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Development and Validation of Pesticide Residues Determination Method in Fruits and Vegetables through Liquid and Gas Chromatography Tandem Mass Spectrometry (LC-MS/MS and GC-MS/MS) Employing Modified QuEChERS Method and a Centrifugal Vacuum Concentrator

Styliani E. Romniou 1, Konstantina Nana 2, Marilena Dasenaki 1, Efstratios Komaitis 2,∗ and Charalampos Proestos 1,∗

1 Food Chemistry Laboratory, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis, 15784 Athens, Greece
2 Analytical Chemistry Laboratory, Hellenic Research & Innovation Centre-Institute of Food Safety, 128 Kifisou Avenue Str., 12131 Athens, Greece
∗ Correspondence: skomaitis@hriclabs.gr (E.K.); harpro@chem.uoa.gr (C.P.)

Abstract: Pesticides are used for controlling organisms, weeds and animals, causing damage to plants. Although the use of pesticides is a prerequisite for producing safe food, their accumulation makes their rapid determination necessary to avoid negative impacts on human health. The aim of this study was to develop reliable and robust analytical methods for the determination of pesticide residues in fruits and vegetables, validated according to SANTE/12682/2019 guidance. Five different categories of fruits and vegetables were selected (apple, orange, onion, lettuce, tomato). The sample preparation was based on QuEChERS methodology, slightly modified in the clean-up step, and appropriate d-SPE reagents were selected for each commodity. A Multi-Tube Vortexer was used for better agitation. In the final step, the extract was split in two: one part was acidified and injected in LC-MS/MS and the other part was evaporated in a centrifugal vacuum concentrator, and reconstituted and injected in GC-MS/MS. With the centrifugal vacuum concentrator used instead of nitrogen stream, more pesticides were determined, while sensitivity and repeatability increased. Validation results satisfied the SANTE/12682/2019 guidelines for approximately 220 analytes for each commodity. LOQ was set at 0.010 mg/kg for all analytes. Successful external quality assessment (proficiency testing) proved that the methods are fit for purpose.

Keywords: modification of QuEChERS method; LC-MS/MS; GC-MS/MS; SANTE/12682/2019; centrifugal vacuum concentrator evaporation advantages

1. Introduction

Pesticides are chemical compounds widely used in agriculture, mainly for protecting plant damage that may be caused by other unwanted plants, organisms or animals, for stimulating plant growth processes or enhancing the action of other substances. In this respect, the main advantages of pesticide use are: (a) increase of crop yield; (b) protection and maintenance of crops; (c) lower food production cost; and (d) inhibition of natural plant toxins such as tropane and pyrrolizidine alkaloids [1]. There are various types of pesticides. Classification can be based on the purpose of their use, such as insecticides, herbicides, rodenticides, fungicides etc. Pesticides are also classified based on their origin into natural—which are derived from sources in nature (plants, microorganisms)—and synthetic—which are produced from chemical alteration. Synthetic pesticides are the most commonly used worldwide due to their high efficacy and affordability. They are divided into inorganic and organic depending on their chemical structure. Inorganic synthetic...
pesticides are formed by transforming inorganic compounds. Typical examples of this class are pesticides that include inorganic compounds and elements, such as copper, boric acid, silicates, sulfur, and arsenic [2]. Organic synthetic pesticides are chemically modified structures and are considered to be the most potent pesticides. These pesticides are categorized into different chemical groups, with the most common ones being organophosphates, organochlorines, carbamates and pyrethroids [3]. Due to their advantages in agricultural production, more and more pesticides are being used despite their chronic or acute toxicity when employed in concentrations higher than proposed. Exposure to pesticides can occur either directly, via inhalation (sprays), ingestion and dermal contact, usually to people who use them daily, such as farmers, or indirectly to consumers via pesticide residues found in water and food [4]. The contamination of water, soil, air and the accumulation in crops, e.g., fruits and vegetables, pose a potential risk to human health as they can lead to neurological disorders, gastrointestinal dysfunction, genetic disorders, reproductive problems, cardiovascular disorders, cancer and other health problems [5]. Consequently, the determination of pesticides residues in food matrices has become a necessity in view of the toxicity and stability of these chemicals.

Analytical methods for pesticide residue determination must be accurate, sensitive, robust and quick—especially for vulnerable products which are supplied to open markets—and must have fast response for their quick market release. The most common techniques in multiresidue analysis include capillary-GC, HPLC, GC-MS, LC-MS, GC-MS/MS and LC-MS/MS. The choice of the appropriate analytical technique depends mainly on the physicochemical properties of the analytes, as they have different solubilities and volatilities due to their chemical structure. For this reason, mass spectrometric techniques hyphenated to gas or liquid chromatography, principally liquid and gas chromatography tandem mass spectrometry (LC-MS/MS and GC-MS/MS), are mainly used for the identification of pesticide residues, because of their high selectivity and sensitivity, their ability to determine pesticides of all chemical classes, the short analysis time, and the reduced matrix interference due to their quadrupole array [6,7].

In this work, two multiresidue methods were developed and validated for the simultaneous determination of more than 200 pesticides in fruits and vegetables. Sample preparation was based on the QuEChERS method (Quick, Easy, Cheap, Effective, Rugged and Safe), being a combination of salting out solid-liquid extraction (SLE) to an organic solvent (acetonitrile) and a dispersive-SPE (d-SPE) clean-up [8]. This method can be easily modified depending on the sample type and the target-analytes. For example, to improve the extraction of polar organophosphate pesticides, the addition of acetic acid is recommended, and when testing citrus fruits, it is recommended to add aqueous NaOH to reach the desired pH and improve the analysis. In the process of pesticide residue detection in fruits with low pH, there are substances which interfere with extraction, reduce extraction efficiency, and produce matrix effects. By changing the pH value, the matrix effects can be reduced or eliminated by reducing the residue quantity of some substances that cause those effects [9].

The last step of the QuEChERS method is the right preparation of the extract, in order to be injected in LC-MS/MS and GC-MS/MS, which includes acidification for LC-MS/MS analysis and evaporation and reconstitution with another organic solvent for GC-MS/MS analysis, in case GC separation column cannot tolerate the extraction solvent.

When the GC-MS/MS technique is used for the identification of pesticides residues in food, organic solvents are usually evaporated and the solutions are reconstituted with another solvent. Different evaporation methods include heating, gas blowing onto the solvent’s surface, use of a centrifugal force, use of a rotary vacuum evaporator etc. In this study, the acetonitrile was evaporated by means of a centrifugal vacuum concentrator. Extracts were dried or concentrated in centrifugal tubes which were placed in a centrifuge rotor and after the application of high vacuum the solvents were easily and faster evaporated [10].

Thorough optimization was performed in order to choose the optimum method for each commodity. The next step was the validation of these methods according to method
performance criteria set by SANTE/12682/2019 guidance [11]: linearity, specificity, repeatability, reproducibility, sensitivity, recovery, retention time stability, and uncertainty, at two different concentration levels, 0.010 mg/kg (LOQ) and 0.080 mg/kg for both methods, and the participation in proficiency testing as external quality assessment (requirement in standard ISO/IEC 17025).

2. Materials and Methods

2.1. Reagents and Reference Standards

Acetonitrile of >99.95% purity, LC/MS grade, type PESTIPUR for pesticide analysis, acetone, type PESTIPUR for pesticide analysis and hexane, type PESTIPUR for pesticide analysis, were purchased from Carlo Erba (Val-de-Reuil, France). SupelTM QuE Citrate (EN) tubes of 15 mL, SupelTM QuE PSA (EN) Tubes of 15 mL and SupelTM QuE PSA/ENVI-Carb (EN) tubes of 15 mL were obtained from Supelco (Bellefonte, PA, USA). Formic acid of 99% purity, LC/MS grade and cyclohexane of >99.5% purity, Distol-Pesticide Residue grade were purchased from Fisher Scientific (Hampton, NH, USA). Multi-standard solutions of pesticides: parts 1–15 out of 20, 100 mg/L in acetone were purchased from CPA Chem (Bogomilovo, Bulgaria). Standard of piperonyl butoxide 10 mg, standard solution of cis-chlordane 100 µg/mL in cyclohexane, standard solution of cis-chlordane 100 µg/mL in cyclohexane, standard solutions mix14, mix64 and mix118, each 10 µg/mL in cyclohexane, were purchased by Dr. Ehrenstorfer (Augsburg, Germany).

2.2. Sample Collection

In annex A of the SANTE/12682/2019 table named “Commodity groups and representative commodities Vegetable and fruits, cereals and food of animal origin” presents 10 different commodity groups and their relative typical commodity categories within the group and typical representative commodities within the category. They are categorized specially because of the clean-up, or dilution step that may be necessary to reduce matrix interferences and contamination of the analytical instruments, are affected by difference in physicochemical properties (e.g., polarity, solubility, molecular size) between the pesticides and the matrix components (matrix interference). The two main commodities groups of fruits and vegetables that we were interested in being validated were: (1) High water content and (2) High acid content and high water content.

Initial full validation according to this guidance dation must be performed for all analytes within the scope of the method, for at least one commodity from each of the commodity groups

The chosen fruits and vegetables were four from the commodity group “high water content”: apple (pome fruits), onion (alliums), lettuce (leafy vegetables), tomato (fruity vegetables), and one from the commodity group “high acid content and high water content”: orange (citrus fruits). All samples were purchased from local supermarkets.

2.3. Equipment

The laboratory equipment used for the method development and sample analysis was: a Vortex Genie 2 from Scientific Industries, a Multi-tube Vortexer BV1010 BenchMixer purchased from Benchmark (Sayreville, NJ, USA), a centrifuge Rotofix 32A from Hettich, a HyperVAC-LITE Centrifugal Vacuum Concentrator purchased from Gyrozen (Gimpo, South Korea), pipettes, automatic pipettes, centrifuge tubes, pH paper, PTFE syringe filters (diameter 25 mm, pore diameter 0.2 µm) and glass chromatographic vials. The chromatographic systems used for the analysis were: 6460 Triple Quad LC/MS from Agilent (Santa Clara, CA, USA) with a two channel LC Binary pump with vacuum degasser from Edwards (Burgess Hill, United Kingdom) and GC-2010 Plus from Shimadzu (Kyoto, Japan). The mass analyzers and columns used were triple stage quadrupole analyzer G6460A and ZORBAX Eclipse Plus C18 column, 50 m × 2.1 mm, pore diameter 1.8 µm from Agilent (Santa Clara, CA, USA) for LC-MS/MS, and triple quadrupole mass detector
GC-MS-TQ804 and SH-Rxi-5Sil MS column, 30 m × 0.25 mm, pore diameter 0.25 µm, temperature range 320/350 °C from Shimadzu (Kyoto, Japan) for GC-MS/MS.

2.4. Method Optimization—Selection of a Method Suitable for the Purpose for Each Type of Food

The determination methods of pesticide residues in all selected types of fruits and vegetables were based on the QuEChERS method of EN 15662:2008 with some modifications. The basic steps of the experimental procedure of this method are: (1) the extraction, where the analytes are extracted from the substrate with a solvent (acetonitrile) with the help of salts/buffers; and (2) the clean-up step, where suitable d-SPE (dispersive Solid Phase Extraction) reagents are added to overcome matrix interference from other food components (sugars, lipids, sterols, organic acids, proteins, pigments), causing damage to the LC-MS/MS and GC-MS/MS systems, reducing the ionization efficiency of the desired analytes and affecting the detection, i.e., the identification and quantification of the pesticides.

The selection of the appropriate method for each commodity was based on tests at the two steps of the experimental procedure, which is described in Section 2.5. These tests were based on (1) the addition of different reagents in the clean-up step, which means the purification of the solution by dispersive-solid phase extraction (d-SPE), (2) the use of different means of stirring, and (3) the use of different means of evaporating the samples’ extracts before being injected into GC-MS/MS.

For the clean-up step, the selection of the appropriate d-SPE reagents for each type of fruit and vegetable was made after literature research and with the information provided by technical brochures of Supelco (Bellefonte, PA, USA) from where we purchased the pre-weighed salts and d-SPE reagents for the QuEChERS method. Especially, for the determination of pesticide residues in orange, two different combinations of such reagents were tested. After spiking two orange samples with standard pesticide solutions, containing 339 pesticides, at a concentration of 0.010 mg/kg, the experimental procedure was followed as described in Section 2.5 except that in the first sample we added 150 mg PSA (a mixture of primary and secondary amines) and 900 mg MgSO$_4$, whereas in the second sample 150 mg PSA, 15 mg C18 (18 carbon chain adsorbent) and 900 mg MgSO$_4$ were added.

Moreover, after the addition of the d-SPE reagents the samples were stirred using the Multi-Tube Vortexer before being centrifuged. Agitation was set at 2500 rpm for 1 min and compared to manual stirring.

Finally, for the determination of pesticide residues by GC-MS/MS, before sample injection it was necessary to evaporate the acetonitrile added in the first step and reconstitute the solution with acetone:hexane (1:1), as acetonitrile could affect the chromatography column (resolution, drift in retention time). This extra step, that is not proposed in the classic QuEChERS procedure, was added as the “used in GC-MS/MS” column showed low tolerance in large volume of acetonitrile injected through the PTV inlet. More specifically a drift of 0.002 min for every acetonitrile extract injection in retention time was observed in primary experiments. Another alternative was to use a different type of column, that would be tolerant in acetonitrile. That could not be an option, as the Pesticide Smart Database-library of Shimadzu (including proposed retention indices, MRM and optimized voltages) is based on this specific column and such a change would mean much more time consumed for obtaining new retention times and for MS/MS parameters optimization. Possible means of evaporation in the laboratory were either with a stream of nitrogen or with a centrifugal vacuum concentrator. Ten replicates of homogenized apple were spiked with standard pesticide solutions, containing 339 pesticides, at a concentration of 0.010 mg/kg and followed the experimental procedure until the evaporation stage, where the first five samples were evaporated to dryness with nitrogen, while the other five samples were evaporated to dryness in the centrifugal vacuum concentrator. The results of this optimization test are presented in Section 3.1.
2.5. Sample Preparation

First, 10 g of homogenized sample (spiked or unspiked) was weighted into a 50 mL centrifuge tube. Then, 10 mL of acetonitrile was added, and the tube was shaken by hand for 1 min. For the extraction of the analytes, a pre-weighted salt mixture Supel\textsuperscript{TM} QuE Citrate (EN) was added (1 g Sodium Citrate tribasic dehydrate, 0.5 g Sodium citrate dibasic sesquihydrate, 4 g Magnesium Sulfate, 1 g Sodium Chloride) and the tube was vigorously shaken by hand for 1 min. The pH should be 5–5.5, to reduce or eliminate matrix effects, and was checked with pH paper. If needed, 5N NaOH solution was added. After being centrifuged at 4000 rpm for 5 min, 6 mL of the supernatant phase was transferred into a 15 mL centrifuge tube containing a pre-weighted salt mixture Supel\textsuperscript{TM} QuE PSA (EN) (150 mg Supelclean PSA, 900 mg Magnesium Sulfate) in the case that the sample was apple, orange, onion or tomato, and into a 15 mL centrifuge tube containing a pre-weighted salt mixture Supel\textsuperscript{TM} QuE PSA/ENVi-Carb (EN) (150 mg Supelclean PSA, 45 mg Supelclean ENVi-Carb, 900 mg Magnesium Sulfate) when the sample was lettuce. The tubes were stirred vigorously with the Multi-Tube Vortexer for 1 min and were centrifuged at 4000 rpm for 5 min. Approximately 2 mL of the supernatant phase was filtered with a 0.2 µm PTFE syringe filter into a glass chromatographic vial. The final step of sample preparation is different for LC-MS/MS and GC-MS/MS and it is described in Sections 2.6 and 2.7.

2.6. Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS) Analysis

First, 1 mL of the filtered sample was transferred into another glass chromatographic vial. Then, 10 µL of 5% formic acid solution in acetonitrile was added and the vial was vortexed. Next, 5 µL were injected in LC-MS/MS.

The elution was gradient and the composition of the mobile phase was altered during the course of the chromatographic run between 0.1% formic acid solution in water and 0.1% formic acid solution in 95:5 acetonitrile:water, as described in Table 1.

Table 1. LC gradient elution program, A: 0.1% formic acid solution in water, B: 0.1% formic acid solution in 95:5 acetonitrile:water.

| Time (min) | %A | %B | Flow (mL/min) |
|-----------|----|----|---------------|
| 0.00      | 90 | 10 | 0.600         |
| 10.00     | 30 | 70 | 0.600         |
| 15.00     | 10 | 90 | 0.600         |
| 20.00     | 90 | 10 | 0.600         |
| 25.00     | 90 | 10 | 0.600         |

The column temperature was set to 35 ± 0.8 °C, the column pressure was approximately 350 bar and the chromatographic analysis duration was 30 min. The injector’s draw speed was 200 µL/min and eject speed 200 µL/min. MS/MS detector’s scan type was dynamic-MRM mode (d-MRM) and we used an electrospray ionization (ESI) source in positive and negative mode. Gas temperature was set to 325 °C and gas flow to 6 L/min.

2.7. Gas Chromatography with Tandem Mass Spectrometry (GC-MS/MS) Analysis

First, 0.4 mL of the filtered sample were transferred into a 2 mL plastic centrifuge tube. The tube was then placed in the centrifugal vacuum concentrator at 2000 rpm, at 45 °C, for 30 min in the case that the sample was apple, 35 min if it was lettuce or tomato and 60 min if it was orange or onion, to be evaporated to dryness. Before the GC-MS/MS analysis, reconstitution with a mixture of acetone/hexane 1:1 and vortex agitation for 20 s were necessary. The injection volume was 3 µL using a PTV injector in splitless mode. The oven temperature program was 50 °C for 1 min, rate 25 °C/min until 125 °C and rate 10 °C/min until 300 °C with a hold time of 15 min. An electron impact (EI) ion source was used. Ion source temperature was set to 230 °C and transfer line temperature to 300 °C. Chromatographic analysis duration was 25 min.
2.8. Identification of Pesticides

Qualitative determination of pesticide residues in food samples analyzed in LC-MS/MS was carried out through QQQ Quantitative Analysis data processing software (Agilent, Santa Clara, CA, USA), whereas in GC-MS/MS through GC-MS Browser software (Shimadzu, Kyoto, Japan). The identification was performed by comparing the Quantifier ion peak and the Qualifier ion peak for each pesticide on each substrate, comparing the ion ratios of two transitions of samples and matrix-matched standards (±30%), and checking the standard deviation of retention time (±0.1 min). For the LC-MS/MS method development we used the Pesticide t-MRM Database from Agilent (Santa Clara, CA, USA). For the GC-MS/MS methodology a standard alkane solution from Restek (France) was analyzed using a chromatography method with the same analytical conditions (autosampler, temperature program) as the pesticide determination method we developed. After alkane determination, correction in the retention time indices followed, through Pesticide Quick-DB Smart Data Base from Shimadzu (Kyoto, Japan), with proposed MRMs and MS Voltages (collision energies) for the pesticides of interest.

2.9. Quantitative Determination of Pesticide Residues

For the quantification of pesticide residues, we used a matrix-matched calibration curve at 6 levels of concentration: 0.005–0.100 mg/kg for LC-MS/MS analysis, while at 5 levels of concentration: 0.005–0.080 mg/kg for GC-MS/MS analysis, using the precursor ion peak area, which is usually the most abundant transition. The matrix-matched calibration curve was built by spiking samples with different volumes of a multi-standard solution of pesticides in acetonitrile. The calculation of the curve equation was performed through QQQ Quantitative Analysis data processing software and GC-MS Browser software and the calibration graph can be described by the equation y = ax + b, where y is the peak area and x is the pesticide concentration.

The equation used for the calculation of each analyte’s concentration is:

\[ C \text{ (mg/kg)} = \frac{C \text{ (mg/L)}}{\text{dilution factor}} \times \text{dilution factor,} \]

where dilution factor = 1 L/kg.

The expanded uncertainty of the method was set to 50% according to SANTE/12682/2019 guidance.

2.10. Methods Validation

Validation must be performed for all analytes to provide evidence that the method is fit for purpose. At least five replicates are required in the LOQ of the method (the lowest spiked level), and one higher level. Therefore, for the experimental part of the validation what was needed was one blank sample (solvents only), one non-spiked sample (matrix only), five spiked samples at LOQ, five spiked samples at 8×LOQ, and matrix-matched samples (for the calibration curve). The validation parameters checked were linearity (the value \( R^2 \geq 0.98 \) was defined as internal criterion), sensitivity-LOQ, specificity, accuracy (bias), repeatability-precision, reproducibility-precision, ion ratio, retention time and uncertainty. It has to be underlined that, according to SANTE/12682/2019, LOQ is defined as the lowest spike level meeting the identification and method criteria for recovery and precision. This is a common strategy followed when in case of multiresidue methods, so as not to proceed in enormous mathematical calculations. For fruit and vegetables, the most common and lowest MRL for pesticide residues set by the EURL is, for the vast majority, 0.010 mg/kg. In this aspect LOQ was set to 0.010 mg/kg.

2.11. Quality Control

Internal and external quality control were assessed for all methods developed. Internal quality control was performed with a standard solution for checking instrument sensitivity and potential drift and also to build quality control charts (QC Charts Shewhart). The
standard solution was analyzed periodically after every 20 samples and the parameters monitored were the retention time of each analyte (with standard deviation \( \pm 0.1 \) min), the signal-to-noise (\( S/N > 10 \)), the ion ratio stability (\( \pm 30\% \)), the analytes’ concentrations and the recovery (\( \pm 30\% \)). Internal control with quality control charts was performed by monitoring 10% of the identified pesticides on each substrate at LOQ = 0.010 mg/kg. The pesticides for creating QC charts for each fruit and vegetable are presented in Table S1 (in Electronic Supplementary Material).

The QC Charts were filled with 10% of the number of pesticides which met the criteria of SANTE/12682/2019. In QC Charts, the pesticides concentrations (at LOQ = 0.010 mg/kg) were compared to the warning limit that is equal to \( \pm 2SD \) and the action limit that is equal to \( \pm 3SD \), where SD is the standard deviation of the reproducibility experiments.

External quality control was performed by participating in proficiency testing schemes. Apple purée, lime and lettuce samples were distributed by the proficiency scheme organizations. The experimental procedure for each matrix was followed, as described in Sections 2.5–2.7, and z-score was calculated to prove that the proficiency testing schemes were successful, as described in Section 3.2.8.

3. Results
3.1. Optimization Results

Firstly, an appropriate clean-up step was chosen. Different d-SPE reagents were tested in the orange matrix: (1) 150 mg PSA (a mixture of primary and secondary amines) and 900 mg MgSO\(_4\), and (2) 150 mg PSA, 15 mg C18 (18 carbon chain adsorbent) and 900 mg MgSO\(_4\). After processing the results extracted by LC-MS/MS and GC-MS/MS, we came to the conclusion that, in the first case (use of a pre-weighed mixture containing 150 mg PSA and 900 mg MgSO\(_4\)), we had a higher signal (Peak Area) and a higher signal-to-noise ratio (\( S/N \)), and as a result more pesticides could be detected.

Secondly, manual and mechanical stirring was tested. Use of the Multi-Tube Vortexer (2500 rpm for 1 min) provided better mixing of the contents of each centrifuge tube compared to manual agitation, leading to more complete purification of the extract and better phase separation. An even better result in repeatability and recovery of the methods was observed with the pulsing mode of the Multi-Tube Vortexer, namely every 20 s for a total of 1 min. Additionally, with this automatic device, many samples were stirred simultaneously, because it provides many tube positions.

From the comparison of the results given by GC-MS/MS for the ten spiked samples evaporated with different methods, it was concluded that the centrifugal vacuum concentrator is preferred, as it is observed: (1) better signal (Peak Area) (Indicative pesticide examples are presented in Scheme 1 and Table 2), (2) higher \( S/N \) ratio (\( \geq 10 \)) (Table 3), and (3) lower %RSD (Table 4).

Table 2. Comparison of Peak Area and Height values of specific pesticides with the two different means of evaporation used.

| Pesticide     | Evaporation with Nitrogen | Evaporation with the Centrifugal Vacuum Concentrator | Evaporation with Nitrogen | Evaporation with the Centrifugal Vacuum Concentrator |
|---------------|---------------------------|-----------------------------------------------------|---------------------------|-----------------------------------------------------|
| Diphenylamine | 176,255                   | 218,447                                             | 76,694                    | 89,390                                               |
| Di-allate     | 67,137                    | 75,906                                              | 31,043                    | 35,345                                               |
| Metolachlor   | 1,087,219                 | 1,359,908                                           | 449,229                   | 554,375                                              |
| Phenothrin    | 65,104                    | 87,551                                              | 29,501                    | 38,051                                               |
| Tetradifon    | 85,394                    | 113,522                                             | 37,779                    | 48,653                                               |
| Permethrin    | 101,182                   | 146,641                                             | 45,546                    | 60,044                                               |
| Flucythrinate | 72,328                    | 103,736                                             | 30,843                    | 43,334                                               |
Scheme 1. Average Peak Area comparison diagram after evaporating 5 spiked samples at 0.010 mg/kg under nitrogen stream and the other 5 spiked samples at 0.010 mg/kg using the centrifugal vacuum concentrator, for indicative pesticide examples: (1) Methacrifos, (2) Chlorpyrifos methyl, (3) Heptachlor, (4) Pirimiphos methyl, (5) Fenitrothion, (6) Malathion, (7) Chlorpyrifos, (8) Butralin, (9) o, p'-DDT, (10) Aldrin.

Table 3. Comparison of Area and S/N ratios values using the two different evaporation methods.

| Pesticide       | Evaporation with Nitrogen | Evaporation with the Centrifugal Vacuum Concentrator | Evaporation with Nitrogen | Evaporation with the Centrifugal Vacuum Concentrator |
|-----------------|---------------------------|-----------------------------------------------------|---------------------------|-----------------------------------------------------|
| Chlofentazine   | 123,229                   | 89,467                                              | 14.52                     | 739.18                                              |
| Dichlorvos      | 177,582                   | 219,833                                             | 684.27                    | 15,045.65                                           |
| Dichlobenil     | 722,194                   | 875,020                                             | 42,438.89                 | 7625.23                                             |
| EPTC            | 127,803                   | 149,539                                             | 78.81                     | 4504.1                                              |
| Propamocarb     | 4011                      | 4947                                                | 131.27                    | 145.89                                              |
| Mevinphos       | 369,434                   | 522,548                                             | 20.3                      | 3004.61                                             |
| Butylate        | 341,677                   | 419,314                                             | 26.13                     | 7661.01                                             |
| Etridiazole     | 169,289                   | 200,476                                             | 135.82                    | 3704.16                                             |
| Propham         | 360,770                   | 447,460                                             | 8.14                      | 2005.92                                             |
| Methacrifos     | 378,743                   | 439,691                                             | 3407.33                   | 28,314.14                                           |
| 2-Phenylphenol  | 574,019                   | 702,230                                             | 91.14                     | 8280.63                                             |
| Molinate        | 686,144                   | 751,433                                             | 600.98                    | 6.51                                                |
| Tecnazene       | 68,057                    | 81,796                                              | 1173.91                   | 2238.88                                             |
| Propoxur        | 291,132                   | 436,214                                             | 36.42                     | 1136.86                                             |
| Propachlor      | 263,567                   | 300,638                                             | 3,589,033.2               | 4,866,521.21                                        |
| Diphenylamine   | 176,255                   | 218,447                                             | 21.71                     | 9,905,219.14                                        |
| Ethoprophos     | 224,206                   | 268,623                                             | 18.1                      | 17.94                                               |
| Ethalfluralin   | 45,195                    | 54,101                                              | 1.56                      | 1809.48                                             |
| Chlorpropham    | 227,475                   | 305,572                                             | 18.47                     | 948.27                                              |
| Trifluralin     | 128,909                   | 153,185                                             | 4645.11                   | 4945.71                                             |
| Benfluralin     | 98,343                    | 117,948                                             | 4543.35                   | 5216.71                                             |
| Cadusafos       | 520,350                   | 646,087                                             | 1.14                      | 33.58                                               |
| Di-allate       | 187,758                   | 220,277                                             | 2.9                       | 33,846.33                                           |
| alpha-BHC       | 594,646                   | 684,202                                             | 4.15                      | 816.71                                              |
| Hexachlorobenzene| 200,032                  | 241,729                                             | 249.58                    | 5582.26                                              |
| Dicloran        | 66,456                    | 95,659                                              | 14.96                     | 486.46                                              |
| Simazine        | 139,317                   | 190,985                                             | 9.31                      | 1052.16                                             |
| Pesticide                     | Evaporation with Nitrogen | Evaporation with the Centrifugal Vacuum Concentrator | Evaporation with Nitrogen | Evaporation with the Centrifugal Vacuum Concentrator |
|-------------------------------|---------------------------|-----------------------------------------------------|---------------------------|-----------------------------------------------------|
| Dimethipin                   | 21,939                    | 29,746                                               | 1.65                      | 148.78                                               |
| Atrazine                     | 92,624                    | 118,070                                              | 46                        | 1094.27                                              |
| Chlorbufam                   | 32,982                    | 44,504                                               | 133.65                    | 610.67                                               |
| Clomazone                    | 311,198                   | 394,187                                              | 89.12                     | 2244.78                                              |
| beta-BHC                     | 190,185                   | 287,292                                              | 0.88                      | 72.71                                                |
| Quintozene                   | 35,719                    | 39,349                                               | 31.49                     | 585.41                                               |
| Dioxathion                   | 15,595                    | 18,876                                               | 0.09                      | 142.51                                               |
| gamma-BHC (Lindane)          | 236,769                   | 279,907                                              | 1.64                      | 1451.52                                              |
| Terbufos                     | 322,969                   | 366,145                                              | 457.9                     | 4564.78                                              |
| Propyzamide                  | 576,662                   | 712,115                                              | 540.99                    | 293.39                                               |
| Diazinon                     | 81,040                    | 96,787                                               | 2270.11                   | 803.44                                               |
| Teftluithrin                 | 630,360                   | 734,920                                              | 883.37                    | 5745.48                                              |
| Tri-allate                   | 180,277                   | 211,677                                              | 163.58                    | 1092.95                                              |
| Pirimicarb                   | 891,731                   | 1,185,571                                            | 20.05                     | 145.46                                               |
| Formothion                   | 605                       | 1030                                                 | 0.25                      | 0.31                                                 |
| Dimethachlor                 | 260,765                   | 315,437                                              | 43.25                     | 4657.50                                              |
| Dimethenamid                 | 421,469                   | 508,351                                              | 1077.21                   | 12,123.16                                            |
| Acetochlor                   | 153,508                   | 190,791                                              | 9,864,921.36              | 4,370,556.78                                         |
| Chlorpyrifos-methyl          | 242,653                   | 296,685                                              | 22.15                     | 8587.13                                              |
| Vinlozolin                   | 66,822                    | 79,772                                               | 1347                      | 1011.08                                              |
| Parathion-methyl             | 180,138                   | 223,844                                              | 0.55                      | 704.41                                               |
| Alachlor                     | 197,998                   | 248,331                                              | 10.77                     | 5817.62                                              |
| Tolclofos-methyl             | 409,625                   | 501,591                                              | 10.28                     | 2741.49                                              |
| Heptachlor                   | 427,999                   | 487,626                                              | 211.66                    | 7530.75                                              |
| Fenchlorphos                 | 300,382                   | 359,237                                              | 666.08                    | 9595.41                                              |
| Pirimiphos-methyl            | 279,950                   | 335,424                                              | 912.75                    | 2960.56                                              |
| Fenitrothion                 | 139,649                   | 179,941                                              | 28.93                     | 378.75                                               |
| Methiocarb                   | 42,976                    | 74,071                                               | 77.29                     | 145.03                                               |
| Ethofumesate                 | 235,529                   | 282,317                                              | 43.25                     | 1177.81                                              |
| Malathion                    | 362,917                   | 491,875                                              | 2277.68                   | 8416.23                                              |
| Metolachlor                  | 1,087,219                 | 1,359,908                                            | 44.1                      | 4331.05                                              |
| Chlorpyrifos                 | 349,564                   | 449,808                                              | 132.05                    | 13,353.84                                            |
| Thiobencarb                  | 130,079                   | 170,180                                              | 17.25                     | 1470.02                                              |
| Fenthion                     | 273,955                   | 332,114                                              | 33.15                     | 1064.93                                              |
| Aldrin                       | 114,379                   | 138,423                                              | 178.97                    | 1685.69                                              |
| Chlothal-dimethyl            | 228,963                   | 286,051                                              | 43.21                     | 10,912.66                                            |
| Parathion                    | 63,696                    | 84,970                                               | 0.58                      | 112.16                                               |
| Triadimefon                  | 193,938                   | 256,560                                              | 5.34                      | 233.19                                               |
| Dicofol (DCBP)               | 604,319                   | 785,466                                              | 46.18                     | 792.03                                               |
| Butralin                     | 34,777                    | 47,759                                               | 255.2                     | 696.38                                               |
| Pendimethalin                | 56,722                    | 75,396                                               | 513.04                    | 750.50                                               |
| (E)-Chlorfenvinphos           | 10,844                    | 12,776                                               | 0.06                      | 382.05                                               |
| Metazachlor                  | 265,284                   | 333,915                                              | 198.15                    | 2167.63                                              |
| Fipronil                     | 111,767                   | 141,384                                              | 197.91                    | 2542.64                                              |
| Penconazole                  | 321,349                   | 404,029                                              | 430.00                    | 3489.80                                              |
| Chlozolinate                 | 6203                      | 7540                                                 | 62.14                     | 235.58                                               |
| (Z)-Chlorfenvinphos           | 214,413                   | 272,821                                              | 9.34                      | 2617.19                                              |
| Heptachlor-exo-epoxide       | 62,132                    | 82,817                                               | 440.3                     | 2067.10                                              |
| Mecarbam                     | 14,536                    | 19,810                                               | 20.84                     | 42.18                                                |
| Heptachlor-endo-epoxide      | 14,155                    | 17,919                                               | 0.64                      | 133.98                                               |
| Quinalphos                   | 426,954                   | 43,8189                                              | 28.47                     | 6,624,899.58                                         |
| Procymidone                  | 154,130                   | 190,749                                              | 240.30                    | 4055.71                                              |
| Triflumizole                 | 121,005                   | 144,913                                              | 12.43                     | 737.31                                               |
| Zoxamide                     | 40,393                    | 44,048                                               | 1.55                      | 39.33                                                |
Table 3. Cont.

| Pesticide               | Evaporation with Nitrogen | Evaporation with the Centrifugal Vacuum Concentrator | Evaporation with Nitrogen | Evaporation with the Centrifugal Vacuum Concentrator |
|-------------------------|----------------------------|------------------------------------------------------|---------------------------|------------------------------------------------------|
| Methoprene              | 70,923                     | 141,572                                              | 0.18                      | 133,752.88                                           |
| Bromophos-ethyl         | 231,527                    | 286,039                                              | 275.71                    | 3340.63                                              |
| Chlorbenside            | 463,024                    | 568,017                                              | 6593.54                   | 4438.62                                              |
| cis-Chlordane           | 126,575                    | 146,610                                              | 1.13                      | 2381.07                                              |
| trans-Chlordane         | 114,358                    | 146,610                                              | 1.23                      | 2329.68                                              |
| o, p’-DDE               | 4197                       | 5014                                                 | 5.53                      | 16.00                                                |
| Paclobutrazol           | 362,013                    | 453,615                                              | 298.02                    | 12,758.21                                            |
| Mepanipyrim             | 1,051,066                  | 1,410,620                                            | 11.97                     | 6.05                                                 |
| alpha-Endosulfan        | 75,406                     | 88,687                                               | 15.73                     | 778.89                                               |
| Napropamide             | 375,174                    | 518,589                                              | 6.77                      | 1,437,868.74                                         |
| Chlorfenon              | 724,840                    | 936,452                                              | 3.25                      | 9476.61                                              |
| Fludioxonil             | 324,924                    | 436,468                                              | 120.05                    | 3168.12                                              |
| Fenamiphos              | 90,109                     | 114,318                                              | 134.09                    | 659.52                                               |
| Tricyclazole            | 277,480                    | 423,024                                              | 24.19                     | 1134.98                                              |
| Profenofos              | 54,981                     | 72,622                                               | 2714.59                   | 1334.41                                              |
| Oxadiazon               | 199,906                    | 226,688                                              | 541.4                     | 3759.60                                              |
| p, p’-DDE               | 561,385                    | 631,455                                              | 87.06                     | 6112.72                                              |
| Myclobutanil            | 393,777                    | 501,589                                              | 646.84                    | 2266.38                                              |
| Ipvalicarb              | 82,867                     | 117,512                                              | 1.55                      | 151.70                                               |
| Oxylufuron              | 27,241                     | 35,081                                               | 60.89                     | 312.61                                               |
| Flusilazole             | 161,763                    | 202,572                                              | 186.58                    | 7755.73                                              |
| Dieldrin                | 60,014                     | 70,904                                               | 5.76                      | 981.42                                               |
| Endrin                  | 67,017                     | 81,552                                               | 0.78                      | 319.75                                               |
| Bupirimite              | 163,604                    | 203,339                                              | 206.19                    | 2165.78                                              |
| o, p’-DDD               | 51,093                     | 70,606                                               | 4.19                      | 55.95                                                |
| Carboxin                | 546,214                    | 432,900                                              | 1806.28                   | 205.68                                               |
| Chlorfenapyr            | 20,921                     | 24,793                                               | 96.99                     | 334.43                                               |
| Aramite                 | 55,845                     | 67,213                                               | 3.84                      | 1547.28                                              |
| Chlorobenzilate         | 845,151                    | 990,529                                              | 41.43                     | 4577.30                                              |
| beta-Endosulfan         | 38,920                     | 50,948                                               | -                         | 723.86                                               |
| Diniconazole            | 264,523                    | 340,574                                              | 210.68                    | 592.68                                               |
| Oxadixyl                | 353,274                    | 411,206                                              | 111.07                    | 1464.63                                              |
| Ethion                  | 305,498                    | 366,263                                              | 2.41                      | 8.55                                                 |
| o,p’-DDT                | 990,310                    | 1,163,918                                            | 3060.36                   | 35,409,580.79                                        |
| p,p’-DDD                | 1,071,418                  | 1,274,806                                            | 3751.03                   | 41,936,916.5                                         |
| Mepronil                | 1,857,251                  | 2,318,169                                            | 2.84                      | 2106.24                                              |
| Triazophos              | 220,306                    | 290,282                                              | 3.03                      | 149.63                                               |
| Carfentrazone-ethyl     | 158,046                    | 190,676                                              | 21.90                     | 2069.87                                              |
| Benalaxyl               | 317,509                    | 403,508                                              | 2.67                      | 410.06                                               |
| Endosulfan sulfate      | 115,936                    | 143,804                                              | 102.75                    | 534.16                                               |
| Pyraflufen-ethyl        | 95,959                     | 113,102                                              | 145.1                     | 3103.57                                              |
| p,p’-DDT                | 812,240                    | 904,674                                              | 194.72                    | 3419.61                                              |
| Diclofop-methyl         | 172,404                    | 212,563                                              | 164.80                    | 5155.66                                              |
| Propargite              | 320,216                    | 452,987                                              | 0.61                      | 43.36                                                |
| Piperonyl butoxide      | 480,861                    | 599,925                                              | 61.22                     | 26,179.65                                            |
| Resmethrin (Bioresmethrin) | 131,735                | 159,161                                              | 6.47                      | 43.89                                                |
| Bromuconazole           | 198,976                    | 247,694                                              | 1.09                      | 10,877,233.91                                        |
| Tetramethrin            | 136,449                    | 188,587                                              | 0.34                      | 1031.21                                              |
| Bifenazate              | 141,256                    | 180,715                                              | 1429.42                   | 2221.02                                              |
| Methoxychlor           | 644,484                    | 786,627                                              | 641.65                    | 4325.77                                              |
| Etoxazole               | 18,946                     | 25,235                                               | 133.13                    | 1083.36                                              |
| Fenpropathrin           | 76,639                     | 105,178                                              | 99.15                     | 1100.56                                              |
| Fenamidone              | 120,860                    | 161,456                                              | 10.70                     | 2927.11                                              |
| Tebufenpyrad            | 451,206                    | 578,838                                              | 263.05                    | 7435.66                                              |
| Tetradifon              | 85,394                     | 113,522                                              | 22.41                     | 1,416,336.27                                         |

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Table 3. Cont.

| Pesticide          | Evaporation with Nitrogen | Evaporation with the Centrifugal Vacuum Concentrator | Evaporation with Nitrogen | Evaporation with the Centrifugal Vacuum Concentrator |
|--------------------|----------------------------|------------------------------------------------------|----------------------------|------------------------------------------------------|
| Phenothrin         | 206,647                    | 261,617                                              | 3.20                       | 1961.73                                              |
| Phosalone          | 191,137                    | 291,508                                              | 0.07                       | 492.00                                               |
| Triticonazole      | 107,791                    | 143,226                                              | 59.74                      | 238.20                                               |
| Pyriproxyfen       | 356,159                    | 491,117                                              | 1.86                       | 6.24                                                  |
| Cyhalofop-butyl    | 450,839                    | 610,550                                              | 99.31                      | 2014.65                                              |
| Acrinathrin        | 8522                       | 15,309                                               | 0.23                       | 93.69                                                 |
| Amitraz            | 156,383                    | 117,684                                              | 17.29                      | 8369.99                                              |
| Pyrazophos         | 318,943                    | 411,239                                              | 58.66                      | 619.68                                               |
| Fenarimol          | 178,882                    | 228,637                                              | 122.9                      | 10,901.89                                            |
| Spirodiclofen      | 13,423                     | 17,581                                               | 26.36                      | 167.34                                               |
| Permethrin         | 101,182                    | 146,641                                              | 0.20                       | 3,075,240.78                                         |
| Bitertanol         | 49,979                     | 92,576                                               | 0.06                       | 301.76                                               |
| Dioxathion         | 64,501                     | 82,770                                               | 1.73                       | 1709.66                                              |
| Pyridaben          | 720,297                    | 931,972                                              | 26.88                      | 33,742.89                                            |
| Cyfluthrin         | 39,079                     | 57,440                                               | 0.41                       | 0.46                                                  |
| Cypermethrin       | 103,363                    | 143,311                                              | 2.60                       | 2.55                                                  |
| Etofenprox         | 1,681,402                  | 2,165,175                                            | 63.92                      | 30,980.56                                            |
| Pyridalyl          | 207,703                    | 288,298                                              | 37.68                      | 2819.59                                              |
| Flucythrinate      | 72,328                     | 103,736                                              | 0.75                       | 3,956,346.4                                          |
| Flumioxazin        | 25,724                     | 34,851                                               | 17.37                      | 261.49                                               |
| Deltamethrin       | 34,854                     | 28,161                                               | 1.10                       | 585.19                                               |
| Indoxacarb         | 13,754                     | 29,080                                               | 4.71                       | 39.56                                                 |
| Dimethomorph       | 174,667                    | 224,810                                              | 1.10                       | 2657.37                                              |
| Cinicon-ethyl      | 147,692                    | 201,824                                              | 134.31                     | 895.71                                               |
| Propaquizafop      | 9962                       | 13,506                                               | 316.77                     | 486.44                                               |

Table 4. Indicative results of %RSD (repeatability) of the methods for 5 replicates, using the two different evaporation methods. The “-” symbolizes pesticides which did not meet the validation requirements (mentioned in Section 2.10) or were not detected.

| Pesticide          | %RSD (N = 5) |
|--------------------|--------------|
| Methacrifos        | 11.4 | 4.5 |
| Propoxur           | -  | 3.5 |
| Chlorpyrifos methyl| 5.9 | 1.6 |
| Heptachlor         | 5.9 | 0.7 |
| Pirimiphos methyl  | 4.1 | 2.7 |
| Fenitrothion       | -  | 3.5 |
| Malathion          | -  | 2.5 |
| Chlorpyrifos       | 3.6 | 1.4 |
| Butralin           | -  | 3.8 |
| Dieldrin           | 5.1 | 4.9 |
| o, p'-DDD          | -  | 5.0 |
| o, p'-DDT          | 7.7 | 2.6 |
| p, p'-DDT          | 5.3 | 1.9 |
| Methoxychlor       | 6.5 | 1.0 |
| Tebufenpyrad       | -  | 4.9 |
| Phenothrin         | 13.0 | 4.9 |
| Phenothrin         | 3.0 | 2.4 |
| Permethrin         | 7.3 | 6.8 |
| Cypermethrin       | 3.8 | 2.3 |
| Indoxacarb         | -  | 5.5 |
In Table 3, although there are no large differences in Area values, we see much larger differences in signal-to-noise ratios. The ratio S/N must be equal or greater than 10, therefore more pesticides are detected by evaporating the samples with the centrifugal vacuum concentrator.

3.2. Results of Validation Requirements

3.2.1. Linearity

For linearity, six matrix-matched standard solutions were analyzed in LC-MS/MS in order to construct a calibration curve. The concentration of each standard solution was 0.005, 0.010, 0.020, 0.050, 0.080 and 0.100 mg/kg respectively. On the other hand, for GC-MS/MS analysis 5 concentration levels of standards were applied for calibration: 0.005, 0.010, 0.020, 0.050 and 0.080 mg/kg. The value of the correlation coefficient, \( R^2 \), was set to be greater than or equal to 0.98 and this internal criterion was successfully verified (Tables S2–S6 in Electronic Supplementary Material).

3.2.2. Repeatability

Precision under repeatability conditions was evaluated based on the results of independent tests obtained by the same method on identical test samples during the same laboratory day. Specifically, spiked samples were analyzed at two different concentration levels for \( N = 5 \) replicates. The first level of concentration was the 0.010 mg/kg (LOQ) and the second level was 0.080 mg/kg (8 \( \times \) LOQ) (Tables S7–S11 in Electronic Supplementary Material).

3.2.3. Reproducibility-Intermediate Precision

Precision under reproducibility conditions was evaluated, as in repeatability, based on the results of independent tests obtained by the same method on identical test samples on two different laboratory days at two concentration levels for \( N = 10 \) replicates. The first level of concentration was the 0.010 mg/kg (LOQ) and the second level was 0.080 mg/kg (8 \( \times \) LOQ) (Tables S12–S16 in Electronic Supplementary Material).

3.2.4. Sensitivity-LOQ

As LOQ is set the concentration of 0.010 mg/kg, where according to the EURL website [12] this concentration is less than or equal to the MRLs of pesticides on the substrates tested. Additionally, the average signal-to-noise ratio (S/N) for each analyte must be greater than or equal to 10, according to SANTE/12682/2019 guidance [13]. The results of the experiments showed that S/N \( \geq 10 \) for all pesticides and the range of S/N values is from 10 to 50,000.

3.2.5. Specificity

In the methods developed, a triple quadrupole mass analyzer is used, establishing excellent specificity and selectivity due to the two specialized transitions monitored for each pesticide.

3.2.6. Retention Time

The retention time stability was checked by calculating the standard deviation at two concentration levels. First level was LOQ = 0.010 mg/kg and second level was 8 \( \times \) LOQ = 0.080 mg/kg. According to SANTE/12682/2019 guidance [13] the standard deviation in the retention time should be less than 0.1 min in absolute value (SD < \( \pm 0.1 \) min). These values were successfully verified in the results of each analyte (Tables S17–S21 in Electronic Supplementary Material).

3.2.7. Uncertainty

Due to the large number of determined pesticides, the expanded uncertainty is given horizontally to all pesticides and is equal to 50% according to SANTE/12682/2019 guidance.
3.2.8. External Quality Control—Proficiency Testing

For the external quality control apple purée, lime and lettuce samples were analyzed. The experimental procedure for each matrix was followed, as described in Sections 2.5–2.7, and the z-score was calculated to prove that the proficiency testing schemes were successful (Table 5).

Table 5. Proficiency testing schemes results.

| Commodity | LC-MS/MS | GC-MS/MS | Assigned Value (mg/kg) | Assigned Value (mg/kg) | z-Score | z-Score |
|-----------|----------|----------|------------------------|------------------------|---------|---------|
| Apple purée | Pendimethalin | 0.041 | 0.7 | Flucythrinate | 0.042 | 0.0 |
| | Profenofos | 0.119 | −0.5 | HCB | 0.051 | −0.7 |
| | Proquinazid | 0.083 | −0.2 | Oxyfluorfen | 0.051 | 0.4 |
| | Tebufenpyrad | 0.056 | −0.3 | Pendimethalin | 0.041 | −0.6 |
| | | | | Procydmidone | 0.077 | −0.6 |
| | | | | Profenofos | 0.119 | −0.5 |
| | | | | Tebufenpyrad | 0.059 | −0.3 |
| Lime | Ethion | 0.043 | −1.4 | 2-Phenylphenol | 0.092 | 1.0 |
| | Myclobutanil | 0.123 | −0.2 | trans-Chlordane | 0.056 | 1.5 |
| | Phosalone | 0.051 | −1.7 | Chlorpyrifos (ethyl) | 0.107 | −1.0 |
| | Imazalil | 0.169 | −2.3 | Diazinon | 0.061 | −0.2 |
| | | | | Ethion | 0.043 | −0.5 |
| | | | | Myclobutanil | 0.123 | 0.1 |
| | | | | Phosalone | 0.051 | −0.2 |
| Lettuce | Piperonyl butoxide | 0.054 | 0.4 | Piperonyl butoxide | 0.054 | −0.4 |
| | Fluopicolide | 0.042 | −0.1 | Chlorpropham | 0.031 | −1.5 |
| | Oxadixyl | 0.029 | −0.7 | Chlorpyrifos-ethyl | 0.039 | −1.4 |
| | Pancycuron | 0.025 | −0.5 | Deltamethrin | 0.024 | −0.8 |
| | Propmocarb | 0.028 | −0.9 | Fludioxonil | 0.027 | −0.7 |
| | Propyzamide | 0.087 | 0.8 | gamma-HCH | 0.069 | −0.3 |
| | Pirimicarb | 0.062 | −0.9 | Oxadixyl | 0.029 | −0.5 |
| | | | | Procydmidone | 0.060 | −0.9 |
| | | | | Propmocarb | 0.028 | −1.4 |
| | | | | Propyzamide | 0.087 | −0.6 |
| | | | | Pirimicarb | 0.062 | −0.8 |
| | | | | Vinclozolin | 0.094 | −0.9 |

3.3. Pesticides That Satisfied the Validation Criteria

The number of total pesticides which met the above validation criteria in each type of fruit and vegetable tested is shown in Table 6. The total pesticides determined in each commodity by using LC-MS/MS and GC-MS/MS is presented in Tables S22 and S23 (Electronic Supplementary Material).

Table 6. Number of pesticides that satisfied the developed method’s validation criteria, in each commodity tested using the two chromatography techniques.

| Commodity | Total Pesticides LC-MS/MS | Total Pesticides GC-MS/MS | Common Pesticides LC&GC | Overall Pesticides Determined |
|-----------|---------------------------|---------------------------|-------------------------|-----------------------------|
| Apple     | 136                       | 140                       | 58                      | 218                         |
| Orange    | 136                       | 152                       | 66                      | 222                         |
| Onion     | 131                       | 159                       | 80                      | 210                         |
| Lettuce   | 136                       | 127                       | 53                      | 210                         |
| Tomato    | 129                       | 147                       | 64                      | 212                         |

Of the validated pesticides, those common to all substrates studied for LC-MS/MS analysis are 102, whereas for GC-MS/MS analysis are 111 (Table 7).
Table 7. Common validation pesticides in five fruits and vegetables which were studied for LC-MS/MS and GC-MS/MS analysis.

| Common pesticides in 5 commodities |
|-----------------------------------|
| Acetamiprid, Acetochlor, Alachlor, Aludrin, Atrazine, Azinphos-ethyl (Guthion ethyl), Benalaxyl, Bitertanol, Bromuconazole, Bupirimate, Butralin, Cadusafos, Carbadox, Carbofuran, Carboxin, Carfenrazone-ethyl, Chloridazon (Pyrazon), Chloroxuron, Chromafenozide, Clofentizin, Clomazone, Cyazofam, Cymoxanil (Curitate), Desmedipham, Dichlorvos, Dimethenamid (SAN 582H), Dimethomorpf(E), Diniconazole, Dodemorph, EPTC, Ethirimol, Ethopropanol, Etoxazole, Fenamidone, Fenamiphos, Fenamiphos–sulfone, Fenamiphos-sulfoxide, Fenamiphos, Fenarimol, Fenazaquin, Fenhexamid, Fenthion sulfoxide (Mesulfenfos), Fenthion-oxon-sulfone, Fipronil, Fluenoxyuron, Fluometuron, Fluopicidol, Flurtamone, Flusilazole, Forchlorfenuron, Imazalil (Eniconazole), Iprovalicarb, Isoxaben, Lenacil, Methamiphos, Metaflumizone, Metamitron, Methabenzthiazuron, Methiocarb sulfoxide, Methiocarb sulfoxide, Metabolachlor, Metribuzin, Monolinuron, Monuron, Myclobutanil, Naloxone, Oxadixyl, Oxamyl, Oxybenzone, Oxychloron, Pencycuron, Pendimethalin (Penoxalin), Phenmedipham, Phosphamidon, Pirimicarb, Pirimiphos-methyl, Propargite, Propanil, Propoxur, Propyazamide (Primaamide), Proquinoxazin, Prosturicarb, Pyrazophos, Pyridaben, Pyriproxyfen, Quinalphos, Simazine, Spirodiclofen, Spirotriazole, Tepp-A, Thiabendazole, Thiabendazole, Triadimefon, Tri-allate, Triazophos, Trichlorfon (DEP), Tricyclazole, Triflumizole |

Figure 1 presents the Total Ion chromatograms extracted from LC-MS/MS and GC-MS/MS for all pesticides, at a concentration of 0.010 mg/kg simultaneously in the five commodities selected for the validation of the determination methods.

![Figure 1. TIC chromatograms of total pesticides, each at a concentration of 0.010 mg/kg, in all five commodities extracted from: (a) LC-MS/MS; (b) GC-MS/MS (horizontal axes: retention time in minutes, vertical axes: intensity × 10^5).](image-url)
4. Discussion

When developing the methods for determination of pesticide residues in fruits and vegetables using the GC-MS/MS technique, in the additional step, where the extraction solvent (acetonitrile) must be evaporated to dryness and be reconstituted with another solvent (acetone:hexane 1:1), evaporation could be performed either using nitrogen or the centrifugal vacuum concentrator system. The advantages of this system make it more suitable for evaporating the solvent of samples, compared to nitrogen, in all fruits and vegetables studied, as:

- A larger number of pesticides are detected. As presented in Table 4 we were able to determine many pesticides eluting at the beginning of the chromatographic analysis, which are more volatile and more sensitive to analysis, while when using nitrogen evaporation, we could not even detect them;
- According to the S/N values in Table 3, 154 pesticides achieved $S/N \geq 10$ when using the centrifugal vacuum concentrator system. On the contrary, 104 pesticides using nitrogen evaporation satisfied this requirement;
- The sensitivity of the method increases, since in the majority of pesticides the $S/N$ ratio is much higher when applying the centrifugal vacuum concentrator evaporation;
- The analysis time is reduced, especially in a larger number of samples. The evaporation with a centrifugal vacuum concentrator is performed in 30–60 min (depending on the substrate) and all the samples are evaporated at the same time because of the multiple positions of the centrifugal vials it has, while in nitrogen stream the evaporation time of a sample is longer than 40 min and there were only 12 positions to place the samples;
- The cost of analysis is reduced by not consuming nitrogen;
- The cost of analysis and the effort of the analyst are reduced from the non-use of plastic liners. These liners are used to supply nitrogen and need to be thoroughly rinsed with organic solvents, as well as they are usually deformed by excessive use due to their flexibility and need to be replaced with new ones;
- The evaporation with the centrifugal vacuum concentrator achieves greater repeatability and reproducibility in the majority of pesticides determined, in combination with the Multi-Tube Vortexer stirring: $1 < \% \text{RS} \leq 20$ for $N = 5$ replicates and $N = 10$ replicates, as depicted in indicative Table 4.

After validating this LC-MS/MS technique, 136 pesticide residues in apple, orange and lettuce, 141 in onion and 129 in tomato fulfilled the requirements set by SANTE/12682/2019 guidance (linearity, repeatability, reproducibility, sensitivity, % recovery, uncertainty). In the GC-MS/MS technique, after applying the specific evaporation method, 140 pesticide residues in apple, 152 in orange, 157 in onion, 127 in lettuce and 147 in tomato satisfied the same requirements. This explains why the two different methods were developed.

5. Conclusions

Food safety is very important for human health and that makes it increasingly necessary to develop and apply analytical methods by which we can identify hazardous substances, such as pesticide residues. LC and GC-MS methods, involving a simple sample preparation step, represent an interesting approach as they enable the determination of pesticide residues in fruits and vegetables, by using modern devices, such as the Multi-tube vortexer and the centrifugal vacuum concentrator. The evaporation with the centrifugal vacuum concentration system increased sensitivity and led to the determination of significantly more pesticide residues in the GC-MS/MS technique. The validation according to SANTE/12682/2019 guidance demonstrated the ability of the methods for pesticide residues determination in a variety of different commodities from the whole category “Fruits and Vegetables”, highlighting their multi-residual ability and that they are fit for purpose. In this study, 218, 222, 227, 210, 212 pesticide residues were successfully validated in apple, orange, onion, lettuce and tomato matrices, respectively.

In order to improve the methods and to detect as many pesticides as possible, we suggest a modification of GC and LC conditions, an analysis test with another chromatographic
column that will have a different static phase or a larger number of theoretical plates, the selection of appropriate internal standards (I.S.), and in particular deuterium-labeled ones, for pesticides detected but not meeting the validation requirements of the SANTE/12682/2019 guidance, and the extension of the scope of the methods to new pesticides and matrices.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12111936/s1, Table S1: Selected pesticides for creating QC charts for each commodity, Table S2: Validation results for linearity in apple (at LOQ), Table S3: Validation results for linearity in orange (at LOQ), Table S4: Validation results for linearity in onion (at LOQ), Table S5: Validation results for linearity in lettuce (at LOQ), Table S6: Validation results for linearity in tomato (at LOQ), Table S7: Validation results for repeatability in apple (N = 5 replicates at two concentration levels: LOQ & 8 × LOQ), Table S8: Validation results for repeatability in orange (N = 5 replicates at two concentration levels: LOQ & 8 × LOQ), Table S9: Validation results for repeatability in onion (N = 5 replicates at two concentration levels: LOQ & 8 × LOQ), Table S10: Validation results for repeatability in lettuce (N = 5 replicates at two concentration levels: LOQ & 8 × LOQ), Table S11: Validation results for repeatability in tomato (N = 5 replicates at two concentration levels: LOQ & 8 × LOQ), Table S12: Validation results for reproducibility in apple (N = 10 replicates at two concentration levels: LOQ & 8 × LOQ), Table S13: Validation results for reproducibility in orange (N = 10 replicates at two concentration levels: LOQ & 8 × LOQ), Table S14: Validation results for reproducibility in onion (N = 10 replicates at two concentration levels: LOQ & 8 × LOQ), Table S15: Validation results for reproducibility in lettuce (N = 10 replicates at two concentration levels: LOQ & 8 × LOQ), Table S16: Validation results for reproducibility in tomato (N = 10 replicates at two concentration levels: LOQ & 8 × LOQ), Table S17: Validation results for retention time in apple (N = 5 replicates at two concentration levels: LOQ & 8 × LOQ), Table S18: Validation results for retention time in orange (N = 5 replicates at two concentration levels: LOQ & 8 × LOQ), Table S19: Validation results for retention time in onion (N = 5 replicates at two concentration levels: LOQ & 8 × LOQ), Table S20: Validation results for retention time in lettuce (N = 5 replicates at two concentration levels: LOQ & 8 × LOQ), Table S21: Validation results for retention time in tomato (N = 5 replicates at two concentration levels: LOQ & 8 × LOQ), Table S22: Pesticides that satisfied the developed method’s validation criteria, in each commodity tested using LC-MS/MS, Table S23: Pesticides that satisfied the developed method’s validation criteria, in each commodity tested using GC-MS/MS.

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