*Analytical Methods*

**Electronic Supplementary Information – ESI †**

# Optimization and validation of liquid-liquid extraction with low-temperature purification (LLE-LTP) for determining fluopyram fungicide in water samples using HPLC-DAD

Lucas Victor Pereira de Freitas (Orcid 0000-0001-7183-9362),[a] Gleysson de Paula Terra,[b] Silas de Souza Santos,[c] Lázaro Chaves Sicupira (Orcid 0000-0002-4085-2959),[d] Flaviano Oliveira Silvério (Orcid: 0000-0002-5753-8739)[c*]

[a] School of Pharmaceutical Sciences of Ribeirão Preto, Universidade de São Paulo, 14040-903, Ribeirão Preto, São Paulo, Brazil

[b] Department of Chemistry, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, Minas Gerais, Brazil

[c] Institute of Agricultural Sciences, Universidade Federal de Minas Gerais, 39404-547, Montes Claros, Minas Gerais, Brazil

[d] Institute of Engineering, Science and Technology, Universidade Federal dos Vales do Jequitinhonha e Mucuri, 39447-790, Janaúba, Minas Gerais, Brazil

* Corresponding Author
E-mail: flavianosilverio@ufmg.br
https://orcid.org/0000-0002-5753-8739

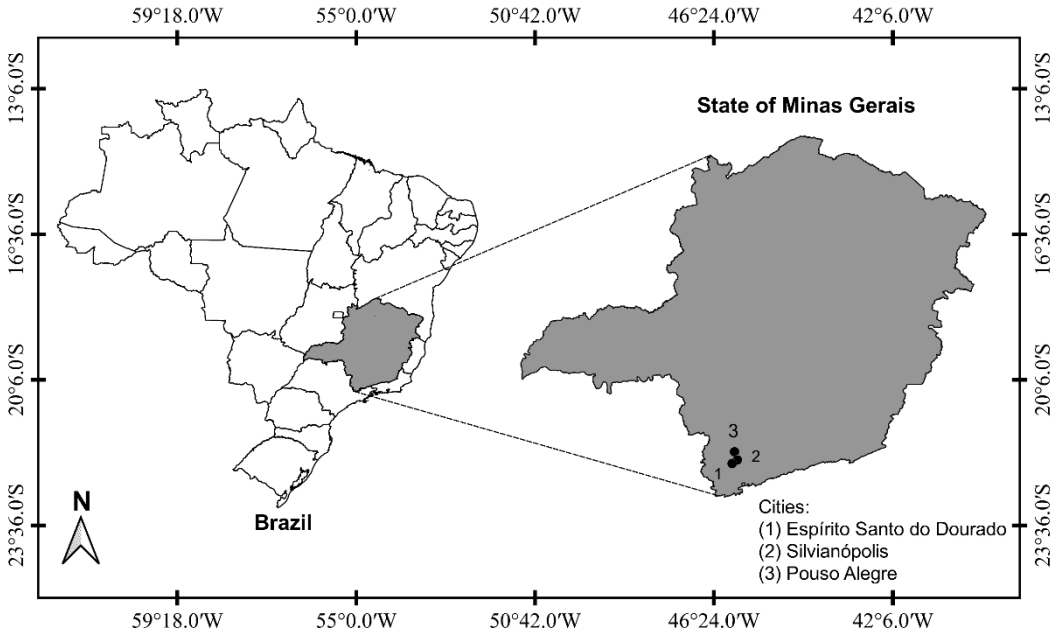


**Fig. S1** Map showing the location of the three Brazilian cities where the water samples were collected.

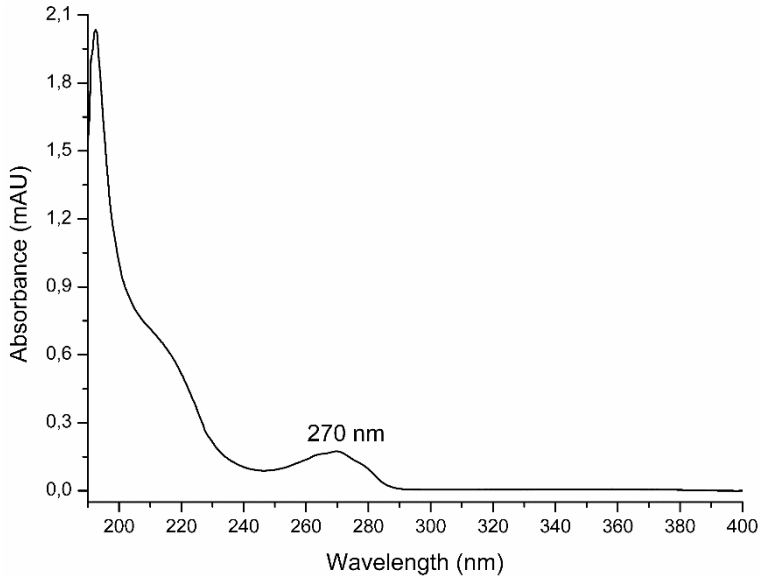


**Fig. S2** Absorption spectrum in the ultraviolet and visible region of the fluopyram standard solution at 1.0 mg $L^{-1}$ in acetonitrile.

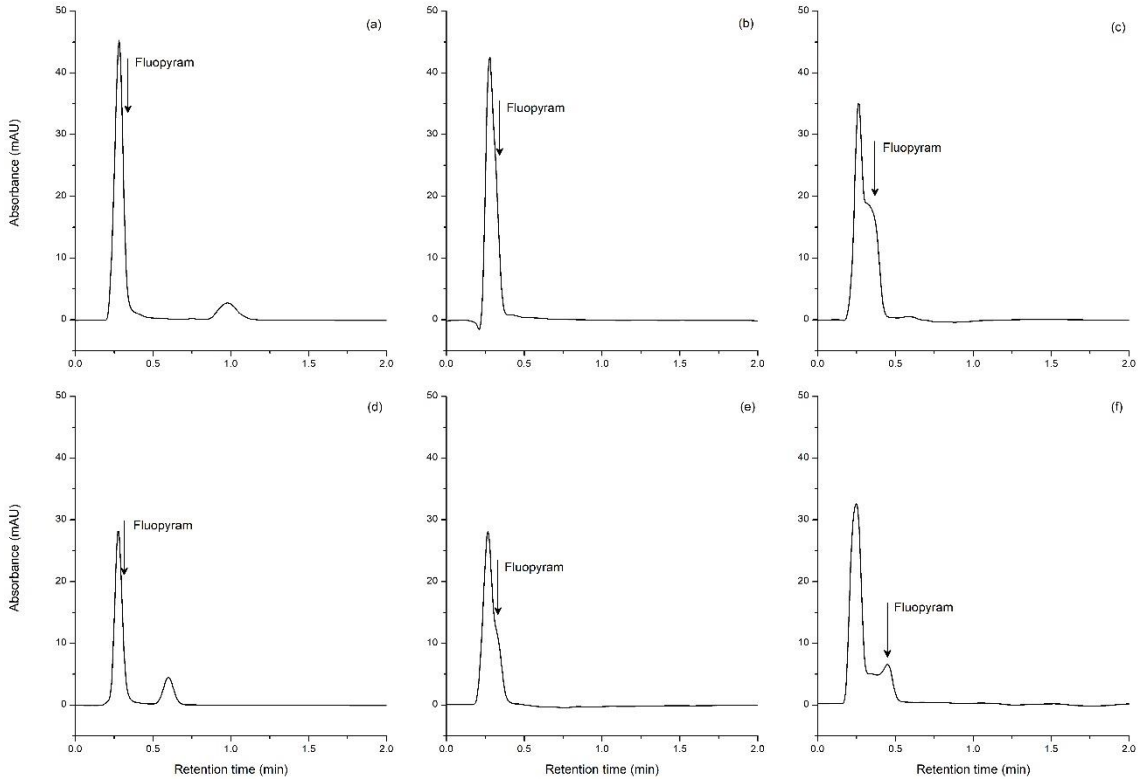


**Fig. S3** Chromatograms of the fluopyram standard solution at 1.0 mg $L^{-1}$ in acetonitrile. Zorbax column (2.1 mm x 50 mm, 1.8 μm); column temperature = 30 ºC; λ = 270 nm; flow rate = 0.5 mL $min^{-1}$; mobile phase (a) ACN:$H_2O$ (100:0 v/v), (b) ACN:$H_2O$ (90:10 v/v), (c) ACN:$H_2O$ (80:20 v/v), (d) MeOH:$H_2O$ (100:0 v/v), (e) MeOH:$H_2O$ (90:10 v/v), and (f) MeOH:$H_2O$ (80:20 v/v). The arrows in the figure indicate the retention time of the fluopyram.

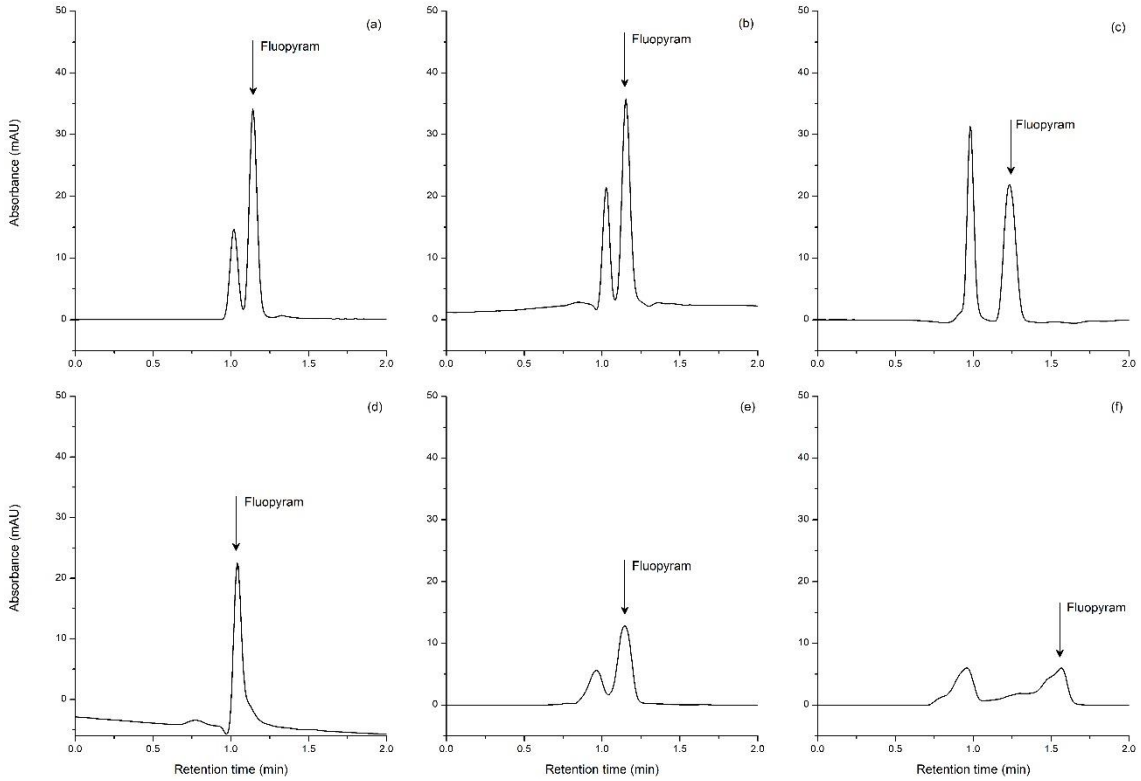


**Fig. S4** Chromatograms of the fluopyram standard solution at 1.0 mg $L^{-1}$ in Acetonitrile. Poroshell column (4.6 mm x 50 mm, 2.7 µm, Agilent Technologies); column temperature = 30 ºC; λ = 270 nm; flow rate = 0.5 mL $min^{-1}$; mobile phase (a) ACN:$H_2O$ (100:0 v/v), (b) ACN:$H_2O$ (90:10 v/v), (c) ACN:$H_2O$ (80:20 v/v), (d) MeOH:$H_2O$ (100:0 v/v), (e) MeOH:$H_2O$ (90:10 v/v), and (f) MeOH:$H_2O$ (80:20 v/v). The arrows in the figure indicate the retention time of the fluopyram.

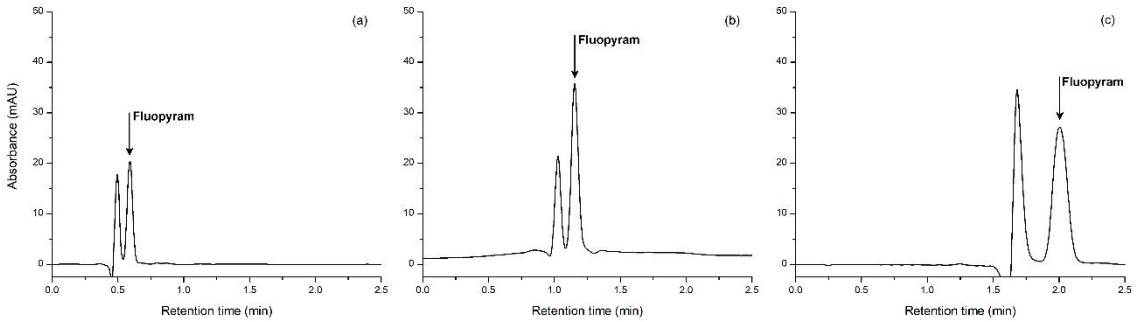


**Fig. S5** Chromatograms of the fluopyram standard solution at 1.0 mg $L^{-1}$ in Acetonitrile. Poroshell column (4.6 mm x 50 mm, 2.7 μm, Agilent Technologies), λ = 270 nm; column temperature = 30 ºC; mobile phase ACN:$H_2O$ (90:10 v/v); flow rate (a) 1.0 mL $min^{-1}$, (b) 0.5 mL $min^{-1}$, and (c) 0.3 mL $min^{-1}$.

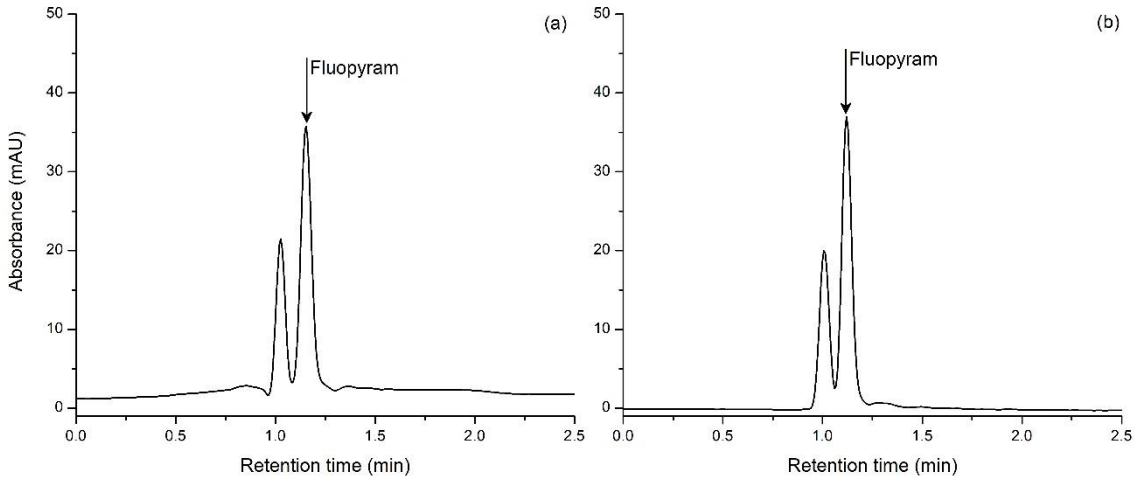


**Fig. S6** Chromatograms of the fluopyram standard solution at 1.0 mg $L^{-1}$ in Acetonitrile. Poroshell column (4.6 mm x 50 mm, 2.7 μm, Agilent Technologies), λ = 270 nm; mobile phase ACN:$H_2O$ (90:10 v/v); flow rate = 0.5 mL $min^{-1}$; column temperature (a) 30 ºC, and (b) 40 ºC.

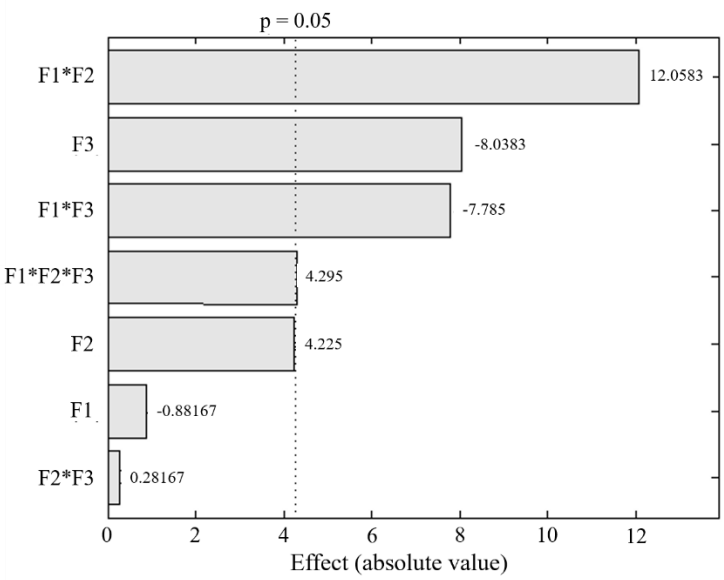


**Fig. S7** Pareto chart of the effects and their interaction in the LLE-LTP of fluopyram at a probability level of 95% ($p \leq 0.05$).

F1, factor 1 (extraction phase: proportion of ACN:EtOAc); F2, factor 2 (ionic strength); F3, factor 3 (sample freezing time).

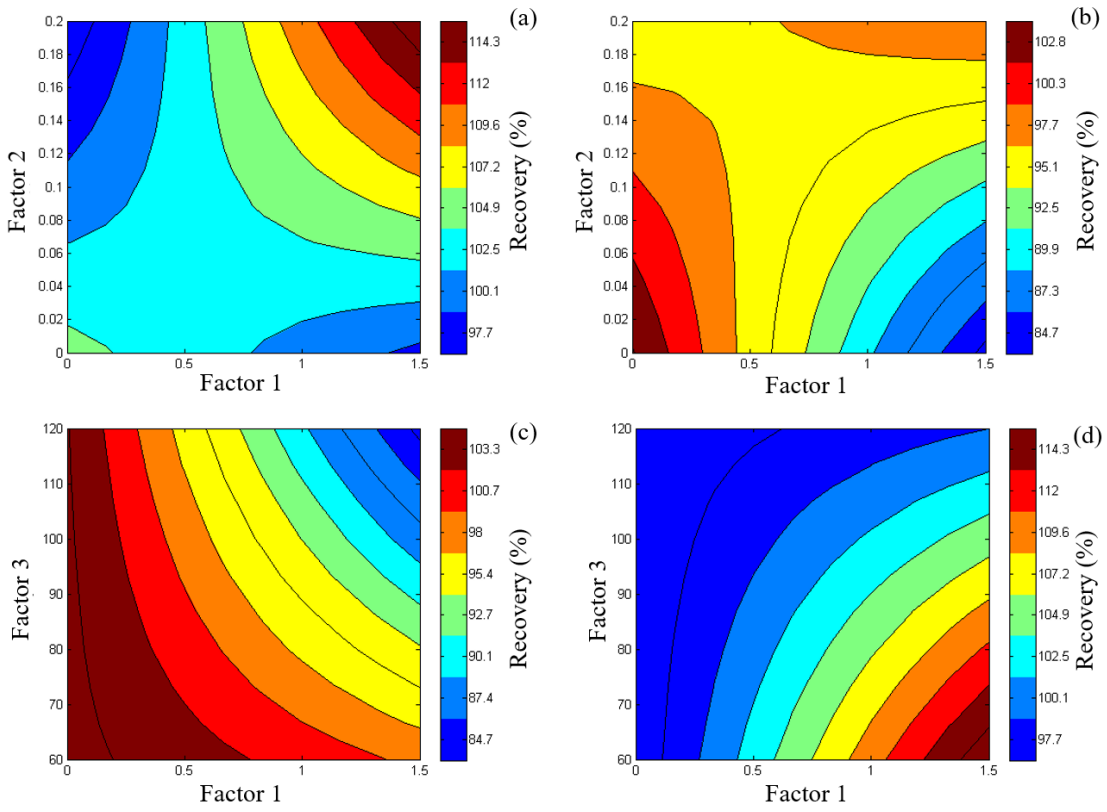


**Fig. S8** Response surface plots for interactive effects between the extraction phase (factor 1) and ionic strength (factor 2) at the time of (a) 60 min and (b) 120 min; and the interactive effects between the extraction phase (factor 1) and the sample freezing time (factor 3) with the ionic strength at (c) level (+) and (d) level (-).

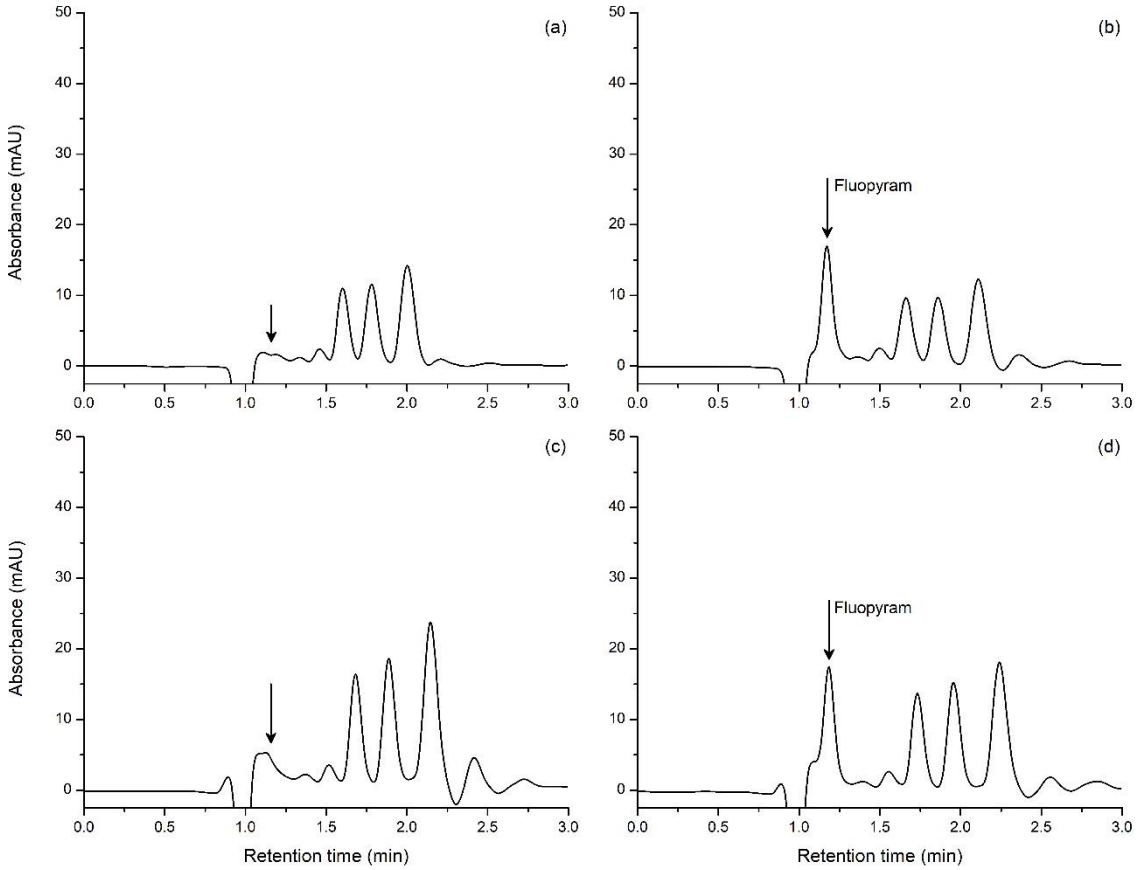


**Fig. S9** Chromatograms of (a) the blank extract and (b) the fortified matrix extract obtained using the extraction phase composed by acetonitrile; and chromatograms of (c) the blank extract and (d) the fortified matrix extract obtained using the extraction phase composed by EtOAc.

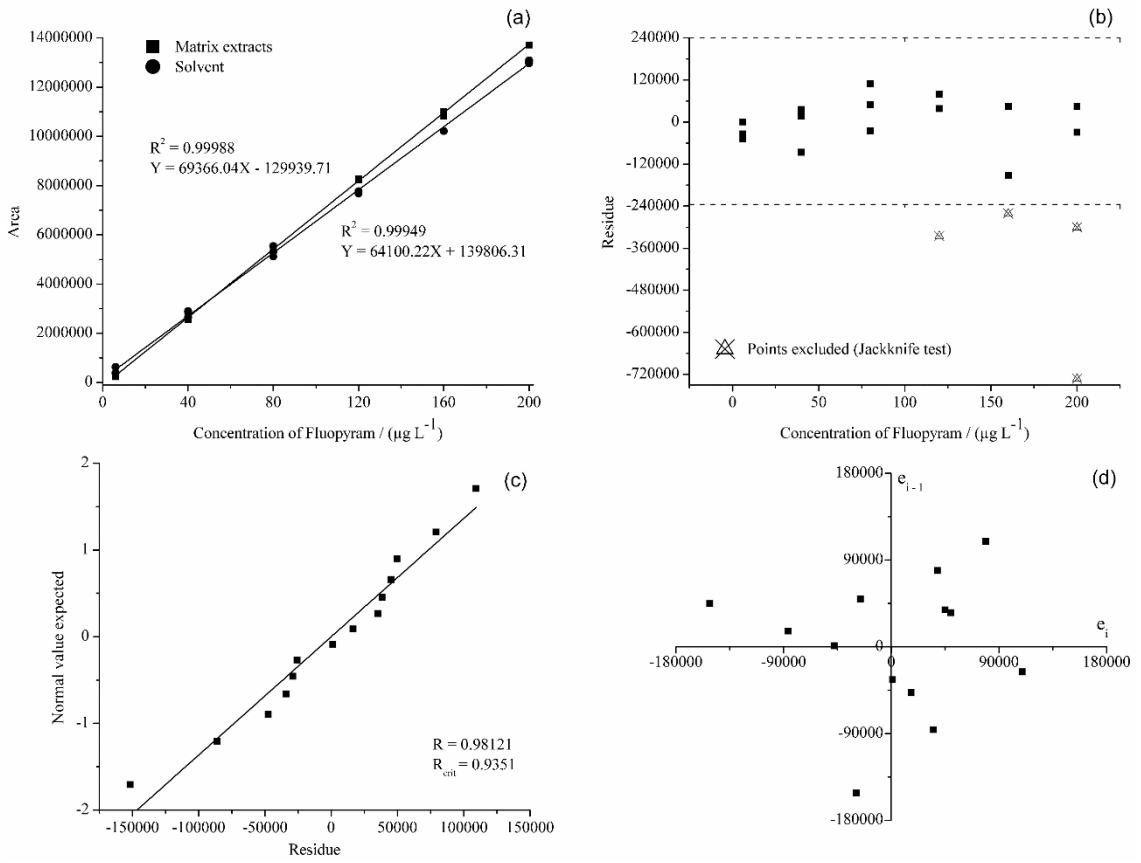


**Fig. S10** (a) analytical curves of fluopyram solutions in solvent and matrix extracts; (b) linear regression residues after the exclusion of the outliers (Jackknife test); (c) normal probability of regression residues (Ryan-Joiner test); (d) autocorrelation of regression residues (Durbin-Watson test).

ei, residue; $R^2$, determination coefficient; R, correlation coefficient of Ryan-Joiner test; d, Durbin-Watson statistics

**Table S1** ANOVA for the significance of regression and deviation from linearity

| | Regression | Deviation from linearity |
|---|---|---|
| n | 14 | 14 |
| $F_{crit}$ | 4.75 | 3.84 |
| F | $4.88 \times 10^4$ | 1.12 |

n, number of observations; $F_{crit}$, F critical; F, ratio between variances