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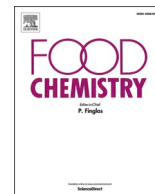
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Development and validation of a highly sensitive method for the analysis of succinate dehydrogenase inhibitors (SDHIs) in plant-based food and beverages

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ABSTRACT

Recently, concerns have been raised over succinate dehydrogenase inhibitor (SDHI) fungicides and their potential adverse effects on the environment and nontarget organisms including humans. Appropriate analysis methods are thus necessary for their proper risk assessment. Here, a highly sensitive method based on QuEChERS and UHPLC-MS/MS was developed and validated for the simultaneous quantification of 12 SDHIs and seven of their metabolites in beverages, fruits and vegetables. The method displayed linearity over more than three orders of magnitude, precision with RSD <20 % and recoveries between 70 and 120 % for all compounds, ensuring robustness across a wide concentration range. Limits of quantification as low as 0.003–0.3 ng/g depending on the matrix and analytes were reached. The method was applied to 28 representative samples of fruits, vegetable, fruit juices, wine and water samples. The results illustrate the reliability of this method for the quantification of SDHI fungicides in a variety of foodstuff matrices.

1. Introduction

Fungicides are pesticides used to control phytopathogenic fungi. Among fungicides, succinate dehydrogenase inhibitors (SDHIs) represent the fastest-growing class in terms of new compounds developed and introduced on the market (Sierotzki & Scalliet, 2013). SDHIs have gained attention due to their unique chemical structures (Fig. 1), high levels of activity, and broad fungicidal spectrum against basidiomycetes, deuteromycetes, and ascomycetes (Veloukas & Karaoglanidis, 2012). SDHIs block the enzyme complex succinate dehydrogenase (SDH), a universal and highly conserved complex which plays a key role in the mitochondrial respiratory chain and the functioning of the Krebs cycle. SDH is essential for cellular energy production, not only in fungi but in all living organisms with mitochondria, including humans (Bénil et al., 2019). In the mitochondrial respiratory chain, electron transport between complex II (SDH) and complex III takes place via co-enzyme Q. SDHIs bind to the co-enzyme Q binding pocket, thereby inhibiting SDH function. Inhibition of SDH leads to an accumulation of superoxide ions (ROS) in the mitochondrial respiratory chain. The disruption of the

Krebs cycle by preventing the transformation of succinate into fumarate, leads to an accumulation of succinate, whose excess in turn causes inhibition of so-called α -KG-dependent enzymes. This deregulation has epigenomic, transcriptional, metabolomic and growth factor-inducing consequences which are potentially involved in carcinogenesis (Bénil et al., 2022; Duarte-Hospital et al., 2023).

In 2023, twenty-four molecules from three generations are listed as SDHIs in the Fungicide Resistance Action Committee (FRAC) classification (FRAC, 2023). In the European Union (EU), ten are authorized (flutolanil, boscalid, penthiopyrad, sedaxane, fluopyram, bixafen, penflufen, fluxapyroxad, benzovindiflupyr, isofetamid), five are no longer approved (carboxin, benodanil, fenfuram, mepronil, isopyrazam) and four are under evaluation (pydiflumetofen, isoflucypram, inpyrfluxam, fluidapyr). SDHI fungicides are widely used to control pathogenic fungi on various crops such as fruits, vegetable, cereals, turfs and ornamentals. Consequently, research on potential food and environmental contaminations is crucial. Analytical methods for the determination of SDHI fungicides in environmental and/or foods matrices are scarce in scientific literature. Most are multi-residue methods which are non-specific to

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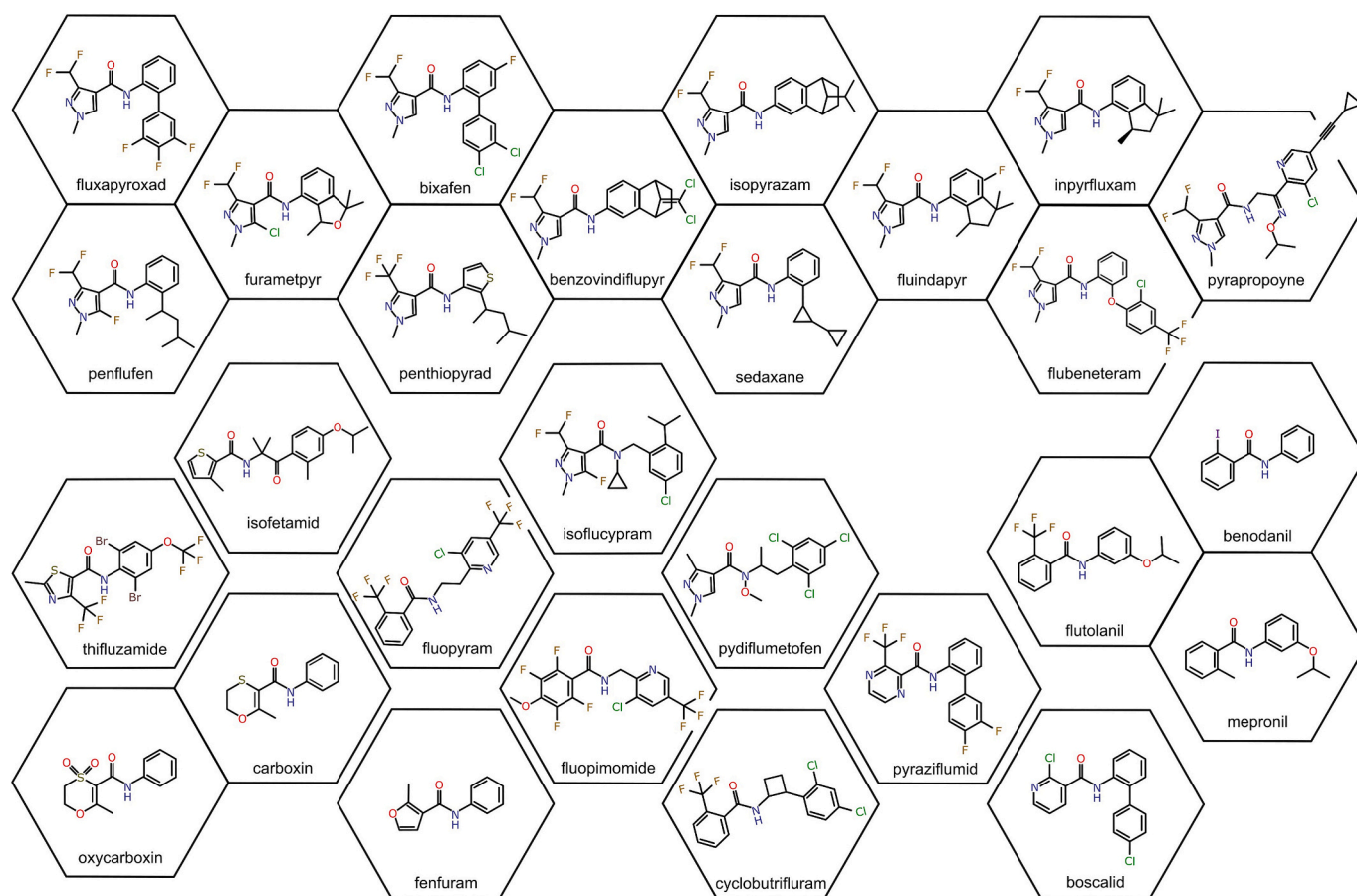


Fig. 1. Structural overview of SDHI fungicides. The graphical representation is based on Chen et al., 2021; FRAC, 2023; Luo & Ning, 2022; Umetsu & Shirai, 2020.

the SDHI family and include only a few selected SDHIs (e.g., boscalid), resulting in relatively low coverage and sensitivity. Over the last decade, more targeted procedures have been developed for the analysis of seven SDHIs in fruits and vegetable crops (Abad-Fuentes et al., 2015; Wu et al., 2019), eight in surface water (Gulkowska et al., 2014), nine in cereals, vegetable and fruits (Tian et al., 2020) and eighteen in cereals (Zhao et al., 2022). These procedures are generally based on a “Quick, Easy, Cheap, Effective, Rugged and Safe” (QuEChERS) extraction method followed by sensitive analysis by ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS).

To our knowledge, there is no analytical method able to monitor all EU approved major SDHIs in a variety of foodstuff matrices, with high sensitivity and including their metabolites. The aim of this study was thus to develop a robust and sensitive method for the simultaneous determination of 12 SDHI fungicides currently or once authorized in the EU and 7 of their metabolites in foodstuffs such as beverages, fruits and vegetable. An approach based on the QuEChERS method coupled with UHPLC-MS/MS was developed. Several parameters for sample preparation, chromatographic separation and mass spectrometric detection were optimized, and the method was validated and applied to the analysis of various foodstuffs.

2. Materials and methods

2.1. Chemicals

Acetonitrile LiChrosolv grade and acetonitrile LC/MS Hipersol Chromanorm were purchased from VWR (Rosny-sous-Bois, France). Ultrapure water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Formic acid and ammonium formate of

ULC/MS grade were purchased from Biosolve (Dieuze, France). All salts used for QuEChERS (anh. MgSO_4 , NaCl, citrate sodium hydrate and disodium hydrogen citrate sesquihydrate) were obtained from Sigma-Aldrich. Isolute® PSA bulk phase was purchased at Biotage (Uppsala, Sweden). C18 (Zeoprep 90) bulk phase was supplied by ZeoChem (Uetikon, Switzerland).

Twelve SDHIs and seven metabolites were considered in this study. The metabolites were selected based on three criteria: i) they are commercially available as reference standards, ii) are included in the definition of pesticide residues (i.e., bixafen BYF00587, boscalid M510F01, fluopyram benzamid, oxycarboxin and penthiopyrad carboxamide), and iii) have been associated with environmental metabolism and possess potential toxicological effects on mammals (i.e., bixafen M44, fluopyram benzamid and fluxapyroxad M700F001). Thirteen certified pesticide standards with purity between 95.0 % to 99.9 % (bixafen, bixafen M44, boscalid, carboxin, fluopyram, fluopyram benzamid, flutolanil, fluxapyroxad, fluxapyroxad M700F001, isofetamid, penthiopyrad, penthiopyrad carboxamid and sedaxane) and three isotopically labelled standards (boscalid-d4, fluopyram-d4 and flutolanil-d7) were purchased from Cluzeau Info Lab (Sainte-Foy-la-Grande, France). Six other certified pesticide standards with purity between 98.7 % and 99.6 % (bixafen BYF00587, boscalid M510F01, isopyrazam, penflufen, oxycarboxin and thifluzamide) were purchased from Sigma-Aldrich (Isle d'Abeau, France). Standard solutions of each chemical were prepared at 1 mg/mL in acetonitrile LiChrosolv grade and stored at $-20\text{ }^\circ\text{C}$.

2.2. Samples

The foodstuff commodities used in this study were tap water, orange

Table 1

Optimized LC-MS/MS parameters for target compounds and their labelled forms including internal standard, ion source, retention time (RT) of each pesticide, MRM transitions and collision energies (CE).

Analyte	Internal standard	Ion source	RT (min)	transition 1 (quantifier)	CE (V)	transition 2 (qualifier)	CE (V)
Bixafen	Fluopyram-d4	ESI+	8.49	414.0 → 394.0	20	414.0 → 374.0	31
Bixafen BYF00587	Fluopyram-d4	ESI+/-	7.95	400.0 → 360.0	31	398.0 → 358.0	-22
Bixafen M44	Fluopyram-d4	ESI-	2.24	161.0 → 141.0	-12	141.0 → 66.0	-25
Boscalid	Boscalid-d4	ESI+	7.92	343.0 → 307.0	29	343.0 → 140.0	33
Boscalid M510F01	Boscalid-d4	ESI+/-	6.22	359.0 → 323.0	29	357.0 → 244.0	-26
Carboxin	Fluopyram-d4	ESI+	6.42	236.1 → 143.1	21	236.1 → 93.1	53
Fluopyram	Fluopyram-d4	ESI+	8.03	397.1 → 208.0	30	397.1 → 145.0	70
Fluopyram benzamide	Fluopyram-d4	ESI+	3.82	190.0 → 170.0	15	190.0 → 130.0	28
Flutolanil	Flutolanil-d7	ESI+	8.30	324.1 → 262.1	27	324.1 → 242.1	37
Fluxapyroxad	Fluopyram-d4	ESI+	7.82	382.1 → 362.1	31	382.1 → 342.1	20
Fluxapyroxad M700F001	Fluopyram-d4	ESI+/-	3.37	177.0 → 137.0	23	175.0 → 91.0	-27
Isofetamid	Flutolanil-d7	ESI+	8.70	360.2 → 182.2	21	360.2 → 125.2	39
Isopyrazam	Fluopyram-d4	ESI+	9.34	360.2 → 320.2	30	360.2 → 258.2	27
Oxycarboxin	Fluopyram-d4	ESI+	4.98	268.1 → 175.1	22	268.1 → 147.1	33
Penflufen	Fluopyram-d4	ESI+	8.40	318.2 → 234.2	25	318.2 → 141.2	43
Penthiopyrad	Fluopyram-d4	ESI+	8.87	360.1 → 276.1	20	360.1 → 236.1	29
Penthiopyrad carboxamide	Fluopyram-d4	ESI+	2.95	194.1 → 174.0	15	194.1 → 154.0	20
Sedaxane	Fluopyram-d4	ESI+	8.12	332.2 → 159.1	28	332.2 → 292.1	23
Thiifluzamid	Flutolanil-d7	ESI-	8.66	526.8 → 125.0	-74	526.8 → 166.0	-26
Boscalid-d4		ESI+	7.89	347.1 → 112.0	55		
Fluopyram-d4		ESI+	8.01	401.1 → 208.0	30		
Flutolanil-d7		ESI+	8.27	331.2 → 283.0	21		

juice, red wine, potato and tomato. Tap water samples were collected in 50 mL Falcon™ tubes in May and July 2022 from three French cities in the Loiret department (Orleans: urban area, St-Cyr-en-Val: peri-urban area and Ruan: intensive agricultural area). Each sampling tube was first rinsed with tap water before sampling. The samples were frozen at -20 °C immediately after collection and until analysis.

Orange juices, red wines and potatoes were obtained from supermarkets in Orleans, France, in April–May 2022. Tomatoes were obtained from supermarkets in Neuchatel, Switzerland, in June 2023. All samples were frozen at -20 °C shortly after their arrival at the laboratory and thawed prior to sample preparation.

2.3. Sample Preparation.

2.3.1. Water samples

Water sample analysis was performed using a “dilute and shoot” procedure. 80 µL of water was diluted with 80 µL of an acetonitrile solution containing an internal standard (IS) mix composed of three labelled SDHIs (10 ng/mL boscalid-d4, 0.5 ng/mL flutolanil-d7 and 0.5 ng/mL fluopyram-d4). The diluted sample was filtered through a 13 mm PTFE hydrophilic 0.22 µm porosity syringe filter (BGB Analytik, Bockten, Switzerland) into an HPLC vial containing a 250 µL conical insert.

2.3.2. Beverage samples

Beverage (orange juice and wine) sample analysis was performed using a QuEChERS procedure. A 6 mL volume of beverage was poured into a 15-mL Falcon™ polypropylene centrifuge tube, followed by the addition of 6 mL acetonitrile solution containing an internal standard (IS) mixture of three labelled SDHIs as described above (10 ng/mL boscalid-d4, 0.5 ng/mL flutolanil-d7 and 0.5 ng/mL fluopyram-d4). The resulting solution was shaken, centrifuged for 4 min at 6000 rpm to ensure deposition of any solid particle that could be present in the sample. Ten mL of the supernatant was pipetted into a new 15 mL tube containing 3.25 g of extraction salts for QuEChERS (2 g MgSO₄, 0.5 g NaCl, 0.5 g sodium citrate dihydrate and 0.25 g sodium citrate sesquihydrate) and cooled in an ice bath in order to prevent possible thermal degradation due to the exothermic reaction generated by the hydration of anhydrous magnesium sulfate (anh. MgSO₄) during the extraction. After the mixture was vigorously shaken by hand for approximately 2 min, the tube was centrifuged at 6000 rpm for 4 min. A volume of 1 mL

of supernatant was transferred into a 2 mL Safe-Lock Eppendorf® tube containing the purification salts (30 mg MgSO₄ and 20 mg C18). After vigorous shaking, the tube was centrifuged for 3 min at 14,000 rpm. An aliquot of 0.5 mL supernatant was then taken and diluted by addition of 0.5 mL of ultrapure water. Finally, the diluted sample was filtered through a 13 mm PTFE hydrophilic 0.22 µm porosity syringe filter (BGB Analytik, Bockten, Switzerland) into an HPLC vial containing 250 µL conical insert.

2.3.3. Fruits and vegetable

Fruits and vegetable (tomatoes, potatoes) were blended and homogenized with a mixer (4 in 1 Moulinex® blender). For samples having water content below 90 % (i.e., potatoes), 3.5 mL of milliQ water was added to 3.5 g of weighed samples. For samples with water content higher than 90 % (i.e., tomatoes), 7 g of samples were used without the addition of water. A volume of 7 mL of acetonitrile containing the internal standard (IS) mixture of three labelled SDHIs (10 ng/mL boscalid-d4, 0.5 ng/mL flutolanil-d7 and 0.5 ng/mL fluopyram-d4) was added to the 15-mL Falcon™ polypropylene centrifuge tube containing either homogenized fruit or vegetable samples. The QuEChERS procedure described above for the beverages was then followed.

2.4. Chromatography and mass spectrometric conditions.

The analysis of SDHIs was performed on a UHPLC-MS/MS system composed of an ACQUITY UPLC I-Class system (Waters, Milford, USA) coupled to a QTRAP 6500+ (AB Sciex, Framingham, USA) equipped with an electrospray ionization source.

Chromatographic separation was carried out on an ACQUITY UPLC HSS T3 column (2.1 × 100 mm, 1.8 µm, Waters) at 30 °C. The analysis was performed in gradient mode using H₂O containing 0.05 % formic acid and 1 mM ammonium formate as mobile phase A, and acetonitrile containing 0.05 % formic acid as mobile phase B. The following gradient elution was used at a flow rate of 0.4 mL/min: 2–85 % B in 10 min, 85–100 % B in 0.5 min hold at 100 % B for 2.0 min and re-equilibration to the initial condition (2 % B) for 3.1 min. The total chromatographic run time was 15.6 min. The injection volume was set to 2.5 µL. Detection was performed in both positive and negative electrospray ionization using polarity switching and scheduled multiple reaction monitoring (sMRM) with a retention time window of 30 s. One quantifier and one qualifier sMRM transition were monitored for each analyte. The

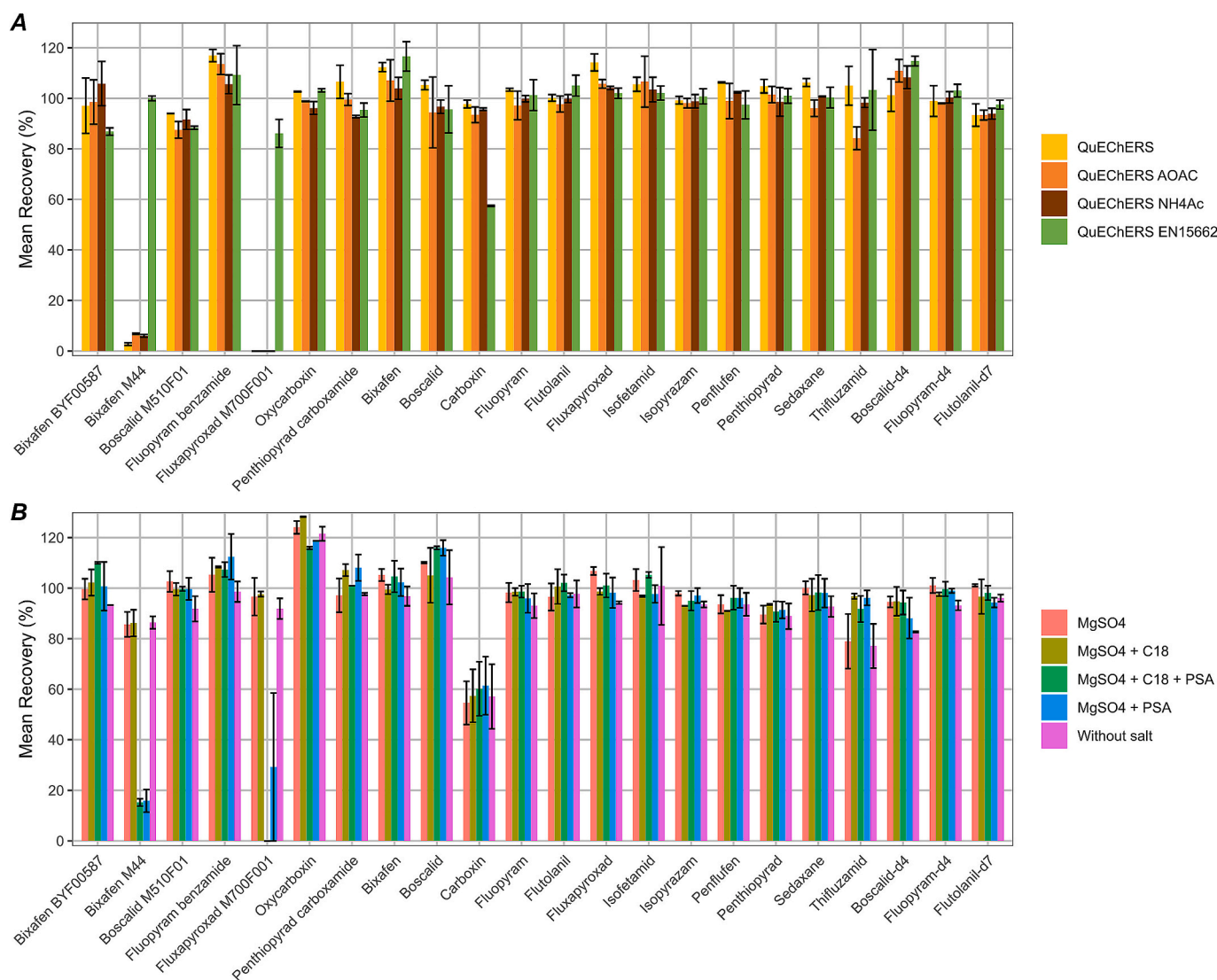


Fig. 2. Effect of different extraction parameters. A. Recoveries after using different modified QuEChERS ($n = 2$). B. Recoveries after using different sorbents following EN15662 ($n = 2$).

retention time, qualifier and quantifier transitions, polarity and collision energy of each analyte are listed in Table 1.

Considering the small number of commercially available isotopically labelled standards for quantification, and to avoid any matrix effects due to the complexity of the matrices, matrix-matched calibration curves were used.

2.5. Method validation and greenness

The method was validated in terms of specificity, precision (intra- and inter-day variability), recovery (accuracy), linearity, limit of detection (LOD), and limit of quantification (LOQ) in accordance with SANTE/12682/2019 method (European Commission, 2019). The validation was done on blank samples of representative beverages (water, orange juice, wine) and food commodities (potatoes and tomatoes). Blank matrices were tested before validation to confirm the absence of interferences or quantifiable trace of SDHIs. Specificity was determined by analyzing blank samples and samples spiked with a known amount of SDHIs. The precision was evaluated using five replicates of blank samples ($n = 5$) spiked at five different concentrations (Supplementary Table S1) within one day (intra-day variation) and over three consecutive days (inter-day variation). The percentage of relative standard

deviation (%RSD) was calculated as the measure of precision. The recovery of the method was evaluated by calculating the percentage of deviation between expected and measured concentrations at the five concentration levels. Linearity was evaluated in matrix solutions using six calibration levels spanning different ranges depending on the analytes and matrices. For example, in water, the tested linearity for carboxin ranged from 0.005 ng/mL to 10 ng/mL, while for bixafen M44 it ranged from 0.05 to 100 ng/mL due to sensitivity differences. The LOQ of each analyte was determined at the lowest concentration in fortified matrices at which precision, recovery (accuracy) and linearity criteria were met for validation. Matrix effects were evaluated in the different matrices by the equation: $ME\% = (\text{peak area of an analyte in a sample matrix} / \text{peak area of the analyte in a solvent solution})$. The Horwitz Ratio (HorRat) was calculated as the ratio of inter-day observed relative standard deviation (RSD%) to the predicted RSD, set at 22 % as concentrations, expressed as mass fraction, were below 1.2×10^{-7} (Horwitz & Albert, 2006). The measurement uncertainty (MU) was based on the contribution from the bias, u' (bias), and the precision from the recovery test, u' (precision), following the approach 1 of SANTE/12682/2019 (eq. 1). In case the number of recovery tests exceeds 9, MU can be approximated to u' (precision), which is equal to the RSD. The expanded uncertainty is then calculated as the overall RSD multiplied by a coverage

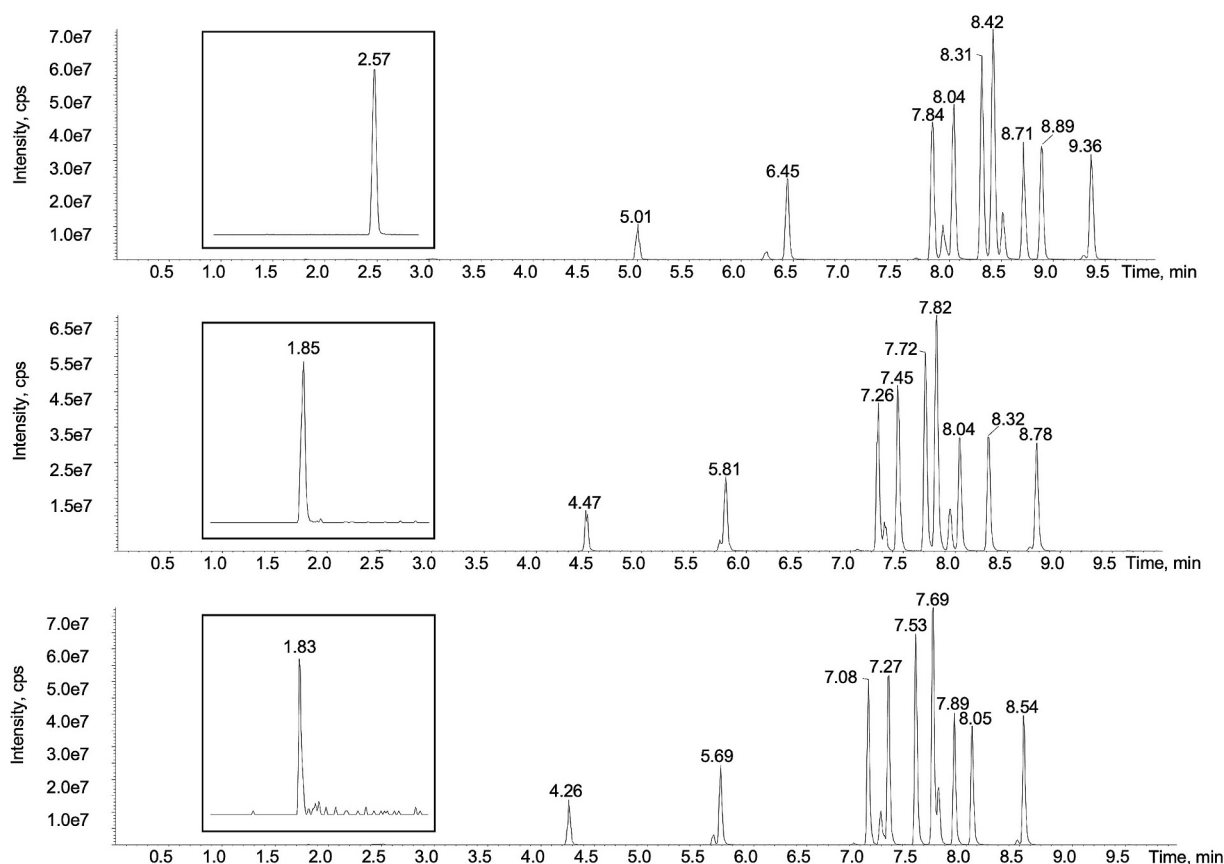


Fig. 3. Chromatograms of a standard mix (5 ng/mL in ACN:H₂O (50:50, v:v)) separated on an HSS T3 column (2.1 × 100 mm; 1.8 μm, top), BEH C18 column (2.1 × 100 mm; 1.7 μm, middle), and Cortecs C18+ column (2.1 × 100 mm, 1.6 μm, bottom). Mobile phases and gradient were kept identical. Insets: zoom on the metabolite bixafen M44.

factor $k = 2$.

$$MU = \sqrt{w(\text{bias})^2 + w(\text{precision})^2} \quad (1)$$

The greenness of the method was evaluated using the analytical GREENness metric (AGREE) approach (Pena-Pereira et al., 2020).

3. Results and discussion

3.1. Method optimization

3.1.1. Optimization of extraction method

In the QuEChERS extraction approach, the extraction solvent, salts and sorbents can be optimized to achieve acceptable recovery and cleanup.

The extraction solvent is one of the primary factors influencing recovery in the QuEChERS method. To extract pesticides, and in particular SDHIs, from aqueous matrices, an overwhelming proportion of studies have used acetonitrile, with a few reporting ethyl acetate and acetone (Banerjee et al., 2012; Chen et al., 2019; Matadha et al., 2021; Valera-Tarifa et al., 2020). Here, acetonitrile was used for its multipurpose characteristics, including coverage of a wide polarity range encompassing both hydrophobic pesticides and their polar metabolites, miscibility with water but easily separated when adding salt, and compatibility with HPLC.

Several procedures based on the QuEChERS method were evaluated on water samples in order to establish the best extraction parameters: original (Anastassiades et al., 2003), citrate known as EN15662 (European Committee for Standardization, 2008), sodium acetate known as AOAC (Lehotay et al., 2010) and ammonium acetate. In the

original QuEChERS procedure, the extraction salt was a mixture of magnesium sulfate (MgSO₄) and sodium chloride (NaCl). Ammonium acetate, sodium acetate and citrate QuEChERS procedures were composed of magnesium sulfate and respectively ammonium acetate (NH₄Ac), sodium acetate (NaOAc) and a mixture of sodium chloride, sodium citrate (Na₃C₆H₅O₇) and disodium citrate sesquihydrate. An extraction recovery between 70 % and 120 % was defined as acceptable. As shown in Fig. 2-A, for all QuEChERS methods used, the recoveries ranged from 80 % to 115 % except for three analytes, fluxapyroxad M700F001, bixafen M44 and carboxin. Fluxapyroxad M700F001 and bixafen M44 reached satisfactory recovery only with the citrate based EN15662 method. However, the recovery of carboxin decreased by a factor of 1.7 with the EN15662 method compared to the others QuEChERS methods. This might be due to the exothermic hydration reaction that can degrade carboxin into carboxin oxide. This hypothesis was confirmed by an HRMS analysis, which detected a peak corresponding to the oxidized carboxin (m/z 252.0695, proposed formula C₁₂H₁₃NO₃S) in the citrate QuEChERS extract solution. In order to reduce the exothermic reaction, tubes containing salt and solution were cooled in an ice bath. This increased the extraction recovery for carboxin to almost 100 %. The citrate based EN15662 was thus selected as the best approach for salting-out liquid-liquid extraction of SDHIs.

3.1.2. Optimization of clean-up procedure

The clean-up step is crucial for ensuring the reduction of matrix co-extractants. Considering that beverage and foodstuff matrices can be rich in lipids, pigments, sugars and other interfering compounds, magnesium sulfate (MgSO₄), primary secondary amine (PSA) and octadecyl silica (C18) were evaluated as purification adsorbents for d-SPE. Anhydrous magnesium sulfate (anh. MgSO₄) is widely used for

Table 2

Summary of validation results for all analytes at 3 to 5 fortification levels and across matrices. Only data at and above the limit of quantification (LOQ) were used to calculate recoveries and precision values. For the complete set of data, see Supplementary Table S1.

Pesticide	LOQ (ng/ml or ng/g)	Intra-day		Inter-day	
		Recovery (%)	Precision (%RSD)	Recovery (%)	Precision (%RSD)
Carboxin	0.01–0.02	85.1–108.1	1.9–14.9	83.6–109.1	2.8–14.8
Oxycarboxin	0.01–0.2	87.3–104.0	1.1–7.5	89.5–101.4	4.0–13.8
Penthiopyrad carboxamid	0.01–0.02	81.0–116.3	0.7–13.2	86.0–105.2	3.7–15.2
Fluopyram benzamide	0.01–0.02	83.6–109.9	1.6–17.4	81.9–113.7	5.4–17.7
Fluopyram	0.01–0.02	78.3–106.4	1.8–13.0	92.2–106.9	3.1–20.8
Flutolanil	0.01–0.02	84.6–106.3	2.1–20.4	88.0–103.6	4.0–16.3
Bixafen	0.01–0.02	83.6–110.4	2.2–17.6	84.1–105.7	4.7–19.9
Fluxapyroxad	0.01–0.02	84.1–104.8	2.0–18.5	87.3–103.9	3.2–17.9
Isopyrazam	0.01–0.02	89.9–108.0	1.3–9.6	91.5–107.5	3.4–15.1
Penthiopyrad	0.01–0.02	85.1–106.8	2.0–7.5	91.4–111.1	3.8–16.1
Isofetamid	0.01–0.2	80.0–111.4	5.8–18.9	82.1–118.8	7.7–20.7
Penflufen	0.01–0.02	84.3–107.6	0.9–11.5	89.4–109.8	2.0–13.5
Sedaxane	0.01–0.1	86.3–111.4	1.7–12.1	91.8–101.6	3.3–18.8
Bixafen BYF00587	0.1–0.4	82.2–111.2	2.3–21.5	84.8–103.6	3.5–18.0
Bixafen M44	0.1–0.2	78.7–121.0	1.4–16.6	73.4–110.4	3.8–23.4
Fluxapyroxad M700F001	0.1–1.0	72.9–113.8	1.5–14.8	82.3–112.2	5.1–15.3
Boscalid M510F01	0.05–0.2	88.2–112.1	2.4–17.5	86.2–114.9	3.5–17.9
Thifluzamid	0.05–0.1	82.1–109.9	1.6–20.3	83.5–115.9	3.0–18.6
Boscalid	0.05–0.1	74.3–117.5	3.3–17.6	80.1–105.3	4.6–17.4

removing water from the organic solvent and/or sample. Primary secondary amine (PSA) is mainly employed to remove acidic compounds such as organic acids, fatty acids and certain sugars. C18 is used to adsorb non-polar compounds such as fat-soluble pigments and lipids. In this work, the clean-up efficiencies of four different mixtures of sorbents (30 mg MgSO₄, 30 mg MgSO₄ + 20 mg C18, 30 mg MgSO₄ + 20 mg PSA and 30 mg MgSO₄ + 20 mg C18 + 20 mg PSA) were assessed. As shown in Fig. 2-B, the extraction recoveries for MgSO₄ ranged from 78.9 to 124 %, for (MgSO₄ + C18) they ranged from 86.2 to 128.2 %, for (MgSO₄ + PSA) from 15.8 to 115.9 %, and for (MgSO₄ + C18 + PSA) from 15.2 to 115.9 %. The recommendation of SANTE/12682/2019 regarding extraction recovery was only satisfied for MgSO₄ and (MgSO₄ + C18). PSA is known to strongly bind to acidic compounds (Kamel, 2010), hence to the carboxylic acid group present in the chemical structure of fluxapyroxad M700F001 and bixafen M44, which resulted in low recoveries. Therefore, a purification based on the combination of MgSO₄ and C18 was finally selected as the cleanup sorbent.

In order to increase the method's sensitivity, we evaluated the possibility of adding a concentration step involving evaporation and reconstitution in a smaller volume. Using centrifugal evaporation at 35 °C, satisfactory recoveries ranging from 80 % to 110 % for all SDHIs were obtained, except for carboxin for which extraction recovery was only 2 %. Decreasing the temperature to 5 °C in the evaporation chamber increased the extraction recovery for carboxin to 20 %, and partial evaporation (leaving a few microliters in the tube) further increased it to 80–100 %. However, partial evaporation can be very tricky to implement for large batches in routine analysis. Therefore, evaporation was not used in further experiments.

3.1.3. Optimization of liquid chromatography

Several chromatographic conditions were studied to achieve the best separation and retention for the SDHIs under investigation. First, three reverse phase chromatographic columns were tested: Waters Cortec C18+ (2.1 × 100 mm; 1.6 μm) for providing excellent peak shape for basic analytes at low pH, Waters BEH C18 (2.1 × 100 mm; 1.7 μm) for its universality and suitability for a diverse range of analytes and Water HSS T3 (2.1 × 100 mm; 1.8 μm) for its high retentivity of polar compounds. Each column was tested with the same mobile phases, i.e. H₂O + 0.05 % formic acid + 1 mM ammonium formate and acetonitrile + 0.05 % formic acid. Overall, the HSS T3 column offered the best compromise in terms of peak distribution along the chromatogram, peak shape and sensitivity (Fig. 3). In particular, it better retained the more

polar metabolites such as bixafen M44. Then, the influence of the mobile phase composition on retention and MS response was studied. The addition of 1 mM ammonium formate to the acidic aqueous mobile phase generally had a positive effect on signal intensity except for the two polar metabolites bixafen M44 and fluxapyroxad M700F001. The volume and the composition of solvent injected was also evaluated. One ng/mL mix solutions were prepared at different percentage of acetonitrile (from 0 % ACN to 100 % ACN) and different volumes were injected in the LC-MS/MS system. Peak intensities and peak shapes were evaluated in order to achieve the best compromise between injected volume, percentage of ACN and compound sensitivity. A clear deterioration of the peak shapes was observed with increasing injection volume and ACN percentage, especially for the most rapidly eluting analytes (Supplementary Fig. S1). The best compromise was obtained for a 2.5 μL injection volume of 50 % ACN.

3.1.4. Optimization of mass spectrometry parameters

The detection of trace levels of pesticides requires a sensitive analytical method. To maximize the sensitivity and selectivity of our analytical method, an optimization of all mass spectrometric parameters was performed (Table 1). For each SDHI, the responses in negative or positive modes were compared and the polarity chosen according to the highest signal-to-noise ratio obtained. For most analytes, the pseudo-molecular ions [M+H]⁺ or [M-H]⁻ were selected. The MRM method was developed using the most sensitive and selective transition of each analyte for quantification, and the second best transition was used for confirmation. Two MRM transitions for each SDHIs and one transition for labelled internal standards were selected, resulting in 41 transitions in total. In order to increase the sensitivity and the dwell time spent on each transition, two acquisition strategies were tested, time segments and scheduled-MRM (sMRM). The sMRM enables optimized cycle time and maximized dwell times during acquisition allowing an increased sensitivity (Lozano et al., 2012). For segmented acquisition, three periods could be set up, however the third period contained 27 transitions due to the concomitant presence of several analytes eluting in the same chromatographic region. This resulted in dwell times as low as 12 ms, which could impact sensitivity and peak shapes at low intensity. Using sMRM with an acquisition window of 30 s around each analyte, the minimum dwell time increased to 25 ms and peak shapes significantly improved for low intensity peaks (Supplementary Figs. S2 and S3). The sMRM mode was thus selected for the SDHIs method.

Table 3
Comparison of the method described here with other methods in the academic literature.

Matrix	Number of SDHIs and names	Extraction	Analysis	Recovery	LOD	LOQ	References
Surface water	8 bixafen, boscalid, fluopyram, flutolanil, fluxapyroxad, isopyrazam, penflufen, and penthiopyrad	SPE	LC-MS/MS	84–100 %	0.1–1 ng/L	0.5–5 ng/L	Gulkowska et al., 2014
Fruits and vegetables (apple, strawberry, tomato, and spinach)	7 fluxapyroxad, boscalid, fluopyram, penflufen, bixafen, penthiopyrad, and isopyrazam	QuEChERS	UHPLC-MS/MS	81–136 %	0.8–2 µg/kg	10 µg/kg	Abad-Fuentes et al., 2015
Fruits (watermelon)	7 isopyrazam, fluopyram, pydiflumetofen, boscalid, penthiopyrad, fluxapyroxad, and thifluzamide	d-SPE-DLLME	UHPLC-MS/MS	72.0–111.6 %	0.2–0.4 µg/kg	0.1 µg/kg	Wu et al., 2019
Cereals, vegetables and fruits (grape, potato, rice, corn, wheat and soybean)	9 bixafen, sedaxane, benzovindiflupyr, isopyrazam, penthiopyrad, flutolanil, fluopyram, penflufen and fluxapyroxad	QuEChERS	UHPLC-MS/MS	75.7–104.2 %	1 µg/kg	10 µg/kg	Tian et al., 2020
Wine	12 benzovindiflupyr, bixafen, boscalid, carboxin, fluopyram, flutolanil, fluxapyroxad, isopyrazam, mepronil, penflufen, penthiopyrad and sedaxane	QuEChERS	UHPLC-MS/MS	84–108 %	0.3 µg/kg	1 µg/kg	Schusterova et al., 2021
Cereals (rice, sorghum wheat, buckwheat, maize, millet, oat, glutinous rice)	18 carboxin, oxycarboxin, mepronil, flutolanil, benodanil, fenfuram, boscalid, thifluzamide, benzovindiflupyr, furametpyr, penthiopyrad, isopyrazam, fluxapyroxad, bixafen, sedaxane, penflufen, fluopyram, isofetamid and pydiflumetofen	QuEChERS	UHPLC-MS/MS	79.6–126.2 %	0.01–2.96 µg/L	10 µg/L	Zhao et al., 2022
Fruits and vegetables (tomato and potato)	12 + 7 metabolites bixafen, boscalid, carboxin, fluopyram, flutolanil, fluxapyroxad, isofetamid, isopyrazam, penflufen, penthiopyrad, sedaxane, thifluzamide, bixafen BYF00587, bixafen M44, boscalid M510F01, fluopyram benzamide, fluxapyroxad M700F001, oxycarboxin and penthiopyrad carboxamide	QuEChERS	UHPLC-MS/MS	71.0–117.5 %	0.003–0.13 ng/g	0.01–0.4 ng/g	This study
Beverage (tap water, orange juice and wine)				73.7–118.8 %	0.003–0.067 ng/mL	0.01–0.2 ng/mL	

3.2. Method validation and greenness

The method developed was validated to verify its applicability in routine analysis and ensure the reliability of the results. The method specificity, precision (intra- and inter-day variability), recovery (accuracy), linearity and limits (LOD and LOQ) were calculated to evaluate the performance of the method. In addition, the HorRat and the measurement uncertainty were calculated. Method validation was carried out in representative beverages (Milli-Q water, orange juice and red wine) and food commodities with water content under 90 % (potatoes) and above 90 % (tomatoes). The results are shown in Table 2 and Supplementary Table S1.

Analysis of blank matrices did not present any interferences at the retention times for targeted SDHIs, except for oxycarboxin in wine, demonstrating the specificity of the method. Indeed, a significant amount of oxycarboxin was present in the supposedly blank wine sample used for validation, which does not allow a proper validation for this molecule. Precision and recovery data were obtained for all SDHIs spiked into beverage and food commodities at concentrations ranging from 0.01 to 200 ng/mL ($n = 5$ at each concentration). The results summarized in Supplementary Table S1 show that, with the exception of the lowest concentrations in complex matrices, precision and recoveries were all within the norm recommendations (precision below 20 %RSD and recovery between 70 % and 120 %). Indeed, at low spiked concentrations, the noise generated by the matrices or the presence of interferences greatly reduced the precision and recoveries for a number of molecules, especially metabolites. For example, in tomato, the recovery and precision (%RSD) for fluxapyroxad M700F001 reached 150 % and 93 %, respectively, for the lowest concentration of 0.1 ng/g. Consequently, while its LOQ in water was as low as 0.1 ng/mL, it increased to 1 ng/g in tomato. In agreement with SANTE/12682/2019, LOQs were determined as the lowest analyte concentration successfully validated, i. e. meeting the requirement of an acceptable recovery within the range of

70–120 % and an RSD <20 %. LODs were estimated at one third of the LOQs. Table 2 and Supplementary Table S1 show that the LOQs values were in the range of 0.01 to 0.2 ng/mL in beverages and from 0.01 to 0.4 ng/g in food-based products, except for the metabolite fluxapyroxad M700F001 which had an LOQ of 1 ng/mL in wine and 1 ng/g in tomato.

Different calibration curves were calculated using a matrix-matched standard calibration approach in blank samples. Satisfactory linearities ($R^2 > 0.99$) were obtained for all compounds in the range of 0.01 to 20 ng/mL, 0.05 to 100 ng/mL (boscalid M510F01, thifluzamid and boscalid) and 0.1 to 200 ng/mL (bixafen BYF00587, bixafen M44, fluxapyroxad M700F001), using linear regressions weighted by 1/x. Matrix effect was found to vary depending on the analytes and matrices. No matrix effect was observed in water. By contrast, a significant matrix effect (i.e. superior to ± 20 %) was detected for three SDHIs metabolites (fluxapyroxad M700F001, penthiopyrad carboxamide, fluopyram benzamide) in orange juice, for 59 % of all SDHIs in tomatoes and for 70 % of all SDHIs in wine. In general, the more polar metabolites suffered from higher matrix effects than the less polar parent pesticides. Altogether, these results highlight the variable complexity of the different matrices and the need for matrix-matched calibration.

In order to assess the reproducibility of the method, the HorRat was calculated. HorRat values, with some exceptions at low concentrations, were below 0.5 for all SDHIs in various matrices (Supplementary Table S1). Thus, the methodology presents satisfactory reproducibility.

The estimation of the measurement uncertainty was carried out following the “top-down” model by using the data from intra-day validation of 17 SDHIs, at the 0.05 ng/g fortification level in all matrices (water, orange juice, tomato, wine and potato). The overall mean recovery (98.7 %) and RSD (15.7 %) demonstrated the fitness of the method. When considering a coverage factor (k) of 2, the expanded uncertainty was 31.3 %, which is below the ISO/IEC 17025 and SANTE/12682/2019 recommendation not to exceed the default value of 50 %.

The recovery and sensitivity of our method were compared with

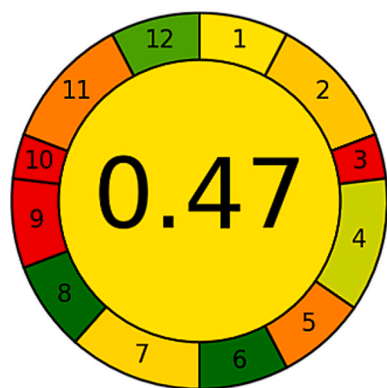


Fig. 4. Analytical greenness score obtained by AGREE software for the developed method.

other methods from the scientific literature reported for the determination of SDHIs in foodstuff matrices and beverages (Table 3). All the methods present comparable performances in terms of recovery. However, our method exhibits enhanced sensitivity, reaching LOD/LOQ up to an order of magnitude lower than other methods for given analytes and matrices.

The method greenness was evaluated using the AGREE software based on the estimation of 12 principles of green analytical chemistry combined into three main criteria: sample preparation (sample pretreatment used, sample size, number of distinct steps), reagents and solvents used (waste amount, bio-based reagents, toxic reagents, safety for the operator), and instrumentation (in-situ measurement, automated and miniaturized method, derivatization, multi-analytes/parameter method, energy used). Using this approach, our method obtained a score of 0.47 (Fig. 4), which is in alignment with standard LC-MS/MS methodologies.

3.3. Application of the methods to real samples

In order to verify the effectiveness of the method and its applicability in routine analysis, we applied it to the analysis of 28 samples (four water samples, six orange juices, six wines, eight potatoe samples, and four tomatoe samples) collected in France and Switzerland (Supplementary Table S2). No SDHI was detected in water samples, and only fluopyram benzamid was quantified from 0.11 to 0.55 ppb in orange juice. Three wine samples contained boscalid at concentrations ranging from 0.09 to 0.96 ppb, four wine samples contained fluopyram and fluopyram benzamid at concentrations between 0.02 and 1.39 ppb and between 0.03 and 0.43 ppb, respectively. In potatoes, bixafen was found in only one sample at 0.02 ppb. In tomatoes, fluopyram, fluopyram benzamid and bixafen M44 were quantified at levels between 0.98 and 34.0 ppb, 0.32 and 57.4 ppb, and at 0.20 ppb, respectively. All these values are below the maximum residue limits set by the European Commission.

4. Conclusion

In this study, a simple and reliable method was established for the determination of 12 SDHI fungicides and 7 of their metabolites in five representative beverage and food matrices (water, orange juice, red wine, potatoes and tomatoes). The proposed method shows excellent sensitivity and acceptable levels of linearity, repeatability, reproducibility, and accuracy for the 19 targeted analytes. The method will be used for future risk assessment studies on SDHIs and will provide a strong basis for the integration of these analytes into broader multi-residue methods.

CRedit authorship contribution statement

Camille Pasquet: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis. **Sylvie Guinchard:** Investigation. **Marie Villier:** Investigation. **Eléa Guyaz:** Investigation. **Jean-Marc Bonmatin:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Gaetan Glauser:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2025.144532>.

Data availability

Data will be made available on request.

References

- Abad-Fuentes, A., Ceballos-Alcantarilla, E., Mercader, J. V., Agulló, C., Abad-Somovilla, A., & Esteve-Turrillas, F. A. (2015). Determination of succinate-dehydrogenase-inhibitor fungicide residues in fruits and vegetables by liquid chromatography-tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, 407(14), 4207–4211. <https://doi.org/10.1007/s00216-015-8608-3>
- Anastassiades, M., Lehotay, S. J., Stajnbaher, D., & Schenck, F. J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and “dispersive solid-phase extraction” for the determination of pesticide residues in produce. *Journal of AOAC International*, 86(2), 412–431. <https://doi.org/10.1093/jaoac/86.2.412>
- Banerjee, K., Utture, S. C., Dasgupta, S., Kandaswamy, C., Pradhan, S., Kulkarni, S., & Adsule, P. (2012). Multiresidue determination of 375 organic contaminants including pesticides, polychlorinated biphenyls and polyaromatic hydrocarbons in fruits and vegetables by gas chromatography-triple quadrupole mass spectrometry with introduction of semi-quantification. *Journal of Chromatography A*, 1270, 283–295. <https://doi.org/10.1016/j.chroma.2012.10.066>
- Bénit, P., Goncalves, J., El Khoury, R., Rak, M., Favier, J., Gimenez-Roqueplo, A. P., & Rustin, P. (2022). Succinate dehydrogenase, succinate, and Superoxides: A genetic, epigenetic, metabolic. *Environmental Explosive Crossroad. Biomedicines*, 10(8), 1–21. <https://doi.org/10.3390/biomedicines10081788>
- Bénit, P., Kahn, A., Chretien, D., Bortoli, S., Huc, L., Schiff, M., et al. (2019). Evolutionarily conserved susceptibility of the mitochondrial respiratory chain to SDHI pesticides and its consequence on the impact of SDHIs on human cultured cells. *PLoS One*, 14(11), Article e0224132. <https://doi.org/10.1371/journal.pone.0224132>
- Chen, W., Wei, L., Li, X., Ma, H., Lou, T., Zhang, P., & Yang, G. (2021). Point mutations in FgSdhC2 or in the 5' untranslated region of FgSdhC1 confer resistance to a novel succinate dehydrogenase inhibitor Flubeneteram in fusarium graminearum. *Journal of Agricultural and Food Chemistry*, 69(44), 13006–13019. <https://doi.org/10.1021/acs.jafc.1c04363>
- Chen, X., He, S., Gao, Y., Ma, Y., Hu, J., & Liu, X. (2019). Dissipation behavior, residue distribution and dietary risk assessment of field-incurred boscalid and pyraclostrobin

- in grape and grape field soil via MWCNTs-based QuEChERS using an RRLC-QqQ-MS/MS technique. *Food Chemistry*, 274, 291–297. <https://doi.org/10.1016/j.foodchem.2018.08.136>
- Duarte-Hospital, C., Tête, A., Debizet, K., Imler, J., Tomkiewicz-Raulet, C., Blanc, E. B., & Bortoli, S. (2023). SDHi fungicides: An example of mitotoxic pesticides targeting the succinate dehydrogenase complex. *Environment International*, 108219. <https://doi.org/10.1016/j.envint.2023.108219>
- European Commission. (2019). *SANTE/12682/2019: Analytical quality control and method validation procedures for pesticide residues analysis in food and feed*.
- European Committee for Standardization. (2008). *European standard EN 15662, foods of plant origin—Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE-QuEChERS-method*. Brussels, Belgium: European Committee for Standardization.
- FRAC. (2023). Fungicide Resistance Action Committee Fungicide Mode of Action Poster 2023. Retrieved from <https://www.frac.info/publications/downloads>.
- Gulkowska, A., Buerge, I. J., & Poiger, T. (2014). Online solid phase extraction LC-MS/MS method for the analysis of succinate dehydrogenase inhibitor fungicides and its applicability to surface water samples. *Analytical and Bioanalytical Chemistry*, 406(25), 6419–6427. <https://doi.org/10.1007/s00216-014-8073-4>
- Horwitz, W., & Albert, R. (2006). The Horwitz ratio (HorRat): A useful index of method performance with respect to precision. *Journal of AOAC International*, 89, 1095–1109. <https://doi.org/10.1093/jaoac/89.4.1095>
- Kamel, A. (2010). Refined methodology for the determination of neonicotinoid pesticides and their metabolites in honey bees and bee products by liquid chromatography-tandem mass spectrometry (LC-MS/MS). *Journal of Agricultural and Food Chemistry*, 58(10), 5926–5931. <https://doi.org/10.1021/jf904120n>
- Lehotay, S. J., Son, K. A., Kwon, H., Koesukwiwat, U., Fu, W., Mastovska, K., ... Leepipatiboon, N. (2010). Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables. *Journal of Chromatography A*, 1217(16), 2548–2560. <https://doi.org/10.1016/j.chroma.2010.01.044>
- Lozano, A., Rajski, L., Belmonte-Valles, N., Uclés, A., Uclés, S., Mezcuca, M., & Fernández-Alba, A. R. (2012). Pesticide analysis in teas and chamomile by liquid chromatography and gas chromatography tandem mass spectrometry using a modified QuEChERS method: Validation and pilot survey in real samples. *Journal of Chromatography A*, 1268, 109–122. <https://doi.org/10.1016/j.chroma.2012.10.013>
- Luo, B., & Ning, Y. (2022). Comprehensive overview of Carboxamide derivatives as succinate dehydrogenase inhibitors. *Journal of Agricultural and Food Chemistry*, 70, 957–975. <https://doi.org/10.1021/acs.jafc.1c06654>
- Matadha, N. Y., Mohapatra, S., & Siddamalliah, L. (2021). Distribution of fluopyram and tebuconazole in pomegranate tissues and their risk assessment. *Food Chemistry*, 358, 8. <https://doi.org/10.1016/j.foodchem.2021.129909>
- Pena-Pereira, F., Wojnowski, W., & Tobiszewski, M. (2020). AGREE—Analytical GREENness metric approach and software. *Analytical Chemistry*, 92, 10076–10082. <https://doi.org/10.1021/acs.analchem.0c01887>
- Schusterova, D., Hajslova, J., Kocourek, V., & Pulkrabova, J. (2021). Pesticide residues and their metabolites in grapes and wines from conventional and organic farming system. *Foods*, 10(2), 307. <https://doi.org/10.3390/foods10020307>
- Sierotzki, H., & Scalliet, G. (2013). A review of current knowledge of resistance aspects for the next-generation succinate dehydrogenase inhibitor fungicides. *Phytopathology*, 103(9), 880–887. <https://doi.org/10.1094/PHYTO-01-13-0009-RVW>
- Tian, F., Qiao, C., Luo, J., Guo, L., Pang, T., Pang, R., & Xie, H. (2020). Development of a fast multi-residue method for the determination of succinate dehydrogenase inhibitor fungicides in cereals, vegetables and fruits by modified QuEChERS and UHPLC-MS/MS. *Journal of Chromatography B*, 1152, Article 122261. <https://doi.org/10.1016/j.jchromb.2020.122261>
- Umetsu, N., & Shirai, Y. (2020). Development of novel pesticides in the 21st century. *Journal of Pesticide Science*, 45(2), 54–74. <https://doi.org/10.1584/JPESTICS.D20-201>
- Valera-Tarifa, N. M., Santiago-Valverde, R., Hernández-Torres, E., Martínez-Vidal, J. L., & Garrido-Frenich, A. (2020). Development and full validation of a multiresidue method for the analysis of a wide range of pesticides in processed fruit by UHPLC-MS/MS. *Food Chemistry*, 315, Article 126304. <https://doi.org/10.1016/j.foodchem.2020.126304>
- Veloukas, T., & Karaoglanidis, G. S. (2012). Biological activity of the succinate dehydrogenase inhibitor fluopyram against *Botrytis cinerea* and fungal baseline sensitivity. *Pest Management Science*, 68, 858–864. <https://doi.org/10.1002/ps.3241>
- Wu, J., Zhi, S., Jia, C., Li, X., Zhu, X., & Zhao, E. (2019). Dispersive solid-phase extraction combined with dispersive liquid-liquid microextraction for simultaneous determination of seven succinate dehydrogenase inhibitor fungicides in watermelon by ultra high performance liquid chromatography with tandem mass sp. *Journal of Separation Science*, 42(24), 3688–3696. <https://doi.org/10.1002/jssc.201900862>
- Zhao, H., Li, M., Liu, X., Yang, J., Li, X., Chen, J., & Li, Z. (2022). Simultaneous determination of succinate-dehydrogenase-inhibitor fungicide traces in cereals by QuEChERS preparation and UPLC-MS/MS analysis. *Food Chemistry*, 396, Article 133708. <https://doi.org/10.1016/j.foodchem.2022.133708>