactory recovery and reproducibility confirming excellent accuracy and precision of method. Results demonstrated detection of fludioxonil in rice samples collected from Badin, Multan, Hyderabad, Lahore, Jahania and Sarghoda were found to be below its MRL level whereas its concentration was high in Khanewal and Gularchi samples. However, it was not detected in rice sample collected from city Makhdumpur. It is concluded that the proposed method can be applied in the laboratories those are not equipped with recent extraction techniques and modern instruments.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authorship contribution statement

Saeeda Nadir Ali was the incharge of overall direction and planning. Saeeda Nadir Ali and Sajid Iqbal conceived and planned the experiments. Zahoor Ul Hussain Awan and Farah Kishwar provided rice samples after their pre- treatment. Aneeqa Khalid and Sadia Ansari performed the experiments. Amtul Qayoom interpreted the experimental data. Aneeqa Khalid wrote the manuscript in consultation with Saeeda Nadir Ali and Amtul Qayoom. Philippe Daniel verified the analytical method and discussed the analytical results, provided feedback and approved the version to be published.

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