

## Article

# A New LC-MS Method for Evaluating the Efficacy of Pesticide Residue Removal from Fruit Surfaces by Washing Agents

Magdalena Zarebska <sup>1,\*</sup>, Zofia Hordyjewicz-Baran <sup>1</sup>, Tomasz Wasilewski <sup>2</sup>, Ewa Zajszy-Turko <sup>1</sup>  
and Natalia Stanek <sup>1</sup>

<sup>1</sup> Łukasiewicz Research Network-Institute of Heavy Organic Synthesis “Blachownia”, Energetyków 9, 47-225 Kedzierzyn-Kozle, Poland; zofia.hordyjewicz@icso.lukasiewicz.gov.pl (Z.H.-B.); ewa.zajszy@icso.lukasiewicz.gov.pl (E.Z.-T.); natalia.stanek@icso.lukasiewicz.gov.pl (N.S.)

<sup>2</sup> Department of Industrial Chemistry, Faculty of Chemical Engineering and Commodity Science, Kazimierz Pulaski University of Technology and Humanities in Radom, Chrobrego 27, 26-600 Radom, Poland; tomasz.wasilewski@uthrad.pl

\* Correspondence: magdalena.zarebska@icso.lukasiewicz.gov.pl; Tel.: +48-691-606-660

**Abstract:** Modern agriculture uses pesticides to improve the quality and quantity of crops. However, pesticide residues can remain on agricultural products, posing very serious risks to human health and life. It is recommended to wash fruits and vegetables before consumption. To assess the removal efficacy of pesticide residue, a sensitive and reliable method based on ultrahigh-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) was developed and optimized for the simultaneous determination of four pesticide residues (acetamiprid, boscalid, pyraclostrobin, and pendimethalin). Isotope-labeled standards were used to validate the method in terms of recovery, linearity, matrix effects, precision, and sensitivity. The mean recovery values for both low-quality control (LQC) and high-quality control (HQC) transitions were in the range of 89–105%, and the intra-day precision was less than 13.7%. The limits of detection (LOD) and quantification (LOQ) were 0.003 mg/kg and 0.01 mg/kg, respectively. The proposed method is suitable for evaluating the quality of detergents for removing pesticide residues from fruit surfaces.

**Keywords:** UHPLC-ESI-MS/MS; validation; human health; fruits washing; pesticides residue removal; washing detergents



**Citation:** Zarebska, M.; Hordyjewicz-Baran, Z.; Wasilewski, T.; Zajszy-Turko, E.; Stanek, N. A New LC-MS Method for Evaluating the Efficacy of Pesticide Residue Removal from Fruit Surfaces by Washing Agents. *Processes* **2022**, *10*, 793. <https://doi.org/10.3390/pr10040793>

Academic Editors: Ofelia Anjos, Eugenia Gallardo and Raquel P. F. Guiné

Received: 25 March 2022

Accepted: 15 April 2022

Published: 18 April 2022

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Pesticides are commonly used to control plant diseases, weeds, and insect pests and to regulate plant growth to secure the quantity and quality of produce. The use of pesticides in agriculture has a theoretically positive dimension—it is responsible for an increase in farm productivity and allows products to be transported from distant places. However, pesticide residues can remain on agricultural products, causing great harm to consumers related to food-borne illnesses such as carcinogenicity, neurotoxicity, reproductive disorders, and cell dysplasia [1–3]. Despite the introduced regulations related to the controlled use of pesticides, pesticide residues remain a serious problem affecting the safety of fruits and vegetables.

Apples are extensively consumed in many countries and are considered valuable, with beneficial effects on human health [4–6]. Poland produces over 3 million tons of apples annually, which puts this country in fourth place in the world. Apple orchards in Poland cover an area of about 180 thousand ha, and harvested apples are destined for domestic fresh consumption (15–35%), export (13–32%), and processing (48–58%) [7]. On the other hand, apple tree cultivation requires the use of plant protection agents at various stages of plant development, which may constitute a hazard to human health [8,9]. Particularly, the high consumption of pesticides in Poland is noted in horticultural production (e.g., apples and pears) [10,11].

European Union regulations define the maximum residue limit (MRL) for pesticides in food of plant origin [12]. The values of the MRL are updated based on current safety data on the application of such pesticides. Monitoring of pesticide residues in food conducted in Poland in the years 2004–2007 by the State Sanitary Inspection revealed MRL exceedance in 2.3% of analyzed apple samples [13]. According to the Ministry of Health Report [14], a total of 29 pesticide residues were found in all 85 analyzed apple samples in Poland in 2017, whereas in four samples, there were five instances of exceeded MRL values. Residues of at least one pesticide were detected in 71% of the apples under research, and residues of at least two pesticides were noted in 39% of the samples. Boscalid was one of the most frequently detected pesticides (22% of the samples).

Pesticide residues in plant material are determined using advanced analytical methods, e.g., gas chromatography [15–17], near-infrared (NIR) spectroscopy [18], and surface-enhanced Raman scattering (SERS) mapping [19]. Techniques such as LC-MS/MS have become the main technique for accurate and simultaneous determination of pesticides in food samples [16,20]. The majority of procedures use the reversed-phase high-performance liquid chromatography (HPLC) technique with gradient elution [21,22]. The mobile phase consists of a mixture of acetonitrile, methanol, and water in various volume ratios [23,24]. Various additives, such as organic acid (formic acid [16,21] and acetic acid [25]), ammonium salts (ammonium formate [22] or ammonium acetate [26,27]), or a combination of both substances [28], are applied to the mobile phase to improve the ionization capacity. One of the key technological revolutions in LC was the introduction of ultrahigh-performance liquid chromatography (UHPLC). The solid-phase particle size was reduced from 5  $\mu\text{m}$  to less than 2  $\mu\text{m}$ , allowing for better resolution with shorter run times [29,30]. In addition, coupling UHPLC with MS/MS for food analysis provides higher sensitivity, better peak capacity, higher speed and better resolution [31–33].

Pesticide residues, in addition to appearing at very low concentrations, are often associated with matrices that interfere with their analysis, so the selection of an appropriate pretreatment method becomes particularly important [34]. The most widely used method for the isolation of trace contaminants in complex food and plant samples is the Quick, Cheap, Effective, Rugged, and Safe sample preparation method (QuEChERS) [35–38], which allows both the sample amount and toxic solvents to be minimized. Currently, the commonly applied methods include the European Committee for Standardization (CEN) Method 15662:2008 [39] and AOAC Official Method 2007.01 [40].

Washing is the most common and direct form of preparing food for consumption and removing contaminants in fruits and vegetables [41,42]. Washing with tap water has a limited effect on removing pesticide residues because many pesticides are hydrophobic [43]. Therefore, various detergent solutions are used to leach pesticides from fruits and vegetables. Their effectiveness in removing different types of pesticides is the subject of extensive studies. Knowledge of the effect of home processing on the level of pesticide residues in fruits is necessary to reduce dietary risk, especially among fruits consumed with the peel directly after washing, such as apples.

The aim of this study was to develop and validate in accordance with SANCO [44,45] guidelines the UHPLC-MS/MS method for evaluating the removal efficiency of pesticide residues from the surface of apples by washing products. Common pesticides in crop protection products (acetamiprid, boscalid, pyraclostrobin, and pendimethalin) were selected. The use of new solutions such as columns with smaller particle sizes or an optimal mobile phase modifier for tested pesticides allowed the development of an economical and sensitive method that can be suitable for evaluating the quality of detergents for removing pesticide residues from fruit and vegetable surfaces.

## 2. Materials and Methods

### 2.1. Plant Materials

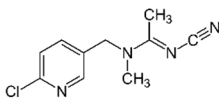
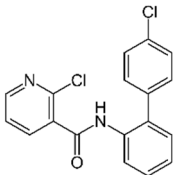
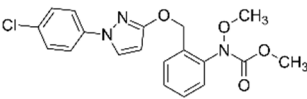
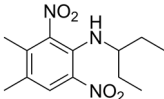
Apples (*Malus domestica* 'Champion') were purchased from the local food market. Checking the apples for the presence of pesticides proved that they had not been previously treated with

the pesticides under consideration. Fruits were selected based on uniform size and ripeness and were stored at 4 °C in a refrigerator before use for the selected pesticide application.

## 2.2. Chemicals and Reagents

Raw materials used in commercial household products were used to develop detergents for fruits: Laureth-4 (Rokanol L4, PCC Rokita SA, Brzeg Dolny, Poland), Laureth-10 (Rokanol L10, PCC Rokita SA, Brzeg Dolny, Poland), benzyl alcohol, ethylhexylglycerin, tocopherol (Euxyl K900, Schülke & Mayr GmbH, Norderstedt, Germany), sodium citrate (Krachemia, Kraków, Poland), and distilled water. A Supel QuE Citrate Extraction Tube (4 g MgSO<sub>4</sub>, 1 g NaCl, 0.5 g NaCitrate dibasic sesquihydrate, 1 g NaCitrate tribasic dehydrate) and a Supel QuE PSA (EN) Tube (150 mg Supelclean PSA, 900 mg MgSO<sub>4</sub>) as well as acetamiprid, boscalid, pendimethalin, and pyraclostrobin as PESTANAL analytical standards were purchased from Merck (Darmstadt, Germany). The main properties of considered pesticides are summarized in Table 1. Acetamiprid-d<sub>3</sub>, boscalid-d<sub>4</sub>, pyraclostrobin-d<sub>6</sub>, and pendimethalin-d<sub>5</sub> were isotope-labeled internal standards obtained from Toronto Research Chemicals (Toronto, ON, Canada). All standards were >95% pure. Acetonitrile of LC-MS grade was supplied by J.T. Baker (Avantor, Radnor, PA, USA). Ammonium formate of LC-MS grade was supplied by Fisher Scientific (Waltham, MA, USA), dry ice was supplied by Cryopoland (Bielsko-Biala, Poland), and ultrapure water with resistance < 18 MΩ cm came from the Direct-Q Water Purification System (Merck, Darmstadt, Germany).

**Table 1.** Main properties of considered pesticides.

Pesticide	Acetamiprid	Boscalid	Pyraclostrobin	Pendimethalin
Chemical structure				
Chemical formula	C <sub>10</sub> H <sub>11</sub> ClN <sub>4</sub>	C <sub>18</sub> H <sub>12</sub> Cl <sub>2</sub> N <sub>2</sub> O	C <sub>19</sub> H <sub>18</sub> ClN <sub>3</sub> O <sub>4</sub>	C <sub>13</sub> H <sub>19</sub> N <sub>3</sub> O <sub>4</sub>
Molecular mass, Da	222	342	387	281
Category	Insecticide	Fungicide	Fungicide	Herbicide
Chemical group	Neonicotinoid	Carboxamide	Strobilurin	Dinitroaniline
Mode of action	Systemic	Systemic	Systemic	Non-systemic
Molar mass, g/mol	222.67	343.21	387.82	281.31
Threshold of toxicological concern *	High (Class III)			
MRL for apple, mg/kg	0.4	3	0.5	0.05
Water solubility 20 °C, mg/L	2950	4.6	1.9	0.33
Log Kow in 20 °C pH 7	0.8	2.96	3.99	5.2
pKa	0.7	Not applicable	−0.23	2.8

\* Cramer class; MRL: maximum residue level, Kow: octanol/water partition coefficient.

## 2.3. Sample Preparation

A procedure for preparing apple samples for testing is graphically presented in Supporting Materials Figure S1. This procedure included immersion in pesticide solution, washing with detergent, washing with tap water to remove the detergent, and the extraction of ground apples using the QuEChERS method.

### 2.3.1. Sample Spiking with Pesticides

Five apples were used for each application experiment as a representative fruit sample. Soaking was chosen as a model for pesticide application because this model is the closest to a field application [5]. Apples were immersed in 2 L of a solution containing 50 mg/L of each tested pesticide: acetamiprid, boscalid, pyraclostrobin, and pendimethalin dissolved in a solution of tap water and acetonitrile maintained at room temperature. Fruits were soaked for 5 min in a baker filled with an application bath to appropriately distribute pesticides on the surface of the apples, as presented in Figure S1a. After the spiking process, the samples were left to dry for 24 h at room temperature, awaiting subsequent washing.

### 2.3.2. Washing Procedure

The idea of preparing a model detergent was based on the specific properties of non-ionic surfactants with a polyoxyethylene chain in their molecule. These compounds may interact with hydrophilic or lipophilic analytes and cause their solubilization in the washing bath. Additionally, non-ionic surfactants tend to separate out from the bulk solution forming micelles with high aggregation numbers when heated to or above their critical temperature. Under such conditions, the solution becomes cloudy, visible to the naked eye. This property is utilized in cloud point extraction (CPE), which makes non-ionic surfactants suitable for the effective extraction and isolation of pesticide residues during fruit washing. Consumer habits based on the preparation of a warm washing bath were considered in the development of a method to evaluate the effectiveness of fruit washing. It was assumed that fruits in domestic conditions are washed at about 37 °C. Based on our own experience and literature reports, the model detergent was designed in such a way that in the conditions of the washing process, the surfactant composition would be in the direct vicinity of the cloud point; hence the developed model surfactant system was characterized by a cloud point of about 45 °C. The effect of such a condition is the high surface and solubilization activity of surfactants. A similar mechanism is used in an efficient cloud point extraction method [46,47].

A model detergent (MD) formulation was prepared and used to develop a method for determining the effectiveness of fruit detergents in removing pesticide residues. The detailed list of ingredients used in the MD for washing the fruits under study is given in Table 2.

**Table 2.** Formulations of model detergent for fruits.

Name According to INCI Nomenclature	Concentration, wt.%
	MD
Laureth-4	3.8
Laureth-10	6.2
Aqua	to 100
Sodium citrate	5.0
Benzyl alcohol Benzoic acid Dehydroacetic acid Tocopherol	0.5

The production of the MD required the use of an appropriate procedure. In the first step, Laureth-10, benzyl alcohol, benzoic acid, dehydroacetic acid, and tocopherol were dissolved in water and thoroughly mixed (temperature 22 °C). Then, Laureth-4 was added and dispersed in the prepared solution, followed by the addition of sodium citrate. The mixture was thoroughly mixed until a clear solution was obtained. The model detergent was used to develop a method for determining the effectiveness of specialized fruit detergents in removing pesticide residues.

Fruits with implemented pesticides were washed for 15 min in a washing bath, which was a 10% aqueous solution of the MD. Two liters of washing bath at 37 °C were used for each washing time. Apples were placed in a beaker with the washing agent, and the bath was stirred using a mechanical stirrer (CAT, R50D; M. Ziperer GmbH, Ballrechten-Dottingen, Germany) at 210 rpm for the appropriate time, as shown in Figure S1b. After washing was completed, apples were drained on a sieve and rinsed with two 3 L portions of cold tap water for detergent residue removal (Figure S1c).

The pesticides applied and non-washed apples were prepared for pesticide removal calculations. As the reference sample, applied apples washed for 15 min with 2 L of tap water as a washing agent were used.

After the rinse process, the apples were left to dry at room temperature for 4 h. The dried samples were packed in separate plastic self-seal bags and stored in a freezer at −18 °C (Freezer CF3100, RedFox, Ruzyně, Czech Republic) until they were deep-frozen, at which point they awaited further preparation.

### 2.3.3. QuEChERS Extraction

Each deep-frozen fruit sample was homogenized by grinding with dry ice in a laboratory knife mill (Cutter Mixer R5 Plus, Robot Coupe, Palinges, France). The entire contents of the laboratory mill were placed in separate, self-sealing plastic bags and stored for 24 h in a freezer at <−18 °C until all dry ice was sublimated, and then extraction was performed.

The procedure of extraction for the determination of pesticide residues was based on European Standard EN 15662:2008 [39]. An amount of 10 g ± 0.1 g of homogenized fruit sample was separately weighted in 50 mL Falcon tubes. An aliquot of 10 mL of acetonitrile was added to the sample. The centrifuge tubes were shaken for 1 min automatically (Bench-Mixer XL multi-tube vortexer, Benchmark Scientific, Sayreville, NJ, USA) as presented in Figure S1d. Subsequently, a liquid-liquid partitioning step was performed by adding a buffer-salt mixture (4 g ± 0.2 g of magnesium sulfate anhydrous, 1 g ± 0.05 g of sodium chloride, 0.5 g ± 0.03 g NaCitrate dibasic sesquihydrate, 1 g ± 0.05 g NaCitrate tribasic dehydrate). The centrifuge tube was shaken automatically for 1 min at 2500 rpm. The obtained extract was centrifuged at 5000 rpm for 5 min (Universal 320R centrifuge, Andreas Hettich GmbH & Co., Tuttlingen, Germany). After centrifugation, 6 mL of obtained supernatant was transferred into 15 mL d-SPE centrifuge tubes containing 150 mg Supelclean PSA (primary secondary amine) and 900 mg MgSO<sub>4</sub>. The tube was shaken automatically for 1 min and centrifuged at 5000 rpm for 5 min. The extract was filtered through a 0.22 µm Nylon syringe filter.

### 2.4. LC-MS/MS Analysis

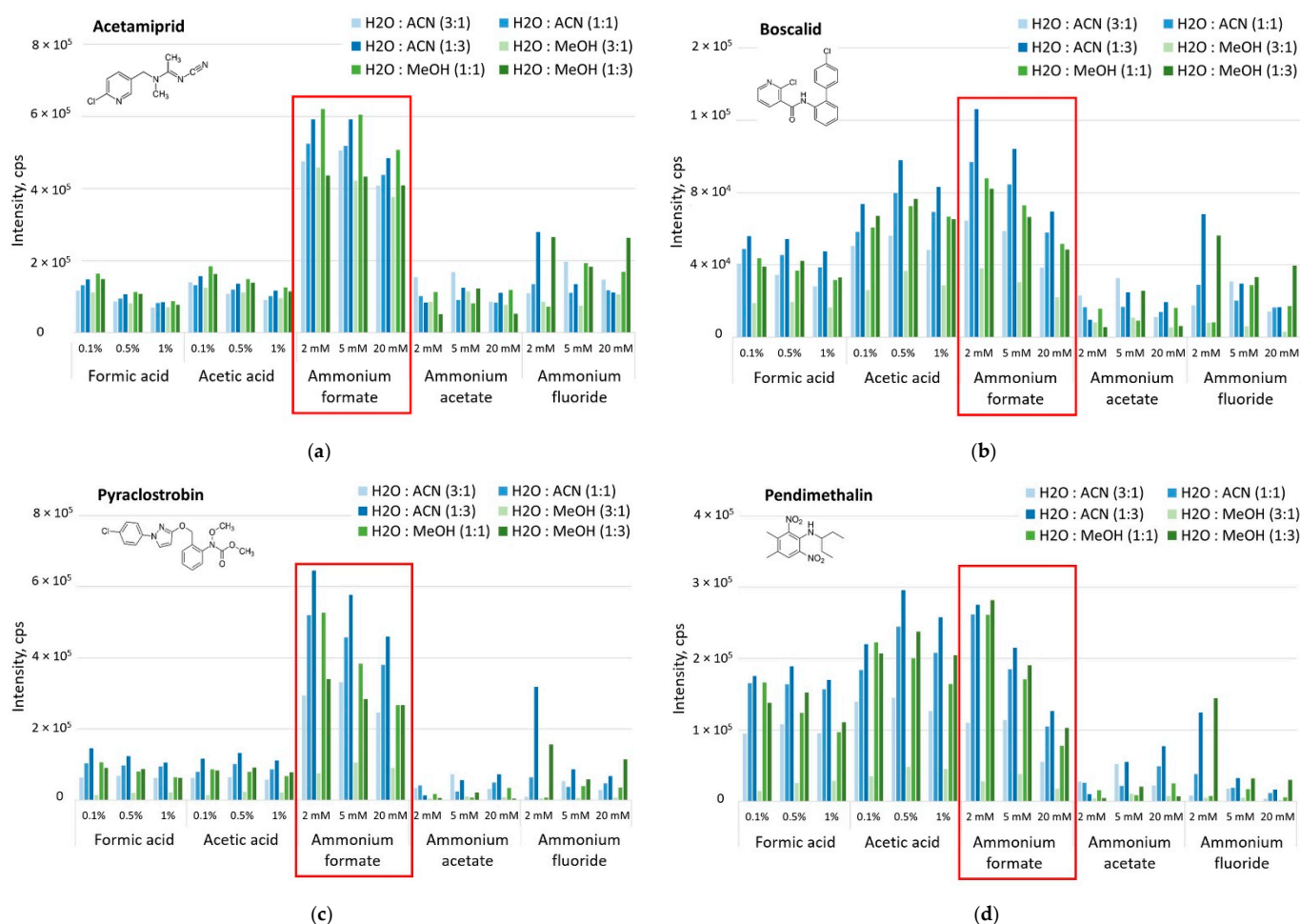
All compound characteristics and ion source parameters were optimized and presented in the following sections. Simultaneous analysis of four pesticides and their isotope-labeled counterparts was performed in three independent replicates of each tested extract. The extract solutions filtered through the 0.2 µm syringe filters were separated using an ultrahigh-performance liquid chromatography system, UHPLC (Sciex ExionLC AD, AB Sciex, Concord, ON, Canada), equipped with a reverse-phase pre-column and column (Synergi Fusion RP, 2.5 µm, 100 Å, 50 × 2 mm, Phenomenex, Torrance, CA, USA) maintained at 25 °C. The mobile phase consisted of 2 mM ammonium formate in water as solvent A and 2 mM ammonium formate in acetonitrile as solvent B. The gradient elution conditions were as follows: 0.0–0.5 min 40% B, 0.5–3.0 min 40–100% B, 3.0–4.0 min 100% B, 4.0–4.1 min 100–40% B, and 4.1–5.0 min 40% B. The flow rate of the mobile phase was 0.5 mL/min, and the injection volume was 10 µL. The MS/MS detection was performed using a triple quadrupole mass spectrometer (4500 QTRAP, AB Sciex, Concord, ON, Canada), equipped with an electrospray ionization source (ESI) working in positive-scan mode. Multiple reaction monitoring (MRM) was used to detect and quantify selected pesticides. The data were processed using Analyst ver.1.7.2.

### 2.4.1. MS/MS Parameter Optimization

For the optimization of compounds' characteristic parameters, the MRM transition conditions for each analyte were determined by step changes in potentials and energy of relevant parameters: declustering potential (DP), collision energy (CE), collision cell exit potential (CXP), entrance potential (EP).

Acetamiprid, boscalid and pyraclostrobin contain chlorine in their structure; therefore, it was necessary to take into account the isotopic distribution in the choice of the precursor ion describing the MRM pair for the deuterated analogues of these pesticides.

Because pesticide residues are trace contaminants of fruits, to obtain maximum signal intensity for the tested pesticides, the ionization efficiency of the tested compounds was increased by selecting the optimal composition of the sample solvent. The solvents were chosen to correspond to the eluents and their modifiers used in UHPLC-MS/MS (type and ratio of water-methanol, water-acetonitrile), additives modifying the mobile phase (formic acid, acetic acid, ammonium formate, ammonium acetate, ammonium fluoride), and the concentration of the mobile phase modifying the additive (0.1%, 0.5%, 1% for acids, 2 mM, 5 mM, 20 mM for salts). Such an experiment gave information on which solvent is the most proper for target analysis. For this purpose, the experiment in the flow injection analysis (FIA) mode by direct injection that bypassed the chromatography column at 100% elution with water for one pair of MRMs was run. The signal intensity for each pesticide along with each combination of considered parameters is presented in Figure 1.

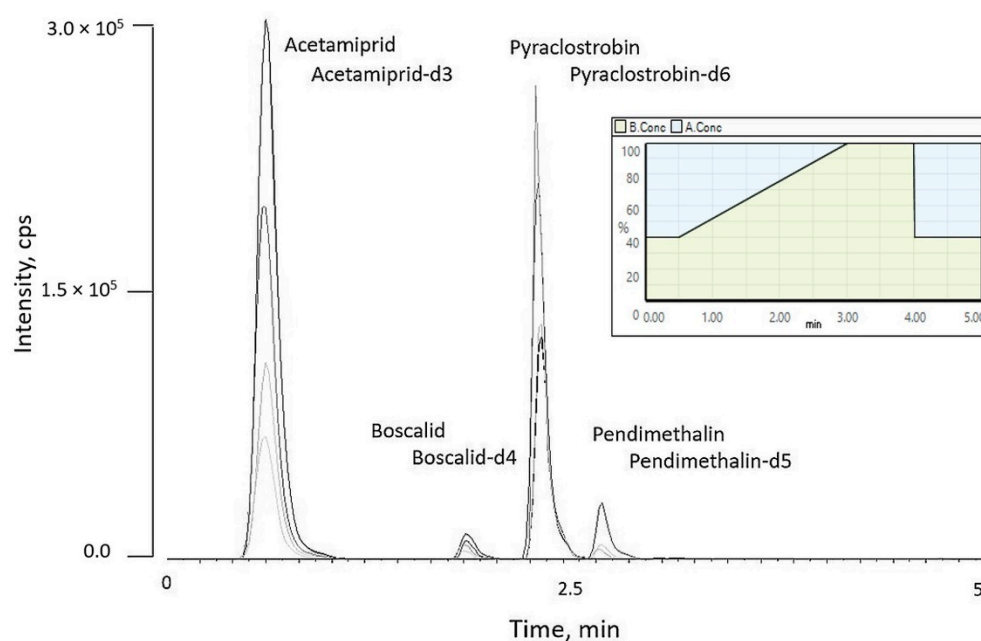


**Figure 1.** Intensity for: (a) acetamiprid, (b) boscalid, (c) pyraclostrobin, and (d) pendimethalin during ionization matrix experiment.

The ion source parameters depend on the solvent and the flow rate of the mobile phase. Therefore the selection of the ion source parameters was performed by FIA with the UHPLC system. Using a concentration of solvents and their modifier selected in the previous section, optimization of the ion source was carried out by stepping over the entire voltage range for ion spray (IS), the temperature range for gas temperature (TEM) and the pressure ranges for ion source nebulizing gas (GS1), drying gas (GS2) and curtain gas (CUR).

#### 2.4.2. Chromatographic Condition Optimization

For the chromatographic separation, the UHPLC system equipped with a new generation of a column filled with modified ultra-pure silica with a small grain size was used. This solution made it possible to work with higher solvent flow rates, allowing analysis times to be reduced to 5 min without compromising signal intensity. Using the most optimal mobile phase (water:acetonitrile and the addition of 2 mM ammonium formate) selected in the Ionization Efficiency Optimization section, chromatographic conditions were optimized by choosing an appropriate eluent gradient. Extract solutions were separated using a UHPLC equipped with a reverse-phase pre-column and column. Pesticides were eluted in the following order: 0.62 min, acetamiprid, acetamiprid-d3; 1.82 min, boscalid, boscalid-d4; 2.26 min, pyraclostrobin, pyraclostrobin-d6; 2.63 min, pendimethalin, pendimethalin-d5. As a result of the optimization of the chromatographic conditions, a very good separation of the analyzed pesticides on the chromatographic column was obtained, as shown in Figure 2.



**Figure 2.** UHPLC-MS/MS-extracted ion chromatogram (XIC) in positive ion-mode for pesticide mixtures with overlapped gradient profile.

#### 2.4.3. Quantitative Analysis

Standard stock solutions (1 mg/mL) of each pesticide and their isotope-labeled counterparts were prepared by dissolving 10 mg of individual compounds in 10 mL of acetonitrile. For pesticides, working standard solutions (0.04, 0.02, 0.01, 0.005, 0.003, 0.001, 0.0006, 0.0003  $\mu\text{g}/\text{mL}$ ) were prepared by mixing each standard stock solution and diluting them with acetonitrile for the solvent-matched calibration curve and with apple extract for the matrix-matched calibration curve. For the isotope-labeled standards (IS), 2  $\mu\text{g}/\text{mL}$  solution was prepared by mixing individual pesticide isotopes and diluting them with acetonitrile. All the standard solutions were stored at 4 °C.

For sample preparation, 100  $\mu\text{L}$  of apple extract obtained from QuEChERS, 50  $\mu\text{L}$  of 2  $\mu\text{g}/\text{mL}$  IS mixture, 600  $\mu\text{L}$  of acetonitrile and 250  $\mu\text{L}$  of 8 mM ammonium formate in water were transferred to an amber glass chromatographic vial. The mixture was hand-shaken gently and subjected to LC-MS/MS analysis.

Quantification was performed using linear regression with weighting over the required concentration range from 0.0003 to 0.04  $\mu\text{g}/\text{mL}$ . From the standard calibration curve (ckk: pesticide concentration from the calibration curve,  $\mu\text{g}/\text{mL}$ ), pesticide concentration in the plant material ( $C$ ,  $\text{mg}/\text{kg}$ ) in the samples was calculated using Analyst ver.1.7.2. and Microsoft Excel spreadsheets for both ion transitions, where  $V$  represented the volume of solvent used for QuEChERS extraction in  $\text{mL}$ , and  $m$  represented the mass of the plant material in  $\text{g}$  (Equations (1) and (2)).

$$\text{ckk, } \mu\text{g}/\text{mL} = \frac{\frac{\text{pesticide peak area}}{\text{IS peak area}} - \text{intercept}}{\text{slope}} \quad (1)$$

$$C, \text{mg}/\text{kg} = \frac{\text{ckk} \times V \times \text{dilution}}{m} \quad (2)$$

### 2.5. Validation of the UHPLC-MS/MS Method

All the validation parameters described in the following subchapters are included in Table 3.

**Table 3.** Validation parameters for acetamiprid, boscalid, pendimethalin and pyraclostrobin in apple matrix.

Validation Parameter	Acetamiprid		Boscalid		Pendimethalin		Pyraclostrobin		Acceptance Criterion	
	223 > 126	223 > 99	343 > 140	343 > 112	282 > 212	282 > 91	388 > 105	388 > 120		
SST	2.2	3.4	6.0	4.0	5.2	9.6	1.5	1.9	<10%	
Selectivity	$\Delta t_{\text{R}}$ , min	LQC	0.02		0.05		0.03		0.04	< $\pm 0.1$ min
		HQC	0.01		0.03		0.02		0.03	
	conf. IRD, %	LQC	4.1		−0.8		−8.3		−8.9	$\pm 30\%$
		HQC	4.0		−1.4		−11		−6.3	
	$S/N \geq 3$	30.8	6.4	12.5	8.5	49	37.2	18.5	9.5	$\geq 3$
Matrix effect, %	LQC	−4	1	0	0	−1	2	−8	1	negligible < 20%
	HQC	1	2	−2	3	−2	3	−2	5	
Precision, %	LQC	3.6	5.8	6.9	6.1	8.5	13.7	10.0	3.7	RSD < 20%
	HQC	1.1	3.2	4.8	3.9	2.7	2.3	2.1	3.0	
Accuracy, %	LQC	98	98	103	90	99	97	89	94	70–110%
	HQC	101	99	99	105	105	99	103	99	
Linearity	R	0.9997	0.9999	0.9990	0.9963	0.9986	0.9979	0.9996	0.9991	R > 0.99
	Calibr. curve equation	$y = 38.4x + 0.00194$ $y = 38.3x - 0.00064$		$y = 52.4x + 0.00564$ $y = 48.3x + 0.0141$		$y = 147x + 0.00147$ $y = 38.4x + 0.00085$		$y = 15.4x + 0.00672$ $y = 15.7x + 0.00732$		
	Calibr. range	0.003–0.4 $\text{mg}/\text{kg}$ (0.0003–0.04 $\mu\text{g}/\text{mL}$ )								
LOD	0.003 $\text{mg}/\text{kg}$ (0.0003 $\mu\text{g}/\text{mL}$ )								<30% LOQ	
LOQ	0.01 $\text{mg}/\text{kg}$ (0.001 $\mu\text{g}/\text{mL}$ )								0.01 $\text{mg}/\text{kg}$	
Stability, %	LQC	2.9	5.7	−7.2	−2.3	2.2	−5.3	−1.8	−3.2	<10%
	HQC	2.0	−2.0	7.6	−2.6	3.6	0.5	3.7	−1.2	

SST—system suitability test;  $t_{\text{R}}$ —retention time; LQC—low-quality control = 0.01  $\text{mg}/\text{kg}$ ; HQC—high-quality control = 0.1  $\text{mg}/\text{kg}$ ;  $S/N$ —signal-to-noise ratio; RSD—residual standard deviation; R—correlation coefficient; LOD—limit of detection; LOQ—limit of quantification; Conf. IRD, —confirmation ion ratio difference;  $\Delta t_{\text{R}}$ —retention time difference.

#### 2.5.1. System Suitability Test

The system suitability test (SST) was performed to verify that the system was functioning properly at the beginning of each analytical sequence. For this purpose, a reference



solution with a concentration corresponding to HQC (0.1 mg/kg) was injected for each pesticide tested in five replications. The RSD% of the area was calculated for the first and second MS/MS ion transition.

### 2.5.2. Selectivity

Confirmation of the ability to detect the correct analyte (analyte identity) and confirmation that the analyte signal is quantified correctly and does not interfere with any other compound was done by observing the two MS/MS ion transitions specific to a given pesticide (Table 3). The mass spectrum showing precursor ions  $[M + H]^+$  for pesticides and their deuterated counterparts is shown in Figure 3.

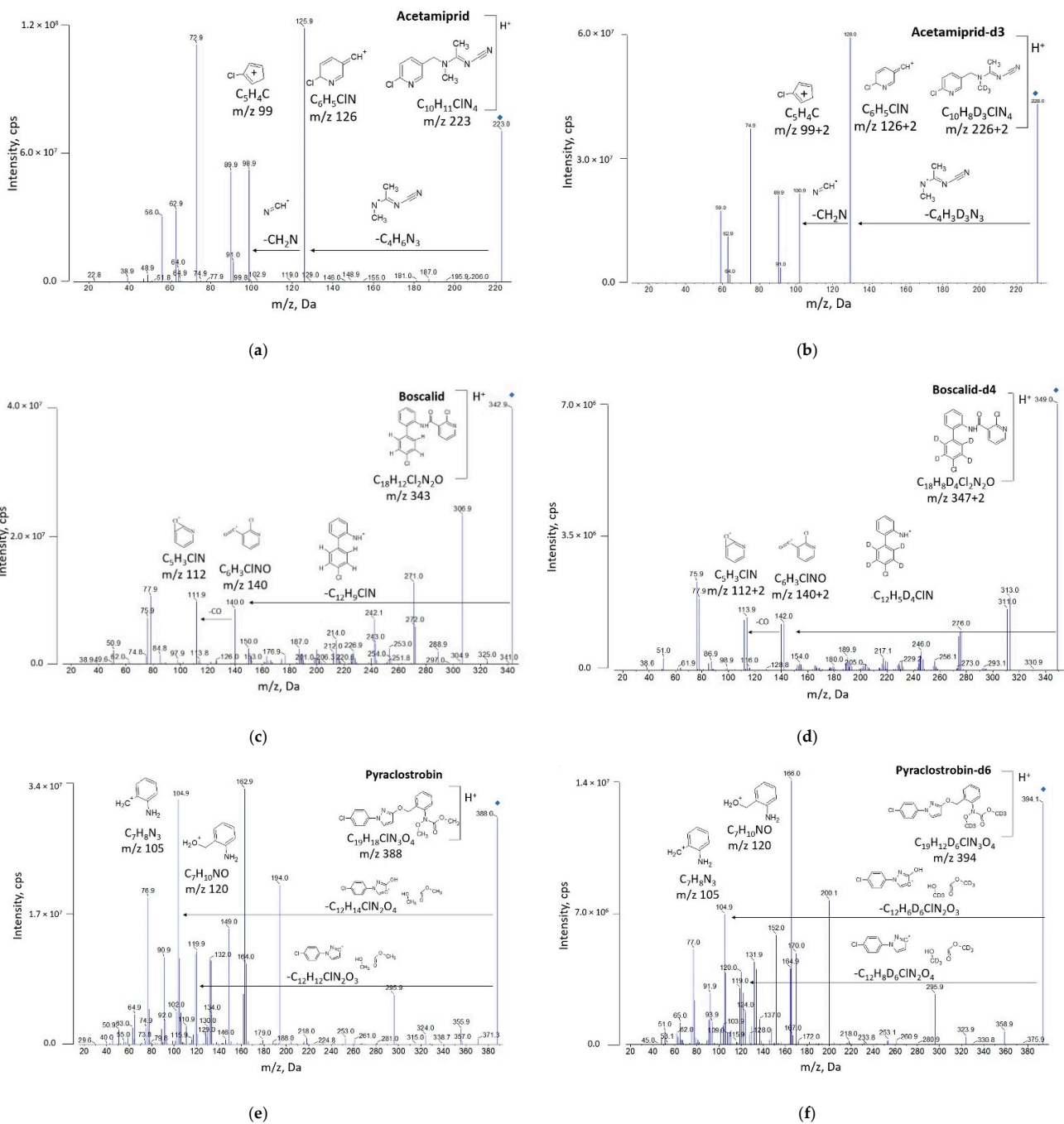
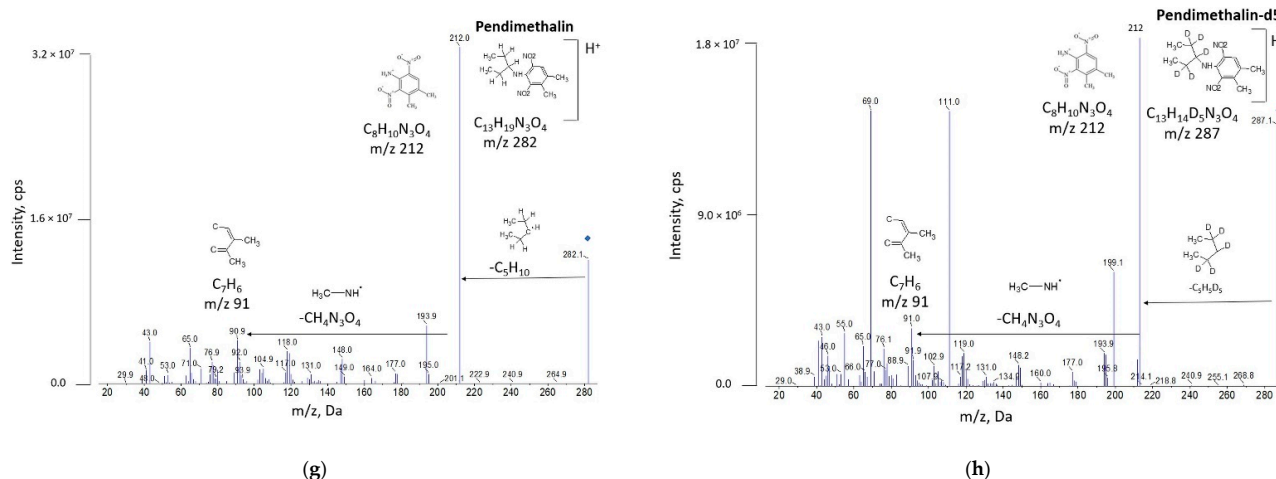


Figure 3. Cont.



**Figure 3.** MS<sup>2</sup> fragmentation by collision-induced dissociation (CID) mass spectra for: (a) acetamiprid, (c) boscalid, (e) pyraclostrobin, (g) pendimethalin, and their isotope-labeled counterparts: (b) acetamiprid-d3, (d) boscalid-d6, (f) pyraclostrobin-d6 and (h) pendimethalin-d5.

Two solvent blanks, two reagents blanks, and three control specimens (apples without pesticide treatments) were extracted and analyzed according to the method to investigate the presence of residue and/or background interference at the retention time of the analyte.

A series of standard solutions prepared in a solvent (acetonitrile) and an apple matrix were separated on the column, corresponding to two concentration levels: LQC (0.01 mg/kg) and HQC (0.1 mg/kg). By monitoring the MS/MS ion transitions for the tested pesticides, the confirmation ion ratios were calculated and compared (Equation (3)).

$$\text{Confirmation Ion Ratio} = \frac{\text{Peak Area of Confirmation Ion Transition}}{\text{Peak Area of Quantification Ion Transition}} \times 100 \quad (3)$$

Confirmation ion ratio differences were calculated as the percentage difference from the mean ratio of confirmation ions for the standards in the solvent as per Equation (4), where CIR Diff. represents the confirmation ion ratio difference; St. CIR represents the average standard confirmation ion ratio; and Sam. CIR represents the sample confirmation ion ratio.

$$\text{CIR Diff., \%} = \frac{\text{St. CIR} - \text{Sam. CIR}}{\text{St. CIR}} \times 100 \quad (4)$$

### 2.5.3. Matrix Effect

The influence of the matrix caused by apple sample extracts on the tested pesticide during the analysis was determined. The matrix effect (ME) was assessed by comparing the ratio of the analyte peak area/IS peak area of the standards at two concentration levels: LQC (0.01 mg/kg) and HQC (0.1 mg/kg) in the plant matrix with the analogous ratio of the selected standards in the pure solvent according to Equation (5). A negative value of the matrix effect indicates matrix suppression, and a positive value indicates matrix enhancement.

$$\text{ME [\%]} = \left( \left( \frac{\frac{\text{analyte peak area}}{\text{IS peak area}} \text{ in matrix}}{\frac{\text{analyte peak area}}{\text{IS peak area}} \text{ in solvent}} \right) - 1 \right) \times 100 \quad (5)$$

### 2.5.4. Accuracy and Precision

Accuracy was determined by enhancing the extract samples with known amounts of the analyte at two concentration levels: LQC (0.01 mg/kg) and HQC (0.1 mg/kg), and then determining the recoveries after performing the extraction procedure. Precision was determined based on repeatability (relative standard deviation—RSD). Five recovery

determinations were performed at LQC and HQC for the apple matrix. For the fortified sample, the percent recovery was calculated from the following Equation (6):

$$\text{Recovery, \%} = \frac{\text{Analyte in Enhanced Sample, mg/kg}}{\text{Enhancement Level, mg/kg}} \times 100 \quad (6)$$

### 2.5.5. Linearity

Certified pesticide standards and their isotopically labeled analogues were used as internal standards (IS) for calibration. The linearity of the detector response to the test pesticide was demonstrated by a single injection of the calibration standards in solvent (acetonitrile) at eight concentration levels ranging from 0.0003  $\mu\text{g/mL}$  to 0.04  $\mu\text{g/mL}$ , which corresponds to 0.003 mg/kg to 0.4 mg/kg. A weighted curve (1/x) was used.

### 2.5.6. Limit of Detection and Limit of Quantification

The limit of detection (LOD) is the lowest concentration of a substance that is detectable by a given measurement procedure.

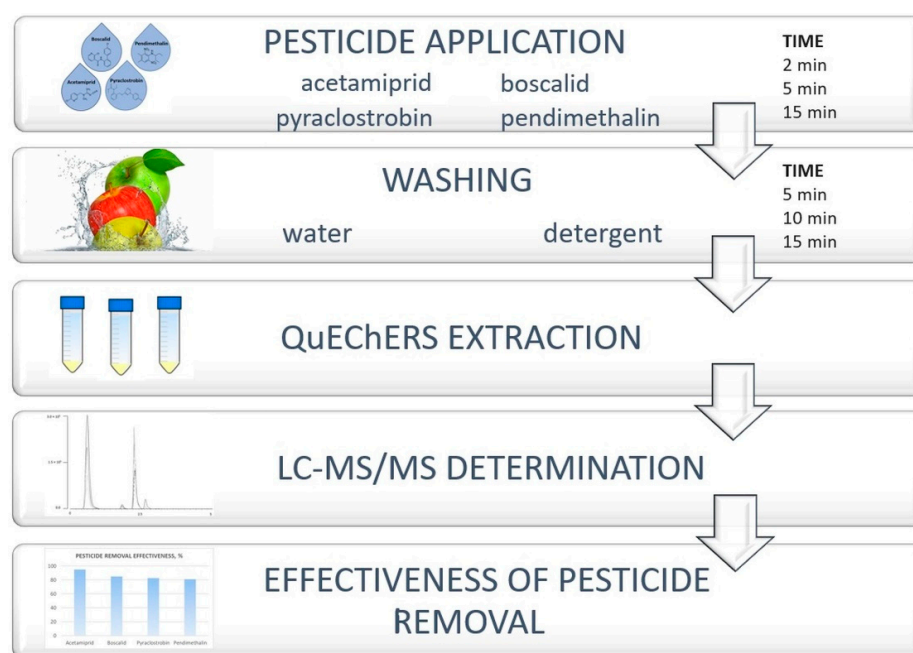
The limit of quantification (LOQ) determines the lowest concentration of an analyte that can be determined using a given measurement procedure. The LOQ of a method was defined as the lowest concentration of an analyte at which the method was successfully validated.

### 2.5.7. Stability

The solutions used to prepare the LQC and HQC standards on the plant matrix were stored in a refrigerator at  $5 \pm 4$  °C, and, at the end of the experimental phase, the solutions were re-analyzed by injection five times and compared with freshly prepared solutions. Assuming that the mean value of the initial determination is 100%, the percentage difference was calculated.

### 2.6. Pesticide Removal Efficacy Study

The developed method was applied to evaluate the removal efficacy of pesticide residues by the model detergent and water. This research stage aimed to select the parameters of the analytical procedure to evaluate the pesticide removal efficacy during the optimized cycle, according to the scheme shown in Figure 4.



**Figure 4.** Scheme of procedure for evaluating the effectiveness of pesticide removal.

Apples were selected as plant material for this stage of the study because they are a commonly consumed fruit in Poland and are the most frequently found to have the highest concentrations of pesticide residue during control.

The timing of 2, 5, and 15 min applications of a mixture of selected pesticides to apples was analyzed. A known amount of analytical standards of the most commonly used pesticides to protect the selected fruit (the insecticide acetamiprid, two fungicides: boscalid and pyraclostrobin, and the herbicide pendimethalin) was artificially applied to the selected plant material under laboratory conditions.

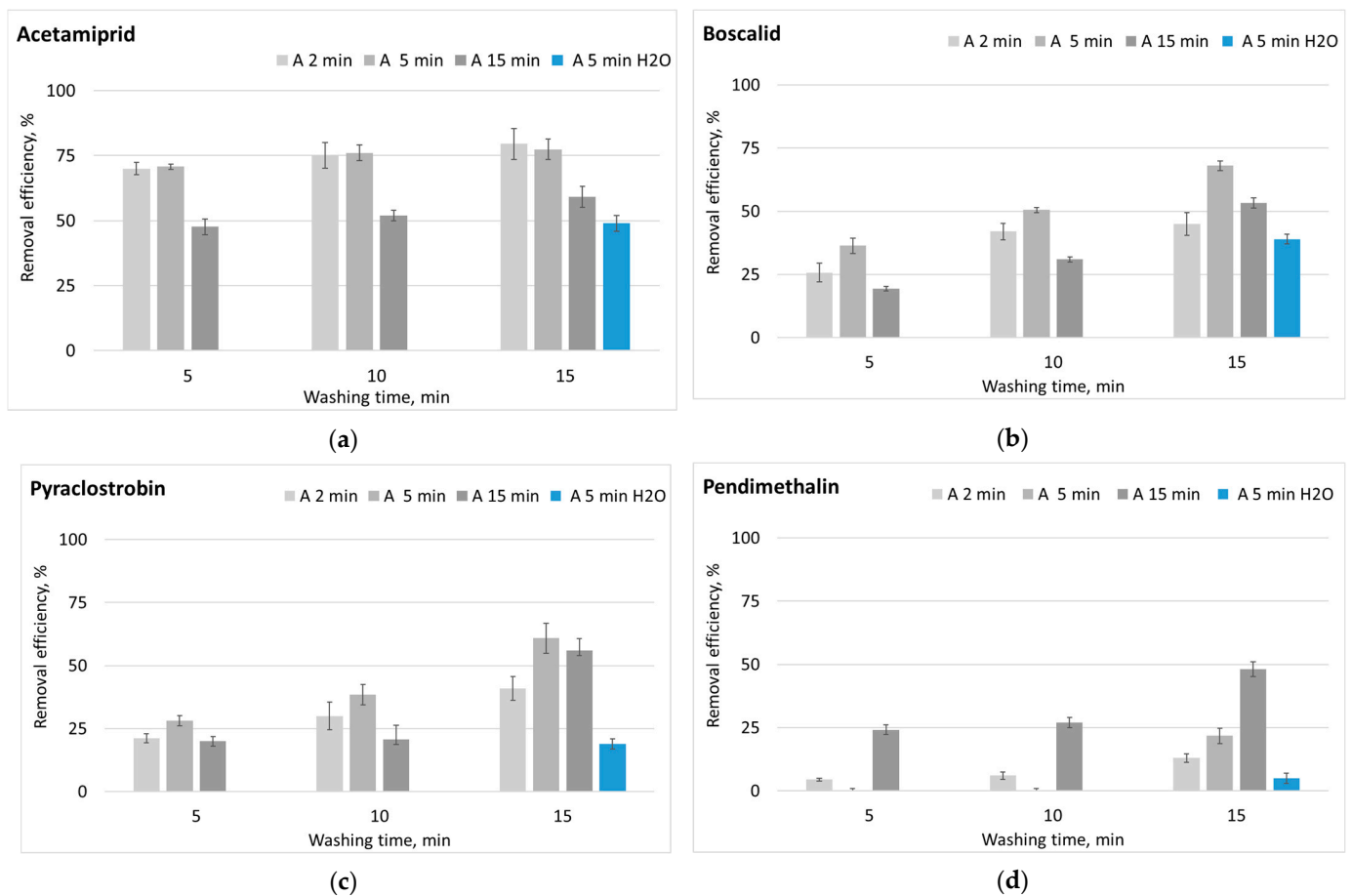
Then, the 5, 10 and 15 min times were analyzed for washing with the use of the model detergent, and the results were compared with water washing.

The proposed approach provides a very high ability to leach pesticides from the surface of fruits.

The removal efficiency of pesticide residues from the surface of the tested fruits was calculated according to Equation (7).

$$\text{Removal efficiency, \%} = \frac{\text{pesticide quantity in unwashed apple} - \text{pesticide quantity in washed apple}}{\text{pesticide quantity in unwashed apple}} \quad (7)$$

The results of the washing efficiency using the model detergent are summarized in Figure 5. Additionally, the results obtained with the 15 min water washing were referred to as a comparison.



**Figure 5.** The removal efficiency of (a) acetamiprid, (b) boscalid, (c) pyraclostrobin, and (d) pendimethalin at different pesticide application times and at different wash times with the model detergent with reference to 15 min water washing (blue bar). Mean  $\pm$  standard deviation (SD),  $n = 3$ . A 2 min—2 min application and MD washing, A 5 min—5 min application and MD washing, A 15 min—15 min application and MD washing, A 5 min H<sub>2</sub>O—5 min application and water washing.

### 2.7. Statistical Analysis

All UHPLC-MS/MS results in the study are expressed as the mean from three independent replicates ( $n = 3$ )  $\pm$  the standard deviation. Calculations were performed using the software Statistica ([www.StatSoft.pl](http://www.StatSoft.pl), accessed on 24 March 2022) ver. 10. A correlation matrix was used to find a significant correlation between the considered variables. Differences were considered significant when the  $p$ -value was  $<0.05$ .

## 3. Results and Discussion

### 3.1. Development of UHPLC-MS/MS Method

For the development of the UHPLC-MS/MS method, the MS/MS parameters, as well as chromatographic separation, were optimized. The MS/MS was working in MRM mode. The protonated molecule of each pesticide (parent ion) was generated in the ion source by ESI and was then isolated by the first quadrupole according to the mass/charge ( $m/z$ ) ratio and subjected to collision-induced dissociation (CID), which occurs in the collision cell (second quadrupole). The resulting fragmentation ions were separated according to their  $m/z$  ratio in the third quadrupole, and the fragmentation spectra were presented to confirm the chemical structures of the compounds of interest. For four pesticides and four isotope-labeled counterparts, the compounds' characteristic parameters such as DP, CE, EP, and CXP were optimized by a step-change in potentials as a result of instrument tuning and summarized in Table S1.

Although LC/MS/MS tools are very powerful, the most pernicious fundamental problem with powerful methods is their susceptibility to matrix effects that adversely affect quantification when analyzing complex samples [48]. Quantification of the tested pesticides was performed using the corresponding deuterium-labeled internal standards. Considering isotopic distribution, to avoid false-positive signals between the pesticide and isotope analog pair, a less intense third isotope peak was chosen as a quantitative ion for acetamiprid and boscalid internal standards (Table S2). This ensured the highest possible repeatability and guarantee of analytical quality by compensating for errors due to analyte recovery during sample preparation and due to instrument variability. However, the use of isotopically labeled internal standards is not common practice among other researchers, and this solution has not been used to validate the quality of other methods for evaluating the effectiveness of washing agents in removing pesticides from fruit or vegetable surface [15–17,20,49]. Similarly, deuterium-labeled internal standards were not used in the LC-MS/MS determination of multiple pesticide residues in fruit samples, including apples [50,51]. This approach makes our method more reliable in terms of eliminating unwanted matrix effects.

By comparing the ionization efficiency of the organics-water mixture, it was shown that acetonitrile causes an improvement in the signal intensity of the analyzed pesticides compared to methanol. The ratio of 75% acetonitrile and 25% water was found to be the most effective. On the other hand, the addition of ammonium formate as a phase modifier caused the greatest increase in signal intensity for the studied compounds in contrast to ammonium acetate, which proved to be the least efficient in terms of ionization. The highest ionization efficiency was observed for a concentration of ammonium formate of 2 mM, and further increases in its concentration resulted in a signal decrease. Based on the optimization, the solvent combination consisted of water:acetonitrile (25:75,  $v/v$ ), and the addition of 2 mM ammonium formate was used to maximally increase the ionization efficiency for the tested group of pesticides. The choice of this parameter allowed the sensitivity of the developed method to be increased. Yang et al. [19] used 0.1% formic acid in water and 0.1% formic acid in acetonitrile for the gradient elution program, while Bakırcı et al. [20] performed a gradient elution with eluent A composed of 5% methanol and 95% 2 mM ammonium acetate solution and eluent B composed of 95% methanol and 5% 2 mM ammonium acetate solution. Furthermore, 0.1% formic acid and water in the ratio of 4:6 was applied by Wu et al. [16]. However, this type of comparison of optimal solvent composition and concentration as well as the type and concentration of additives modifying

this solvent has not been previously shown by authors of other method validation work for evaluating the effectiveness of washing pesticide residues from fruit or vegetable surfaces.

Considering the chosen solvent, the optimal ion source parameters were selected by scanning the corresponding temperatures (TEM), pressures (GS1, GS2, CUR), and voltages (IS) stepwise. The optimized parameters of the ionization source were as follows: IS 5200 V, TEM 550 °C, GS1 60 psi, GS2 40 psi, and CUR 30 psi.

As a result of the optimization of the chromatographic conditions, a very good separation of the analyzed pesticides on the chromatographic column was obtained. By using UHPLC and a new generation of small grain size columns, the analysis time was shortened to 5 min, which was reduced by four times in comparison with analyses using traditional chromatographic columns while at the same time lowering both the amount of solvents used and the waste generated. Tian et al. [30] showed that under optimized conditions, with the 2.7 µm particle size, the analysis time was reached in less than 8 min, including cleaning and re-equilibration time for eleven fungicides in different matrices with acceptable levels of linearity, repeatability, accuracy, and precision. Dong et al. [29] used a column with a 1.7 µm particle size, and the achieved analysis time was less than 3 min for five target compounds. Additionally, UHPLC columns improved chromatographic performance and scale compatibility with MS/MS, making them an ideal choice for the separation of pesticide residues [52]. Different works dealing with bioanalytical and metabolites analysis, as well as environmental separations, compared columns packed with 5 µm and sub-2 µm supports and demonstrated that the latter clearly reduced the analysis time with comparable efficiency. Generally, the separation was achieved 5–20 times faster than in conventional HPLC [53,54].

### 3.2. Analytical Method Validation

The QuEChERS and UHPLC-MS/MS methods for the determination of pesticides in the apples were validated. Method validation was evaluated for the system suitability test, selectivity, matrix effect, accuracy and precision, linearity, limits of detection and quantification, and stability. The results were in accordance with the requirements of the SANCO/825/00 rev. 8.1 [45] and SANCO/3029/99 ver.4 [44] guidelines.

In the system suitability test, for both ion transitions of the studied pesticides, the RSD% value did not exceed the assumed acceptance criteria of 10%. The system was found to be working properly, and further actions proceeded. For both ion mass transitions of the solvent blank, reagent blank, and control specimens, interferences were not observed in the retention time of the studied compounds, proving the selectivity of the method. The retention time of the analyte in extracts corresponded to that of the calibration standards with a tolerance of  $\leq \pm 0.1$  min. Additionally, confirmation ion ratios for the analyte in all samples were within  $\pm 30\%$  of the average found for the standards. The effect of the matrix on the detection of acetamiprid, boscalid, pyraclostrobin and pendimethalin in apple extracts was below 20% and therefore not considered significant. The use of an internal standard allowed the matrix effect to be eliminated. The mean recovery values at LQC and HQC levels for both ion transitions for all pesticides were in the range of 70–110% and therefore complied with the assumed acceptance criteria. For both ion transitions of the four pesticides tested, all precision values based on the relative standard deviation at LQC and HQC fortification levels were  $< 20\%$ , which also met the SANCO requirements. For all pesticides, both calibration curves for both MRM transitions were linear with correlation coefficients (R) greater than 0.99. In the conducted study, a LOD of  $< 30\%$  LOQ (0.003 mg/kg) was determined for all pesticides, meeting the established acceptance criterion. Furthermore, the chromatographic peaks at LOD for apples, for both mass transitions, were more than three times the signal-to-noise ratio ( $S/N > 3$ ). A LOQ of 0.01 mg/kg was validated for all four considered pesticides as the lowest concentration of an analyte at which the method was successfully validated. The difference between stored and freshly prepared solutions did not exceed 10%. The results showed that the

matrix-prepared solutions of all pesticides were stable when tested under refrigerated conditions for at least 1 day.

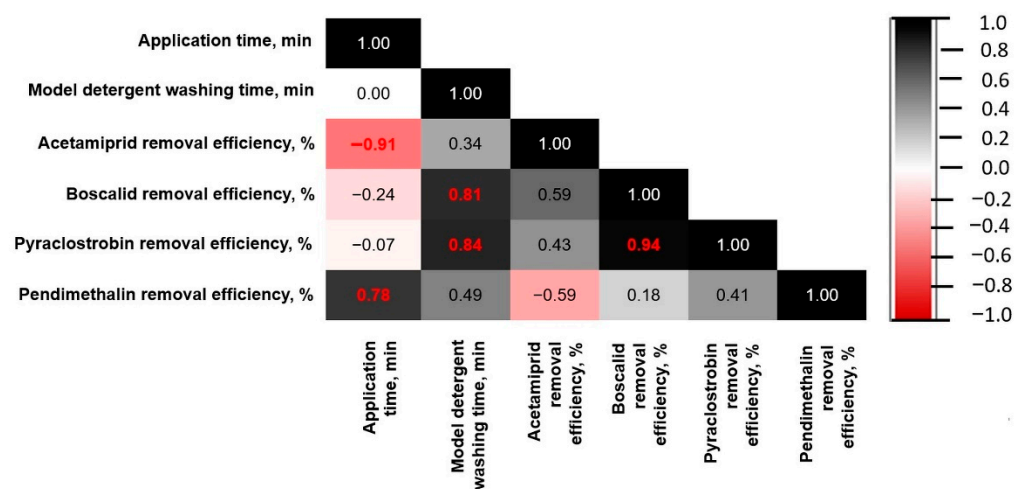
The obtained results showed that the developed method, in combination with extraction and purification by QuEChERS, is sensitive, highly specific, and selective and can be used for the determination of residues of selected pesticides (acetamiprid, boscalid, pyraclostrobin, and pendimethalin) in apples as a representant of the plant origin food. The final determination of pesticide residues in the obtained apple extracts was performed using the UHPLC-MS/MS technique, which is considered to be ideal for food analysis and allows the detection of analytes in a complex matrix at very low levels [55–58].

The determination of pesticide residues in food matrices is a challenge especially because of the low concentration of analytes and large amounts of interfering substances that can be co-extracted with analytes and, in most cases, negatively affect the analysis results. Currently, the QuEChERS method and its modifications are applied to a wide variety of matrices and compounds. Various combinations can be used depending on the type of matrix, chromatographic technique, and the compounds to be analyzed. Authors reported that the cleanup in the QuEChERS method for the determination of pesticide residues in fruits and vegetables helped reduce the amount of co-extracted matrix from the extracts and the matrix effect. In recent years, ultra-high performance liquid chromatography (UHPLC) has shown a variety of advantages compared to traditional high-performance liquid chromatography (HPLC). Coupling UHPLC with MS for food analysis provides higher sensitivity, better peak capacity, higher speed, better resolution, and reduced chromatographic run time because the particle size of the stationary phase is much smaller [31–33]. With all these considerations, there is an assumption that there is the potential of UHPLC coupled with the MS/MS technique for applicability in the routine analysis of fruit pesticides.

### 3.3. Pesticide Residuals Removal

The analytical procedure for the selection of pesticide application times and washing times using a model washing detergent was developed. The optimized process for assessing the effectiveness of removing pesticide residues from the surface of apples using the model detergent was shown.

To find significant relationships between the variables considered, a correlation matrix was used to interpret the data obtained, taking into account the statistical significance of the results (Figure 6).



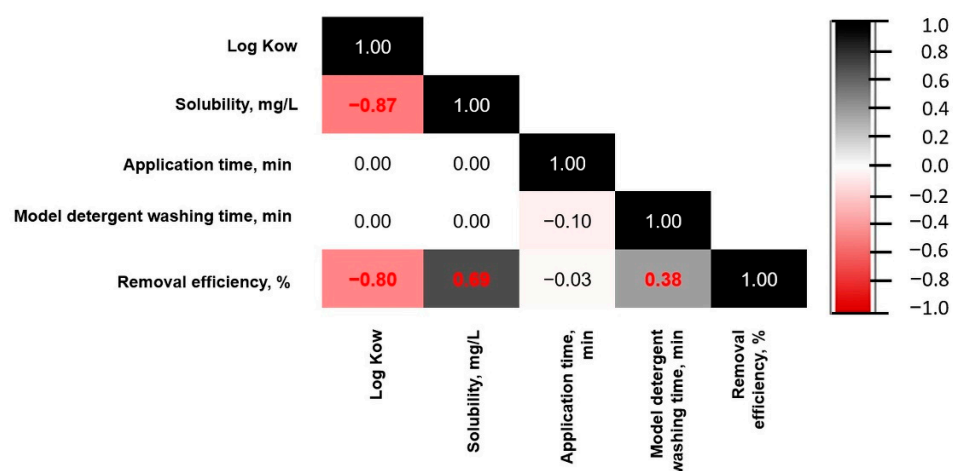
**Figure 6.** Correlations between pesticide application time, model detergent washing time, and amount of pesticide removed. Statistically significant coefficients highlighted in red ( $p < 0.05$ ).

This analysis allowed us to observe that an increase in the application time with the pesticide mixture resulted in a decrease in the washing efficiency of acetamiprid from the apple surface in contrast to pendimethalin, whereas with an increase in its application

time, its washing efficiency increased. Moreover, the application time did not have a statistically significant effect on the change in the amount of boscalid and pyraclostrobin. For the experiments conducted, it does not seem reasonable to increase the time of pesticide application, as a 5-min application allows changes in pesticide removal to be tracked.

The study also concluded that prolonging the washing time with the model detergent allowed more efficient removal of boscalid and pyraclostrobin from the surface of the applied apples, while the degree of removal of acetamiprid and pendimethalin did not differ significantly in the considered washing times. No plateau of the washing time curve was observed. For further studies on the evaluation of the effectiveness of washing out pesticide residues from the surface of apples, it was therefore decided to choose a washing time of 15 min, as its extension does not seem to be practical in terms of consumer use of the liquids.

Based on a correlation analysis of selected physicochemical properties (Table 1) of the pesticides included in the study, the dependence of pesticide removal ability from the apple surface on the solubility of pesticides in water was shown in Figure 7. The pesticides with better water solubility showed a higher wash-off rate.



**Figure 7.** Correlations between individual parameters. Statistically significant coefficients are highlighted in red ( $p < 0.05$ ).

In addition, a correlation of the n-octanol-water partition coefficient (log Kow) of the considered pesticides with the degree of their removal from the apple surface was found. The pesticides with a lower log Kow value (acetonitrile and boscalid) represented a higher removal efficiency. Pendimethalin and pyraclostrobin, which represented higher values of the partition coefficient and thus were more hydrophobic, washed off worse. This may be due to the fact that on the surface of the apple skin, there is a wax that helps, among others, to protect the fruit from excessive loss of moisture, helps to maintain firmness, and naturally slows down the spoilage process. Sometimes the fruit is coated with an additional portion of high-quality edible wax such as shellac or carnauba wax to reinforce the fruit's natural wax coating and prolong its shelf life. These waxes are edible and safe to eat [59]; however, due to their hydrophobic properties, they more easily trap hydrophobic pesticides (pendimethalin, pyraclostrobin), causing them to wash off the fruit surface more poorly. Such observations on the analysis of the relationships between the physicochemical properties of the tested pesticides are in agreement with other literature reports [49]. Valverde et al. also reported that pyridaben and tralomethrin were quickly absorbed and strongly retained by the waxes of pepper skin owing to their high liposolubility. Elimination by water washing only was impossible [60,61]. Similarly, Boulaid et al. concluded that negligible elimination by washing was due to the high liposolubility of pyrifenoxy, pyridaben, and tralomethrin; these pesticides were quickly absorbed and



strongly retained by the waxes of tomato skin [61,62]. Additionally, inversely related log Kow to aqueous solubility was also noted [63].

For comparison purposes, the most effective 15-min washing of apples with water after a prior 5-min pesticide application was chosen. The removal efficiency of water washing for all pesticides was found to be significantly worse compared to the model washing detergent, which is in agreement with other authors [16].

The removal efficiencies of acetamiprid, boscalid, pyraclostrobin, and pendimethalin during a 15-min wash with water were 49, 39, 19, and 5%, respectively. During the same washing time, in a bath using 10% solution of model detergent, 77, 68, 61, and 22% of acetamiprid, boscalid, pyraclostrobin, and pendimethalin were removed, respectively. The difference in detergent and water removing efficiency was the highest for pyraclostrobin (42%), followed by boscalid and acetamiprid (28% and 29%) and lowest for pendimethalin (17%).

The removal efficiency of pesticide residues, especially with hydrophobic properties, by washing with pure water is relatively low. The use of surfactant solutions provides an opportunity to leach particularly hydrophobic pesticide residues from the surface of fruits and then transport them to the bulk phase of the bath through a micellar solubilization process. The obtained results indicated that the surface and solubilization activity of surfactants helps in removing pesticide residues from the surface of fruits. Wu et al. [16] described the effect of removing 10 typical pesticide residues from kumquat, cucumber, and spinach using tap water, micron calcium solution, alkaline electrolyzed water, ozonated water, active oxygen, and sodium bicarbonate. The efficiency of tap water in removing the investigated pesticide residues from cucumber was less than 35%. Washing with a 2% active oxygen solution and micron calcium solution for 20 min resulted in a loss of 40–63% and 35–86% of pesticides, respectively. Rao et al. [64] have studied the removal of pesticide residues from brinjal by dipping it in 0.1% sodium bicarbonate. The results showed that 20% to 60% of the residues of the pesticides were removed. The studies on the removal of pesticides from food products revealed a trend in the search for washing fruits and vegetable products based mainly on surfactants or various types of salts as well as substances with an alkalizing or acidifying effect in the washing process [16,64–66].

#### 4. Conclusions

In this paper, the possibility of applying the UHPLC-MS/MS technique for evaluating the removal efficacy of pesticide residues from fruit surfaces by dedicated washing products was established.

As a result, an analytical procedure for the quantitative and qualitative determination of the residues of four pesticides, acetamiprid, boscalid, pyraclostrobin, and pendimethalin, was developed and validated in an apple matrix. The use of a new generation of columns shortened the analysis time by four times, improving the economics of the assay. A selected mobile phase modifier increased the sensitivity of the method, and isotope-labeled internal standards ensured the highest analytical quality. It was also confirmed that the removal efficacy of pesticides by washing with water alone is lower than with dedicated washing detergents. Furthermore, the dependence of the ability to remove pesticides from the surface of apples on the water solubility of pesticides has been demonstrated. Additionally, the correlation of log Kow of considered pesticides with pesticides removal was shown.

The obtained results indicate that washing samples in a detergent solution can effectively reduce pesticide residues in fruits and provide people with a healthier diet. The developed method can be successfully applied to evaluate the washing efficacy of fruits in assessing the developed detergents.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/pr10040793/s1>, Table S1: Mass spectrometer operating parameters used for the determination of selected pesticides; Table S2: isotopic distributions for individual pesticides and their deuterated analogues: (a) acetamiprid, (b) boscalid, (c) pyraclostrobin, (d) pendimethalin; Figure S1: Sample preparing procedure: (a) immersion in a pesticides solution,

(b) detergent washing, (c) tap water washing for detergent removal, (d) QuEChERS extraction of the ground apples.

**Author Contributions:** Conceptualization M.Z., Z.H.-B. and T.W.; Data curation M.Z., Z.H.-B., T.W. and E.Z.-T.; Formal analysis M.Z., Z.H.-B., T.W., N.S. and E.Z.-T.; Methodology M.Z., Z.H.-B., T.W., N.S. and E.Z.-T.; Project administration M.Z., Z.H.-B. and T.W.; Visualization M.Z.; and Writing—original draft M.Z., Z.H.-B., T.W., N.S. and E.Z.-T.; Writing—review & editing M.Z., Z.H.-B., T.W., N.S. and E.Z.-T. All authors have read and agreed to the published version of the manuscript.

**Funding:** The research was funded by the European Union from the European Regional Development Found under the Intelligent Development Operational Program 2014–2020. Project implemented as part of a competition of National Centre for Research and Development: Application Project, grant number POIR.04.01.04-00-0112/19-00.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

- Bassil, K.; Vakil, C.; Sanborn, M.; Cole, D.; Kaur, J.; Kerr, K. Cancer Health Effects of Pesticides: Systematic Review. *Can. Fam. Phys.* **2007**, *53*, 1704–1711.
- Mostafalou, S.; Abdollahi, M. Pesticides and Human Chronic Diseases: Evidences, Mechanisms, and Perspectives. *Toxicol. Appl. Pharmacol.* **2013**, *268*, 157–177. [[CrossRef](#)]
- Bjørning-Poulsen, M.; Andersen, H.R.; Grandjean, P. Potential Developmental Neurotoxicity of Pesticides Used in Europe. *Environ. Health* **2008**, *7*, 50. [[CrossRef](#)]
- Lončarić, A.; Matanović, K.; Ferrer, P.; Kovač, T.; Šarkanj, B.; Skendrović Babojelić, M.; Lores, M. Peel of Traditional Apple Varieties as a Great Source of Bioactive Compounds: Extraction by Micro-Matrix Solid-Phase Dispersion. *Foods* **2020**, *9*, 80. [[CrossRef](#)]
- Slavin, J.L.; Lloyd, B. Health Benefits of Fruits and Vegetables. *Adv. Nutr.* **2012**, *3*, 506–516. [[CrossRef](#)]
- Wallace, T.C.; Bailey, R.L.; Blumberg, J.B.; Burton-Freeman, B.; Chen, C.-Y.O.; Crowe-White, K.M.; Drewnowski, A.; Hooshmand, S.; Johnson, E.; Lewis, R.; et al. Fruits, Vegetables, and Health: A Comprehensive Narrative, Umbrella Review of the Science and Recommendations for Enhanced Public Policy to Improve Intake. *Crit. Rev. Food Sci. Nutr.* **2020**, *60*, 2174–2211. [[CrossRef](#)]
- Żurawicz, E.; Kubik, J.; Lewandowski, M.; Rutkowski, K.P.; Zmarlicki, K. The apple industry in Poland. *Acta Hort.* **2019**, *1261*, 13–20. [[CrossRef](#)]
- Łozowicka, B.; Kaczyński, P.; Rutkowska, E.; Jankowska, M.; Hrynko, I. Evaluation of Pesticide Residues in Fruit from Poland and Health Risk Assessment. *Agric. Sci.* **2013**, *4*, 106–111. [[CrossRef](#)]
- Kowalska, G.; Kowalski, R. Pestycydy—Zakres i Ryzyko Stosowania, Korzyści i Zagrożenia—Praca Przeglądowa. Pesticides—Scope and Risk of Use, Benefits and Hazards. A Review. *Ann. Hort.* **2020**, *29*, 5–25. [[CrossRef](#)]
- Jankowska, M.; Łozowicka, B.; Kaczyński, P. Comprehensive Toxicological Study over 160 Processing Factors of Pesticides in Selected Fruit and Vegetables after Water, Mechanical and Thermal Processing Treatments and Their Application to Human Health Risk Assessment. *Sci. Total Environ.* **2019**, *652*, 1156–1167. [[CrossRef](#)]
- Szpyrka, E.; Slowik-Borowiec, M.; Matyaszek, A.; Podbielska, M.; Rupa, J. Pesticide residues in raw agricultural products from the south-eastern region of Poland and the acute risk assessment. *Rocz. Panstw. Zakł. Hig.* **2016**, *67*, 237–245. [[PubMed](#)]
- Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on Maximum Residue Levels of Pesticides in or on Food and Feed of Plant and Animal Origin and Amending Council Directive 91/414/EEC (Text with EEA Relevance). Available online: <https://www.legislation.gov.uk/eur/2005/396/contents> (accessed on 12 April 2022).
- Góralczyk, K.; Struciński, P.; Korcz, W.; Czaja, K.; Hernik, A.; Snopczyński, T.; Ludwicki, J. The Survey of Pesticide Residues in Food of Plant Origin in Poland, 2004–2007. *Rocz. Panstw. Zakładu Zakł. Hig.* **2009**, *60*, 113–119.
- Analiza Potencjalnego Zagrożenia Zdrowia Konsumentów Wynikającego z Obecności Pozostałości Pestycydów w Żywności Dostępnej na Polskim Rynku w Roku 2017, Narodowy Instytut Zdrowia Publicznego PZH—Państwowy Instytut Badawczy 31.12.2019. Available online: <https://www.pzh.gov.pl/wp-content/uploads/2020/03/Raport-Analiza-potencjalnego-zagro%C5%BCenia-zdrowia-konsument%C3%B3w-...-pozosta%C5%82o%C5%9Bci-pestycyd%C3%B3w-2017-na-stron%C4%99.pdf> (accessed on 21 December 2021).
- Łozowicka, B.; Jankowska, M. Comparison of the effects of water and thermal processing on pesticide removal in selected fruit and vegetables. *J. Elem.* **2016**, *21*, 99–111.
- Wu, Y.; An, Q.; Li, D.; Wu, J.; Pan, C. Comparison of Different Home/Commercial Washing Strategies for Ten Typical Pesticide Residue Removal Effects in Kumquat, Spinach and Cucumber. *Int. J. Environ. Res. Public Health* **2019**, *16*, 472. [[CrossRef](#)] [[PubMed](#)]
- Wang, Z.; Huang, J.; Chen, J.; Li, F. Effectiveness of Dishwashing Liquids in Removing Chlorothalonil and Chlorpyrifos Residues from Cherry Tomatoes. *Chemosphere* **2013**, *92*, 1022–1028. [[CrossRef](#)]
- Soltani Nazarloo, A.; Rasooli Sharabiani, V.; Abbaspour Gilandeh, Y.; Taghinezhad, E.; Szymanek, M.; Sprawka, M. Feasibility of Using VIS/NIR Spectroscopy and Multivariate Analysis for Pesticide Residue Detection in Tomatoes. *Processes* **2021**, *9*, 196. [[CrossRef](#)]

19. Yang, T.; Doherty, J.; Zhao, B.; Kinchla, A.J.; Clark, J.M.; He, L. Effectiveness of Commercial and Homemade Washing Agents in Removing Pesticide Residues on and in Apples. *J. Agric. Food Chem.* **2017**, *65*, 9744–9752. [[CrossRef](#)]
20. Bakırcı, G.T.; Hışıl, Y. Fast and Simple Extraction of Pesticide Residues in Selected Fruits and Vegetables Using Tetrafluoroethane and Toluene Followed by Ultrahigh-Performance Liquid Chromatography/Tandem Mass Spectrometry. *Food Chem.* **2012**, *135*, 1901–1913. [[CrossRef](#)]
21. Han, Y.; Zou, N.; Song, L.; Li, Y.; Qin, Y.; Liu, S.; Li, X.; Pan, C. Simultaneous Determination of 70 Pesticide Residues in Leek, Leaf Lettuce and Garland Chrysanthemum Using Modified QuEChERS Method with Multi-Walled Carbon Nanotubes as Reversed-Dispersive Solid-Phase Extraction Materials. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2015**, *1005*, 56–64. [[CrossRef](#)]
22. Botero-Coy, A.M.; Marín, J.M.; Serrano, R.; Sancho, J.V.; Hernández, F. Exploring Matrix Effects in Liquid Chromatography–Tandem Mass Spectrometry Determination of Pesticide Residues in Tropical Fruits. *Anal. Bioanal. Chem.* **2015**, *407*, 3667–3681. [[CrossRef](#)]
23. Golge, O.; Kabak, B. Evaluation of QuEChERS Sample Preparation and Liquid Chromatography-Triple-Quadrupole Mass Spectrometry Method for the Determination of 109 Pesticide Residues in Tomatoes. *Food Chem.* **2015**, *176*, 319–332. [[CrossRef](#)] [[PubMed](#)]
24. Mol, H.G.J.; Zomer, P.; García López, M.; Fussell, R.J.; Scholten, J.; de Kok, A.; Wolheim, A.; Anastassiades, M.; Lozano, A.; Fernandez Alba, A. Identification in Residue Analysis Based on Liquid Chromatography with Tandem Mass Spectrometry: Experimental Evidence to Update Performance Criteria. *Anal. Chim. Acta* **2015**, *873*, 1–13. [[CrossRef](#)] [[PubMed](#)]
25. Núñez, O.; Gallart-Ayala, H.; Ferrer, I.; Moyano, E.; Galceran, M.T. Strategies for the Multi-Residue Analysis of 100 Pesticides by Liquid Chromatography–Triple Quadrupole Mass Spectrometry. *J. Chromatogr. A* **2012**, *1249*, 164–180. [[CrossRef](#)] [[PubMed](#)]
26. Li, W.; Morgan, M.K.; Graham, S.E.; Starr, J.M. Measurement of Pyrethroids and Their Environmental Degradation Products in Fresh Fruits and Vegetables Using a Modification of the Quick Easy Cheap Effective Rugged Safe (QuEChERS) Method. *Talanta* **2016**, *151*, 42–50. [[CrossRef](#)] [[PubMed](#)]
27. Prodhon, M.; Papadakis, E.-N.; Papadopoulou-Mourkidou, E. Analysis of Pesticide Residues and Their Variability in Cabbage Using QuEChERS Extraction in Combination with LC-MS/MS. *Food Anal. Methods* **2016**, *9*, 3470–3478. [[CrossRef](#)]
28. Stachniuk, A.; Szmagara, A.; Czaczk, R.; Fornal, E. LC-MS/MS Determination of Pesticide Residues in Fruits and Vegetables. *J. Environ. Sci. Health Part B* **2017**, *52*, 446–457. [[CrossRef](#)]
29. Dong, F.; Chen, X.; Liu, X.; Xu, J.; Li, Y.; Shan, W.; Zheng, Y. Simultaneous Determination of Five Pyrazole Fungicides in Cereals, Vegetables and Fruits Using Liquid Chromatography/Tandem Mass Spectrometry. *J. Chromatogr. A* **2012**, *1262*, 98–106. [[CrossRef](#)]
30. Tian, F.; Qiao, C.; Luo, J.; Guo, L.; Pang, T.; Pang, R.; Li, J.; Wang, C.; Wang, R.; Xie, H. 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/M.S. *J. Chromatogr. B* **2020**, *1152*, 122261. [[CrossRef](#)]
31. Concha-Meyer, A.; Grandon, S.; Sepúlveda, G.; Diaz, R.; Yuri, J.A.; Torres, C. Pesticide Residues Quantification in Frozen Fruit and Vegetables in Chilean Domestic Market Using QuEChERS Extraction with Ultra-High-Performance Liquid Chromatography Electrospray Ionization Orbitrap Mass Spectrometry. *Food Chem.* **2019**, *295*, 64–71. [[CrossRef](#)]
32. Stringhini, F.; Ribeiro, L.; Rocha, G.; Kuntz, J.; Zanella, R.; Prestes, O.; Adaime, M. Dilution of QuEChERS Extracts Without Cleanup Improves Results in the UHPLC-MS/MS Multiresidue Analysis of Pesticides in Tomato. *Food Methods* **2021**, *14*, 1511–1523. [[CrossRef](#)]
33. Guo, J.; Tong, M.; Tang, J.; Bian, H.; Wan, X.; He, L.; Hou, R. Analysis of Multiple Pesticide Residues in Polyphenol-Rich Agricultural Products by UPLC-MS/MS Using a Modified QuEChERS Extraction and Dilution Method. *Food* **2019**, *274*, 452–459. [[CrossRef](#)] [[PubMed](#)]
34. Hajslová, J.; Zrostlíková, J. Matrix Effects in (Ultra)Trace Analysis of Pesticide Residues in Food and Biotic Matrices. *J. Chromatogr. A* **2003**, *1000*, 181–197. [[CrossRef](#)]
35. Anastassiades, M.; Lehotay, S.; Štajnbaher, D.; Schenck, F. Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and “Dispersive Solid-Phase Extraction” for the Determination of Pesticide Residues in Produce. *J. AOAC Int.* **2003**, *86*, 412–431. [[CrossRef](#)] [[PubMed](#)]
36. Lehotay, S.; Kok, A.; Hiemstra, M.; Bodegraven, P. Validation of a Fast and Easy Method for the Determination of Residues from 229 Pesticides in Fruits and Vegetables Using Gas and Liquid Chromatography and Mass Spectrometric Detection. *J. AOAC Int.* **2005**, *88*, 595–614. [[CrossRef](#)]
37. Lehotay, S.; Mastovska, K.; Lightfield, A. Use of Buffering Other Means to Improve Results of Problematic Pesticides in a Fast Easy Method for Residue Analysis of Fruits Vegetables. *J. AOAC Int.* **2005**, *88*, 615–629. [[CrossRef](#)]
38. Nguyen, T.D.; Lee, B.S.; Lee, B.R.; Lee, D.M.; Lee, G.-H. A Multiresidue Method for the Determination of 109 Pesticides in Rice Using the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) Sample Preparation Method and Gas Chromatography/Mass Spectrometry with Temperature Control and Vacuum Concentration. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 3115–3122. [[CrossRef](#)]
39. EN 15662:2008—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. Available online: <https://standards.iteh.ai/catalog/standards/cen/9f9e56e8-ac1c-4f3e-9f91-23d42703dd8a/en-15662-2008> (accessed on 6 March 2022).

40. Lequang, H. AOAC Official Method 2007.01 Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate Gas Chromatography/Mass Spectrometry and Liquid Chromatography/Tandem Mass Spectrometry First Action 2007. Available online: [https://www.academia.edu/33471645/AOAC\\_Official\\_Method\\_2007\\_01\\_Pesticide\\_Residues\\_in\\_Foods\\_by\\_Acetonitrile\\_Extraction\\_and\\_Partitioning\\_with\\_Magnesium\\_Sulfate\\_Gas\\_Chromatography\\_Mass\\_Spectrometry\\_and\\_Liquid\\_Chromatography\\_Tandem\\_Mass\\_Spectrometry\\_First\\_Action\\_2007](https://www.academia.edu/33471645/AOAC_Official_Method_2007_01_Pesticide_Residues_in_Foods_by_Acetonitrile_Extraction_and_Partitioning_with_Magnesium_Sulfate_Gas_Chromatography_Mass_Spectrometry_and_Liquid_Chromatography_Tandem_Mass_Spectrometry_First_Action_2007) (accessed on 6 February 2022).
41. Kaushik, G.; Satya, S.; Naik, S.N. Food Processing a Tool to Pesticide Residue Dissipation—A Review. *Food Res.* **2009**, *42*, 26–40. [[CrossRef](#)]
42. Hao, J.; Liu, H.; Chen, T.; Zhou, Y.; Su, Y.-C.; Li, L. Reduction of Pesticide Residues on Fresh Vegetables with Electrolyzed Water Treatment. *J. Food Sci.* **2011**, *76*, C520–C524. [[CrossRef](#)]
43. Qi, H.; Huang, Q.; Hung, Y.-C. Effectiveness of Electrolyzed Oxidizing Water Treatment in Removing Pesticide Residues and Its Effect on Produce Quality. *Food Chem.* **2018**, *239*, 561–568. [[CrossRef](#)]
44. European Commission. *Technical Material and Preparations: Guidance for Generating and Reporting Methods of Analysis in Support of Pre- and Post-Registration Data Requirements for Annex II (Part A, Section 4) and Annex III (Part A, Section 5) of Directive 91/414. SANCO/3029/99-Rev.4*; European Commission: Brussels, Belgium, 2000.
45. European Commission. *Guidance Document on Residue Analytical Methods. SANCO/825/00-Rev. 8.1*; European Commission: Brussels, Belgium, 2010.
46. Melnyk, A.; Namieśnik, J.; Wolska, L. Theory and Recent Applications of Coacervate-Based Extraction Techniques. *Trends Anal. Chem.* **2015**, *71*, 282–292. [[CrossRef](#)]
47. Arya, S.S.; Kaimal, A.M.; Chib, M.; Sonawane, S.K.; Show, P.L. Novel, Energy Efficient and Green Cloud Point Extraction: Technology and Applications in Food Processing. *J. Food Sci. Technol.* **2019**, *56*, 524–534. [[CrossRef](#)] [[PubMed](#)]
48. Trufelli, H.; Palma, P.; Famigliani, G.; Cappiello, A. An Overview of Matrix Effects in Liquid Chromatography-Mass Spectrometry. *Mass Spectrom. Rev.* **2011**, *30*, 491–509. [[CrossRef](#)] [[PubMed](#)]
49. Rodrigues, A.A.Z.; de Queiroz, M.E.L.R.; Neves, A.A.; de Oliveira, A.F.; Prates, L.H.F.; de Freitas, J.F.; Heleno, F.F.; Faroni, L.R.D. Use of Ozone and Detergent for Removal of Pesticides and Improving Storage Quality of Tomato. *Food Res. Int.* **2019**, *125*, 108626. [[CrossRef](#)] [[PubMed](#)]
50. Christia, C.; Bizani, E.; Christophoridis, C.; Fytianos, K. Pesticide Residues in Fruit Samples: Comparison of Different QuEChERS Methods Using Liquid Chromatography–Tandem Mass Spectrometry. *Environ. Sci. Pollut. Res.* **2015**, *22*, 13167–13178. [[CrossRef](#)]
51. Mandal, S.; Poi, R.; Ansary, I.; Hazra, D.K.; Bhattacharyya, S.; Karmakar, R. Validation of a Modified QuEChERS Method to Determine Multiclass Multipesticide Residues in Apple, Banana and Guava Using GC–MS and LC–MS/MS and Its Application in Real Sample Analysis. *SN Appl. Sci.* **2020**, *2*, 188. [[CrossRef](#)]
52. Masiá, A.; Blasco, C.; Picó, Y. Last Trends in Pesticide Residue Determination by Liquid Chromatography–Mass Spectrometry. *Trends Environ. Anal. Chem.* **2014**, *2*, 11–24. [[CrossRef](#)]
53. Nguyen, D.T.-T.; Guillaume, D.; Rudaz, S.; Veuthey, J.-L. Fast Analysis in Liquid Chromatography Using Small Particle Size and High Pressure. *J. Sep. Sci.* **2006**, *29*, 1836–1848. [[CrossRef](#)]
54. Walter, T.H.; Andrews, R.W. Recent Innovations in UHPLC Columns and Instrumentation. *Trends Anal. Chem.* **2014**, *63*, 14–20. [[CrossRef](#)]
55. Wang, J.; Chow, W.; Chang, J.; Wong, J.W. Ultrahigh-Performance Liquid Chromatography Electrospray Ionization Q-Orbitrap Mass Spectrometry for the Analysis of 451 Pesticide Residues in Fruits and Vegetables: Method Development and Validation. *J. Agric. Food Chem.* **2014**, *62*, 10375–10391. [[CrossRef](#)]
56. Stachniuk, A.; Fornal, E. Liquid Chromatography-Mass Spectrometry in the Analysis of Pesticide Residues in Food. *Food Anal. Methods* **2016**, *9*, 1654–1665. [[CrossRef](#)]
57. Botitsi, H.V.; Garbis, S.D.; Economou, A.; Tsipi, D.F. Current Mass Spectrometry Strategies for the Analysis of Pesticides and Their Metabolites in Food and Water Matrices. *Mass Spectrom. Rev.* **2010**, *30*, 907–939. [[CrossRef](#)] [[PubMed](#)]
58. Kittlaus, S.; Schimanke, J.; Kempe, G.; Speer, K. Assessment of Sample Cleanup and Matrix Effects in the Pesticide Residue Analysis of Foods Using Postcolumn Infusion in Liquid Chromatography–Tandem Mass Spectrometry. *J. Chromatogr. A* **2011**, *1218*, 8399–8410. [[CrossRef](#)] [[PubMed](#)]
59. Dozwolone Substancje Dodatkowe. Dz.U.2010.232.1525-OpenLEX. Available online: <https://sip.lex.pl/akty-prawne/dzu-dziennik-ustaw/dozwolone-substancje-dodatkowe-17665215> (accessed on 8 March 2022).
60. Valverde, A.; Aguilera, A.; Rodríguez, M.; Boulaid, M.; Soussi-El Begrani, M. Pesticide Residue Levels in Peppers Grown in a Greenhouse after Multiple Applications of Pyridaben and Tralomethrin. *J. Agric. Food Chem.* **2002**, *50*, 7303–7307. [[CrossRef](#)] [[PubMed](#)]
61. Chung, S.W. How Effective Are Common Household Preparations on Removing Pesticide Residues from Fruit and Vegetables? A Review: Removal of Pesticide Residues. *J. Sci. Food Agric.* **2018**, *98*, 2857–2870. [[CrossRef](#)]
62. Boulaid, M.; Aguilera, A.; Camacho, F.; Soussi, M.; Valverde, A. Effect of Household Processing and Unit-to-Unit Variability of Pyrifenoxy, Pyridaben, and Tralomethrin Residues in Tomatoes. *J. Agric. Food Chem.* **2005**, *53*, 4054–4058. [[CrossRef](#)]
63. Finizio, A.; Vighi, M.; Sandroni, D. Determination of N-Octanol/Water Partition Coefficient (Kow) of Pesticide Critical Review and Comparison of Methods. *Chemosphere* **1997**, *34*, 131–161. [[CrossRef](#)]
64. Rao, C.S.; Bhushan, V.S.; Reddy, H.; Darsi, R.; Aruna, M.; Ramesh, B. Risk Mitigation Methods for Removal of Pesticide Residues in Brinjal for Food Safety. *Univers. J. Agric. Res.* **2014**, *2*, 279–283. [[CrossRef](#)]

65. Tomer, V.; Sangha, J.; Singh, B.; Takkar, R. Efficacy of Processing Treatments on Cypermethrin Residues in Okra (*Abelmoschus Esculentus*). *Nutr. Food Sci.* **2014**, *44*, 545–553. [[CrossRef](#)]
66. Yu-shan, Z.; Xiao-peng, L.I.; Hong-mei, L.I.U.; Yao-kun, Z.; Fan-fei, Z.; Qin-jie, Y.U.; Hao, L.I.; Jian-wen, C. Study on Universal Cleaning Solution in Removing Blended Pesticide Residues in Chinese Cabbage. *J. Environ. Chem. Ecotoxicol.* **2013**, *5*, 202–207. [[CrossRef](#)]