Mass Spectrometry
DOI: 10.5702/massspectrometry.A0172

Vol. 14 (2025), A0172

Technical Report

Stir Bar Sorptive Extraction (SBSE)-HPLC-Tandem MS-Based Method for Multi-Residue Determination of Pesticides in Drinking Water

Alex Affricano*, Silvia Serra, Alice Di Bernardo, Riccardo Aigotti, Francesco Floris, Federica Dal Bello, and Claudio Medana

Department of Molecular Biotechnology and Health Sciences (MBC), University of Torino, Via Nizza 52, 10125 Torino, Italy

Pesticide residues in water contamination represent a significant public and political issue due to their harmful effects on the environment, biodiversity, and human health, even at low concentrations. Pesticides are chemically heterogeneous, covering a wide range of Log$K_{o/w}$ values. Therefore, developing sensitive methods to detect a broad spectrum of hazardous chemicals in aqueous matrices is challenging. Gas and liquid chromatography/high-performance liquid chromatography-mass spectrometry (GC/HPLC-MS) are established tools but typically require pre-concentration steps. Stir bar sorptive extraction (SBSE) is a green, simple, automatable, and HPLC-compatible technique. This study presents a multi-residue method for determining 131 pesticides in mineral water using SBSE followed by HPLC-tandem MS. The selected pesticides, from various chemical classes, were evaluated in fortified ultra-pure and mineral water samples. The method demonstrated excellent sensitivity, with lower limits of quantification ranging from 20 to 50 ng/L for all analytes, enabling detection at trace levels. Selectivity was high (SEL% <20%), and reproducibility was confirmed with RSD% values below 20%. Intra- and interday precision tests revealed RSD% values from 0.23% to 19.81%. Trueness was validated with BIAS% below 20% at all concentrations. Uncertainty values were acceptable, with U% ranging from 1.44% to 49.24%. This SBSE-HPLC-tandem MS method is a robust, efficient, and reliable alternative to traditional approaches for routine monitoring of pesticide residues in drinking water, with quantification limits below regulatory requirements. It offers a suitable tool for public health applications, ensuring reliable pesticide detection in complex water matrices.

Please cite this article as: Mass Spectrom (Tokyo) 2025; 14(1): A0172

Keywords: SBSE**,** HPLC-MS/MS, pesticides, drinking water

(Received February 18, 2025; Accepted March 17, 2025; advance publication released online March 27, 2025)

1. INTRODUCTION

The use of pesticides is very common in modern agriculture, as it is a tool that provides excellent control for plant protection from infestations and diseases. Recent global studies showed that annual pesticide usage has almost doubled over the last 30 years, soaring to nearly 3 million tons in 2020.[1] However, only 1% of total pesticides are effectively used to control plant infections, while the huge amount of remaining pesticide residues penetrates and reaches nontarget plants, ecosystems, soil, air, and water, with deleterious effects on the environment.[2]

Because of their capability to mimic endogenous compounds (*e.g.*, hormones), it has been well demonstrated that acute and chronic exposures to pesticides may cause detrimental effects on the endocrine system,[3] human reproductive issues, an increased risk of some types of tumors (*e.g.*, breast, prostate, and brain cancer), neurotoxicity, and the potential development of neurodegenerative diseases.[4–6] Therefore, despite the success of pesticide application in terms of food security, the intensive use of pesticides poses a serious threat to human health.

The EU Pesticides Database lists more than 1378 active ingredients, 466 of which have been approved by the European Union.[7] They are characterized by an extreme variety of chemical and functional groups that correspond to very strong differences in chemical–physical properties, including ionizability, stability, and Log$K_{o/w}$ values. This high variability influences the possibility of having an analytical method capable of monitoring them simultaneously.

---

*Correspondence to: Alex Affricano, *Department of Molecular Biotechnology and Health Sciences (MBC), University of Torino, Via Nizza 52, 10125 Torino, Italy*, e-mail: alex.affricano@unito.it

As regards pesticide determination in mineral water, normative legislation varies across different regions and countries, with regulations established at the federal,[7)] regional,[8,9)] and national[10)] levels to ensure the safety and quality of mineral water products.

The European Water Framework Directive 2000/60/EC provides for the establishment and implementation of programs to monitor water status (Article 8). In particular, to verify the chemical status of water bodies, the determination of the concentration of potentially hazardous substances is required, followed by a comparison with an environmental quality standard defined as the concentration of pollutants that must not be exceeded to preserve the environment and human health. To this end, European Directive 2020/2184 (ex 98/83/EC) has been published to set maximum residue limits (MRLs) of pesticides as guidelines for their analytical determination. MRLs for drinking water are set at a limit of 100 ng/L for each pesticide or its metabolite, except for organochlorides (limit of 30 ng/L). The limit for the sum of the set and quantitatively detected pesticides and their metabolites is 500 ng/L.[11)]

Chromatographic separation coupled with mass spectrometry (GC/HPLC-MS) is recognized as a highly sensitive and specific technique for environmental organic trace contaminants and is the most commonly used technique by laboratories dealing with pesticide analysis.[12,13)] Nevertheless, at least one sample pre-treatment and extraction step is necessary to detect these very low concentrations.

For this purpose, solid-phase extraction (SPE) is usually employed,[14)] although in recent decades there has been an increase in the use of microextraction techniques (*e.g.*, SPME and SBSE) due to their advantages in reducing or even eliminating the use of toxic organic solvents and reducing the required sample volumes.[15)]

Solid phase microextraction (SPME) is a microextraction technique that eliminates solvents, simplifying the process by integrating sampling, extraction, concentration, and sample introduction in a single step.[16)] However, it faces limitations such as short fiber lifespan, high cost, fragility, carryover effects, and lack of selectivity in complex matrix analyte extraction. The type of absorbent polymer used as coating is crucial, impacting recovery and selectivity. SPME coupled with GC-MS has been applied for the simultaneous extraction of pesticides with similar characteristics but has limits in multi-residue pesticide analysis due to different fiber affinities required.[17)] Commercially available fibers have a restricted polarity range, reducing applicability for simultaneously extracting compounds with different polarities. Additionally, most fiber coatings exhibit low chemical resistance to nonpolar organic solvents and low thermostability. The amount of extracting phase in SPME fiber is limited (*e.g.*, a 100-µm-thick fiber corresponds to about 0.5 µL volume), resulting in low extraction efficiency for partially water-soluble solutes.

Stir bar sorptive extraction (SBSE) allows overcoming these limitations. It is a technique for extracting and enriching not only thermosensitive but also thermoresistant organic compounds from aqueous matrices onto a polymer coating. The extraction employs magnetic stir bars encapsulated in a glass jacket and coated with an absorbing layer (0.5–1 mm thick) of polydimethylsiloxane (PDMS), providing an extracting phase volume 50–250 times greater than that of SPME fibers.[18)] This extraction technique allows working without organic solvents, making the extraction method environmentally friendly and reducing potential systematic errors by the operator. Additionally, it enables quantitative analysis over a wide linear range, ensuring very low detection limits. Similarly to SPME, SBSE is an equilibrium technique based on the solute partition coefficient between the polymeric phase and the aqueous phase. Unlike adsorption-based techniques, where the analyte interacts with active sites on a surface, here the solute migrates into the absorbing phase and, as a consequence, in SBSE, not only the surface but also the volume of the phase is relevant.[19)]

Another advantage of SBSE is that it can be directly interfaced not only with GC-MS but also with HPLC-MS by using thermodesorption and microvolume liquid desorption for analyte recovery from the stir bar. This makes SBSE an extremely interesting technique for multi-residue analysis, allowing the determination of both thermosensitive and thermostable analytes thanks to its dual interfacing capability while still ensuring excellent sample enrichment performance.[20–23)]

Herein, we aimed to set up a multi-residue method based on SBSE for the determination *via* HPLC-tandem MS of pesticides in mineral water. In particular, 131 thermostable pesticides, whose determination is required by European health control authorities, were selected. They fall into a range of chemical classes, with triazines, organophosphates, carbamates, amides, and triazoles being the most prevalent groups. After its implementation and validation, the method was applied to real mineral water samples collected from various springs in Central and Northern Italy.

2. MATERIALS AND METHODS

2.1. Chemicals

Pesticide standards of analytical grade were purchased from Lab Instruments (Bari, Italy) and CPA-Chem (Bogomilovo, Bulgaria). Furthermore, deuterated pesticide standards were obtained from Restek Corporation (Milan, Italy). All the pesticide standards were purchased in acetonitrile, acetone, or toluene and subsequently diluted in methanol or methanol/water (50:50, v/v). Each pesticide standard solution contains only the analyte or a variable number of components, including the pesticides of interest for this work (see Supporting Information—Table S1 for the list of pesticides and further details). Ultrapure water was produced using a VWR apparatus (Milan, Italy). Methanol LC-MS grade and chloroform were purchased from VWR Chemicals (Leuven, Belgium), while formic acid and ammonium formate were bought, respectively, from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, MO, USA).

2.2. SBSE extraction protocol

PDMS-coated stir bars with 47 µL of phase volume were purchased from Gerstel (Mülheim an der Ruhr, Germany). The volume of the PDMS layer (47 µL) depends on the specific stir bar used and is determined by the product of the film thickness (0.5 mm, in this case) and the length of the stir bar (20 mm, in this case). Before performing SBSE extraction, stir bars underwent a conditioning step. Stir bars were conditioned by immersing them in a methanol/chloroform (50:50, v/v) solution for 30 min in an ultrasonic bath and drying them with a lint-free tissue before the extraction step. In the optimized procedure, aliquots of 40 mL of water samples were placed in amber glass vials and sealed with a screw cap. This allowed for maintaining a volume phase ratio (β factor) of 833 while also ensuring a significant concentration factor before the analytical phase. To check the method at the extraction level, each water sample was spiked with three deuterated standards (Diazinon-d_{10}, Carbaryl-d_7, and Atrazine-d_5) at a constant concentration (50 ng/L). Then, stir bars were added and stirred at a rate of 700 rpm for 4 h at room temperature. Several samples were processed simultaneously using a 15-position magnetic stirrer (Thermo Scientific, Waltham, MA, USA). After the SBSE step, the stir bars were removed with forceps, dried with a lint-free tissue, and placed in a glass reduction unit inside a 1.5 mL vial for the liquid desorption step. Then, 200 µL of a methanol/water (50:50, v/v) solution was added, and the sealed vials with stir bars were placed in an ultrasonic bath for 10 min at room temperature. The desorption solution was spiked with another two deuterated pesticides (Diuron-d_6 and Dimethoate-d_6) at a concentration of 10 µg/L to evaluate the method at the injection level. After removing the stir bar using a specific magnetic rod, the samples were stored at 4°C while stir bars were reconditioned by washing them using the same washing solvent and conditions as in the conditioning step.

2.3. Instrumentation

Separation and analysis of all analytes and samples were achieved using an HPLC-TQ MS system, which consisted of a Shimadzu LCMS-8045 platform (Shimadzu Corporation, Kyoto, Japan). The qualitative and quantitative post-run analysis of the samples was performed using Shimadzu's LabSolutions software platform.

Chromatographic separation was carried out using a reverse-phase octadecyl silica column (Raptor C18, 2.7 µm, 150 × 3.0 mm; Restek, Bad Homburg, Germany). The oven temperature was set at 40°C. A volume of 10 µL of each sample was injected into the LC system, and compounds were separated with a gradient of aqueous methanol. Eluent A consisted of water with 0.2% formic acid and 0.1% ammonium formate, whereas eluent B consisted of methanol with 0.2% formic acid and 0.1% ammonium formate. After injection, the composition changed from 50% eluent A to 95% eluent B within 20 min and was then held at 95% eluent B for 7 min. Subsequently, the initial running condition (50% eluent A) was restored in 1 min, and the column was re-equilibrated for 8 min, with an overall run time of 36 min. The flow rate was 0.175 mL/min.

The measurements were performed in multiple reaction monitoring in electrospray-positive and electrospray-negative ion mode (ESI+/−). Method optimization was performed by direct injection of standard mixtures of pesticides. The nebulizing gas flow was set at 3 L/min while the heating and drying gas flows were set at 10 L/min. The interface temperature was kept at 300°C. To increase sensitivity and specificity, some detection parameters, such as Q1 pre-bias (voltage that promotes the ionization of the precursor ion), Q3 pre-bias (voltage that promotes the ionization of the product ion), and collision energy, were optimized for each analyte. Detection parameters, precursor ions, and product ions used for each pesticide are listed in Table 1. The method was scheduled, and a time window of 3 min was maintained for each pesticide. This strategy ensures that the maximum dwell time does not exceed the range of 1–21 ms.

2.4. Method validation

The following paragraph describes the process of creating calibration curves in the 2–200 ng/L range and validating the calibration model. The analysis was automated to iterate on all pesticides and was performed using Python (version 3.12). The analysis process followed guidelines from the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). This research will mainly follow a revised version of the approach shown in the literature.[24,25] Among the calibration models, we considered only linear models. The following steps were performed:

- Heteroscedasticity detection using the White test.
- Selection of the weighting scheme that generated the smallest spread of weighted normalized variances. The following weighting schemes were considered: 1, $1/x$, $1/x^2$, and $1/\sqrt{x}$.
- Calculation of the parameters of the chosen regression model: intercept, slope, and Pearson squared correlation coefficient (R^2).
- Validation of the model through the Kolmogorov–Smirnov test for the analysis of the normality of residuals.

The results can be seen in Supporting Information—Table S2.

The method validation was performed following Eurachem guidelines.[26] The calibration curves were obtained using a pesticide-free water sample by performing a standard addition method. The different validation parameters were calculated in agreement with the validation protocol, which involves obtaining three sets of replicates per day over three different days.

The calibration curves were obtained in fortified purified water after verifying that there are no relevant differences compared to mineral water (Student's t-test evaluation; see Section 3.4). The inter- and intraday replicates were performed by fortifying the sample matrices with different known concentrations of pesticide analytes and a constant concentration of extraction spiked standards (50 ng/L) and syringe spiked standards (10 µg/L) added, respectively, before and after the extraction step. The standard addition was performed at 5–7 levels from 20 up to 200 ng/L (equivalent to 2 times the MRL), depending on the analyte. In any case, the number of calibration levels always remained above the minimum required number ($l = 4$).

Specifically, the parameters evaluated were as follows: selectivity (SEL%), limit of detection (LOD), lower limit of quantification (LLOQ), trueness (BIAS%), precision (RSD%), and expanded uncertainty (U%). In addition, repeatability at extraction and injection levels was evaluated on extraction spiked ($\mathrm{RSD_{surr}}$%) and syringe spiked standard ($\mathrm{RSD_{is}}$%) signal, respectively.

3. RESULTS

The total ion chromatograms are presented in Fig. 1, highlighting the chromatographic peaks associated with each compound. To generate these chromatograms, a pesticide-free water sample was fortified with all pesticides at a concentration of 200 ng/L, and the most intense SRM transition

Table 1. Experimental details (time window, event time, transitions, dwell time, collision energy, and Q1 and Q3 pre-bias) of all the evaluated pesticides in HPLC-MS^2. HPLC-MS^2, high-performance liquid chromatography-tandem mass spectrometry.

Pesticide name	Acquisition time, start (s)	Acquisition time, end (s)	Event time (s)	First transition (*m/z*→*m/z*)	Dwell time (1) (ms)	Q1 pre-bias (1) (V)	Collision energy (1) (V)	Q3 pre-bias (1) (V)	Second transition (*m/z*→*m/z*)	Dwell time (2) (ms)	Q1 pre-bias (2) (V)	Collision energy (2) (V)	Q3 pre-bias (2) (V)
2,6-Dichlobenzamide	5.058	8.058	0.014	190.0→163.0	4.0	−10.0	−22.0	−28.0	190.0→136.0	4.0	−14.0	−29.0	−23.0
Ametoctradin	17.977	20.977	0.024	276.0→176.0	9.0	−12.0	−39.0	−29.0	276.0→149.0	9.0	−11.0	−37.0	−24.0
Ametryn	8.001	11.001	0.012	228.1→186.1	3.0	−10.0	−18.0	−18.0	228.1→96.0	3.0	−12.0	−18.0	−21.0
Atrazine	10.345	13.345	0.018	216.0→174.0	3.0	−14.0	−18.0	−17.0	216.0→104.0	3.0	−10.0	−29.0	−15.0
Azinophos-ethyl	15.168	18.168	0.024	346.0→97.0	3.0	−20.0	−32.0	−15.0	346.0→160.0	3.0	−10.0	−10.0	−15.0
Azinophos-methyl	12.072	15.072	0.024	318.0→160.0	5.0	−12.0	−13.0	−14.0	318.0→125.0	5.0	−15.0	−27.0	−19.0
Azoxystrobin	12.603	15.603	0.024	404.0→372.0	3.0	−12.0	−15.0	−23.0	404.0→344.1	3.0	−16.0	−24.0	−15.0
Benalaxyl	17.430	20.430	0.024	326.2→148.2	9.0	−12.0	−22.0	−13.0	326.2→208.2	9.0	−10.0	−16.0	−20.0
Bifenazate	15.176	18.176	0.024	301.2→198.1	9.0	−10.0	−9.0	−12.0	301.2→170.1	9.0	−10.0	−19.0	−16.0
Bixafen	16.542	19.542	0.024	414.0→394.0	9.0	−12.0	−14.0	−25.0	414.0→266.0	9.0	−22.0	−27.0	−27.0
Boscalid	13.656	16.656	0.024	343.0→307.0	5.0	−13.0	−19.0	−20.0	343.0→140.0	5.0	−12.0	−24.0	−22.0
Bromuconazole	16.997	19.997	0.024	378.0→70.0	9.0	−12.0	−26.0	−26.0	378.0→159.0	9.0	−15.0	−22.0	−20.0
Bupirimate	12.970	15.970	0.024	317.0→166.0	5.0	−12.0	−25.0	−30.0	317.0→237.2	5.0	−16.0	−20.0	−10.0
Buprofezin	19.725	22.725	0.024	306.0→201.0	3.0	−12.0	−12.0	−12.0	306.0→86.0	3.0	−19.0	−24.0	−14.0
Cadusafos	18.925	21.925	0.024	271.1→159.1	5.0	−10.0	−14.0	−15.0	271.1→97.0	5.0	−14.0	−36.0	−14.0
Carbaryl	8.583	11.583	0.012	202.1→145.1	3.0	−10.0	−10.0	−24.0	202.1→127.1	3.0	−11.0	−25.0	−21.0
Carbendazim	2.474	5.474	0.012	192.1→160.1	3.0	−10.0	−17.0	−15.0	192.1→132.1	3.0	−10.0	−28.0	−21.0
Carbofuran	7.699	10.699	0.012	222.3→165.1	3.0	−14.0	−13.0	−16.0	222.3→123.1	3.0	−14.0	−21.0	−20.0
Carfentrazone-ethyl	17.186	20.186	0.024	429.1→412.0	9.0	−14.0	−12.0	−26.0	429.1→346.0	9.0	−11.0	−30.0	−15.0
Chlorotoluron	10.036	13.036	0.018	212.9→72.1	3.0	−10.0	−24.0	−28.0	212.9→46.1	3.0	−10.0	−16.0	−20.0
Chlorpropham	10.805	13.805	0.010	214.0→172.0	2.0	−14.0	−13.0	−15.0	214.0→136.0	2.0	−15.0	−16.0	−13.0
Chlorpyrifos	22.034	25.034	0.024	350.0→198.0	9.0	−10.0	−19.0	−18.0	350.0→97.0	9.0	−12.0	−34.0	−14.0
Clodimafop-propargyl	16.887	19.887	0.024	349.9→266.1	9.0	−13.0	−14.0	−28.0	349.9→91.2	9.0	−10.0	−28.0	−16.0
Clomazone	12.397	15.397	0.008	240.0→125.1	1.0	−18.0	−19.0	−20.0	240.0→89.0	1.0	−30.0	−16.0	−14.0
Cloquintocet-mexylhexyl ester	20.805	23.805	0.024	336.1→238.1	9.0	−11.0	−16.0	−24.0	336.1→179	9.0	−14.0	−12.0	−14.0
Cyazofamid	15.598	18.598	0.024	325.1→108.1	9.0	−10.0	−12.0	−17.0	325.1→44.2	9.0	−17.0	−53.0	−13.0
Cyflufenamid	18.247	21.247	0.024	413.0→295.2	9.0	−10.0	−16.0	−13.0	413.0→241.0	9.0	−10.0	−23.0	−24.0
Cyproconazole	14.061	17.061	0.012	291.9→70.1	1.0	−23.0	−39.0	−21.0	291.9→125.0	1.0	−24.0	−30.0	−24.0
Cyprodinil	13.567	16.567	0.008	226.1→77.1	1.0	−16.0	−33.0	−25.0	226.1→93.1	1.0	−15.0	−26.0	−13.0
Diazinon	17.85	20.85	0.024	304.9→169.2	5.0	−11.0	−21.0	−15.0	304.9→96.9	5.0	−10.0	−32.0	−16.0
Dichlorvos	7.696	10.696	0.016	221.0→109.0	5.0	−11.0	−18.0	−18.0	221.0→127.0	5.0	−9.0	−14.0	−15.0
Difenoconazole	19.084	22.084	0.024	406.1→251.1	3.0	−13.0	−25.0	−24.0	406.1→152.0	3.0	−11.0	−53.0	−24.0
Diflubenzuron	16.310	19.310	0.024	311.1→158.1	9.0	−17.0	−14.0	−15.0	311.1→141.1	9.0	−18.0	−36.0	−20.0
Diflufenican	19.609	22.609	0.024	395.1→266.2	9.0	−12.0	−22.0	−15.0	395.1→245.9	9.0	−12.0	−37.0	−22.0

(Continued)

Pesticide name	Acquisition time, start (s)	Acquisition time, end (s)	Event time (s)	First transition (*m/z*→*m/z*)	Dwell time (1) (ms)	Q1 pre-bias (1) (V)	Collision energy (1) (V)	Q3 pre-bias (1) (V)	Second transition (*m/z*→*m/z*)	Dwell time (2) (ms)	Q1 pre-bias (2) (V)	Collision energy (2) (V)	Q3 pre-bias (2) (V)
Dimethenamid	13.426	16.426	0.024	278.0→246.0	5.0	−10.0	−14.0	−25.0	278.0→168.1	5.0	−15.0	−24.0	−27.0
Dimethomorph	14.121	17.121	0.024	388.2→301.1	5.0	−12.0	−21.0	−19.0	388.2→165.1	5.0	−12.0	−32.0	−15.0
Dithianon	7.930	10.930	0.012	297.0→201.0	3.0	−11.0	−18.0	−19.0	297.0→269.0	3.0	−21.0	−28.0	−20.0
Emamectin	19.539	22.539	0.024	886.4→158.2	9.0	−22.0	−40.0	−14.0	886.4→82.2	9.0	−22.0	−55.0	−13.0
Ethiofencarb	9.894	12.894	0.012	225.9→107.1	3.0	−10.0	−16.0	−17.0	225.9→164.1	3.0	−14.0	−8.0	−15.0
Ethion	21.280	24.280	0.024	385.0→143.0	9.0	−12.0	−25.0	−12.0	385.0→97.0	9.0	−12.0	−43.0	−16.0
Ethirimol	3.989	6.989	0.012	210.2→140.2	3.0	−11.0	−22.0	−23.0	210.2→98.1	3.0	−11.0	−26.0	−16.0
Ethoprophos	15.790	18.790	0.035	243.0→131.0	4.0	−12.0	−21.0	−20.0	243.0→158.0	4.0	−15.0	−24.0	−25.0
Etoxazole	22.183	25.183	0.024	360.0→141.0	5.0	−11.0	−30.0	−23.0	360.0→113.0	5.0	−11.0	−55.0	−18.0
Fenamidone	13.428	16.428	0.024	312.1→92.1	9.0	−13.0	−25.0	−14.0	312.1→236.2	9.0	−10.0	−14.0	−23.0
Fenamiphos	16.537	19.537	0.024	304.0→217.0	9.0	−12.0	−23.0	−21	304.0→202.0	9.0	−23.0	−35.0	−19.0
Fenbuconazole	16.091	19.091	0.024	337.0→125.1	5.0	−14.0	−28.0	−22.0	337.0→70.1	5.0	−17.0	−20.0	−27.0
Fenothiocarb	16.812	19.812	0.024	254.1→72.1	9.0	−10.0	−18.0	−28.0	254.1→160.1	9.0	−10.0	−9.0	−28.0
Fenoxaprop-P-ethyl	20.266	23.266	0.024	362.1→288.1	9.0	−11.0	−17.0	−29.0	362.1→121.2	9.0	−11.0	−29.0	−19.0
Fenoxycarb	16.620	19.620	0.024	301.9→88.1	9.0	−12.0	−21.0	−15.0	301.9→116.2	9.0	−13.0	−11.0	−18.0
Fenpropidin	10.432	13.432	0.008	274.1→147.2	1.0	−13.0	−26.0	−13.0	274.1→117.1	1.0	−10.0	−53.0	−18.0
Fenpyrazamine	14.419	17.419	0.024	332.0→230.0	9.0	−10.0	−19.0	−22.0	332.0→304.0	9.0	−12.0	−14.0	−19.0
Fluazinam	21.029	24.029	0.024	463.0→416.0	9.0	17.0	19.0	12.0					
Flufenacet	15.618	18.618	0.024	364.1→152.1	5.0	−11.0	−18.0	−14.0	364.1→194.2	5.0	−12.0	−10.0	−19.0
Fluopicolide	14.337	17.337	0.024	383.0→173.1	9.0	−12.0	−21.0	−16.0	383.0→109.1	9.0	−10.0	−55.0	−17.0
Fluopyram	15.395	18.395	0.024	397.0→208.0	9.0	−15.0	−22.0	−20.0	397.0→173.0	9.0	−16.0	−29.0	−16.0
Flusilazole	16.619	19.619	0.024	315.9→247.2	9.0	−14.0	−18.0	−10.0	315.9→165.1	9.0	−11.0	−25.0	−15.0
Folpet	14.877	17.877	0.024	313.0→260.0	9.0	−12.0	−20.0	−29.0	313.0→130.0	9.0	−20.0	−12.0	−21.0
Furathiocarb	20.499	23.499	0.024	383.2→195.1	9.0	−12.0	−18.0	−18.0	383.2→252.1	9.0	−12.0	−12.0	−15.0
Heptenophos	11.636	14.636	0.012	251.0→127.0	3.0	−10.0	−13.0	−20.0	251.0→108.9	3.0	−10.0	−27.0	−16.0
Hexaconazole	18.125	21.125	0.024	314.3→70.1	9.0	−10.0	−23.0	−29.0	314.3→159.0	9.0	−21.0	−34.0	−14.0
Hexythiazox	21.734	24.734	0.024	353.1→228.1	9.0	−11.0	−17.0	−22.0	353.1→168.1	9.0	−12.0	−27.0	−28.0
Indoxacarb	19.086	22.086	0.024	527.9→203.0	3.0	−20.0	−40.0	−20.0	527.9→249.0	3.0	−22.0	−16.0	−23.0
Iprovalicarb	15.225	18.225	0.024	321.0→119.2	5.0	−12.0	−20.0	−19.0	321.0→144.1	5.0	−13.0	−15.0	−13.0
Isoproturon	10.881	13.881	0.018	207.0→72.1	3.0	−15.0	−21.0	−27.0	207.0→46.2	3.0	−14.0	−17.0	−19.0
Isopyrazam	19.150	22.150	0.024	360.0→244.0	9.0	−10.0	−22.0	−25.0	360.0→250.0	9.0	−12.0	−32.0	−23.0
Isoxaben	13.965	16.965	0.024	333.7→165.1	9.0	−12.0	−16.0	−15.0	333.70→150.1	9.0	−12.0	−38.0	−14.0
Isoxadifen-ethyl	16.884	19.884	0.024	313.0→232.0	9.0	−24.0	−20.0	−22.0	313.0→204.0	9.0	−19.0	−29.0	−20.0
Isoxaflutole	10.812	13.812	0.008	360.0→251.0	1.0	−27.0	−18.0	−24.0	360.0→220.0	1.0	−21.0	−14.0	−17.0
Linuron	13.049	16.049	0.309	249.0→160.0	10.0	−10.0	−17.0	−15.0	249.0→182.0	10.0	−12.0	−16.0	−18.0
Malathion	14.306	17.306	0.012	331.0→127.0	1.0	−10.0	−15.0	−22.0	331.0→99.0	1.0	−13.0	−23.0	−15.0

(Continued)

Table 1. Continued

Pesticide name	Acquisition time, start (s)	Acquisition time, end (s)	Event time (s)	First transition (*m/z*→*m/z*)	Dwell time (1) (ms)	Q1 pre-bias (1) (V)	Collision energy (1) (V)	Q3 pre-bias (1) (V)	Second transition (*m/z*→*m/z*)	Dwell time (2) (ms)	Q1 pre-bias (2) (V)	Collision energy (2) (V)	Q3 pre-bias (2) (V)
Mandipropamid	13.589	16.589	0.024	412.0→328.1	3.0	−10.0	−13.0	−21.0	412.0→125.0	3.0	−11.0	−40.0	−20.0
Mefenpyr	13.048	16.048	0.024	317.0→166.0	9.0	−12.0	−24.0	−15.0	317.0→150.0	9.0	−12.0	−21.0	−25.0
Mefenpyr-diethyl	18.877	21.877	0.024	390.0→327.0	9.0	−11.0	−16.0	−30.0	390.0→190.0	9.0	−19.0	−46.0	−16.0
Mepanipyrim	15.020	18.020	0.024	224.1→106.1	9.0	−13.0	−26.0	−17.0	224.1→79	9.0	−16.0	−31.0	−14.0
Metalaxyl	11.044	14.044	0.012	280.2→220.2	1.0	−15.0	−12.0	−21.0	280.2→192.2	1.0	−17.0	−19.0	−11.0
Metazachlor	10.351	13.351	0.015	278.0→134.0	2.0	−21.0	−25.0	−12.0	278.0→210.0	2.0	−28.0	−10.0	−20.0
Metconazole	18.283	21.283	0.024	320.0→70.2	9.0	−12.0	−25.0	−27.0	320.0→125.1	9.0	−12.0	−38.0	−20.0
Methidathion	11.532	14.532	0.024	302.90→145.0	9.00	−12.0	−9.0	−26.0	302.90→85.10	9.0	−12.0	−22.0	−13.0
Methiocarb	13.176	16.176	0.018	225.9→169.1	3.0	−10.0	−9.0	−15.0	225.90→107.1	3.0	−11.0	−16.0	−18.0
Methoxyfenozide	13.960	16.960	0.024	369.0→313.2	9.0	−15.0	−8.0	−14.0	369.0→149.1	9.0	−15.0	−19.0	−13.0
Metobromuron	10.119	13.119	0.018	259.0→170.0	3.0	−10.0	−19.0	−27.0	261.0→172.0	3.0	−12.0	−32.0	−13.0
Metolachlor	15.851	18.851	0.024	284.0→252.0	5.0	−11.0	−16.0	−25.0	284.0→176.0	5.0	−21.0	−25.0	−30.0
Metrafenone	18.554	21.554	0.024	408.9→209.2	9.0	−12.0	−15.0	−12.0	408.9→227.1	9.0	−16.0	−20.0	−22.0
Molinate	14.914	17.914	0.008	187.9→126.2	1.0	−23.0	−13.0	−28.0	187.9→98	1.0	−21.0	−26.0	−16.0
Myclobutanil	14.639	17.639	0.024	289.1→70.1	5.0	−12.0	−22.0	−30.0	289.1→125.0	5.0	−17.0	−33.0	−19.0
Oxadiazon	21.198	24.198	0.024	345.0→303.0	5.0	−14.0	−13.0	−30.0	345.0→220.0	5.0	−25.0	−24.0	−21.0
Paclobutrazol	14.133	17.133	0.008	294.1→70.2	1.0	−11.0	−35.0	−10.0	294.1→125.1	1.0	−12.0	−29.0	−22.0
Parathion-methyl	12.054	15.054	0.018	264.0→232.0	3.0	−12.0	−14.0	−14.0	264.0→125.0	3.0	−15.0	−22.0	−10.0
Penconazole	17.446	20.446	0.024	284.1→70.1	5.0	−15.0	−16.0	−27.0	284.1→159.0	5.0	−16.0	−31.0	−27.0
Pethoxamid	15.699	18.699	0.024	296.0→131.2	3.0	−11.0	−21.0	−12.0	296.0→91.2	3.0	−11.0	−39.0	−14.0
Phenmedipham	11.413	14.413	0.008	318.2→93.1	1.0	−18.0	−41.0	−15.0	318.2→181.0	1.0	−15.0	−22.0	−25.0
Phosmet	12.071	15.071	0.024	318.0→160.0	5.0	−18.0	−11.0	−28.0	318.0→133.0	5.0	−16.0	−35.0	−20.0
Phosphamidon	6.263	9.263	0.012	300.0→174.0	3.0	−11.0	−14.0	−16.0	300.0→127.0	3.0	−11.0	−25.0	−20.0
Picoxystrobin	16.661	19.661	0.024	368.1→145.1	9.0	−11.0	−21.0	−13.0	368.1→205.1	9.0	−11.0	−10.0	−20.0
Pinoxaden	18.421	21.421	0.024	401.0→317.0	3.0	−10.0	−23.0	−19.0	401.0→57.0	3.0	−10.0	−32.0	−19.0
Piperonyl butoxide	21.262	24.262	0.024	356.0→177.0	9.0	−13.0	−13.0	−17.0	356.0→119.0	9.0	−14.0	−35.0	−19.0
Pirimicarb	3.984	6.984	0.016	239.0→72.1	5.0	−12.0	−23.0	−29.0	239.0→182.2	5.0	−17.0	−15.0	−16.0
Pirimiphos-ethyl	20.341	23.341	0.024	334.0→198.0	9.0	−12.0	−22.0	−19.0	334.0→182.0	9.0	−13.0	−21.0	−17.0
Pirimiphos-methyl	17.576	20.576	0.024	306.0→108.0	5.0	−11.0	−32.0	−17.0	306.0→164.0	5.0	−12.0	−23.0	−29.0
Prochloraz	16.662	19.662	0.024	376.0→308.0	9.0	−12.0	−12.0	−13.0	376.0→70.1	9.0	−12.0	−26.0	−26.0
Propaquizafop	20.752	23.752	0.024	444.0→100.0	9.0	−10.0	−22.0	−16.0	444.0→299.0	9.0	−14.0	−24.0	−30.0
Propazine	13.64	16.64	0.018	229.9→146.1	3.0	−10.0	−23.0	−23.0	229.9→188.2	3.0	−10.0	−16.0	−18.0
Propiconazole	17.867	20.867	0.024	342.1→159.0	5.0	−14.0	−28.0	−26.0	342.1→69.2	5.0	−10.0	−20.0	−27.0
Propoxur	7.774	10.774	0.014	209.9→111.1	4.0	−16.0	−13.0	−17.0	209.9→168.1	4.0	−16.0	−8.0	−16.0
Propyzamide	14.282	17.282	0.010	256.0→190.0	2.0	−25.0	−13.0	−12.0	256.0→173.0	2.0	−10.0	−21.0	−29.0

(Continued)

Pesticide name	Acquisition time, start (s)	Acquisition time, end (s)	Event time (s)	First transition (*m/z*→*m/z*)	Dwell time (1) (ms)	Q1 pre-bias (1) (V)	Collision energy (1) (V)	Q3 pre-bias (1) (V)	Second transition (*m/z*→*m/z*)	Dwell time (2) (ms)	Q1 pre-bias (2) (V)	Collision energy (2) (V)	Q3 pre-bias (2) (V)
Pyraclostrobin	18.179	21.179	0.024	388.0→194.1	5.0	−15.0	−13.0	−19.0	388.0→163.1	5.0	−12.0	−24.0	−15.0
Pyraflufen-ethyl	17.415	20.415	0.024	412.9→339.1	9.0	−12.0	−20.0	−20.0	412.9→253.1	9.0	−10.0	−36.0	−27.0
Pyridaphenthion	14.767	17.767	0.024	340.9→189.2	9.0	−13.0	−22.0	−17.0	340.9→205.1	9.0	−11.0	−22.0	−19.0
Pyrimethanil	10.194	13.194	0.018	200.1→107.1	3.0	−21.0	−25.0	−17.0	200.1→82.2	3.0	−11.0	−27.0	−14.0
Pyriproxyfen	21.493	24.493	0.024	322.1→96.1	9.0	−12.0	−14.0	−15.0	322.1→227.1	9.0	−14.0	−15.0	−22.0
Quinalphos	16.953	19.953	0.024	298.6→163.1	9.0	−19.0	−24.0	−30.0	298.6→147.1	9.0	−11.0	−22.0	−28.0
Quinoxyfen	21.740	24.740	0.024	308.0→197.0	9.0	−10.0	−33.0	−18.0	308.0→162.0	9.0	−10.0	−49.0	−15.0
Quizalofop-P-ethyl	20.220	23.220	0.024	373.0→299.0	9.0	−15.0	−20.0	−30.0	373.0→271.0	9.0	−15.0	−24.0	−26.0
Simazine	7.847	10.847	0.024	201.9→124.2	5.0	−17.0	−18.0	−21.0	201.9→132.1	5.0	−15.0	−18.0	−12.0
Spinosad	16.436	19.436	0.024	732.3→142.2	9.0	−28.0	−33.0	−13.0	732.3→98.1	9.0	−22.0	−55.0	−15.0
Spiroxamine	11.958	14.958	0.010	298.2→144.2	2.0	−13.0	−22.0	−29.0	298.2→100.2	2.0	−23.0	−30.0	−15.0
Tebuconazole	17.293	20.293	0.024	308.0→70.1	5.0	−12.0	−25.0	−25.0	308.0→125.0	5.0	−11.0	−35.0	−22.0
Tebufenozide	16.693	19.693	0.024	353.1→133.1	5.0	−19.0	−22.0	−20.0	353.1→105.1	5.0	−13.0	−41.0	−16.0
Tebufenpyrad	20.593	23.593	0.024	334.2→117.1	9.0	−10.0	−37.0	−10.0	334.2→145.1	9.0	−13.0	−27.0	−13.0
Terbuthylazine	13.467	16.467	0.309	230.0→174.0	100.0	−16.0	−16.0	−16.0	230.0→104.0	100.0	−16.0	−33.0	−16.0
Terbuthylazine-desethyl	8.679	11.679	0.024	201.9→146.1	5.0	−15.0	−16.0	−24.0	201.9→104.0	5.0	−14.0	−26.0	−16.0
Terbutryn	10.731	13.731	0.018	242.0→186.2	3.0	−17.0	−19.0	−18.0	242.0→68.1	3.0	−16.0	−44.0	−25.0
Tetrachlorvinphos	16.958	19.958	0.024	367.0→127.0	9.0	−11.0	−13.0	−11.0	367.0→242.0	9.0	−23.0	−28.0	−24.0
Tetraconazole	15.852	18.852	0.024	372.0→159.0	5.0	−14.0	−35.0	−29.0	372.0→70.1	5.0	−22.0	−22.0	−27.0
Tolclofos-methyl	12.882	15.882	0.024	303.0→271.0	5.0	−21.0	−11.0	−17.0	303.0→269.0	5.0	−10.0	−5.0	−11.0
Triadimefon	14.637	17.637	0.024	294.1→69.2	5.0	−10.0	−22.0	−27.0	294.1→197.1	5.0	−16.0	−15.0	−19.0
Triadimenol	15.04	18.04	0.016	296.2→70.2	1.0	−15.0	−21.0	−28.0	296.2→43.2	1.0	−11.0	−38.0	−15.0
Triazophos	14.933	17.933	0.024	314.0→162.0	9.0	−12.0	−19.0	−29.0	314.0→119.0	9.0	−12.0	−34.0	−21.0
Trifloxystrobin	19.270	22.270	0.024	409.0→186.1	9.0	−12.0	−18.0	−18.0	409.0→206.1	9.0	−12.0	−13.0	−21.0
Zoxamide	17.751	20.751	0.024	336.0→187.0	5.0	−13.0	−25.0	−17.0	336.0→159.0	5.0	−11.0	−42.0	−25.0
Atrazine-d5	11.777	14.777	0.018	221.0→179.0	3.0	−22.0	−18.0	−17.0					
Carbamyl-d7	9.511	12.511	0.018	208.9→152.3	3.0	−14.0	−11.0	−19.0					
Diazinon-d10	18.580	21.580	0.018	315.2→170.2	3.0	−16.0	−21.0	−16.0					
Dimethoate-d6	4.821	7.821	0.018	236.1→205.1	6.0	−20.0	−10.0	−21.0					
Diuron-d6	12.277	15.277	0.018	238.9→78.1	6.0	−23.0	−26.0	−30.0					

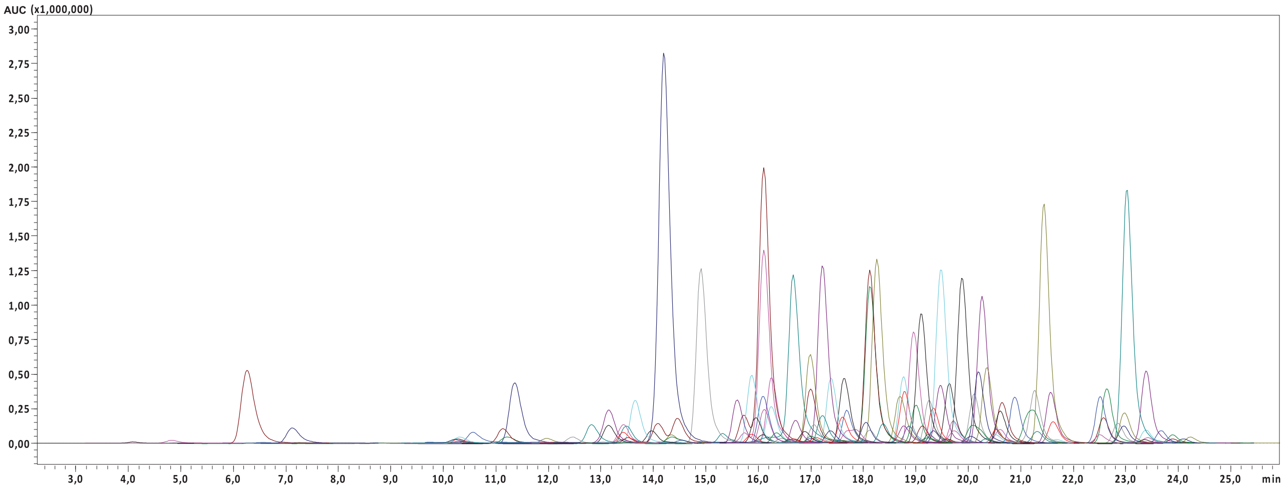

Fig. 1. Total ion chromatograms obtained by SBSE-HPLC-MS^2 of a purified water sample fortified at 200 ng/L. AU, area under the curve; SBSE-HPLC-MS^2, stir bar sorptive extraction-high-performance liquid chromatography-tandem mass spectrometry.

(used for quantification) was chosen for each analyte. According to the Commission Implementing Regulation (EU) 2021/808,[27] an analyte is identified above the LOQ in a sample if certain criteria are met: at least three identification points must be achieved, which include the presence of the two selected product ions. Furthermore, the ratio between the quantification and confirmation transitions in the sample should closely match that of the previously injected standard within the specified percentage. Consequently, two transitions were selected for each compound.

3.1. LOD, LOQ, LLOQ, and selectivity (SEL%)

LOD and LOQ were determined, respectively, by three and ten times the signal-to-noise ratio, expressed as the absolute value of analyte concentration. LLOQ was defined as the experimentally determined lowest analyte concentrations. The ability of the analytical method to uniquely identify the analyte of interest while discriminating against potential matrix interferences was evaluated under selectivity (SEL%). SEL% was measured as the ratio between the signal of four individual pesticide-free matrices and that of spiked matrices at the concentration representing the LLOQ of each pesticide. According to validation guidelines, a maximum threshold value of 20% was set. The results are shown in Table 2.

Considering a signal-to-noise ratio of at least 10 as a necessary requirement, approximately 56% (73 of 131) of the pesticides have a LLOQ of 20 ng/L, 35% (46 of 131) have a LLOQ of 30 ng/L, while the remaining 9% (12 of 131) have a LLOQ of 50 ng/L. Regarding LOQ and LOD, the values were within the range of 0.04–18.17 and 0.01–5.99 ng/L, respectively. With regard to selectivity, values are always below the threshold (SEL% <20%) and range from 0.1% to 19.8%.

3.2. Trueness (BIAS%), precision (RSD%), and expanded uncertainty (U%)

Intra- and interday trueness (BIAS%) and precision (RSD%) were calculated at three levels (low, medium, and high). While the high and medium levels were set at 200 and 100 ng/L, respectively, for all analytes, the low level depended on the LLOQ of the pesticide (20, 30, or 50 ng/L). As an additional parameter to assess the quality of the method, expanded uncertainty (U%) was calculated from the standard uncertainty by multiplying it by a coverage factor k (for $\alpha = 5\%$, $k = 2$). Values could be below 20% for BIAS% and RSD%, and below 50% for U%. The values obtained for each individual pesticide are presented in Supporting Information—Table S3.

The method demonstrated high reproducibility across all three levels evaluated, with RSD% values consistently below 20% in both intra- and interday tests. Intra- and interday test also highlighted that the proposed method has very good trueness, with BIAS% falling below the threshold (BIAS% <20%) across all three levels evaluated. In addition, U% values were below 50% for each pesticide in both the intra- and interday tests. The range of values obtained from the inter- and intraday tests for each parameter is summarized in Table 3.

3.3. Repeatability (RSD$_{std}$%)

For the extraction and syringe spiked standards, intra- and interday repeatability were evaluated using the entire data set of the validation protocol. The RSD% of extraction and syringe spiked standards remains always below 10% (1.46%–5.78% for intraday RSD% and 1.58%–7.02% for interday RSD%), further confirming the good repeatability of the method at both the extraction and instrumental levels (Supporting Information—Table S4). In particular, the two syringe spiked standards added at the injection level in the chromatographic system demonstrated lower RSD% values both intra- and interday compared to the extraction spiked standards. This result is attributable to the fact that the extraction spiked standards underwent an additional analytical step, having been added before the extraction step.

3.4. Sample analysis

The method was applied to detect and quantify pesticide residues in 30 different mineral water samples collected from different springs in central-northern Italy. Traces of six of the targeted pesticides (pyrimethanil, trifloxystrobin, fenoxaprop-P-ethyl, pirimiphos-ethyl, cloquintocet-mexylhexyl ester, and piperonyl butoxide) were detected in some of the

Table 2. Selectivity (SEL%), LOQ, LOD, and LLOQ obtained for each pesticide with the optimized SBSE-HPLC-MS method.

Pesticide name	SEL%	LOQ (ng/L)	LOD (ng/L)	LLOQ (ng/L)
2,6-Dichlobenzamide	4.05	6.85	2.26	30
Ametoctradin	0.53	1.34	0.44	50
Ametrina	0.53	1.17	0.39	30
Atrazine	1.58	2.37	0.78	20
Azinophos-ethyl	1.58	2.74	0.91	30
Azinophos-methyl	1.13	1.42	0.47	20
Azoxystrobin	1.90	0.94	0.31	30
Benalaxyl	1.85	2.20	0.73	20
Bifenazate	0.79	0.86	0.28	20
Bixafen	0.51	0.77	0.26	20
Boscalid	0.72	1.70	0.56	20
Bromuconazole	4.56	3.21	1.06	20
Bupirimate	15.81	6.71	2.22	20
Buprofezin	5.26	0.57	0.19	20
Cadusafos	2.62	1.57	0.52	20
Carbaryl	1.88	4.11	1.36	20
Carbendazim	8.54	10.19	3.36	20
Carbofuran	1.38	4.32	1.43	50
Carfentrazone-ethyl	0.88	1.37	0.45	30
Chlorotoluron	2.98	13.10	4.32	30
Chlorpropham	2.97	9.89	3.26	30
Chlorpyrifos	12.82	5.75	1.90	20
Clodimafop-propargyl	1.03	1.27	0.42	20
Clomazone	1.11	1.93	0.64	30
Cloquintocet-mexylhexyl ester	1.42	0.93	0.31	20
Cyazofamid	0.74	0.79	0.26	30
Cyflufenamid	1.62	3.07	1.01	30
Cyproconazole	6.53	11.07	3.65	20
Cyprodinil	4.17	6.93	2.29	30
Diazinon	1.63	2.35	0.78	20
Dichlorvos	9.60	17.09	5.64	20
Difenoconazole	4.40	7.82	2.58	20
Diflubenzuron	0.96	1.52	0.50	30
Diflufenican	7.53	7.11	2.35	20
Dimethenamid	1.91	1.51	0.50	30
Dimethomorph	3.30	3.73	1.23	30
Dithianon	3.74	7.79	2.57	20
Emamectin	7.89	1.04	0.34	20
Epoxiconazole	0.40	1.23	0.41	30
Ethiofencarb	4.18	6.56	2.16	30
Ethion	19.77	1.77	0.58	20
Ethirimol	3.71	1.02	0.34	30
Ethoprophos	0.40	0.59	0.19	30
Etoxazole	4.76	4.77	1.57	20
Fenamidone	0.45	0.34	0.11	30
Fenamiphos	0.25	0.90	0.30	30
Fenbuconazole	3.48	3.15	1.04	30
Fenothiocarb	0.26	0.21	0.07	50
Fenoxaprop-P-ethyl	8.63	9.41	3.11	20
Fenoxycarb	2.07	0.56	0.19	20
Fenpropidin	2.57	1.37	0.45	20
Fenpyrazamine	1.02	0.82	0.27	20
Fluazinam	3.67	14.33	4.73	50
Flufenacet	0.21	0.09	0.03	30
Fluopicolide	3.59	1.04	0.34	50
Fluopyram	0.29	1.27	0.42	30
Flusilazole	0.88	0.57	0.19	20
Folpet	0.62	0.42	0.14	50
Furathiocarb	2.08	2.55	0.84	20
Heptenophos	2.32	1.46	0.48	30
Hexaconazole	1.67	6.58	2.17	30
Hexythiazox	11.56	15.76	5.20	20
Indoxacarb	18.65	1.60	0.53	20
Iprovalicarb	1.81	2.18	0.72	20
Isoproturon	0.64	0.30	0.10	20
Isopyrazam	1.14	1.13	0.37	20
Isoxaben	1.53	2.98	0.98	20
Isoxadifen-Ethyl	3.06	3.65	1.20	20
Isoxaflutole	2.99	2.18	0.72	30
Linuron	3.60	5.29	1.75	20
Malathion	0.58	0.32	0.10	50
Mandipropamid	0.71	1.08	0.36	20
Mefenpyr	0.86	0.86	0.28	20
Mefenpyr diethyl	4.38	2.38	0.79	20
Mepanipyrim	4.14	0.67	0.22	20
Metalaxyl	6.85	3.95	1.30	20
Metazachlor	0.99	0.07	0.02	20
Metconazole	0.14	2.41	0.80	50
Methidathion	0.49	1.13	0.37	20
Methiocarb	2.29	0.11	0.04	30
Methoxyfenozide	1.10	6.33	2.09	50
Metobromuron	1.15	3.47	1.14	30
Metolachlor	0.45	0.92	0.30	30
Metrafenone	2.06	3.03	1.00	20
Molinate	6.25	4.44	1.47	20
Myclobutanil	1.90	4.03	1.33	20
Oxadiazon	10.45	7.79	2.57	30
Paclobutrazol	2.67	3.45	1.14	30
Parathion-methyl	7.27	6.20	2.05	20
Penconazole	0.71	0.67	0.22	20
Pethoxamid	0.21	0.46	0.15	30
Phenmedipham	1.12	5.14	1.70	30
Phosmet	3.20	1.16	0.38	20
Phosphamidon	18.18	9.94	3.28	30
Picoxystrobin	0.74	0.09	0.03	30
Pinoxaden	2.91	8.20	2.71	30
Piperonyl butoxide	7.40	1.41	0.46	20
Pirimicarb	2.10	3.62	1.20	20
Pirimiphos-ethyl	1.59	2.19	0.72	20
Pirimiphos-methyl	7.47	1.39	0.46	20
Prochloraz	1.54	1.64	0.54	30
Propaquizafop	3.02	1.55	0.51	20
Propazine	0.87	2.40	0.79	30
Propiconazole	1.84	1.23	0.41	20
Propoxur	2.82	4.89	1.61	20
Propyzamide	3.96	3.03	1.00	30
Pyraclostrobin	0.77	0.47	0.16	20
Pyraflufen-ethyl	0.83	1.46	0.48	30
Pyridaphenthion	0.29	0.28	0.09	30
Pyrimethanil	14.35	18.17	5.99	20
Pyriproxyfen	5.20	4.17	1.38	20
Quinalphos	3.78	2.87	0.95	20
Quinoxyfen	13.57	10.33	3.41	20
Quizalofop-P-ethyl	4.05	1.06	0.35	20
Simazine	5.77	8.97	2.96	20
Spinosad	2.46	0.28	0.09	20
Spiroxamine	0.42	0.04	0.01	20
Tebuconazole	1.06	3.25	1.07	50
Tebufenozide	0.91	3.39	1.12	50
Tebufenpyrad	7.00	14.32	4.73	20
Terbuthylazine	0.42	0.79	0.26	30
Terbuthylazine-desethyl	3.79	9.70	3.20	20
Terbutryn	0.32	0.29	0.10	20
Tetrachlorvinphos	0.82	1.04	0.34	30
Tetraconazole	0.71	0.59	0.20	30
Tolclofos-methyl	17.31	11.79	3.89	20
Triadimefon	7.86	12.88	4.25	20
Triadimenol	3.10	8.66	2.86	30
Triazophos	0.26	0.40	0.13	50
Trifloxystrobin	2.26	1.19	0.39	20
Zoxamide	0.79	2.38	0.79	30

(Continued)

LLOQ, lower limit of quantification; LOD, limit of detection; LOQ, limit of quantitation; SBSE-HPLC-MS, stir bar sorptive extraction-high-performance liquid chromatography-mass spectrometry.

Table 3. Summary of the ranges of BIAS%, RSD%, and U% values obtained from the intra- and interday tests.

		Intraday ($n = 3$)	Interday ($n = 3$, $x = 3$)
Low level (LLOQ)	BIAS%	3.12–19.79	1.95–19.92
	RSD%	1.07–19.81	1.42–19.55
	U%	7.21–48.12	6.91–49.24
Medium level (100 ng/L)	BIAS%	0.81–19.85	1.34–19.87
	RSD%	0.27–15.49	0.59–17.95
	U%	2.68–45.69	4.26–47.79
High level (200 ng/L)	BIAS%	0.48–18.06	0.53–19.78
	RSD%	0.37–14.21	0.24–16.77
	U%	1.44–45.58	1.59–46.15

LLOQ, lower limit of quantification.

spring water samples, but only at concentrations below their LLOQ (LLOQ = 20 ng/L) (Supporting Information—Table S5).

To ensure the quality of the results and evaluate the stability of the proposed method, the variation in terms of area for deuterated extraction spiked and syringe spiked standards from validation replicates was evaluated in every batch of samples. To evaluate whether the observed differences were statistically significant, a two-tailed, type 2 Student's *t*-test was performed. It allows for comparing the means of two independent groups while accounting for the possibility of differences in either direction. This test assumes equal variances between the groups and is suitable for determining if the observed differences are statistically significant without presupposing the direction of the effect.

The differences observed in area values were not statistically significant (Supporting Information—Table S6), as determined by the results of the Student's *t*-test. The *p* values obtained were all greater than 0.05, indicating that the variations between the groups were likely due to random chance rather than a true effect. Therefore, we could not reject the null hypothesis and concluded that the differences are not significant, providing further confirmation of the method's applicability to real samples.

4. DISCUSSION

The development of the SBSE-HPLC-MS/MS method addressed several challenges in multi-residue pesticide analysis, particularly for complex aqueous matrices like drinking water. The Blue Applicability Grade Index[28] evaluation of our method resulted in a score of 65%, which demonstrates a good level of practicability and sustainability, particularly when compared to traditional SPE methods. While the sample volume required for our method is 40 mL, this is significantly lower than the 0.5–1 L often used in SPE workflows,[17,29] making it more environmentally friendly. Additionally, the SBSE technique eliminates the need for large volumes of organic solvents during extraction, further reducing the environmental impact. In our protocol, the desorption step requires 200 μL of solvent. This amount is relatively low compared to standard approaches, enhancing the method's sustainability. Overall, our method provides a clear advantage in terms of minimizing resource use, waste production, and energy consumption, representing a more sustainable alternative for multi-residue pesticide analysis in water.

4.1. SBSE extraction parameters

Several studies have linked this equilibrium to octanol–water partition coefficients ($K_{o/w}$).[30] The partition coefficient between PDMS and water ($K_{PDMS/w}$) is defined as described in Eq. (1).

$$\frac{m_{PDMS}}{m_0} = \frac{\left[\frac{K_{PDMS/W}}{\beta}\right]}{1+\left[\frac{K_{PDMS/W}}{\beta}\right]} \qquad (1)$$

Recovery, expressed as the ratio of the amount of solute extracted in the PDMS phase (m_{PDMS}) to the amount of solute originally present in water (m_0) is determined by the partition constant $K_{PDMS/w}$ and the β factor, as described in Eq. (2).

$$K_{O/W} = K_{PDMS/W} = \frac{C_{PDMS}}{C_W} = \left[\frac{m_{PDMS}}{m_W}\right]\cdot\left[\frac{V_W}{V_{PDMS}}\right] = \beta\cdot\frac{m_{PDMS}}{m_W}$$

$$\beta = \frac{V_W}{V_{PDMS}} \qquad (2)$$

From this equation, it is evident that extraction efficiency increases with increasing $K_{PDMS/w}$ and decreases with increasing solute polarity. Moreover, the greater the amount of PDMS used, the higher the extraction efficiency, as the β factor decreases.

Thus, the parameters that influence the extractive capacity of the technique are essentially the β factor and the partition coefficient. The greater the β factor, the longer it will take to reach the partition equilibrium of the analyte between PDMS and water.

In summary, the extraction recovery depends on the amount of analyte and the partition coefficient, as they influence the time required to reach partition equilibrium. At equilibrium, the recovery depends on the partition coefficient, and the maximum extraction recovery depends on the partition coefficient when the extraction time, as a function of the β factor, is sufficient to reach partition equilibrium. Therefore, extraction recovery can be improved by working on the type of stir bar, sample volume, exposure time, and stirring speed.

Thus, the extraction and desorption parameters were selected with reference to the β factor, focusing on achieving good analytical performance for the target pesticides. Given the experimental conditions (47 μL PDMS phase volume of the stir bar in a 40 mL water volume), the β factor was 833. Using representative compounds like atrazines and organophosphates from the main pesticide classes in our set, this β factor offers an effective balance.[31] It achieved a substantial amount of extracted substance while ensuring an adequate concentration factor for the subsequent liquid desorption step. As regards stirring speed, it was maintained at 700 rpm. Although, the higher the stirring speed, the greater the mass transfer, it is advisable not to exceed the range of 600–800 rpm, as higher speeds may compromise the integrity of the stir bar's coating.[32]

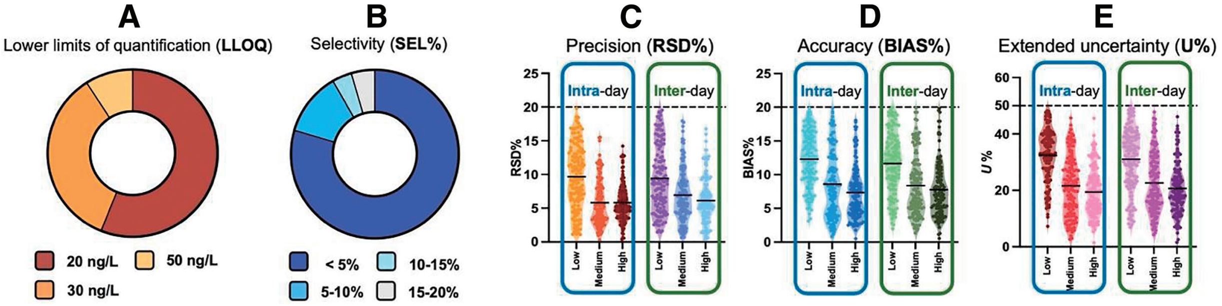

Fig. 2. Graphical summary of validation results, including the LLOQ (**A**), SEL% (**B**), RSD% (**C**), BIAS% (**D**), and U% (**E**). LLOQ, lower limit of quantification.

4.2. Validation results

The results demonstrated the good sensitivity of the analytical method, as evidenced by the distribution of LLOQs (Fig. 2A). They were in the range 20–50 ng/L for all the analytes. Highlighting the method's capability to detect pesticides at trace levels. Additionally, the excellent selectivity (SEL% <20%) across all pesticides further supported the method's reliability, making it suitable for accurate quantification in complex matrices (Fig. 2B). These findings underscored the method's applicability in routine monitoring of pesticide residues in environmental samples.

The method demonstrated high reproducibility, with RSD% values below 20% across all tested concentration levels, confirming its robustness and reliability. Both intra- and interday tests revealed that the method maintained excellent precision, as indicated by the low RSD% values, which ranged from 0.23% to 19.81% (Fig. 2C). This level of consistency suggested that the method was capable of delivering reliable results over time, making it suitable for routine analysis.

Moreover, the trueness of the method was well established, with BIAS% values remaining below the 20% threshold across all levels (Fig. 2D). This confirmed that the method provided measurements with minimal systematic errors. The range of BIAS% values, from 0.48% to 19.91%, reflected the method's capacity to accurately quantify pesticides across different concentrations and conditions.

Extended uncertainty values, which were below 50% for each pesticide in both intra- and interday tests, further supported the method's validity (Fig. 2E). The observed range of U% values, from 1.44% to 49.24%, demonstrated that the method's uncertainty was within acceptable limits, ensuring confidence in the results.

Overall, the data indicated that the method was not only precise and accurate but also reliable, with manageable levels of uncertainty. This performance was crucial for the effective monitoring of pesticide residues in drinking water, ensuring that the method met the rigorous standards required for environmental and public health safety.

5. CONCLUSION

A comprehensive multi-residue method was established for the rapid and simultaneous identification of 131 pesticides in mineral water utilizing SBSE extraction paired with HPLC-tandem MS. This green method limits solvent usage, offering an environmentally friendly, automatable alternative to conventional approaches. The entire analytical process was assessed through various validation parameters following Eurachem guidelines. The method demonstrated good selectivity, precision, and trueness, with quantification thresholds below required regulatory limits for all the tested analytes. Applied to mineral water, this approach effectively demonstrated its reliability and relevance for consistent monitoring, enabling robust analysis in a biologically relevant matrix for public health applications.

ABBREVIATIONS

HPLC-MS, high-performance liquid chromatography-mass spectrometry; PDMS, polydimethylsiloxane; SBSE, stir bar sorptive extraction; SPME, solid phase microextraction.

AUTHOR CONTRIBUTIONS

Alex Affricano: formal analysis, data curation, validation, writing—original draft. Silvia Serra: investigation. Alice Di Bernardo: investigation. Riccardo Aigotti: investigation. Francesco Floris: methodology, writing. Federica Dal Bello: writing—review and editing. Claudio Medana: conceptualization, writing—review and editing, supervision.

DATA AVAILABILITY STATEMENT

Datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

COMPETING INTEREST

The authors declare that there are no competing interests.

REFERENCES

1) F. A. O. Pesticides Use. Food and Agriculture Organization of the United Nations, 2024. Available from: https://www.fao.org/faostat/en/#data/RP.
2) M. F. F. Bernardes, M. Pazin, L. C. Pereira, D. J. Dorta. Impact of pesticides on environmental and human health. *Toxicology Studies—Cells, Drugs and Environment.* InTech, 2015.
3) J. G. Vos, E. Dybing, H. A. Greim, O. Ladefoged, C. Lambré, J. V. Tarazona, I. Brandt, A. D. Vethaak. Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. *Crit. Rev. Toxicol.* 30: 71–133, 2000.
4) T. M. A. Pedroso, M. Benvindo-Souza, F. de Araújo Nascimento, J. Woch, F. G. Dos Reis, D. de Melo E Silva . Cancer and occupational exposure to pesticides: A bibliometric study of the past 10 years. *Environ. Sci. Pollut. Res. Int.* 29: 17464–17475, 2022.

5) E. Elmorsy, A. Al-Ghafari, H. Al Doghaither, M. Salama, W. G. Carter. An investigation of the neurotoxic effects of malathion, chlorpyrifos, and paraquat to different brain regions. *Brain Sci.* 12: 975, 2022.

6) V. P. Kalyabina, E. N. Esimbekova, K. V. Kopylova, V. A. Kratasyuk. Pesticides: Formulants, distribution pathways and effects on human health—A review. *Toxicol. Rep.* 8: 1179–1192, 2021.

7) U. S. Food and Drug Administration (FDA). Pesticide Analytical Manual Volume II (PAM 2), vol. II, 2002.

8) U. S. EPA. Method 8270E, Revision 6, June 2018, Final Update VI to the Third Edition of the Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, EPA publication SW-846.

9) U. S. EPA. Method 8321B, Revision 6, June 2018, Final Update VI to the Third Edition of the Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, EPA publication SW-846.

10) L. Lucentini, M. Patriarca. Per la Sottocommissione del Comitato permanente di Studio sulle Acque del Ministero della Salute (ex art. 9 DM 26 marzo 1991) (Ed.), Metodi analitici per il controllo delle acque da destinare e destinate al consumo umano ai sensi del DL.vo 31/2001 e s.m.i. Metodi chimici, Roma: Istituto Superiore di Sanità, 2019. (Rapporti ISTISAN 19/7).

11) European Parliament and Council. Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption (Text with EEA relevance) (recast). Available from: https://eur-lex.europa.eu/eli/dir/2020/2184/oj.

12) A. Páleníková, G. Martínez-Domínguez, F. J. Arrebola, R. Romero-González, S. Hrouzková, A. G. Frenich. Multifamily determination of pesticide residues in soya-based nutraceutical products by GC/MS-MS. *Food Chem*. 173: 796–807, 2015.

13) Y. Picó, C. Blasco, G. Font. Environmental and food applications of LC-tandem mass spectrometry in pesticide-residue analysis: An overview. *Mass Spectrom. Rev.* 23: 45–85, 2004.

14) A. M. Rodrigues, V. Ferreira, V. V. Cardoso, E. Ferreira, M. J. Benoliel. Determination of several pesticides in water by solid-phase extraction, liquid chromatography and electrospray tandem mass spectrometry. *J. Chromatogr. A* 1150: 267–278, 2007.

15) I. Domínguez, R. Romero González, F. J. Arrebola Liébanas, J. L. Martínez Vidal, A. Garrido Frenich. Automated and semi-automated extraction methods for GC-MS determination of pesticides in environmental samples. *Trends Environ. Anal. Chem.* 12: 1–12, 2016.

16) X. Li, P. Gan, R. Peng, C. Huang, H. Yu. Determination of 23 organophosphorous pesticides in surface water using SPME followed by GC-MS. *J. Chromatogr. Sci.* 48: 183–187, 2010.

17) R. I. Bonansea, M. V. Amé, D. A. Wunderlin. Determination of priority pesticides in water samples combining SPE and SPME coupled to GC-MS. A case study: Suquía River basin (Argentina). *Chemosphere* 90: 1860–1869, 2013.

18) N. Ochiai, K. Sasamoto, H. Kanda, E. Pfannkoch. Sequential stir bar sorptive extraction for uniform enrichment of trace amounts of organic pollutants in water samples. *J. Chromatogr. A* 1200: 72–79, 2008.

19) F. David, N. Ochiai, P. Sandra. Two decades of stir bar sorptive extraction: A retrospective and future outlook. *Trends Analyt. Chem.* 112: 102–111, 2019.

20) S. Nakamura, S. Daishima. Simultaneous determination of 64 pesticides in river water by stir bar sorptive extraction and thermal desorption-gas chromatography-mass spectrometry. *Anal. Bioanal. Chem.* 382: 99–107, 2005.

21) P. Grossi, I. R. B. Olivares, D. R. de Freitas, F. M. Lancas. A novel HS-SBSE system coupled with gas chromatography and mass spectrometry for the analysis of organochlorine pesticides in water samples. *J. Sep. Sci.* 31: 3630–3637, 2008.

22) C. Blasco, G. Font, Y. Picó. Comparison of microextraction procedures to determine pesticides in oranges by liquid chromatography-mass spectrometry. *J. Chromatogr. A* 970: 201–212, 2002.

23) Y. Wang, M. He, B. Chen, B. Hu. Hydroxyl-containing porous organic framework coated stir bar sorption extraction combined with high performance liquid chromatography-diode array detector for analysis of triazole fungicides in grape and cabbage samples. *J. Chromatogr. A* 1633: 461628, 2020.

24) B. Desharnais, F. Camirand-Lemyre, P. Mireault, C. D. Skinner. Procedure for the selection and validation of a calibration model I-Description and application. *J. Anal. Toxicol.* 41: 261–268, 2017.

25) B. Desharnais, F. Camirand-Lemyre, P. Mireault, C. D. Skinner. Procedure for the selection and validation of a calibration model II-Theoretical basis. *J. Anal. Toxicol.* 41: 269–276, 2017.

26) B. Magnusson, U. Örnemark (eds.). Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, 2nd ed., 2014. ISBN 978-91-87461-59-0. Available from: www.eurachem.org.

27) European Commission. Commission Implementing Regulation (EU) 2021/808 of 22 March 2021 laying down rules for the application of Regulation (EU) No 1306/2013 of the European Parliament and of the Council with regard to the systems for the identification and registration of animals, and repealing Commission Regulation (EC) No 911/2004. *Off. J. Eur. Union. L* 180: 14–44, 2021.

28) N. Manousi, W. Wojnowski, J. Płotka-Wasylka, V. Samanidou. Blue applicability grade index (BAGI) and software: A new tool for the evaluation of method practicality. *Green Chem* 25: 7598–7604, 2023.

29) N. Dujaković, S. Grujić, M. Radišić, T. Vasiljević, M. Laušević. Determination of pesticides in surface and ground waters by liquid chromatography–electrospray–tandem mass spectrometry. *Anal. Chim. Acta* 678: 63–72, 2010.

30) F. M. Lancas, M. E. C. Queiroz, P. Grossi, I. R. B. Olivares. Recent developments and applications of stir bar sorptive extraction. *J. Sep. Sci.* 32: 813–824, 2009.

31) F. David, P. Sandra. Stir bar sorptive extraction for trace analysis. *J. Chromatogr. A* 1152: 54–69, 2007.

32) I. Aparicio, J. Martín, J. L. Santos, J. L. Malvar, E. Alonso. Stir bar sorptive extraction and liquid chromatography-tandem mass spectrometry determination of polar and non-polar emerging and priority pollutants in environmental waters. *J. Chromatogr. A* 1500: 43–52, 2017.