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Extraction Method for Determining Florpyrauxifen-Benzyl Herbicide in Rice

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Abstract

Florpyrauxifen-benzyl is an active ingredient used as herbicide to control weeds in rice cultivation. So far, there are few studies related to the extraction and determining methodologies for this compound. Therefore, this work aimed to optimize and validate solid–liquid extraction with low temperature purification (SLE-LTP) using high-performance liquid chromatography coupled to a diode array detection (HPLC–DAD) to determine the florpyrauxifen-benzyl in rice samples. The results showed that the optimal chromatographic conditions were obtained using the Kinetex column, temperature of 25 °C, mobile phase composition of methanol:water (78:22 v/v), flow rate of 0.3 mL min^{-1}, and 260 nm as the wavelength. The optimum extraction conditions were obtained using acetonitrile and water (2:1) as extraction phase, followed by clean-up of extracts with alumina. The recovery percentage achieved was close to 100%, with relative standard deviation (RSD) less than 9.0%. The result showed a significant matrix effect (184.3%), but the methodology was precise, accurate, linear, and selective, with a quantification limit of 5.0 µg kg^{-1}.

Keywords Florpyrauxifen-benzyl · SLE-LTP · Arylpicolinate herbicide · Rice · Synthetic auxin

Introduction

The active ingredient florpyrauxifen-benzyl was recently introduced into the world market to combat weeds that affect rice, sugarcane, corn, and soybean crops (USA 2017a; APVMA 2018; MDAR and MassDEP 2019; ANVISA 2022). This compound is a synthetic auxin from the arylpicolinate chemical group and can be applied to the shoots of weeds, being able to move through the phloem and accumulate in regions that interrupt the growth processes in susceptible plants (USA 2017a; APVMA 2018; MDAR and MassDEP 2019).

These characteristics led to florpyrauxifen-benzyl being authorized for use and commercialization in Brazil in 2019 (ANVISA 2022). However, Brazilian legislation has classified it as a dangerous product for the environment at level III, but has not yet completed the classification in terms of toxicity to humans (ANVISA 2022). The maximum residue limit (MRL) determined for this herbicide by Brazilian legislation (ANVISA 2022) was 10 µg kg^{-1}.

Despite this scenario, studies on extraction methods for monitoring this active principle in environmental and food matrices are still rare, probably due to its recent insertion in the herbicide world market (Vieira et al. 2022; Lopes et al. 2023; Zhou et al. 2022; Arena et al. 2018). So far, only the QuEChERS (quick, easy, cheap, effective, rugged and safe) method has been successfully optimized and validated for monitoring florpyrauxifen-benzyl in rice (Zhou et al. 2022). Therefore, there is a demand for developing new extraction methods which are easy to perform, with reduced use of samples and reagents, and that are sensitive and efficient. In turn, solid–liquid extraction with low temperature purification (SLE-LTP) has been successfully applied to several herbicides, including monitoring florpyrauxifen-benzyl in soil and water (Vieira et al. 2022;

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Lopes et al. 2023). This methodology has stood out for removing interferents which are trapped in the purification at low temperature, functioning as a previous clean-up of the extracts and streamlining the process, reducing the use of organic solvents and the final cost (Ramalho et al. 2020; Pereira et al. 2020).

Considering the representativeness of rice as one of the most consumed cereals in the world (EMBRAPA 2021), and the scarcity of studies involving the development of extraction methods for this herbicide, this study aimed to optimize and validate the SLE-LTP methodology to determine florpyrauxifen-benzyl herbicide in rice samples using high-performance liquid chromatography coupled with diode array detection (HPLC–DAD).

Materials and Methods

Rice Samples

The samples used in this study were obtained using commercial rice free of florpyrauxifen-benzyl. The samples were ground and stored in covered glass vials and kept at room temperature.

Reagents and Solutions

HPLC grade methanol was obtained from F. Maia (Belo Horizonte, Brazil). P.A. acetonitrile solvent was obtained from Êxodo Científica (Sumaré, Brazil), and ethyl acetate was purchased from Dinâmica (Indaiatuba, Brazil). All solvents were filtered through polytetrafluoroethylene (PTFE) membranes with 0.2 µm pores purchased from Filtrilo (Colombo, Brazil). Formic acid was purchased from Sigma-Aldrich (St. Louis, USA), and hydrochloric acid was purchased from Anidrol (Diadema, Brazil). The florpyrauxifen-benzyl standard was purchased from LGC Dr. Ehrenstorfer (Augsburg, Germany).

The standard stock solution was prepared in acetonitrile at a concentration of 20 mg L^{-1}. The working solution was prepared at a concentration of 5 mg L^{-1} by diluting the stock solution in acetonitrile. All solutions were kept at -20 °C. Next, the following adsorbents were evaluated in the clean-up step of the extracts: florisil (60–100 mesh) purchased from Mallinckrodt Baker (Phillipsburg, USA); silica gel 60 (230–400 mesh) from Macherey–Nagel (Düren, Germany); primary and secondary amine (PSA) and octadecyl functionalized silica gel (C18) from Sigma-Aldrich (St. Louis, USA); alumina from Agilent Technologies (St. Clair, USA) and activated carbon that were purchased from Vetec (Rio de Janeiro, Brazil).

Equipment

The equipment used in this study includes a vortex from Scilogex (Rocky Hill, USA), a vacuum pump from Prismatec (Itu, Brazil), a centrifuge from Kindly (São Paulo, Brazil), an analytical scale from Shimadzu (Barueri, Brazil), and a Cary 50 spectrophotometer from Agilent Technologies (St. Clair, USA).

Chromatographic Analyses

Chromatographic analyses were performed on a high-performance liquid chromatograph coupled to a diode array detector (HPLC–DAD, model 1290, Agilent Technologies, St. Clair, USA). The injection volume used was 10 µL, and the chromatographic columns evaluated were Poroshell (St. Clair, USA) and Kinetex (Torrance, USA). The evaluated chromatographic conditions can be seen in Table 1.

Optimization of the Extraction Method

This extraction method is based on adding 4.0 g of rice sample to a 22 mL glass vial and then adding 72 µL of working solution containing florpyrauxifen-benzyl at a concentration of 5 mg L^{-1}. The mixture was kept at rest for 1 h to allow complete integration of the analyte with the matrix. Next, 4 mL of water was added, and the system was vortexed for 30 s, then 8 mL of extractor phase was added, and the system was vortexed again for another 30 s. The system was subsequently kept at -20 °C for 1 h for complete freezing of the aqueous phase and the matrix. Next, 4 mL of the extract and 200 mg of the adsorbent were added in a glass tube to clean-up the extract. This system was vortexed for 60 s and centrifuged for 5 min at 4000 rpm. After this step, 3 mL of the extract were completely evaporated, resuspended in 400 µL of methanol, and stored at -20 °C until analysis by HPLC–DAD. The SLE-LTP was optimized by evaluating the compositions of the extracting phases shown in Table 2.

Clean-Up of the Extracts

An additional clean-up step was inserted in this study due to the presence of several interferents in the matrix. Therefore, 50 mg and 100 mg of adsorbent per mL of the rice matrix extract obtained through optimized SLE-LTP were used. The following adsorbents were evaluated: silica gel, silica gel functionalized with octadecyl (C18), florisil, activated charcoal (Act. Charc), alumina, and primary and secondary amine (PSA).

Table 1 Evaluated chromatographic conditions

Parameters	
Wavelength (λ)/nm	243
	260
Chromatographic column	**Kinetex (C18)**
	(100 A, 150 mm × 4.60 mm, 5 µm, Phenomenex)
	Poroshell 120 EC-C18
	(50 mm × 4.60 mm, 2.7 µm, Agilent)
Mobile phase composition in isocratic mode	Acetonitrile:water[a]
	Methanol:water[a]
	(Acetonitrile:water)[a] acidified with 0.1% (v/v) formic acid
	(Methanol:water)[a] acidified with 0.1% (v/v) formic acid
Flow rate/(mL min − 1)	0.2
	0.3
	0.5
	0.8
Column temperature/°C	**25**
	30

[a]100:0; 90:10; 85:15; 80:20; **78:22**; and 75:25 (% v/v); in bold, the optimal chromatographic conditions

Table 2 Composition of the extracting phases evaluated

Experiment	Extracting phases	Proportion/mL
A	**ACN[a]:H₂O**	**8:4**
B	ACN + FA[b]:H₂O	8:4
C	ACN + HCl[c]:H₂O	8:4
D	ACN + EAC[d]:H₂O	(6.5 + 1.5):4

[a]Acetonitrile; [b]0.1% (v/v) formic acid solution; [c]0.1% hydrochloric acid solution (v/v); [d]ethyl acetate; in bold, the optimal extraction conditions

Method Validation

Validation was performed by evaluating the selectivity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, linear range, and matrix effect results according to the SANTE (2021) protocol. Statistical tests were performed considering a significance level of 0.05.

Selectivity

The method selectivity was evaluated by comparing the chromatograms of the rice matrix extract fortified with florpyrauxifen-benzyl at a concentration of 90.0 µg kg⁻¹ with the chromatogram of the rice matrix extract free from florpyrauxifen-benzyl (blank extract), in six independent replicates (SANTE 2021). The absence of peaks in the blank extract chromatogram at the same retention time as florpyrauxifen-benzyl indicates that the method was selective.

Limit of Detection (LOD) and Quantification (LOQ)

The LOD and LOQ values were determined through rice matrix fortification experiments with the smallest amount of florpyrauxifen-benzyl that can be detected and quantified, respectively, using the optimized conditions in the SLE-LTP. The LOD and LOQ were the florpyrauxifen-benzyl concentrations which generated chromatographic peaks with an area three and ten times larger, respectively, than the baseline noise chromatographic area in the same florpyrauxifen-benzyl retention time of the matrix extract chromatogram from florpyrauxifen-benzyl free rice (blank extract) (SANTE 2021). The LOQ was confirmed as the lowest fortification concentration evaluated by six independent repetitions which meet the established criteria, meaning with a relative standard deviation of less than 20% and recovery rates between 70 and 120% (SANTE 2021).

Precision and Accuracy

Precision and accuracy were evaluated through recovery and fortification experiments of florpyrauxifen-benzyl in the rice matrix. Precision was determined under repeatability conditions using a concentration of 90 µg kg⁻¹, with seven independent replicates, in which relative standard deviations (RSD) less than 20% were considered acceptable. Accuracy was evaluated using concentrations of 5.0, 90.0, and 150.0 µg kg⁻¹ with three independent replicates for each level, and recovery rates with values between 70 and 120% were considered acceptable (SANTE 2021). These validation parameters were determined on the same day, as recommended by the Sante protocol.

Linear Range

The linear range was evaluated using analytical calibration curves of the rice matrix extract fortified with florpyrauxifen-benzyl at six concentration levels: 5.0, 30.0, 60.0, 90.0, 120.0, and 150.0 µg kg^{-1}, with three independent repetitions for each level. Linear regression parameters were estimated using the ordinary least squares (OLS) method with a maximum exclusion of 22.2% of the data (Jacknife test). The linear regression residuals were evaluated by the Ryan-Joiner parameter normality test, homoscedasticity by the Brown-Forsythe test and independence by the Durbin-Watson test. Analysis of variance (ANOVA) was applied to the analytical curve data to verify the significance of the regression parameters and linear deviation (Souza and Junqueira 2005; Bazilio et al. 2012).

Matrix Effect

The matrix effect was evaluated using two analytical calibration curves, one in solvent (methanol) and the other in rice matrix extract, both containing florpyrauxifen-benzyl in concentrations of 5.0, 30.0, 60.0, 90.0, 120.0, and 150.0 µg kg^{-1} and in independent triplicates for each concentration. The two analytical curves were evaluated according to the linear procedure, and the matrix effect was determined by Eq. (1).

$$\text{Matrix effect}(\%) = 100 * \left(\frac{I_{\text{matrix}}}{I_{\text{solvent}}} - 1\right) \tag{1}$$

in which I_{matrix} is the angular coefficient of the analytical curve in the rice matrix extract fortified with the herbicide and I_{solvent} is the angular coefficient of the analytical curve for the compound in methanol solvent.

The result found was interpreted as follows: low matrix effect (values found between -20 and $+20\%$); medium matrix effect (values found between -50 and -20% or between $+20$ and $+50\%$); and high matrix effect (values found below -50 and above $+50\%$) (Tomasini et al. 2012; Economou et al. 2009).

Results and Discussion

Optimization of Chromatographic Conditions

Florpyrauxifen-benzyl is the herbicide indicated for irrigated rice cultivation. Therefore, the use of this pesticide in agriculture can promote the contamination of water resources, soil, and rice (USA 2017a; APVMA 2018; MDAR and MassDEP 2019). Therefore, monitoring of this compound must occur in these three matrices, as carried out by a group of Chinese researchers who used the QuEChERS method followed by UPLC-QTOF-MS/MS analysis (Zhou et al. 2022). Similarly, our research group has also developed extraction methodologies to determine this compound in water (Vieira et al. 2022) and soil (Lopes et al. 2023), and in this study, the rice matrix was the target of our research. In these three studies, chromatographic conditions were evaluated, and the optimal conditions in each study can be seen in Table 3.

Therefore, initially, these chromatographic conditions were applied to determine this herbicide in rice matrix extracts. However, the obtained chromatograms can be observed in Figure S1.

It is possible to observe that, the chromatograms obtained presented many interfering peaks from the rice matrix, hampering to determine the florpyrauxifen-benzyl peak.
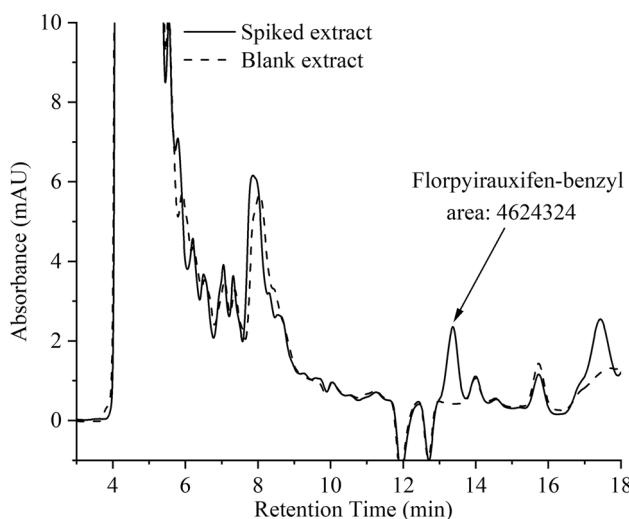
Table 3 Comparative study of the chromatographic conditions for determining florpyrauxifen-benzyl

Parameters	Vieira et al. 2022	Lopes et al. 2023	Zhou et al. 2022			This study
	Water	Soil	Water	Soil	Rice	Rice
Technique	HPLC–DAD[a]	HPLC–DAD	UPLC-QTOF-MS/MS[b]			HPLC–DAD
Chromatographic column	Poroshell C18	Poroshell C18	Waters Cortecstm UPLC C18			Kinetex C18
Column temperature (°C)	30	30	40			25
Elution	Isocratic	Isocratic	Isocratic			Isocratic
Mobile phase composition (% v/v)	ACN[c]:water (100:0)	(ACN:water) with 0.1% FA[d] (85:15)	ACN:water with 0.1% FA (60:40)			Methanol:water (78:22)
Flow (mL min^{-1})	0.4	0.3	0.3			0.3
Injection volume (µL)	20	10	4			10
Wavelength (nm)	243	243	-			260
Retention time (min)	1.5	2.63	5.60			13.02

[a]*HPLC–DAD*, high-performance liquid chromatography with diode array detection; [b]*UPLC-QTOF-MS/MS*, ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry; [c]*ACN*, acetonitrile; [d]*FA*, formic acid

Therefore, a new optimization of the chromatographic conditions was necessary aiming to determine florpyrauxifen-benzyl in rice extracts. The evaluated parameters can be seen in Table 1, and the obtained chromatograms can be seen in Fig. 1.

The chromatogram obtained showed the florpyrauxifen-benzyl peak at a longer retention time (13.02 min) than

Fig. 1 Chromatogram of the matrix extract fortified with florpyrauxifen-benzyl for a concentration of 90 µg kg^{-1} (—) and chromatogram of the florpyrauxifen-benzyl free matrix extract (blank extract) (- -). Chromatographic conditions: Kinetex column, injection volume = 10 µL, flow rate = 0.3 mL min^{-1}, T = 25 ℃, λ = 260 nm, and methanol:water (78:22) mobile phase

previous works (1.5 to 5.60 min), however, free of rice matrix interferers. Therefore, this chromatography condition was used in this study. It is important to highlight that interference-free peak was only possible using 260 nm as the wavelength, despite 243 nm being the wavelength of maximum absorption (Figure S2).
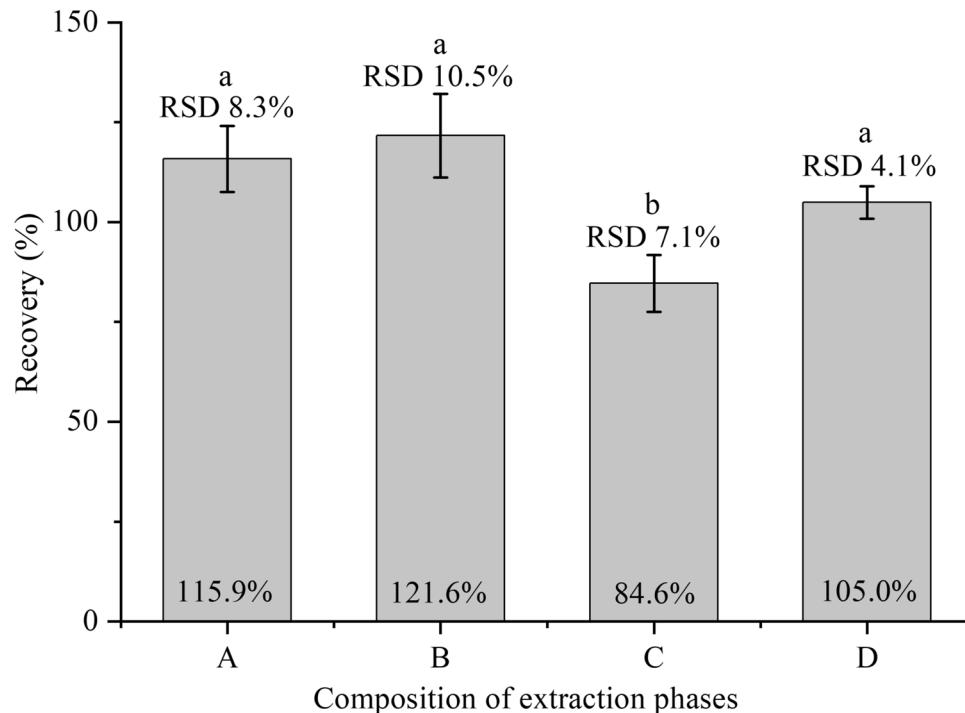
The next step of this study was to determine the optimal extraction conditions using the SLE-LTP method.

Optimization of the Extraction Method

Four extracting phases were evaluated in this study, as can be seen in Table 2. The recovery rates and relative standard deviations (RSD) obtained in each extracting phase are shown in Fig. 2.

The results revealed that only the extracting phase consisting of acetonitrile acidified with hydrochloric acid and water differed statistically from the other three extracting phases by the Tukey's test. Therefore, the chromatograms of the other three extracting phases were compared in aiming to select the chromatogram with the largest chromatographic area for the florpyrauxifen-benzyl peak and the lowest number of matrix interferents. The chromatograms obtained in the evaluation are shown in Figure S3.

The results showed that the chromatogram obtained using the extractor phase consisting of acetonitrile:water (Figure S3(a)) showed greater chromatographic area for the florpyrauxifen-benzyl peak; therefore, this extractor phase was defined for the next step of this study. This extractor phase composition was recently employed to extract

Fig. 2 Florpyrauxifen-benzyl extraction percentages in four different extractor phase compositions. Bars followed by the same letter do not differ statistically from one another by Tukey's test at a 5% significance level. **A** acetonitrile:water (8:4 v/v), **B** acetonitrile acidified with 0.1% (v/v) formic acid:water (8:4 v/v), **C** acetonitrile acidified with 0.1% (v/v) hydrochloric acid:water (8:4 v/v), **D** acetonitrile + ethyl acetate:water (6.5 + 1.5:4 v/v)

florpyrauxifen-benzyl in water, soil, and rice matrices (Vieira et al. 2022; Zhou et al. 2022) and has been successfully used to extract other environmental contaminants such as furans (2,3,7,8-TCDF), dioxins (2,3,7,8-TCDD) (Sicupira et al. 2019, 2023), and organochlorine pesticides (Mesquita et al. 2018).

One of the main features of SLE-LTP is the prior cleaning of extracts during the freezing process. However, in this study, an additional clean-up step for the rice matrix was necessary, because a yellowish extract was observed during the drying step of the extracts, indicating a greasy appearance. Therefore, six adsorbents were evaluated, and the recovery rates and chromatograms profile obtained in each clean-up condition are shown in Fig. 3 and Fig. S4, respectively.

The results indicated that activated charcoal completely adsorbed florpyrauxifen-benzyl (see Figure S4(c)), resulting in a chromatogram without a signal of this compound, i.e., it not interesting for this study. The chromatograms obtained for the extracts after clean-up with C18, alumina, and PSA showed similar profiles in the florpyrauxifen-benzyl peak region, as can be seen in Figure S4(a), (d), and (e). However, only the chromatogram of the extract submitted to clean-up with alumina resulted in a more horizontal baseline, a florpyrauxifen-benzyl peak without interferences, greater absolute chromatographic area for the florpyrauxifen-benzyl peak, adequate recovery rate, and relative standard deviation. Therefore, alumina was defined for the clean-up of the extracts in this study.

The amount of adsorbent per mL of matrix extract was also evaluated in this study. In this sense, masses of 50 and 100 mg of alumina were evaluated for the clean-up of rice extracts. The relative recovery rate and standard deviation obtained can be seen in Figure S5.

The results indicated that recovery rates did not differ statistically by the Tukey's test at the 5% significance level. Therefore, the mass of adsorbent chosen to clean-up the rice extracts was defined as 50 mg of adsorbent per mL of matrix extract.

A comparative study was carried out between the three extraction methods' developments in previous studies and the method optimized in this study, as can be seen in Table 4.
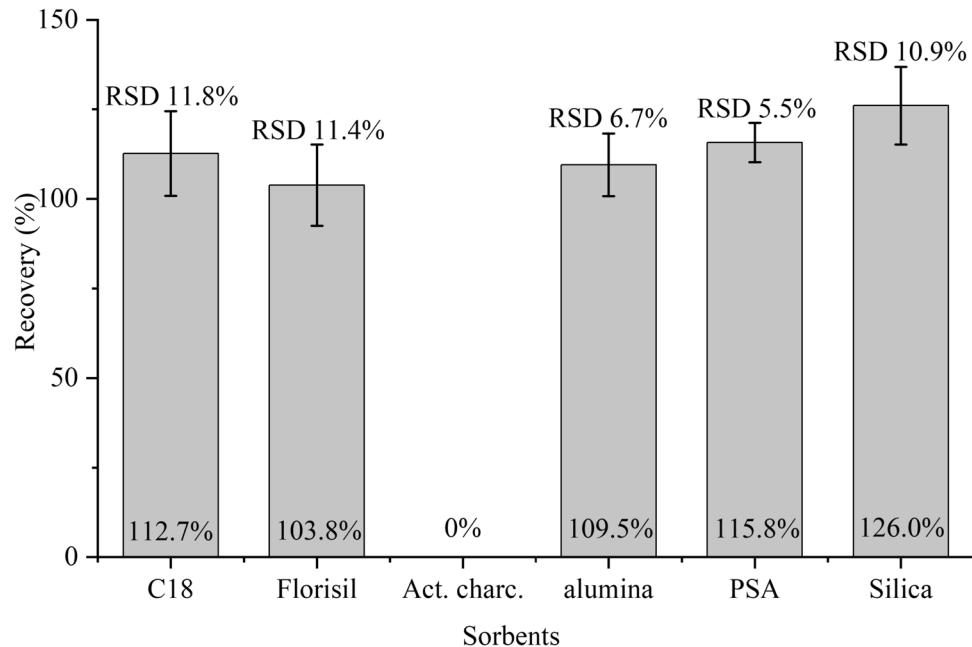
It is possible to observe that the four extraction methods used acetonitrile as extraction phase and presented recovery rates close to 100% and relative standard deviations below 20%. Similarly, all extracting methods presented LOD and LOQ values lower than the LMR (10 µg kg^{-1} in rice). The clean-up step was observed for a three matrices using the Quechers method, but only in the rice matrix using the SLE-LTP method. This result again reveals the previous clean-up carried out during the freezing phase of the SLE-LTP. Table 4 also revealed that LLE-LTP and SLE-LTP presented the lowest extractor phase volume and sample amount in relation to QuEChERS method, indicating a positive factor for these two methods.

After this step, the analytical validation of the extraction method was conducted according to the SANTE protocol (SANTE 2021).

Method Validation

The optimized methodology was validated out using the six figures of merit: selectivity, limits of detection (LOD)

Fig. 3 Florpyrauxifen-benzyl extraction percentages from the rice matrix using the SLE-LTP method under optimized conditions: acetonitrile:water (8:4 v/v) extracting phase for six different clean-up conditions (octadecyl functionalized silica gel (C18), florisil, activated charcoal (Act. Charc), alumina, primary and secondary amine (PSA), and silica gel), all at a concentration of 50 mg of adsorbent per mL of rice matrix extract

Table 4 Comparative study of the extraction methods developments in previous studies and the method optimized in this study

Parameters	Vieira et al. 2022	Lopes et al. 2023	Zhou et al. 2022			This study
	Water	Soil	Water	Soil	Rice	Rice
Extraction method	LLE-LTP[a]	SLE-LTP[b]	QuEChERS[c]			SLE-LTP
Extraction phase (mL)	ACN[d] (8:4)	ACN 0.1% FA[e]:water (8:4)	ACN:water (20:5)			ACN:water (8:4)
Volume of sovent (mL)	8	8	20			8
Mass or volume of sample (g or mL)	4	4	5			4
Clean-up step	-	-	5 mg GCB[f]	15 mg GCB	20 mg GCB	50 mg Alumina
Recovery rate (%)	103.3–105.6	108.3–109.1	95.35–112.63			100.4–118.3
RSD[g] (%)	0.2–5.1	1.86–9.30	0.54–8.31			5.66–16.73
LOD[h] ($\mu g\ L^{-1}$)	2	5	0.42	0.48	0.81	3
LOQ[i] ($\mu g\ L^{-1}$)	4	20	0.94	1.31	1.58	5

[a]*LLE-LTP*, liquid–liquid extraction with low temperature purification; [b]*SLE-LTP*, solid–liquid extraction with low temperature purification; [c]*QuEChERS*, quick, easy, cheap, effective, rugged and safe; [d]*ACN*, acetonitrile; [e]*FA*, formic acid; [f]*GCB*, graphitized carbon black; [g]*RSD*, relative standard deviation; [h]*LOD*, limit of detection; [i]*LOQ*, limit of quantification

and quantification (LOQ), linear range, precision, accuracy, and matrix effect (SANTE 2021).
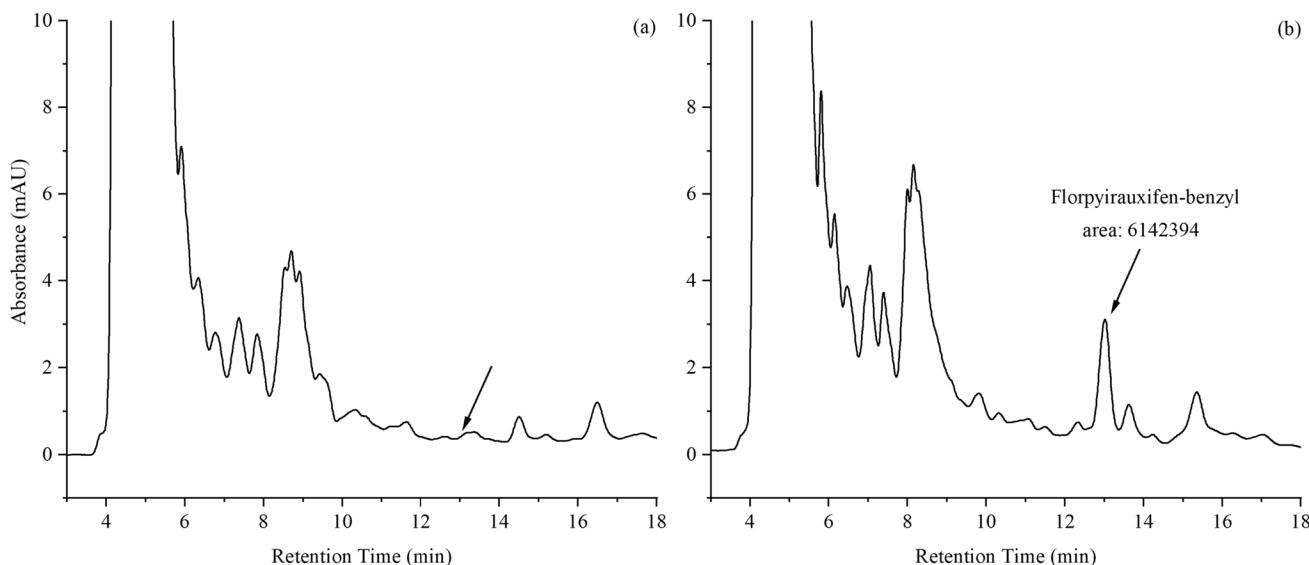
Selectivity

The optimized methodology selectivity was confirmed by analyzing the chromatograms of the rice florpyrauxifen-benzyl free matrix extract (blank extract) and of the matrix extract fortified with florpyrauxifen-benzyl. The obtained chromatograms can be seen in Fig. 4.

The method was considered selective as no interference peaks were observed in the same florpyrauxifen-benzyl retention time.

Limit of Detection (LOD) and Quantification (LOQ)

The LOD and LOQ values achieved by the optimized methodology can be seen in Table 5. These values are below the maximum residue limit (MRL) of florpyrauxifen-benzyl in rice defined by Brazilian (ANVISA 2022) and American (USA 2017b) legislations, which were 10 and 300 $\mu g\ kg^{-1}$,


Fig. 4 A Chromatogram of the analyte-free matrix extract (blank extract) and **b** chromatogram of the matrix extract fortified with flor-pyrauxifen-benzyl at a concentration of 90 $\mu g\ kg^{-1}$. Chromatographic conditions: Kinetex column, injection volume = 10 μL, methanol:water (78:22) mobile phase, T = 25 ℃, flow rate = 0.3 mL min^{-1}, and λ = 260 nm

Table 5 Results of the analytical validation study

Linear range µg kg⁻¹	Line equation	R^{2c}	Recovery ± RSD[d] (%)			LOD[e]	LOQ[f]
			5.0 µg kg⁻¹[a]	90.0 µg kg⁻¹[b]	150.0 µg kg⁻¹[a]	µg kg⁻¹	
5.0–150.0	$y = 51770x - 205839$	0.9933	100.4 ± 16.73	118.3 ± 5.66	114.3 ± 10.75	3.0	5.0

[a]Average of 3 replicates; [b]mean of 7 replicates; [c]coefficient of determination; [d]relative standard deviation; [e]limit of detection; [f]limit of quantification

respectively. Similarly, the values reached in this study were also lower than the MRL defined by the European (Arena et al. 2018) and Australian (APVMA 2018) legislations, which define 20 µg kg⁻¹.

Precision and Accuracy

Precision and accuracy were simultaneously confirmed by fortification and recovery experiments of the rice samples. The results in Table 5 reveal that RSD values were less than 20% and recovery rates were between 70 and 120%. These values demonstrated that the precision and accuracy of the optimized method is in accordance with the guidelines of the SANTE analytical validation protocol (SANTE 2021).

Linear Range

The linear range of the optimized method was determined using six equidistant concentrations with three independent replicates for each level. The linear range includes the LOQ concentration and the fortification concentration values of the sample, which was 90.0 µg kg⁻¹, as can be seen in Figure S6(a).

The ordinary least squares (OLS) method was used to estimate the linear regression parameters, thus obtaining the slope, intersection, and determination coefficient (R^2) values, as can be seen in Table 5. The R^2 value was greater than 0.99, indicating the variability of the collected data explained by the regression model.

The Jacknife test was applied to the regression residuals, and the outliers found were excluded, as can be seen in Figure S6(b). Then, the normality, homoscedasticity, and independence of the regression residuals were evaluated.

The Ryan-Joiner test was used to assess the normality of the regression residuals (Figure S6(c)). The correlation coefficient (R) found was greater than the critical correlation coefficient ($R_{crit.}$) obtained by polynomial interpolation ($R = 0.9826 > R_{crit.}$ 0.9351). Thus, we can conclude that the residuals followed normal distribution at a significance level of 0.05.

The Brown-Forsythe test, which determines the existence of differences between the residual variances through an adaptation of the Levene test, was used to assess the homoscedasticity of the regression residuals. The statistical value for Levene's T (LT) found was less than the critical value at the significance level of 0.05 (LT = 1.54 < $T_{crit. (0.975)}$ = 2.18), thereby confirming homoscedasticity.

The Durbin-Watson test was used to assess the independence of the regression residuals, the statistical value (d) calculated for the test was greater than the value calculated for the upper critical limit (ud) ($d = 2.71 >$ ud = 1.35), and no autocorrelation was observed at the 0.05 significance level. There was a random distribution of the residuals in the four quadrants, demonstrating their independence, as can be seen in Figure S6(d).

The linearity evaluation followed the procedures proposed by Souza and Junqueira (2005) and Bazílio et al. (2012). Data were fitted to the linear model for the evaluated range from 5.0 to 150.0 µg kg⁻¹ (Table 5), in which significant regression and non-significant linear deviation were observed at the significance level of 0.05. Thus, it was concluded that the OLS method was adequate for the data studied.

Matrix Effect

The calculated matrix effect in determining florpyrauxifen-benzyl in rice was 184.3%. This value is classified as a very high matrix effect. This result was different from that found in a recent study using the same analysis and detection system (HPLC–DAD) for determining florpyrauxifen-benzyl in soil, as a low matrix effect was verified (− 3.34%) (Lopes et al. 2023), and there was no matrix effect in the analyzes of this herbicide for the water matrix (Vieira et al. 2022).

It is noteworthy that rice is considered a complex matrix due to its chemical composition being rich in carbohydrates, lipids, and pigments that can be co-extracted together with the target analyte and thus interfere with the chromatographic signal (Pinho et al. 2009). This can be proven through several previous studies that reported a significant matrix effect for the rice matrix (Rebelo et al. 2016; Mardani et al. 2021; Li et al. 2019; Mondal et al. 2017; Sefiloglu et al. 2021). Different analysis methods were used in these studies to quantify pesticides in rice, such as cyclosulfamuron, thiobencarb, cycloxydim, imazapyr, imazapic (Rebelo et al. 2016), and foramsulfuron (Mardani et al. 2021) herbicides; tetraniliprole (Li et al. 2019), cartap, fenpropathrin (Mardani et al. 2021), malathion (Mondal et al. 2017), and chlorpyrifos (Sefiloglu et al. 2021) insecticides; and

prochloraz (Mardani et al. 2021) fungicide. All of these studies prove that the rice matrix commonly presents high matrix effect.

Conclusions

The SLE-LTP methodology was optimized and validated for determining florpyrauxifen-benzyl in rice samples using HPLC–DAD. This methodology proved to be easy to perform, fast, sensitive, and effective for determining florpyrauxifen-benzyl in rice samples, as the quantification limit found was lower than the MRL defined by Brazilian and international legislation for the rice matrix. Therefore, this methodology proved to be a promising alternative for monitoring florpyrauxifen-benzyl in rice samples.

Declarations

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