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Matrix-Matching as an Improvement Strategy for the Detection of Pesticide Residues

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Abstract: More than 90% of the pesticides residues in apples are located in the peel. We developed a gas chromatography/ion trap tandem mass spectrometry method for investigating all detectable residues in the peel of 3 apple varieties. Sample preparation is based on the use of the Quick Easy Cheap Effective Rugged and Safe method on the whole fruit, the flesh, and the peel. Pesticide residues were quantified with solvent-matched and matrix-matched standards, by spiking apple sample extracts. Matrix effects dependent on the type of extract (fruit, flesh, or peel) and the apple variety were detected. The best data processing methods involved normalizing matrix effect rates by matrix-matched internal/external calibration. Boscalid, captan, chlorpyrifos, fludioxonil, and pyraclostrobin were the most frequently detected pesticides. However, their concentrations in the whole fruit were below European maximum residue levels. Despite negative matrix effects, the residues in peel were detected at concentrations up to 10 times higher than those in whole fruits. Consequently, other pesticide residues present at concentrations below the limit of quantification in the whole fruit were detected in the peel.

Keywords: apple, GC–MS/MS, matrix effects, pesticides, residues

Practical Application: The analytical method presented could be extended to the determination of pesticide residues in apple peel extracts, provided that matrix-matched calibration is used to compensate for the matrix effect.

Introduction

Pesticides are of considerable importance in crop production, and are widely used to fight pests and diseases. However, their widespread use in large amounts has led to environmental contamination. Consumers are very concerned about the health risks associated with the presence of pesticide residues in their food (Bro-Rasmussen 1996). Pesticide control has thus become an important issue for the food industry. European Directive 2009/128/CE has drastically limited the amounts of pesticide residues permitted in fruits, with the aim of encouraging good agricultural practice and ensuring food safety.

Sensitive and robust analytical techniques are required to cover all the various chemical classes of pesticides with different physico-chemical properties used. One common analytical approach is based on generic, low-selectivity sample preparation techniques combined with highly selective instrumental analysis. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) procedure is a widely used generic sample preparation method for the extraction of pesticides from fruits, vegetables, and crop products. It involves rapid extraction in acetonitrile, followed by a clean-up step based on dispersive solid-phase extraction with a primary secondary amine (PSA) sorbent, C18 and/or GCB sorbents, plus anhydrous $MgSO_4$ for the elimination of water. Many applications have been successfully validated for a large number of pesticides in complex matrices: honey (Bargańska and others 2013), baby food (Georgakopoulos and others 2011), milk (Jeong and others 2012), rice (Hou and others 2013), farming foodstuffs (Lesueur and others 2008), fruit/vegetables (Lu and others 2012), shrimps (Omar and others 2013), and field soil (Zhang and others 2012).

Pesticides are usually analyzed by liquid chromatography combined with mass spectrometry (Bargańska and others 2013; Sinha and others 2012), but they may also be analyzed by gas chromatography (GC) coupled to mass spectrometry (MS) or electron capture detection (ECD; Balinova and others 2007; Furlani and others 2011). Published quantitative MS applications have included the use of a single quadrupole or ion trap analyzer operating in selected ion-monitoring (SIM) mode (Chen and others 2011; Cunha and Fernandes 2011; Abdulra'uf and Tan 2013), and more selective techniques, such as tandem mass spectrometry (MS/MS) with the use of an ion trap or triple quadrupole, with selected reaction monitoring (SRM) to improve both selectivity and sensitivity (Fillâtre and others 2011; Martins and others 2012; Chertaa and others 2013). Many studies have focused on the determination of pesticide residues in apples (Štajnbaher and Zupančič-Kralj 2008; Cunha and others 2009; Cervera and others 2010; Sinha and others 2012; Chertaa and others 2013; Abdulra'uf and Tan 2013), but the methods developed generally involved the optimization of multiresidue analyses for the study of a large number of pesticides rather than the simultaneous analysis of specific targeted pesticides as reported here. Previous studies have shown that the chromatographic response for pesticide residues may differ considerably between extract matrices (Poole 2007).

The aim of this study was, therefore, to develop a specific GC-MS/MS analytical method for the 10 target pesticides (boscalid, captan, chlorpyrifos-ethyl, dithianon, flonicamid, fludioxonil, pyraclostrobin, pirimicarb, thiacloprid, thiamethoxam) most frequently used to treat apples in the orchards of South-West France. Orchards may be undergo more than 10 phytochemical treatments during the growth cycle, up until harvest, not only to fight pests and diseases, but also to improve fruit preservation. Propargite, a molecule definitively banned in 2013, was also added

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to the list of compounds tested, to assess the potential persistence of this molecule.

Materials and Methods

Target apple varieties

Apple varieties. Three varieties of apples, referred to here as VAR1, VAR2, and VAR3, were chosen from those most widely grown in France and most popular with consumers. These 3 varieties are harvested in different seasons, from the end of summer (early harvest) to the end of winter (late harvest), and are therefore subject to different pest risks. All the varieties were treated according to the seasonal pest risk and the sensitivity of the variety concerned: VAR1 is the most resistant of the 3 varieties, whereas VAR2 is more sensitive to pest stress. VAR3 is the most fragile and is often systematically treated. Experiments were carried out with apples that had been sprayed on the tree and collected from the orchard in August (VAR1), October (VAR2), or November (VAR3). They were stored in a cold room until processing.

Surface characterization. Samples of fruit cuticle were examined using cryofixation and low-temperature scanning electron microscopy (SEM). Micrographs were obtained with a Quanta 250 FEG FEI microscope.

The contact angles of water droplets were measured on apple cuticles with a goniometer at room temperature (Digidrop, GBX).

Target pesticides

The choice of pesticides for this study was based on a number of factors, including the magnitude of pest risks, pesticide efficiency, the variety of fruit and its resistance, the harvest period, and the persistence of the pesticide. Eleven pesticides were studied: the 10 most frequently used on the selected apple varieties and another molecule that was recently banned (Table 1).

Standards and reagents

Pesticide-grade ethyl acetate and acetonitrile were obtained from Sigma-Aldrich (Saint-Quentin-Fallavier, France). The analytical standards used for boscalid (99.9%), captan (99.6%), chlorpyrifos-ethyl (99.9%), dithianon (97.4%), flonicamid (91.9%), fludioxonil (99.9%), pirimicarb (98.5%), propargite (99.5%), pyraclostrobin (99.9%), thiacloprid (99.9%), thiamethoxam (99.7%), thiamethoxam-d3 ($\geq$ 98%), and tris(1,3-dichloro-2-propyl)phosphate (TDCPP; 96%) were the "Analytical Standard Pestanal" standards, as supplied by Sigma–Aldrich (Schnelldorf, Germany). The characteristics of the targeted compounds are summarized in Table 1. Thiamethoxam-d3 was used as an isotopically labeled injection internal standard (IL-IS) and tris(1,3-dichloro-2-propyl)phosphate (TDCPP) was used as a procedural internal standard (P-IS).

The QuEChERS (mixture of $MgSO_4$, sodium chloride, disodium citrate, and disodium hydrogen citrate; Q-Sep Kit 26235), and a mixture of $MgSO_4$, PSA, and C18 (tubes 26221 + 26125), was purchased from Restek (Lisses, France).

Sample preparation

Sample preparation. Three composite samples of 2 kg of whole fruits were collected for each variety, and 1 kg of each was ground in a food processor. The other kilo was peeled, and the peel and the flesh were ground separately. The processed samples were then frozen and stored until extraction. Samples were identified as follows: FRUITVAR1, 2 or 3; FLESHVAR1, 2 or 3; and PEELVAR1, 2 or 3, for the fruit, flesh, and peel extracts, respectively, of each apple variety.

Extraction and clean-up. A modified version of the QuEChERS method (based on AFNOR NF EN 15662 and Anastassiades 2003) was used to obtain pesticide extracts. Homogenized sample (10 g) was mixed with 550 ng P-IS in 10 mL of acetonitrile and subjected to extraction with the QuEChERS Restek Q-Sep™ salts kit. The resulting supernatant was transferred to the Restek dSPE Q-Sep™ adsorbent kit. The entire purified supernatant was recovered and concentrated to dryness under a nitrogen stream. The dry extracts were dissolved in a final volume of 500 μL ethyl acetate supplemented with 1000 ng IL-IS and passed through a polytetrafluoroethylene (PTFE) filter with 0.22 μm pores.

Preparation of standards and calibration curves

Preparation of solvent-matched standards. Stocks were prepared at a concentration of around 100 ng/μL in ethyl acetate and frozen. Stock standard mixture solutions were prepared in 500 μL of ethyl acetate and contained 80 to 8000 ng of all target pesticides, 550 ng of P-IS, and 1000 ng of IL-IS. Six-point calibration curves were obtained by plotting the analytes/internal standard ratio against the concentration of analytes.

These solutions were also used to optimize mass spectrometry detection.

Preparation of matrix-matched standards. Matrix-matched standards at 8 concentration levels were prepared by spiking apple sample extracts from each variety. Apple sample extracts were prepared as described in the sample preparation paragraph, except that P-IS was introduced with the stock standard mixture solutions of the target pesticides and IL-IS was introduced during dry extract recovery. The concentrations of the standards were the same as those for the solvent-matched standards. The final solutions were passed through a PTFE filter with 0.22 μm pores.

Gas chromatography-mass spectrometry

Analyses were performed in an UltraTRACE gas chromatograph with a split/splitless injector, coupled to an ITQ900 ion trap mass spectrometer (Thermo Scientific, Courtaboeuf, France). The RXI-5Sil-MS column (30 m × 0.25 mm ID × 0.25-μm film thickness; Restek, France) was heated as follows: 40 °C (2 min), 220 °C at 30 °C/min, 260 °C at 5 °C/min, and 280 °C (5 min) at 20 °C/min. Helium (99.999%) was used as the carrier gas, at a flow rate of 1 mL/min, and as the collision gas in the ion trap chamber. The mass spectrometer was operated in electron impact mode (70eV). The sample (1 μL) was injected in the splitless mode (0.75 min) at 300 °C. Data were acquired with Excalibur software.

Pesticide spectra were acquired in the full-scan mode. MS/MS conditions were optimized to achieve a good signal-to-noise ratio for MS/MS detection. Each compound was quantified on the basis of total ion count, from 2 to 4 fragment ions, after fragmentation of the selected precursor by collision-induced dissociation (CID). The precursor ions were chosen on the basis of the MS spectra and published data. Five excitation voltages were studied (0.5, 1, 1.5, 2, and 3 V) for each pesticide and its precursor ion. The optimum values were selected by plotting CID voltages against the precursor and its fragment ion areas in the case of solvent-matched standard solutions, with confirmation on apple matrices.

Validation parameters and quality control

The extraction and analytical methods were carried out in accordance with AFNOR NF EN 15662. The limit of

Table 1–Overview on the characteristics of target pesticides.

Commercial Name	Pesticides	CAS No.	Classification	Use	Chemical formula	MW (g/mol)	EU MRLs (μg/g$_{fruit}$)	Log K_{ow}
BELLIS (BASF AGRO)	Boscalid	188425-85-6	Carboxamide F	c	$C_{18}H_{12}Cl_2N_2O$	343.21	2	2.96
BELLIS (BASF AGRO)	Pyraclostrobin	175013-18-0	Strobilurin F	c	$C_{19}H_{18}ClN_3O_4$	387.82	0.5	3.99
SIGMA DG (ARYSTA LIFESCIENCE)	Captan	133-06-2	Phthalimide F	f	$C_9H_8Cl_3NO_2S$	300.59	3	2.50
PYRINEX ME (MAKHTESHIM)	Chlorpyrifos-Et	39475-55-3	Organothiophosphate I, A, N	C	$C_9H_{11}Cl_3NO_3PS$	350.59	0.5	4.7
DELAN WG (BASF AGRO)	Dithianon	95591-89-2	Quinone F	f	$C_{14}H_4N_2O_2S_2$	296.32	3	3.32
TEPPEKI (ISK BIOSCIENCES)	Flonicamid	158062-67-0	Pyridine I, a	a	$C_9H_6F_3N_3O$	229.16	0.2	−0.24
SAFIR (SYNGENTA)	Fludioxonil	131341-86-1	Phenylpyrrole F	c	$C_{12}H_6F_2N_2O_2$	248.19	5	4.12
PIRIMOR G (SYNGENTA)	Pirimicarb	23103-98-2	Carbamate I, A	a	$C_{11}H_{18}N_4O_2$	238.29	2	1.7
CALYPSO (BAYER)	Thiacloprid	111988-49-9	Neo-nicotinoid I, A, M	a	$C_{10}H_9ClN_4S$	252.72	0.3	1.26
ACTARA (SYNGENTA)	Thiamethoxam	153719-23-4	Neo-nicotinoid I	I, w	$C_8H_{10}ClN_5O_3S$	291.71	0.5	−0.13
	Propargite	2312-35-8	Sulfite		$C_{19}H_{26}O_4S$	350.47	3	5.7

A, acaricide; I, insecticide; N, nematicide; F, fungicide; a, aphicide; M, molluscide; **c**, conservation; **f**, against fleck; **C**, against codling moth; **a**, against aphids; **w**, against wolly apple aphids.

quantification (LOQ), defined as the concentration corresponding to a signal-to-noise ratio of 10, was estimated from the chromatogram for the matrix-matched standards at the lowest calibration level used, for each compound. The limit of detection (LOD) was defined as LOQ/2. Linearity was studied with 6 matrix-matched standards. Recovery and reproducibility were determined at 2 levels of fortification (40 and 200 ng/g–10 replicates each), by adding known quantities of pesticide standard solution to 10 g of homogenized peel.

The relationship between the calibration curve slopes obtained in solvent (S_s) and in matrix (S_m) provide information about the matrix effect (%ME; Moura Andrade and others 2011; Kwon and others 2012):

$$\%ME = \frac{S_m - S_s}{S_s} \times 100 \qquad (1)$$

Statistical analysis

ME data were subjected to analysis of variance (ANOVA), principal component analysis (PCA), and factorial discriminant analysis (FDA; XLSTAT 2015, 2.01; Addinsoft, Paris, France). PCA (6 × 9 matrices) was performed on the mean values for extracts and
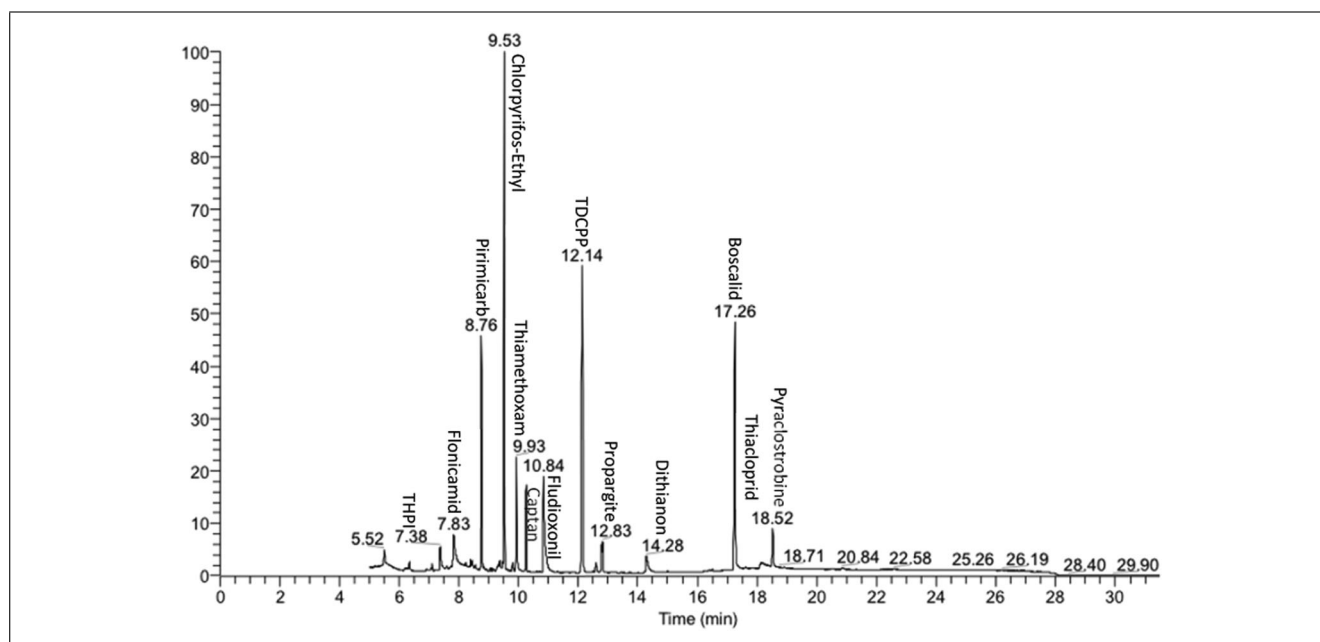

Figure 1–Representative chromatogram of the 11 pesticides, isotopically labeled injection internal standard (IL-IS), and TDCPP (0.01 g/L in ethyl acetate) as a procedural internal standard (P-IS) in scan mode.

Table 2–Retention times (t_R), MS/MS conditions used for apple analysis.

Name	t_R (min)	MS/MS quantification (m/z)			MS/MS confirmation		
		Precursor ion (m/z)	Product ions (m/z)	Excitation voltage (V)	Precursor ion (m/z)	Product ions (m/z)	Excitation voltage (V)
Boscalid	17.25	139.9	76, 112, 140	1	341.8	TIC: 170 to 346	1.25
Captan	10.25	79	51, 79	1	148.9	TIC: 74 to 151	1.25
Chlorpyrifos-Et	9.52	313.65	258, 286, 314	1	285.7	TIC: 142 to 290	1
Dithianon	14.41	207.86	137, 164, 181 192, 208	1.35	263.8	TIC: 131 and 266	1.25
Flonicamid	7.83	173.8	126, 146, 174	1	145.9	TIC: 72 to 150	1.25
Fludioxonil	10.84	181.9	127, 154, 182	1.15	248	TIC: 123 to 250	1.25
Pirimicarb	8.77	166	96, 123, 137, 166	1.25	237.8	TIC: 118 to 240	1.25
Propargite	12.82	135	77, 95, 107, 135	1	349.8	TIC: 174 to 355	1
Pyraclostrobin	18.51	131.9	77, 104, 132	1	163.9	TIC: 81 to 166	0.8
Thiacloprid	18.15	250,85	165, 191, 224, 251	1.15	101	TIC: 50 to 102	1
Thiamethoxam	9.90	211.8	182, 212	1.20	246.8	TIC: 123 to 250	1
Thiamethoxam-d3	9.90	215.1	185, 215	1	185.1	TIC: 92 to 190	1.1
TDCPP	12.13	380.5	159, 271, 367, 381	1.1	268.7	TIC: 89 to 271	1

pesticide replicates, to describe the variation of the ME calculated with (Equation 1). FDA (9 × 6 matrices) was performed on the ME for all pesticides, between apple extracts, on the basis of apple variety and extract origin. A final FDA (18 × 9 matrix) was performed on ME, to study and validate the ME data processing methods.

Results and Discussion

Selection of MS-MS conditions

The total ion chromatogram (TIC) of the 11 pesticides is shown in Figure 1. Captan was degraded by hot splitless injection into tetrahydrophthalimide (THPI) (Banerjee and others 2013). For each target pesticide, the optimized CID voltage is presented in Table 2.

Calibration and impact of variety on matrix effects, evaluated for fruit, flesh, and peel

Four calibration curves were obtained for each pesticide, for each apple variety in solvent, and in real matrices (Figure 2). Experiments were conducted without analyte protectants to evaluate the real ME according to (Equation (1)). GC hot splitless injection is commonly used for the quantitative analysis of trace amounts (Hajslova and Zrostlikova 2003). Unfortunately, ME can affect quantification accuracy, decrease method ruggedness, lower analyte detectability, and may even result in false-positive or false-negative results. This effect may be canceled out if all active sites in the inlet and column likely to lead to analyte adsorption can be avoided. However, as this is difficult to achieve, several strategies for minimizing ME have been suggested, such as the use of analyte protectants (Wang and others 2011) and matrix-matched calibration.
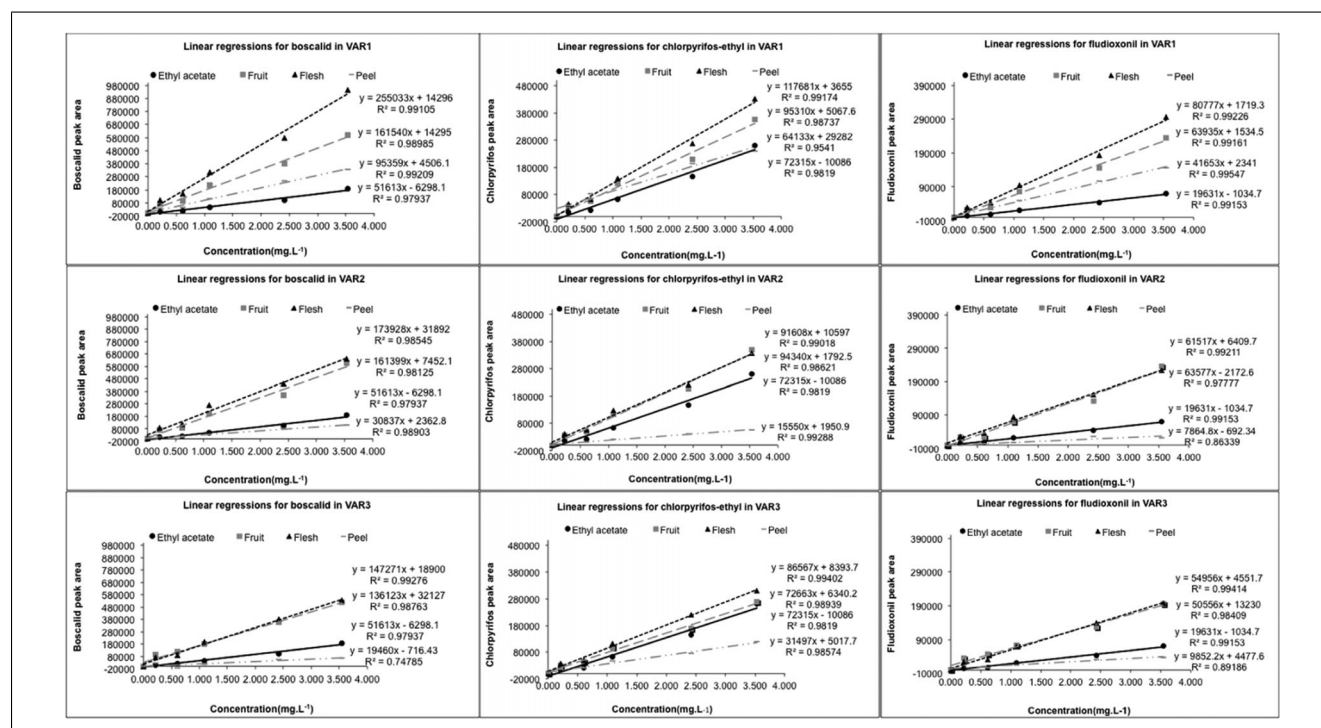

Figure 2–Example of calibration curves for solvent-matched and matrix-matched standards.

For ME values of more than 100, the matrix enhances the signal, leading to an overestimation of pesticide concentrations reflecting the adsorption of coextracted matrix components in the inlet and column. For ME values under 100, the matrix decreases the signal, leading to an underestimation of pesticide concentrations reflecting analyte adsorption onto the active sites of the inlet, column, and/or detector. Analyte responses may also be decreased if nonvolatile matrix components accumulate in the insert liner and/or the column (De Sousa and others 2012).

Kwon and others (2012) showed that the normalization of analyte responses against internal standards decreased the variability of ME for all 38 pesticides they studied by GC, in 20 apple matrices. Moreover, the ME values obtained were all about ±20%. These low values led the authors to question whether matrix-matched calibration was worthwhile. However, it should be borne in mind that the injections in this study were made in the presence of analyte protectants (ethyl glycerol, gulonolactone, D-sorbitol, and shikimic acid). These protectants are known to work well in GC analysis, and they may have had a nonnegligible effect on the pesticide peak areas.

In this study, ME was evaluated for flonicamid, chlorpyrifos, boscalid, fludioxonil, pirimicarb, and propargite in the various apple samples, with and without peak normalization. The trends for ME without normalization depended on both the extract (flesh, whole-fruit, or peel) and variety (Figure 3). Regardless of the pesticide considered, as previously reported by Kwon and others (2012), the normalization of peak areas against IS peak areas smoothed the GC response of the 6 pesticides. Moreover, ME values were much lower after normalization for fruit and flesh extracts, whereas normalization had a much weaker effect for peel extracts. Indeed, ANOVA on ME data showed that normalization

decreased the number of apple extracts considered to induce a significant ME for the 6 pesticides, that is total extracts ($P \leq 0.05$) for nonnormalized data and only PEELVAR3 ($P = 0.049$) and PEELVAR2 ($P = 0.026$) for P-IS- and IL-IS-normalized data, respectively. Anyway, the ME values obtained here in the absence of analyte protectants, were much larger than those calculated by Kwon and others (2012): between −72% and 180%, rather than ± 20%.

The score and loading plots of the PCA model for the ME data highlighted 3 clusters of pesticides (Figure 4) displaying 3 different types of behavior in terms of ME. In all data treatments, fludioxonil and boscalid seemed to behave similarly, but their behavior was different from that of propargite and pirimicarb, which behaved similarly to each other. This difference may reflect the presence (fludioxonil and boscalid) or absence (propargite and pirimicarb) of halogens in the molecule (Table 1).

The FDAs based on the origin of the extract (peel, flesh, and whole fruit) were performed to explain the variation of ME and to determine the impact of the type of extract on ME. All apple extracts were correctly classified in all FDAs.

The results for nonnormalized data showed that apple flesh extracts gave the strongest matrix effect (Figure 5). Indeed, the ME values obtained for fludioxonil and boscalid with flesh extracts were 200% to 400%, whereas the ME values obtained for whole-fruit extracts were only 150% to 200%. For the other pesticides, ME values were below 150% for flesh extracts and 75% for whole-fruit extracts (Figure 3). Moreover, the FDA based on VAR1, VAR2, and VAR3 for nonnormalized data showed that ME did not depend on variety.

Whatever the normalization method used, the difference between peel extracts and flesh and the fruit extracts was more significant than that between flesh and fruit extracts.
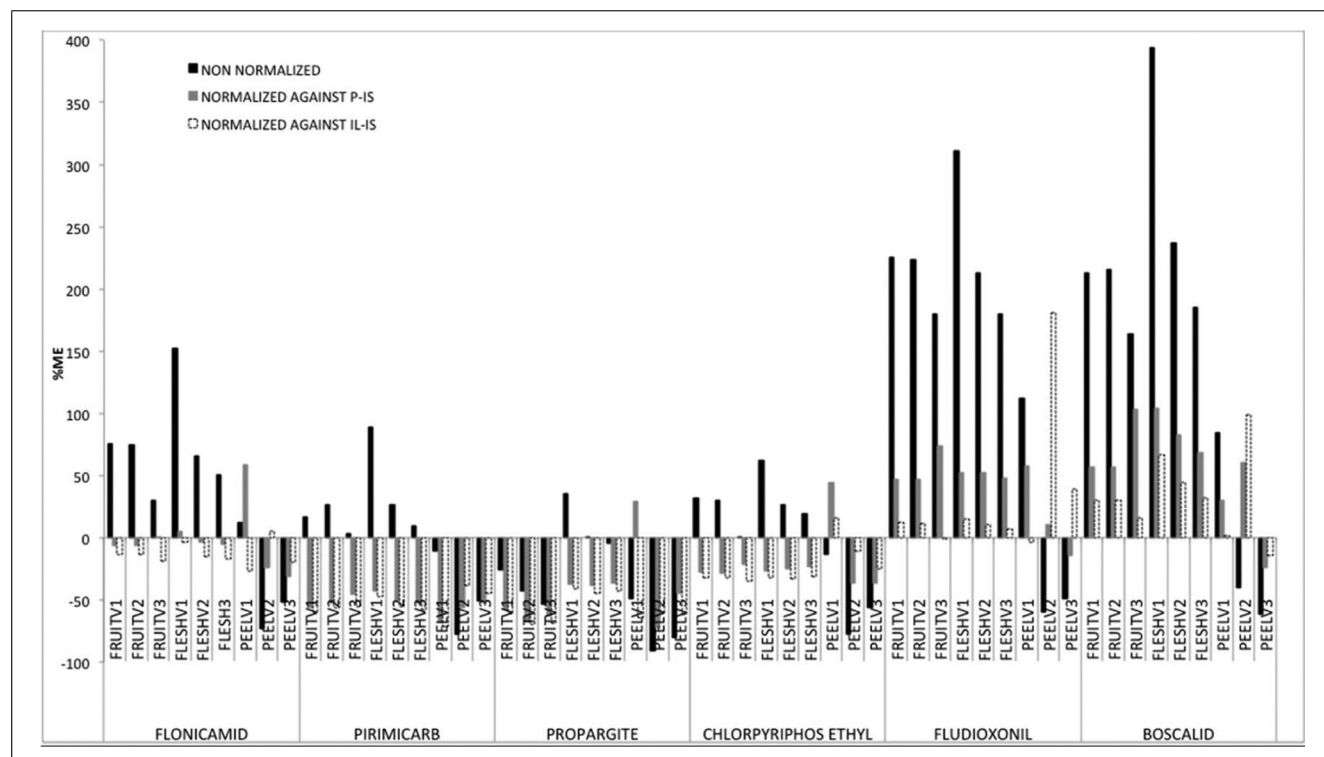

Figure 3–ME calculated with and without normalization against internal standards.

Normalization against P-IS was compared with normalization against IL-IS, by considering 3 groups defined on the basis of ME data-processing methods (non-normalized, normalized against P-IS, and normalized against IL-IS). The normalized ME values were correlated with PEELVAR2 and PEELVAR3, which were significantly discriminant for data normalized against IL-IS and P-IS, respectively (ANOVA). The ME values obtained without normalization differed significantly from those obtained after normalization, but the results obtained indicated that the 2 normalization methods did not differ significantly in their ability to reduce the matrix effect (Figure 6). Thus, P-IL or IL-IS are equally useful for the normalization of results for the GC method described here.

Only a few studies have investigated the correlation between ME and the nature of the coinjected/extracted analytes (De Souza and others 2012; Kowalski and others 2013). The flesh and the whole fruit contain monosaccharides, such as fructose and glucose, together with sucrose and sorbitol, which is used as an analyte protectant (Colin-Henrion 2008; Wang and others 2011). Triterpenoids have been identified as major components of the cuticular waxes in apple peel (Szakiel and others 2012). Preliminary studies to identify the analytes coextracted with pesticides by QuEChERS demonstrated the presence of relevant concentrations of triterpenoids in peel extracts. These molecules were poorly or even not detected in fruit or flesh extracts. They may have been responsible for the negative ME values obtained in these experiments.

Variety was expected to affect the ME. VAR1, which is an early variety, should have a chemical composition different from that of later varieties, particularly as concerns its cuticular waxes. This variety benefits from strong sunlight, favoring the accumulation of large concentrations of sugar and water loss. These factors have an effect on the chemical synthesis of waxes. Structural differences were observed between the 3 apple varieties, in terms of color and epicuticular wax crystallization (Figure 7A). The outer crystal forms protect the fruits against biotic and abiotic stresses.

The cuticle of VAR1 contains epicuticular waxes crystallized as platelets perpendicular to the surface. The wax platelets from VAR2 are parallel to the surface, with some forming tubules. For VAR3, the platelets of crystal waxes are embedded in a smooth continuous film of wax. These different structures result in differences in surface properties, such as wetting: contact angles vary from 80.8° to 104.5° (Figure 7B). VAR1 apples have a more hydrophobic surface than VAR2 and VAR3 apples. The surface of VAR3 apples is the most wettable.

Until 2000, it was taken for granted that the crystalline structure of waxes was correlated with their chemical composition (Belding and others 1998). However, Riedel and others (2003, 2007) demonstrated that crystal formation resulted from a spontaneous phase separation of one highly concentrated constituent from a blend of amorphous waxes.

As a result, differences in metabolism lead to the coextraction of different analytes (or at least different concentrations of analytes) and to different ME values. Further studies of this aspect are currently underway.

Method validation for apple peel

The analytical response was linear across the range studied, with correlation coefficients exceeding 0.98 for all pesticide residues. The calibration data (equation and regression coefficients) for boscalid, chlorpyrifos, and fludioxonil are shown, as an example, in Figure 2.

The limit of quantification (LOQ) was about 0.01 mg/kg for all the compounds studied, and this value is compatible with the requirements of EU legislation concerning the levels of these pesticides permissible in apples (Table 1).

Mean recovery rates of between 71.1% and 119.6% were achieved, with RSDs between 4.1% and 15.1% for all pesticides except dithianon, thiamethoxam, and thiacloprid. These compounds are not easy to analyze by gas chromatography, and liquid chromatography is therefore generally preferred. These results provide evidence that the optimized method achieves acceptable recovery rates, in line with EU guideline criteria
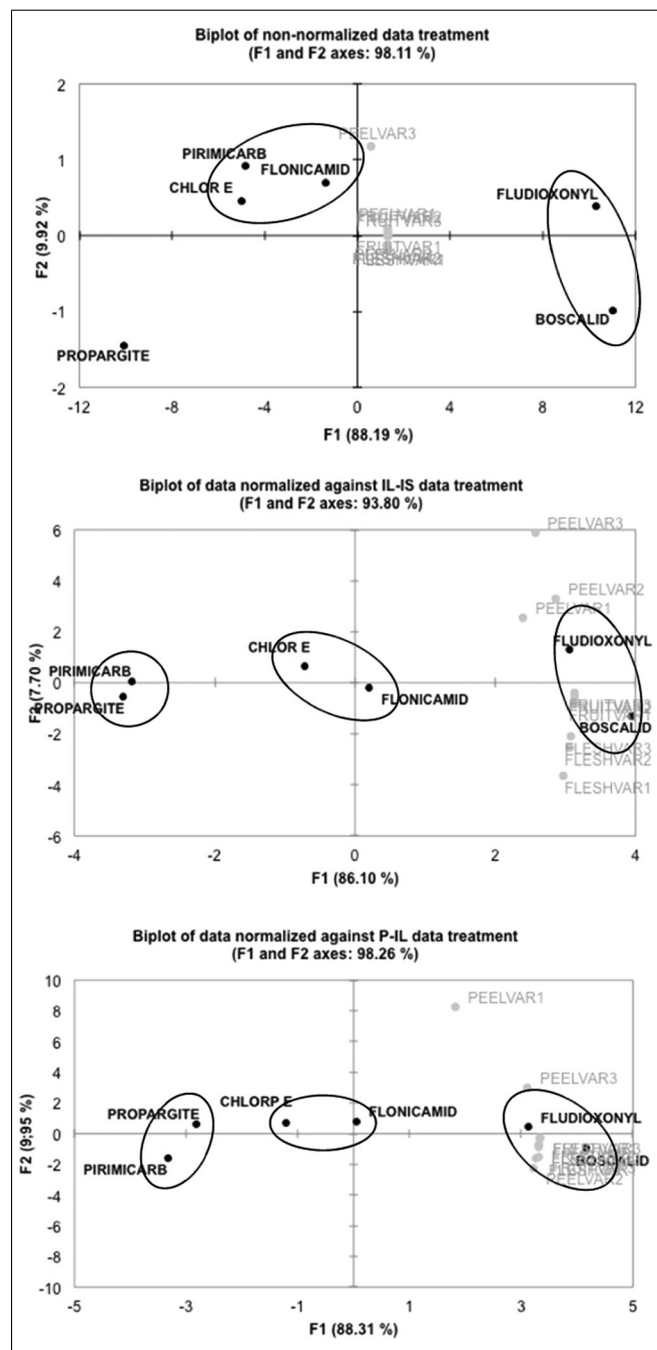

Figure 4–Loading and score plots for the first 2 principal components (F1 and F2) of the PCA models for ME evaluations. F1 and F2 explained (A) 98.11%, (B) 98.26%, and (C) 93.80% of the variation in the dataset.

(SANCO/12571/2013). A practical default range of 70% to 120% with a RSD <20% may be used for individual recoveries in routine multiresidue analysis.

Analysis of apple samples at harvest

The above method was applied to the analysis of the 8 validated pesticides in the 3 varieties of apple just after harvest; we analyzed both the whole fruit and the peel.

The concentrations of the detected pesticides in fruits ranged from 0.003 to 0.2 mg/kg (Figure 8A). Captan, chlorpyrifos, and flonicamid levels were the highest detected in VAR1, as this variety was sprayed with these chemicals a few weeks before the harvest. For VAR2, fludioxonil was the most abundant pesticide. This variety is harvested later than VAR1 and must be sprayed with agents to improve fruit preservation just before harvest (Table 1). VAR3 is also a late variety, and it is also highly sensitive. This variety was therefore treated with large amounts of preservation

Figure 6—Observation plots of FDA on ME based on ME data processing methods. F1 was significantly discriminant ($P = 0.005$) whereas F2 was not ($P > 0.05$).

treatments. The most abundant pesticides detected in the fruit were fludioxonil, boscalid, and pyraclostrobin (Table 1). Captan was also abundant in VAR3. Residues of propargite and pirimicarb were present at concentrations below the detection limit in whole fruits of all 3 varieties. They were therefore not detected in the fruit. The occurrence of pesticide residues in the 3 varieties was highly dependent on the treatments used before harvest: all 3 varieties were treated with SIGMA DG (captan). Only VAR1 was treated with PYRINEX ME (chlorpyrifos-ethyl) and TEPPEKI (flonicamid). VAR2 was sprayed with SAFIR (fludioxonil), as was VAR3, which also received a second fruit–preserving treatment in the form of BELLIS (pyraclostrobin + boscalid). In all cases, pesticide residues in the fruit were below the MRL (Table 1).

Pesticide concentrations were higher in the peel than in the whole fruit, at 0.008 to 0.58 mg/kg (Figure 8B). Sprayed treatments tend to concentrate on the surface of fruits, so they are generally present at concentrations largely above the LOQ of the analytical method, and are therefore easier to detect in peel. The matrix effects observed for peel extracts were generally weak, but globally negative. The concentrations of the 6 pesticides detected were up to 10 times higher in the peel than in the fruit, and some exceeded 0.3 mg/kg (fludioxonil and boscalid in VAR2 and VAR3). These analyses revealed concentration ratios of between less than 100% and 900% between the 2 approaches, and they demonstrated the presence of pesticides in the peel that were not detected in the whole fruit because their concentrations were below the LOQ.

Indeed, in VAR1, the peel contained residues of boscalid and pyraclostrobin, in addition to pirimicarb. The peel of VAR2 contained traces of boscalid and pyraclostrobin, flonicamid, chlorpyrifos, and propargite. The peel of VAR3 contained only the pesticides used for its protection (captan, fludioxonil, boscalid, and pyraclostrobin).

Based on the cropping schedules for the 3 varieties considered, the traces of residues other than those used to treat the orchard concerned may originate from cross-contamination between the 3 orchards or from other crops close to them. The occurrence of propargite, the sale of which has been banned since December 2011, with its use definitively prohibited since 2013, may be
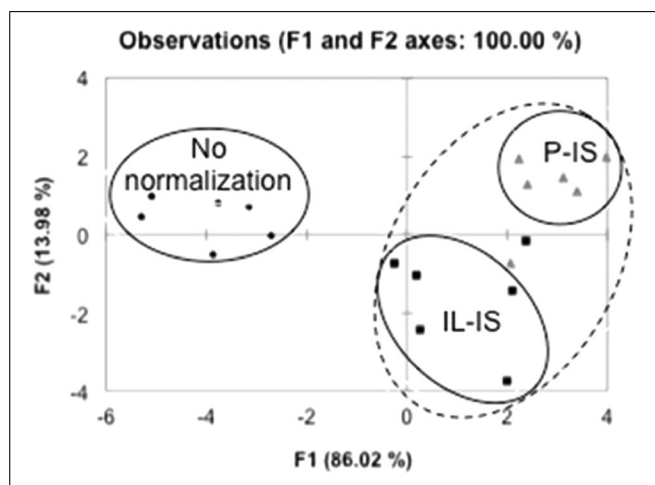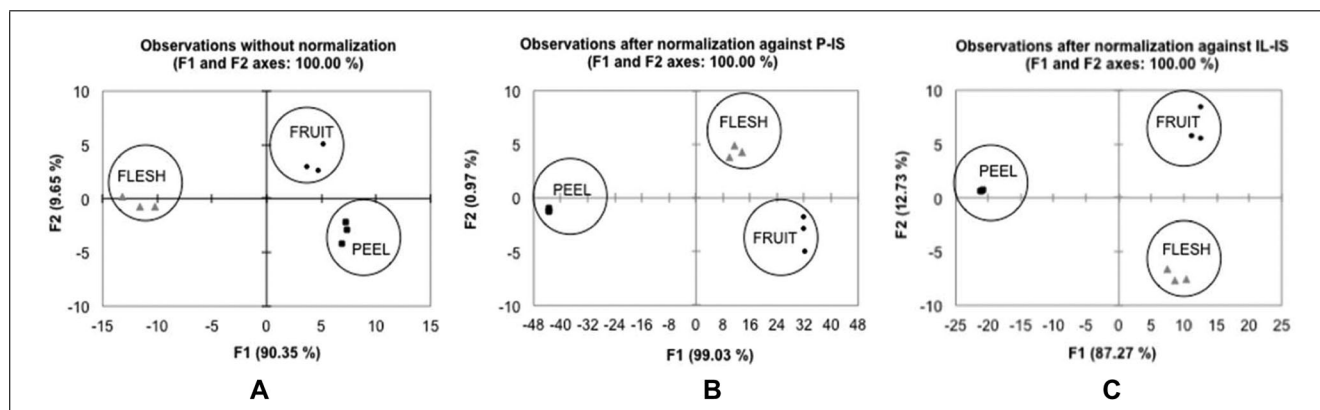

Figure 5—FDA of ME data for 6 pesticides, between apple extracts, according to the origin of the extract (peel, flesh, and fruit). (A) Nonnormalized ME data: 90.35% of the total variance is explained by F1, and F1 is significantly discriminant between extracts ($P = 0.015$), whereas F2 is not ($P > 0.05$). (B) ME data normalized against P-IS: 99% of the total variance of the data after normalization against P-IS is explained by F1, and F1 is significantly discriminant between extracts ($P = 0.000$), whereas F2 is not ($P > 0.05$). (C) ME data normalized against IL-IS: 87.27% of the total variance of the data after normalization against IL-IS is explained by F1, and F1 and F2 are significantly discriminant, $P = 0.001$ and 0.018, respectively.
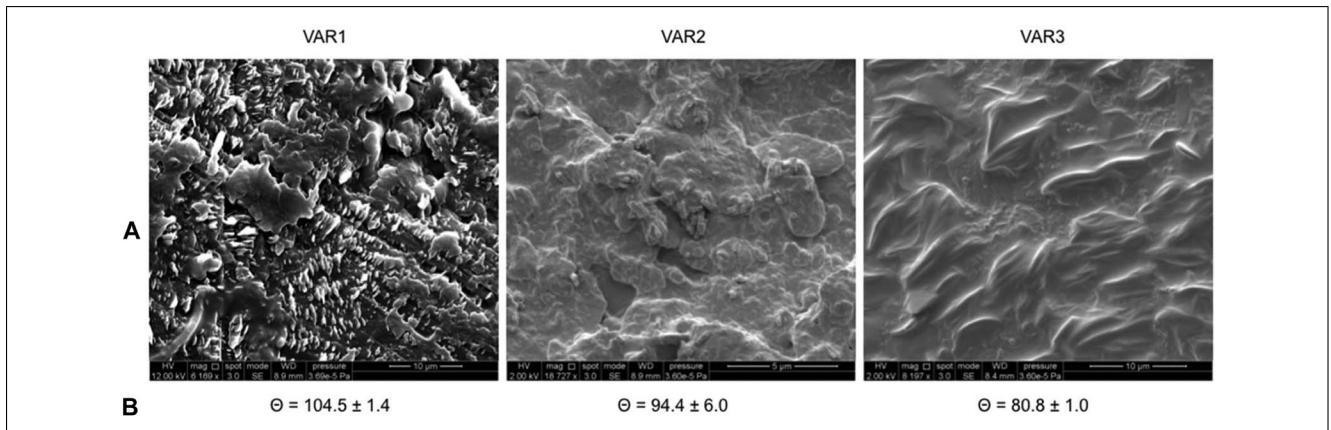
Figure 7–Characterization of the surface of the 3 varieties of apple. (A) Cryo-SEM micrographs of the apple surface. (B) Mean contact angle measurements of water droplets on apple cuticles (at 25 °C; goniometer: Digidrop, GBX; $n = 10$).
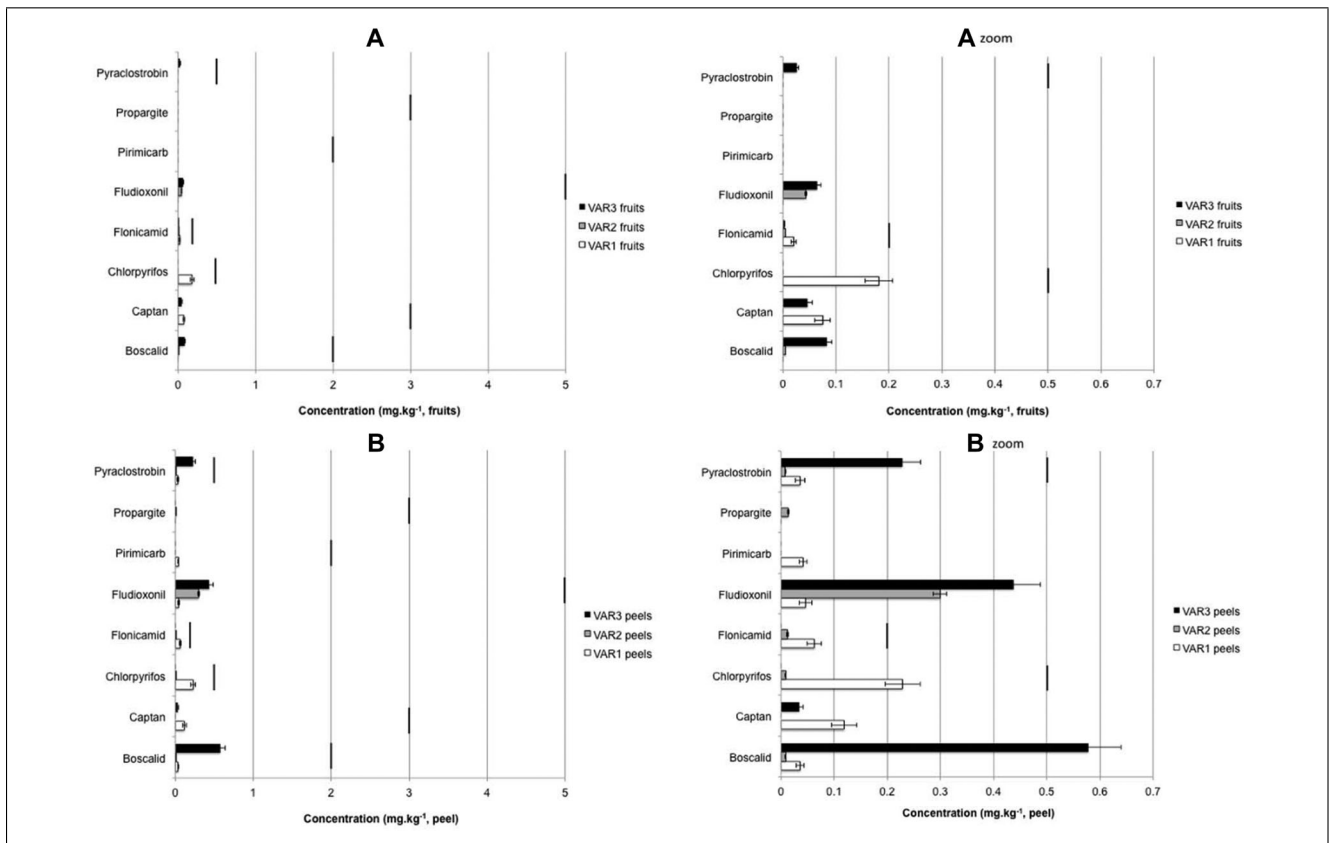

Figure 8–Overview of pesticide residues in harvest apples calculated for the 3 varieties, for the whole fruit (A) and the peel (B) (| EU-MRLs).

explained by the persistence of this molecule. Contamination may also occur from the soil and/or manipulations during harvesting.

Conclusions

A multiresidue method for determining pesticide residues in apples was developed and shown to yield satisfactory results. This method is based on the use of the QuEChERS method coupled to GC-(ITQ) MS/MS. Tandem mass spectrometry is a powerful tool for the identification of pesticide residues, and for analyses in complex matrices, because it facilitates the differentiation of target pesticides from coextracted compounds that might interfere with traces of these pesticides. Nevertheless, matrix in-

terference cannot be totally eliminated by the high specificity of gas chromatography-mass spectrometry GC-MS/MS for some matrix-transition combinations, potentially leading to the over- or underestimation of concentrations.

Matrix effects were largely positive for fruit and flesh extracts but negative for peel extracts. Variety had no influence on the matrix effect in fruit and flesh extracts. However, in peel extracts, variety affected the ME, with a tendency toward positive or less negative values, depending on the pesticide, in VAR1. The nature and concentration of the coextracted analytes in peels from the 3 varieties may account for these differences. Investigations are currently underway to improve our understanding of the role

of coextracted analytes in matrix effects for the same analytical configuration. This investigation could lead to the optimization of purification steps before the analysis of concentrated peel extracts to minimize negative ME. Normalization of the analytical responses of the pesticides to internal standards tended to reduce matrix effects and to minimize their variability between pesticides. However, these effects remained high in peel extracts in the absence of analyte protectants.

Working with the whole fruit matrix is an important approach, as yields concentrations that can be compared with MRLs. In this study, boscalid, captan, chlorpyrifos, fludioxonil, and pyraclostrobin were the most frequently used and, thus, also the most frequently detected pesticides. Their concentrations in whole fruit remained below the maximum residue levels. Analyses of the peel matrix can reveal all the pesticides present on the surface, thanks to the concentration effect from fruit to peel, resulting in concentrations in the peel being more likely to exceed the LOQ. Matrix-matched calibration is a practical and reasonably effective approach that can be used to compensate for matrix effects in GC-MS methods for pesticide residue analysis.

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Authors' Contributions

G. Giacinti, S. Capblancq, C. Raynaud, and V. Simon designed the study, collected data, interpreted the results, and drafted the manuscript.

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