Food Chemistry 525 (2026) 150618

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Pesticide residues in juice and wine: validation of a multi-method and a market survey with consumer risk assessment☆

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ARTICLE INFO

Keywords:
Wine
Juice
Fluorinated pesticides
Pesticide residues
GC–MS/MS

ABSTRACT

An analytical method for determining pesticide residues in fruit juices, vegetable juices, and wine was developed and validated for 32 active compounds, including 11 fluorinated ones. Sample extraction was carried out using a mixture of acetone, dichloromethane, and petroleum ether. The analysis was performed with gas chromatography-tandem mass spectrometry (GC–MS/MS). The method was applied to the analysis of 81 commercial samples. A total of 81 samples were analysed: 34 fruit juices, 6 vegetable juices, and 41 wine samples. After calculating concentrations in raw fruit or vegetables, maximum residue level (MRL) exceedances were found in 4 samples. Calculation of the chronic risk assessment confirmed that for the analysed samples consumer exposure was within acceptable limits. However, due to three active substances, acute exposure could pose a risk.

1. Introduction

Fruit and vegetables are essential components of the human diet due to their nutritional value. Consumers demand that they are not only healthy but also safe foods. Crop protection strategies often require the application of plant protection products (PPPs), which may result in unintended pesticide residues in food. Consequently, monitoring chemical food safety is imperative to protect public health.

The presence of pesticide residues in foods of plant origin has been widely reported, with studies indicating a higher frequency and greater occurrence of multiple residues in fruits than in vegetables. For example, previous studies, such as that by Baša Česnik et al. (2011), have shown that fruits are more likely to contain pesticide residues (74.2% versus 21.0%) and mixtures of compounds (50.2% versus 2.9%) than vegetables. In its latest report, where data for fruit and vegetables were separated (EFSA, 2021), EFSA observed that 69.1% of fruit and 44.6% of vegetables contained pesticide residues. In this year's report, EFSA (2026) found that all fruit matrices inspected in 2024 had more than 50% of samples with multiple residues. More precisely, the percentage of samples with multiple residues was 78% for table grapes, 73.5% for grapefruits, and 66.1% for bananas. These findings are particularly relevant for processed products, such as juices and wines, which are derived directly from fruits and may represent a significant dietary exposure route. In this context, monitoring pesticide residues and evaluating the associated consumer risks remain essential to ensuring food safety.

It is critical to evaluate whether processed fruit and vegetable commodities such as wine and juice exceed the Maximum Residue Levels (MRLs) set only for fruit and vegetables, and whether they are safe for consumers. The literature contains numerous data on pesticide residues in fruit and vegetables (Bakirci et al., 2014; Concha-Meyer et al., 2019; Jankowska et al., 2024; Kumari & John, 2019; Loughlin et al., 2018; Philippe et al., 2021; Qin et al., 2021; Szpyrka et al., 2015), but much less is known about residues in processed fruit. The European Food Safety Authority (EFSA, 2025) reported that, during monitoring of pesticide residues in the European Union (EU) in 2023, only 8.4% of inspected samples were processed food. Of these, 5.6% exceeded MRLs for raw commodities after recalculation using processing factors. Processed products accounted for 6% of all samples with multiple residues. Among processed food with multiple residues, 7.1% were found in red wine. Therefore, EFSA encourages the monitoring of pesticide residues in processed food.

Reliable, validated methods are required for measuring pesticide residues in processed food. Preferably, these methods should achieve low limits of quantification (LOQs) and be capable of both qualitative and quantitative analysis of a high number of active compounds. This paper focuses on the determination of pesticide residues in processed foods such as wine, and fruit and vegetable juices. Numerous analytical methods are reported in the literature. The most frequently used is the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method,

☆ This article is part of a Special issue entitled: '8th IMEKOFOODS' published in Food Chemistry.

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https://doi.org/10.1016/j.foodchem.2026.150618
Received 20 November 2025; Received in revised form 16 July 2026; Accepted 28 July 2026
Available online 31 July 2026

which uses acetonitrile as the extraction solvent. It can be applied to wine (Kosma et al., 2021; Pinheiro et al., 2025; Santana-Mayor et al., 2023; Sykalia et al., 2024) and juices (Gao et al., 2024; Torović et al., 2021; Zhao et al., 2023). The second most common approach is solid-phase extraction (SPE) or dispersive solid-phase extraction (dSPE). This type of extraction can also be used for wine (Fernández-Fernández et al., 2025; Pérez-Mayán et al., 2021) and juices (Farajzadeh et al., 2025; Shamsipur et al., 2016; Zhang et al., 2023). The third approach for extraction of wine is using a mixture of acetone, dichloromethane and petroleum ether (Čuš et al., 2022), while the analysis of juices represents a new extension of this scope. This method uses large volumes of solvents (370 mL per sample) opposite to QuEChERS where 15 ml of solvent is used and requires intensive manual work, as separation is performed in separatory funnels, while QuEChERS uses centrifuge. SPE and dSPE appear less favourable, as analytes may be retained on the solid phase, resulting in lower recoveries. The extraction protocol using a mixture of acetone, dichloromethane and petroleum ether was optimised to reduce organic solvent consumption by seven-fold and separatory funnels were replaced by a centrifuge. Buffering of wine and fruit juices with sodium acetate and acetic acid was performed in a manner similar as in the QuEChERS method (Lehotay, 2007) to achieve better recoveries for certain active substances. The method was extended to juices, demonstrating its robustness. Previous studies (Baša Česnik et al., 2006; Čuš et al., 2022; Skubic et al., 2025) show that using acetonitrile as the extraction solvent results in lower recoveries compared to extraction with a mixture of acetone, dichloromethane and petroleum ether; therefore, this mixture was retained.

Determination of active substances in extracts can be performed using liquid chromatography (LC) or gas chromatography (GC) coupled with mass spectrometry (MS) or tandem mass spectrometry (MS/MS). In the literature, the main instruments reported are GC–MS (Shamsipur et al., 2016; Sykalia et al., 2024; Zhang et al., 2023), LC-MS/MS (Fernández-Fernández et al., 2025; Gao et al., 2024; Pérez-Mayán et al., 2021; Torović et al., 2021; Zhao et al., 2023), and GC–MS/MS (Santana-Mayor et al., 2023). In present study, GC–MS/MS was used, as it enables unequivocal confirmation of active substances. In addition, it is more selective and sensitive than GC–MS, which was used previously.

Present work presents analytical method for determination of 32 active substances, including 11 fluorinated ones: flonicamid, fluazifop-p-butyl, fludioxonil, flufenacet, fluopicolide, fluopyram, flutolanil, lambda-cyhalothrin, tefluthrin, tetraconazole, and trifloxystrobin. All 11 fluorinated substances are, according to the new OECD definition, PFAS, as they contain at least one fully fluorinated methyl or methylene carbon atom (substances containing a CF_3 or CF_2 ($=CF_2$ or $-CF_2-$) group, without H, Cl, Br or I). The selection of active substances was based on those authorised in Slovenia and the EU at present and/or within the past three years. Of these, six were insecticides/acaricides, eight were herbicides, and 18 were fungicides. At the end, juice and wine samples were purchased in shops and analysed to verify the robustness of the method.

2. Materials and methods

2.1. Sample collection

Approximately half of the wine samples were red wines, and the other half were white wines. There are four major juice producers in Slovenia: Fructal, Dana, Moj dan, and Presad. Wine production in Slovenia is divided among three wine-growing regions: Primorska, Podravje, and Posavje, with approximately 250 wine-makers operating in these areas. Consequently, only a few juices and wines are imported into Slovenia, and most of the samples collected were of Slovene origin. The limited number of imported samples restricts the generalisability of the results to other countries. Vegetable juices were rarely available in shops, so only six samples could be collected. Fruit juices were available in two types of packaging: 1 L and 0.2 L (the latter being in the minority), and both were sampled to observe any possible differences between them. Small package juices are mainly consumed by children, who are the most vulnerable group of consumers. Wine samples consisted of one bottle (either 0.75 L or 1 L). One package of juice or one bottle of wine was considered sufficient, as these are homogeneous matrices. Sample size does not affect the analytical procedure.

Upon arrival at the laboratory, samples were transferred to plastic containers and stored at −20 °C until analysis. Samples were analysed within two months of collection.

2.1.1. Juice samples

Forty juice samples were collected in September 2025 from stores: 34 fruit juices and 6 vegetable juices. Five fruit juice samples originated from Austria. The other fruit and vegetable juices were produced in Slovenia. Four fruit juices from Slovenia were packed in 0.2 L volumes. All other fruit and vegetable juices were packed in 1 L volumes. No sample was designated as organic. List of samples is presented in Table 1.

2.1.2. Wine samples

Forty-one wine samples were collected in September and October 2025 from stores: 21 white wines and 20 red wines. Twelve samples were from the Primorska wine-growing region (7 red and 5 white wines), 10 from the Podravje region (4 red and 6 white wines), 10 from

Table 1
Fruit and vegetable juice samples.

Sample designation	Juice description	Origin	Volume (L)
25-4000	40% carrot, 60% apple	Slovenia	1
25-4001	31% carrot, 66% apple, 3% orange	Slovenia	1
25-4002	51% red beet, 49% apple	Slovenia	1
25-4003	98% red beet	Slovenia	1
25-4004	100% red beet	Slovenia	1
25-4005	100% red beet	Slovenia	1
25-4006	100% apple	Slovenia	1
25-4007	100% apple	Slovenia	1
25-4008	100% apple	Slovenia	1
25-4009	57% apple, 43% pear	Slovenia	1
25-4010	100% orange	Slovenia	1
25-4011	57% apple, 43% peach	Slovenia	1
25-4012	100% grape	Slovenia	1
25-4013	100% apple	Austria	1
25-4014	100% apple	Slovenia	1
25-4015	50% apple	Slovenia	1
25-4016	100% apple	Slovenia	1
25-4017	100% apple	Slovenia	1
25-4018	7% apple, 8% strawberry, 5% blueberry	Slovenia	1
25-4019	25% apple, 10% strawberry	Austria	1
25-4020	22% apple, 23% strawberry	Slovenia	1
25-4021	11% apple, 30% strawberry, 4% grape	Slovenia	1
25-4022	13% apple, 30% apricot	Slovenia	1
25-4023	25% apricot	Slovenia	1
25-4024	50% peach	Slovenia	1
25-4025	25% peach	Slovenia	1
25-4026	17% apple, 33% peach	Slovenia	1
25-4027	35% peach	Austria	1
25-4028	8% apple, 9% orange, 25% apricot	Slovenia	1
25-4029	100% orange	Austria	1
25-4030	100% orange	Slovenia	1
25-4031	100% orange	Slovenia	1
25-4032	50% orange	Slovenia	1
25-4033	100% orange	Slovenia	1
25-4034	25% black currant	Austria	1
25-4035	25% black currant	Slovenia	1
25-4036	25% black currant	Slovenia	0.2
25-4037	100% orange	Slovenia	0.2
25-4038	11% apple, 30% strawberry, 4% grape	Slovenia	0.2
25-4039	18% apple, 32% peach	Slovenia	0.2

the Posavje region (6 red and 4 white wines), and 9 were imported. The imported wines originated from Bosnia and Herzegovina (1 white wine), Croatia (1 red and 1 white wine), Italy (2 white wines), Macedonia (1 red and 1 white wine), and Montenegro (1 red and 1 white wine). Only one white wine with designation 25-4593 from Italy was labelled as organic. List of samples is presented in Table 2.

2.2. Chemicals and reagents

The certified standards were obtained from Dr. Ehrenstorfer (Augsburg, Germany). List and purity of standards used is presented in Supplement 1. Acetone p.a., acetone HPLC-grade, dichloromethane p.a., and petroleum ether p.a. were supplied by J.T. Baker (Deventer, Netherlands). All other chemicals (anhydrous CH_3COONa, 100% acetic acid, anhydrous Na_2SO_4) were from Sigma-Aldrich (Steinheim, Germany).

2.3. Determination of pesticide residues in wine and juice

2.3.1. Preparation of solutions

Stock solutions of active substances in HPLC-grade acetone were prepared in 25 mL volumetric flasks at a concentration of 625 μg mL^{-1}. When weighing standards, purity was considered (if the standards had 100% purity, 15.63 ± 0.01 mg would be weighed; for 98.7% purity, 15.83 ± 0.01 mg would be weighed). All standards were completely

Table 2
Wine samples.

Sample designation	Wine description	Origin	Wine-growing region	Volume (L)
25-3980	red wine	Slovenia	Primorska	0.75
25-3981	red wine	Slovenia	Primorska	0.75
25-3982	red wine	Slovenia	Primorska	0.75
25-3983	red wine	Slovenia	Primorska	0.75
25-3984	red wine	Slovenia	Primorska	0.75
25-3985	red wine	Slovenia	Primorska	0.75
25-3986	red wine	Slovenia	Primorska	0.75
25-3987	red wine	Croatia		0.75
25-3988	red wine	Montenegro		0.75
25-3989	red wine	Macedonia		0.75
25-3990	white wine	Slovenia	Primorska	0.75
25-3991	white wine	Slovenia	Primorska	0.75
25-3992	white wine	Slovenia	Primorska	0.75
25-3993	white wine	Slovenia	Primorska	0.75
25-3994	white wine	Slovenia	Primorska	0.75
25-3995	white wine	Bosnia and Herzegovina		0.75
25-3996	white wine	Croatia		0.75
25-3997	white wine	Montenegro		0.75
25-3998	white wine	Macedonia		0.75
25-3999	white wine	Italy		0.75
25-4573	red wine	Slovenia	Posavje	0.75
25-4574	red wine	Slovenia	Posavje	0.75
25-4575	red wine	Slovenia	Posavje	0.75
25-4576	red wine	Slovenia	Posavje	0.75
25-4577	red wine	Slovenia	Posavje	0.75
25-4578	red wine	Slovenia	Posavje	0,75
25-4579	red wine	Slovenia	Podravje	0.75
25-4580	red wine	Slovenia	Podravje	0.75
25-4581	red wine	Slovenia	Podravje	0,75
25-4582	red wine	Slovenia	Podravje	0.75
25-4583	white wine	Slovenia	Podravje	0.75
25-4584	white wine	Slovenia	Podravje	0.75
25-4585	white wine	Slovenia	Podravje	0.75
25-4586	white wine	Slovenia	Podravje	0.75
25-4587	white wine	Slovenia	Podravje	0.75
25-4588	white wine	Slovenia	Podravje	0.75
25-4589	white wine	Slovenia	Posavje	0.75
25-4590	white wine	Slovenia	Posavje	0.75
25-4591	white wine	Slovenia	Posavje	0,75
25-4592	white wine	Slovenia	Posavje	0.75
25-4593	white wine	Italy		1

dissolved in pure acetone. From these 32 stock solutions, three mixed solutions containing all 32 active substances were prepared at concentrations of:

*5 μg mL^{-1} (4 mL of each of 32 standards was transferred with pipette to 500 mL volumetric flask and filled to mark with acetone HPLC-grade),
*1 μg mL^{-1} (100 mL of mixed standard 5 μg mL^{-1} was transferred with pipette to 500 mL volumetric flask and filled to mark with acetone HPLC-grade) and.
*0.1 μg mL^{-1} (50 mL of mixed standard 1 μg mL^{-1} was transferred with pipette to 500 mL volumetric flask and filled to mark with acetone HPLC-grade).

Stock solutions were stored at −20 °C, and mixed solutions were kept in a refrigerator at 4–8 °C. Before use, they were left at room temperature for 1 h.

2.3.2. Extraction procedure

To 15 mL of juice or wine, 25 mL of a mixture of acetone, dichloromethane, and petroleum ether in a 1:2:2 (*v*/v/v) ratio was added. For fruit juice and wine, 2 g of sodium acetate and 0.4 mL of 100% acetic acid were added. The mixture was homogenised for two minutes with a mixer. Centrifugation was carried out for 10 min at 7000 rpm. The organic phase was filtered through black ribbon filter paper containing 30 g of anhydrous Na_2SO_4 into a Soxhlet flask. Extraction of active substances from the remaining aqueous phase was repeated. The solvent in the Soxhlet flask was then evaporated to approximately 2 mL using a rotary evaporator and dried under a nitrogen flow. The dry eluate was dissolved in 1.5 mL of acetone for HPLC using ultrasound. The organic phase was transferred to a vial for GC–MS/MS determination.

Selection of a mixture of acetone dichloromethane and petroleum ether enlarges recoveries not only due to polarity of analytes (dichloromethane and petroleum ether are non-polar and acetone is polar solvent), but also due to interaction with the matrix. During the extraction procedure, 50 mL of solvent was used, which is more than in the QuEChERS method. Our previous experience (Baša Česnik et al., 2006; Čuš et al., 2022) shows that better recoveries are obtained if the extraction is repeated twice. Rotary evaporation was used to concentrate the analytes in the solvent as much as possible and to lower the LOQs. With the exact final volume, no internal standard was needed. Over the years, we have observed that the final volume needs to be at least 1 mL to achieve good recoveries.

2.3.3. Determination with GC–MS/MS

The samples were analysed using a gas chromatograph (Agilent Technologies 8890, Shanghai, China) coupled with a tandem mass spectrometer (Agilent Technologies 7010B, Santa Clara, USA), equipped with a Gerstel 20PRE0795 multipurpose sampler (Gerstel, Sursee, Switzerland) and an HP-5 MS UI column (Agilent Technologies, 30 m, 0.25 mm i.d., 0.25 μm film thickness) with a constant flow of helium at 1.2 mL min^{-1}. The GC oven was programmed as follows: 55 °C for 2 min, then from 55 °C to 100 °C at 20 °C min^{-1}, from 100 °C to 280 °C at 4 °C min^{-1}, and held at 280 °C for 19.75 min. The temperature of the ion source was 230 °C, the auxiliary temperature was 280 °C, and the quadrupole temperature was 150 °C. For qualitative and quantitative determination, the MRM transitions presented in Table 3 were used. For each active substance, two to four transitions were scanned. Calibration was performed with matrix-matched standards.

In the GC–MS/MS method used in the study by Skubic et al. (2025), the oven was programmed differently as in the present study, since only five active substances were measured then: boscalid, kresoxim-methyl, metalaxyl-M, tebuconazole and triadimenol. Separation of the two triadimenol peaks, which were not measured in the present study, required a hold at 180 °C for 20 min. In the study by Skubic et al. (2025) and in

Table 3
MRM transitions and collision energy of active substances sought.

active substance	MRM transitions in *m/z*	CE (V)
azoxystrobin	**344 → 329**.1, 344 → 171.9, 344 → 155.8	10, 40, 40
benthiavalicarb-isopropyl	**181 → 180**, 181 → 126.9, 181 → 83.1	20, 40, 40
boscalid	**140 → 112**, 140 → 76	10, 30
clomazone	204 → 107, **125 → 99**	20, 20
cyprodinil	**225 → 223.7**, 224 → 208.1	20, 20
flonicamid	**174 → 146**, 174 → 126, 174 → 69	10, 20, 40
fluazifop-p-butyl	383 → 282.1, **254 → 146**	10, 20
fludioxonil	248 → 182.1, 248 → 154.1, **248 → 127.1**	10, 20, 30
flufenacet	**151 → 136.1**, 151 → 95.1	10, 30
fluopicolide	347 → 172, **209 → 182**, 173 → 145	30, 20, 10
fluopyram	**173 → 145**, 173 → 95.1	20, 30
flutolanil	**172.8 → 145**, 172.8 → 95, 172.8 → 75	15, 35, 55
iprovalicarb	158 → 98, **158 → 72.1**, 158 → 55.1	10, 10, 20
kresoxim-methyl	206 → 131.1, **206 → 116.1**	10, 10
lambda-cyhalothrin	**181 → 152.1**, 181 → 127.1, 181 → 77.1	20, 30, 40
metazachlor	**209 → 132.1**, 209 → 117.1, 133 → 131.7	20, 40, 20
metribuzin	**198 → 82.1**, 198 → 55.1	20, 40
myclobutanil	**179 → 125**, 179 → 90, 179 → 63	10, 40, 40
napropamide	271 → 72, 128 → 100.1, **128 → 72.1**	20, 10, 10
penconazole	248 → 206.1, 248 → 192.1, **248 → 157.1**	10, 10, 30
pendimethalin	252 → 191.1, **252 → 162.1**, 252 → 106.1	10, 10, 40
pirimicarb	**238 → 166.1**, 166 → 96.1	10, 10
proquinazid	**288 → 245**, 288 → 217, 272 → 216	10, 30, 20
prosulfocarb	**251 → 128.1**, 162 → 91.1, 162 → 65	10, 10, 40
pyraclostrobin	**164 → 132.1**, 164 → 104, 132 → 104	10, 30, 10
pyrimethanil	198 → 183.1, **198 → 118**	20, 40
pyriproxyfen	226 → 186.1, **226 → 77.1**	10, 40
tebuconazole	250 → 153, **250 → 125**, 250 → 70	10, 30, 10
tebufenpyrad	335 → 319.9, 333 → 318.2, **333 → 276.1**	10, 10, 10
tefluthrin	177 → 137, **177 → 127**, 177 → 87.1	20, 20, 40
tetraconazole	**336 → 218.1**, 336 → 164	20, 30
trifloxystrobin	222 → 162.1, 222 → 130, **131 → 116**	10, 10, 20

Bold MRM transitions were used for quantification.

the present study, holds of 20 min and 19.75 min, respectively, at 280 °C were used to measure boscalid in Skubic et al. (2025) and azoxystrobin in the present study. In addition, the long hold at 280 °C helped to prevent carryover effects between injections.

During evaluation of chromatograms, the retention time tolerance for compounds in sample extracts was ±0.1 min compared to matrix-matched standards in the same sequence. Analyte peaks from product ions in the extracted ion chromatogram had to fully overlap. The ion ratio from sample extracts had to be within ±30% (relative) of the average of calibration standards from the same sequence.

2.3.4. Matrices for validation

Validation was conducted on one representative wine, red wine, and on two representative juices: apple juice for fruit juice and carrot juice for vegetable juice. Apple juice is commonly used as an ingredient in various fruit juices. Carrot juice is the most common vegetable juice and was the only organic vegetable juice available. Red wine typically has a higher background than white wine. These are the reasons for selecting these representatives for validation.

Validation on only three representative matrices is a limitation of this study. Nevertheless, it demonstrates the robustness of the method and indicates that it can be applied to other juice and wine matrices. Extending validation by increasing the number of matrices used would provide a broader picture of the suitability and robustness of the method for all wines and for all fruit and vegetable juices. Of particular importance would be extending validation to juice matrices made from guava, coconut or passion fruit, which are exotic fruits with different structures. It would also be interesting to validate a champagne matrix, which contains carbon dioxide.

2.3.5. Matrix effect

Matrix effect was calculated using the following equation:

$$\text{ME} (\%) = (\text{Area MM}/\text{Area STD} - 1) \times 100\%$$

where Area MM is the peak area of the matrix-matched standard, and Area STD is the peak area of the standard in acetone.

2.4. Consumer risk assessment

2.4.1. Chronic exposure

The calculation of long-term exposure was performed using the EFSA PRIMo model revision 3.1. As the model allows input values only for fruit and not for processed products, residues in fruit were calculated using processing factors (Zincke et al., 2024) and, in the case of juices, also the fruit/vegetable portion. Where no processing factors were available, a processing factor of 1 was used. The median residue for fruit was calculated from all determined concentrations. It was compared to the Acceptable Daily Intake (ADI) of a single active compound. Chronic consumer exposure was expressed as a percentage of the ADI. The acceptable limit for long-term exposure is 100% of the ADI.

2.4.2. Acute exposure

The calculation of short-term exposure was performed using the EFSA PRIMo model revision 3.1. Since the model allows input values only for fruit and not for processed products, residues in fruit were calculated using processing factors from EU database (Zincke et al., 2024), and in the case of juices, also the fruit/vegetable portion. Where no processing factors were available, a processing factor of 1 was used. The highest residue for fruit was compared to the Acute Reference Dose (ARfD) of a single active compound. Acute consumer exposure was expressed as a percentage of the ARfD. The acceptable limit for short-term exposure is 100% of the ARfD.

3. Results and discussion

3.1. Performance and validation of the analytical method

Buffering with sodium acetate and acetic acid during extraction procedure resulted in higher recoveries in apple juice for lambda-cyhalothrin (20%) and pyraclostrobin (30%), and in wine for metribuzin (30%) and tefluthrin (20%). In carrot juice, buffering gave lower recoveries for fludioxonil (20%), flufenacet (30%) and pendimethalin (30%); therefore, buffering was not used for vegetable juices.

Blank matrices used for the validation were checked for the presence of pesticide residues and were found to be free of pesticides.

Limits of quantification (LOQs) were set using matrix-matched standards. The signal- to-noise ratio at the LOQ had to be at least 10. All LOQs were set at 0.005 mg L^{-1}.

Linearity was verified with matrix-matched standards using four to five concentration levels, with one calibration point per level. R^2 for the linear curve was calculated. Our R^2 values were mainly equal to or higher than 0.990, except for metribuzin in apple juice (0.987), lambda-cyhalothrin in wine (0.988), and pyraclostrobin in wine (0.978). Lower R^2 values were still acceptable. The linearity range was 0.005 to 0.04 mg L^{-1} for all active substances in carrot juice and apple juice, except for flufenacet, metazachlor, penconazole, proquinazid, and pyrimethanil in apple juice. These active substances, and all active substances in wine, had a linearity range of 0.005 to 0.03 mg L^{-1}.

Accuracy was verified using recoveries. For 10 days, each day two parallel samples of blank matrix spiked at the LOQ with a mixture of pesticides were analysed. From 20 measurements, recoveries and RSDs were calculated for each active substance. According to SANTE guidance (2021), recoveries of 70 to 120% with RSDs ≤20% are acceptable at an LOQ of 0.005 mg L^{-1}. According to SANTE guidance (2020) and recommendations for in-house validation by Alder et al. (2000), recoveries of 60 to 120% with RSDs ≤30% are acceptable at an LOQ of 0.005 mg L^{-1}. All our recoveries during validation were in the range 72.2 to

104.2%, with RSDs from 6.3 to 18.0%. Procedural recoveries (2 parallel samples for each matrix) presented in Supplement 2 were in the range 73.7 to 112.2%.

Measurement uncertainty was calculated from the 20 measurements of matrix spiked at the LOQ mentioned above. From these 20 concentration measurements, the standard deviation of repeatability and the standard deviation of reproducibility were calculated according to ISO 5725 (ISO, 2019). From the standard deviations, the uncertainty of repeatability and the uncertainty of reproducibility were calculated by multiplying the standard deviation of repeatability and the standard deviation of reproducibility by the Student's t factor for 9 degrees of freedom and a 95% confidence level ($t_{95;9} = 2.262$).

$U_r = t_{95;9} \times s_r$; $U_R = t_{95;9} \times s_R$

The measurement uncertainty for pesticide residues should be 50%, as proposed in SANTE guidance (2021). With validation, analysts must demonstrate that their measurement uncertainty is below or equal to the proposed measurement uncertainty. Our measurement uncertainties of repeatability ranged from 7.0% to 26.0%, and our measurement uncertainties of reproducibility ranged from 12.7% to 36.0%.

Matrix effect was also evaluated as suggested in the SANTE guidance (2021). In wine, the observed matrix effect ranged from 3% to 161%, with an average value of 54%. In apple juice, the observed matrix effect ranged from 38% to 363%, with an average value of 129%. In carrot juice, the observed matrix effect ranged from 24% to 393%, with an average value of 130%. The lowest matrix effect was observed in wine. In both juices, it was similar and much higher than in wine. Matrix effects above 50% can cause overestimation of calculated concentrations in samples if standards in acetone are used for calibration. Since most substances had a matrix effect above the tolerable 50%, matrix-matched calibration was the proper approach for calibration compared to the standard in acetone. Considering differences among juices and wine, matrix-matched standards need to be prepared in the same matrix as the samples analysed. A simplified approach could use apple juice as the matrix for fruit juices, carrot juice as the matrix for vegetable juices, and a wine matrix for wine samples. Differences in matrix effects represent limitations in quantification accuracy and robustness of the present method.

SANTE guidance (2021) recommends the use of internal standards, or more precisely more than one internal standard, in multiresidual methods. Selected internal standards should not interfere with the analysis of the target analytes, and they must not be present in the analysed samples. The most elegant solution would be the use of deuterated analytes or analytes in which atoms are substituted with other isotopes. This solution is expensive, time-consuming and often not feasible, since producers of standards do not offer all standards in isotopically labelled form. The limitation of this study is that no internal standards were used. However, it should be noted that checking procedural recoveries in each batch of analyses substantially reduces the need for internal standards. Procedural recoveries outside the limits of 60–140% (SANTE, 2021) are a good marker for quantitatively unreliable results. Moreover, the recommended measurement uncertainty in SANTE guidance (2021) is 50%, meaning that the correct result lies within a wide concentration interval. In the present method, the largest measurement uncertainty of reproducibility was 36%, leading to the conclusion that possible quantification deviations due to the absence of internal standards and high matrix effects could fall within the remaining 14%. Moreover, juice and wine are liquid, homogeneous matrices, meaning that ca 30% of uncertainty due to sample grinding

Table 4
Validation parameters in apple juice.

Active substance	Linearity range (mg L^{-1})	R^2	LOQ (mg L^{-1})	Recovery (%)	RSD[a] (%)	U_r[b] (mg L^{-1})	U_r[c] (%)	U_R[d] (mg L^{-1})	U_R[e] (%)	ME[f] (%)
azoxystrobin	0.005–0.04	0.999	0.005	92.2	10.9	0.0010	19.6	0.0011	22.9	272
benthiavalicarb-isopropyl	0.005–0.04	1.000	0.005	89.8	13.2	0.0007	13.6	0.0014	27.3	270
boscalid	0.005–0.04	1.000	0.005	87.3	12.8	0.0005	9.8	0.0013	25.9	205
clomazone	0.005–0.04	0.995	0.005	92.2	14.4	0.0005	9.8	0.0015	30.9	49
cyprodinil	0.005–0.04	0.990	0.005	90.0	12.7	0.0005	9.0	0.0013	26.4	70
flonicamid	0.005–0.04	0.990	0.005	90.9	13.7	0.0004	7.9	0.0014	28.9	79
fluazifop-p-butyl	0.005–0.04	0.995	0.005	86.8	9.0	0.0005	11.0	0.0009	17.9	105
fludioxonil	0.005–0.04	1.000	0.005	85.8	11.6	0.0008	15.2	0.0011	22.8	170
flufenacet	0.005–0.03	0.991	0.005	89.9	11.6	0.0006	11.9	0.0012	24.0	111
fluopicolide	0.005–0.04	0.992	0.005	89.1	15.8	0.0005	10.6	0.0016	32.6	159
fluopyram	0.005–0.04	0.994	0.005	88.9	10.0	0.0006	12.3	0.0010	20.4	112
flutolanil	0.005–0.04	0.990	0.005	89.9	14.0	0.0005	10.2	0.0009	18.8	158
iprovalicarb	0.005–0.04	0.998	0.005	90.3	11.8	0.0007	13.9	0.0012	24.5	186
kresoxim-methyl	0.005–0.04	0.991	0.005	88.4	10.6	0.0004	8.3	0.0011	21.8	85
lambda-cyhalothrin	0.005–0.04	0.994	0.005	82.0	12.1	0.0009	17.0	0.0011	22.8	172
metazachlor	0.005–0.03	0.992	0.005	89.7	11.6	0.0005	10.0	0.0012	24.1	75
metribuzin	0.005–0.04	0.987	0.005	92.2	11.6	0.0005	10.7	0.0012	24.8	38
myclobutanil	0.005–0.04	0.992	0.005	89.7	13.8	0.0005	9.7	0.0014	28.7	107
napropamide	0.005–0.04	0.991	0.005	89.9	14.0	0.0005	9.2	0.0015	29.1	121
penconazole	0.005–0.03	0.993	0.005	91.2	13.1	0.0006	12.1	0.0014	27.6	55
pendimethalin	0.005–0.04	0.994	0.005	88.4	11.1	0.0008	16.2	0.0011	22.5	39
pirimicarb	0.005–0.04	0.996	0.005	90.8	11.4	0.0007	13.3	0.0012	23.9	57
proquinazid	0.005–0.03	0.998	0.005	89.6	13.9	0.0006	11.5	0.0014	28.9	121
prosulfocarb	0.005–0,04	0.992	0.005	89.6	12.1	0.0004	8.6	0.0013	25.0	58
pyraclostrobin	0.005–0.04	0.993	0.005	84.2	14.5	0.0006	12.0	0.0014	28.2	363
pyrimethanil	0.005–0.03	0.995	0.005	90.7	11.6	0.0004	7.0	0.0012	24.3	57
pyriproxyfen	0.005–0.04	0.997	0.005	86.3	13.3	0.0006	12.7	0.0013	26.5	147
tebuconazole	0.005–0.04	0.993	0.005	88.6	12.9	0.0005	10.6	0.0013	26.5	216
tebufenpyrad	0.005–0.03	0.994	0.005	81.9	8.4	0.0005	10.9	0.0008	15.8	156
tefluthrin	0.005–0.04	0.998	0.005	84.5	13.4	0.0005	10.8	0.0013	26.2	48
tetraconazole	0.005–0.04	0.990	0.005	91.4	13.0	0.0005	10.5	0.0014	27.5	85
trifloxystrobin	0.005–0.04	0.996	0.005	87.9	12.1	0.0007	13.5	0.0012	24.5	182

[a] RSD was obtained during recovery analyses.
[b,c] U_r = uncertainty of repeatability.
[d,e] U_R = uncertainty of reproducibility.
[f] ME = matrix effect.

can be omitted. This can lead to the assumption that a high matrix effect and the absence of internal standards can still make analyses comparable to those of solid and less homogeneous matrices, such as fruit and vegetables.

Results of validation are presented in Tables 4–6.

During the analysis of juice and wine samples, procedural recoveries were measured for all active substances sought. These are presented in Supplement 2.

3.2. Survey of pesticide residues in juice

Survey results are presented in Table 7. In 23 out of 40 samples (57.5%), residues were not found. Seventeen samples (42.5%) were positive. Eight active substances were identified, with concentrations ranging from 0.005 to 0.044 mg L^{-1}. Boscalid was the most frequently determined, found in 9 out of 40 samples (22.5%). The highest number of active substances in a single sample was five, observed in one peach / apple juice sample. The number of samples with multiple residues was 7 (17.5%).

Concentrations of boscalid in juices were 0.005–0.044 mg L^{-1}. Using the uncertainty of reproducibility for boscalid from this study (25.9%), the confidence interval is 0.004–0.055 mg L^{-1}. Concentrations of cyprodinil in juices were 0.008–0.013 mg L^{-1}. Using the uncertainty of reproducibility for cyprodinil from this study (26.4%), the confidence interval is 0.006–0.016 mg L^{-1}. Concentrations of fludioxonil in juices were 0.006–0.044 mg L^{-1}. Using the uncertainty of reproducibility for fludioxonil from this study (22.8%), the confidence interval is 0.005–0.054 mg L^{-1}. Concentrations of fluopyram in juices were 0.005–0.015 mg L^{-1}. Using the uncertainty of reproducibility for fluopyram from this study (20.4%), the confidence interval is 0.004–0.018 mg L^{-1}. The concentration of pyraclostrobin in juice was 0.014 mg L^{-1}. Using the uncertainty of reproducibility for pyraclostrobin from this study (28.2%), the confidence interval is 0.010–0.018 mg L^{-1}. The concentration of pyrimethanil in juice was 0.022 mg L^{-1}. Using the uncertainty of reproducibility for pyrimethanil from this study (24.3%), the confidence interval is 0.017–0.027 mg L^{-1}. Concentrations of tebuconazole in juices were 0.006–0.014 mg L^{-1}. Using the uncertainty of reproducibility for tebuconazole from this study (26.5%), the confidence interval is 0.004–0.018 mg L^{-1}. Concentrations of trifloxystrobin in juices were 0.005–0.008 mg L^{-1}. Using the uncertainty of reproducibility for trifloxystrobin from this study (24.5%), the confidence interval is 0.004–0.010 mg L^{-1}. In conclusion, using the uncertainty of reproducibility from this study to calculate the confidence interval, the concentration range of residues in juice was 0.004–0.055 mg L^{-1}.

Regarding the type of juice, no sample containing red beet (4 samples) or blackcurrant (3 samples) was positive. No sample containing 100% apple (7 samples) was positive. Twelve out of 22 samples containing apple (54.5%) were positive. It is possible that residues in these samples originated from other fruit, not apples. All three samples containing apricot were positive. The only sample containing blueberry was positive. One out of two samples containing carrot (50%) was positive. Two out of nine samples containing orange (22.2%) were positive. All six samples containing peach were positive. The only sample containing pear was positive. All five samples containing strawberry were positive. Two out of three samples containing grapes (66.7%) were positive.

Regarding packaging, 2 out of 4 samples packed in 0.2 L vessels (50%) were positive. Fifteen of 36 juices packed in 1 L vessels (41.7%) were positive. Assuming that children are consumers of 0.2 L packed juices, they are no less exposed to pesticide residues than if they consume 1 L packages. Due to the small data set of 0.2 L packed juices,

Table 5
Validation parameters in carrot juice.

Active substance	Linearity range (mg L^{-1})	R^2	LOQ (mg L^{-1})	Recovery (%)	RSD[a] (%)	U_r[b] (mg L^{-1})	U_r^c (%)	U_R[d] (mg L^{-1})	U_R^e (%)	ME[f] (%)
azoxystrobin	0.005–0.04	1.000	0.005	87.1	16.0	0.0010	19.3	0.0016	32.1	207
benthiavalicarb-isopropyl	0.005–0.04	0.997	0.005	82.5	13.8	0.0006	11.6	0.0013	26.4	182
boscalid	0.005–0.04	0.999	0.005	78.4	9.9	0.0006	12.0	0.0009	17.8	185
clomazone	0.005–0.04	0.997	0.005	76.2	9.6	0.0006	12.6	0.0008	16.8	46
cyprodinil	0.005–0.04	0.999	0.005	78.5	8.8	0.0005	10.3	0.0008	15.9	80
flonicamid	0.005–0.04	0.995	0.005	72.2	7.7	0.0005	10.4	0.0006	12.7	71
fluazifop-p-butyl	0.005–0.04	0.999	0.005	76.7	10.5	0.0005	10.7	0.0009	18.6	126
fludioxonil	0.005–0.04	1.000	0.005	78.8	10.3	0.0005	10.5	0.0009	18.7	186
flufenacet	0.005–0.04	0.993	0.005	79.0	9.0	0.0005	9.7	0.0008	16.4	84
fluopicolide	0.005–0.04	0.996	0.005	78.5	9.6	0.0007	14.0	0.0009	17.1	162
fluopyram	0.005–0.04	0.998	0.005	78.1	9.6	0.0004	8.6	0.0009	17.3	121
flutolanil	0.005–0.04	0.999	0.005	77.3	11.0	0.0006	11.5	0.0010	19.5	169
iprovalicarb	0.005–0.04	0.997	0.005	83.8	10.9	0.0007	13.8	0.0010	21.0	231
kresoxim-methyl	0.005–0.04	0.996	0.005	77.1	9.8	0.0004	7.8	0.0009	17.5	104
lambda-cyhalothrin	0.005–0.04	0.998	0.005	83.0	13.1	0.0007	14.1	0.0013	25.0	191
metazachlor	0.005–0.04	0.995	0.005	76.6	9.3	0.0005	9.5	0.0008	16.3	76
metribuzin	0.005–0.04	0.991	0.005	77.6	16.0	0.0007	13.6	0.0014	28.6	24
myclobutanil	0.005–0.04	0.998	0.005	77.1	8.7	0.0005	9.2	0.0008	15.4	113
napropamide	0.005–0.04	0.999	0.005	77.3	10.2	0.0009	17.7	0.0009	18.1	134
penconazole	0.005–0.04	0.998	0.005	78.1	9.2	0.0005	9.8	0.0008	16.5	58
pendimethalin	0.005–0.04	0.996	0.005	75.3	9.1	0.0005	9.3	0.0008	15.7	57
pirimicarb	0.005–0.04	0.997	0.005	78.7	9.8	0.0005	10.1	0.0009	17.8	57
proquinazid	0.005–0.04	0.998	0.005	80.4	11.4	0.0005	10.1	0.0011	21.1	126
prosulfocarb	0.005–0.04	0.992	0.005	104.2	10.7	0.0007	13.8	0.0012	24.9	63
pyraclostrobin	0.005–0.04	1.000	0.005	90.7	14.7	0.0008	16.4	0.0015	30.7	393
pyrimethanil	0.005–0.04	0.995	0.005	78.8	10.5	0.0006	12.3	0.0010	19.0	49
pyriproxyfen	0.005–0.04	0.999	0.005	76.7	11.9	0.0006	11.7	0.0011	21.0	167
tebuconazole	0.005–0.04	0.999	0.005	76.9	10.2	0.0005	10.2	0.0009	18.1	210
tebufenpyrad	0.005–0.04	0.997	0.005	77.8	8.7	0.0007	13.6	0.0008	15.4	161
tefluthrin	0.005–0.04	0.998	0.005	73.8	13.3	0.0005	9.8	0.0011	22.7	58
tetraconazole	0.005–0.04	0.999	0.005	78.1	9.9	0.0006	11.4	0.0009	17.8	80
trifloxystrobin	0.005–0.04	0.994	0.005	91.6	12.7	0.0006	12.5	0.0013	26.9	176

[a] RSD was obtained during recovery analyses.
[b,c] U_r = uncertainty of repeatability.
[d,e] U_R = uncertainty of reproducibility.
[f] ME = matrix effect.

Table 6
Validation parameters in wine.

Active substance	Linearity range (mg L^{-1})	R^2	LOQ (mg L^{-1})	Recovery (%)	RSD[a] (%)	U_r[b] (mg L^{-1})	U_r^c (%)	U_R[d] (mg L^{-1})	U_R^e (%)	ME[f] (%)
azoxystrobin	0.005–0.03	0.999	0.005	92.0	11.8	0.0005	10.9	0.0013	25.0	65
benthiavalicarb-isopropyl	0.005–0.03	0.991	0.005	93.1	11.8	0.0006	12.2	0.0013	25.3	39
boscalid	0.005–0.03	0.991	0.005	94.5	9.3	0.0009	17.2	0.0010	20.1	7
clomazone	0.005–0.03	0.995	0.005	84.8	11.6	0.0010	20.7	0.0011	22.3	78
cyprodinil	0.005–0.03	0.999	0.005	87.1	9.9	0.0010	19.5	0.0010	19.6	46
flonicamid	0.005–0.03	0.994	0.005	84.5	12.5	0.0008	15.3	0.0012	24.3	26
fluazifop-p-butyl	0.005–0.03	1.000	0.005	89.9	11.4	0.0007	13.6	0.0012	23.6	19
fludioxonil	0.005–0.03	0.995	0.005	87.9	8.5	0.0008	15.7	0.0008	16.9	111
flufenacet	0.005–0.03	0.995	0.005	84.4	18.0	0.0013	26.0	0.0017	34.8	7
fluopicolide	0.005–0.03	0.997	0.005	91.5	8.5	0.0009	17.5	0.0009	18.1	134
fluopyram	0.005–0.03	0.997	0.005	87.9	6.3	0.0006	12.5	0.0007	13.0	17
flutolanil	0.005–0.03	0.996	0.005	87.9	6.4	0.0006	12.6	0.0007	13.1	51
iprovalicarb	0.005–0.03	1.000	0.005	88.5	13.4	0.0008	16.2	0.0014	27.3	83
kresoxim-methyl	0.005–0.03	0.999	0.005	89.6	9.2	0.0007	14.3	0.0009	18.9	51
lambda-cyhalothrin	0.005–0.03	0.988	0.005	90.3	17.0	0.0008	16.9	0.0018	35.5	39
metazachlor	0.005–0.03	0.994	0.005	84.6	11.6	0.0007	13.4	0.0011	22.7	32
metribuzin	0.005–0.03	1.000	0.005	93.5	12.1	0.0007	13.4	0.0018	36.0	3
myclobutanil	0.005–0.03	0.995	0.005	88.4	7.9	0.0007	14.1	0.0008	15.8	46
napropamide	0.005–0.03	0.997	0.005	88.3	10.6	0.0007	13.9	0.0011	21.5	42
penconazole	0.005–0.03	0.997	0.005	87.2	9.9	0.0009	17.7	0.0010	19.6	23
pendimethalin	0.005–0.03	1.000	0.005	85.8	14.9	0.0006	12.2	0.0015	29.7	28
pirimicarb	0.005–0.03	0.995	0.005	85.7	7.9	0.0006	12.3	0.0008	15.5	32
proquinazid	0.005–0.03	0.996	0.005	86.3	14.0	0.0007	13.7	0.0014	27.8	111
prosulfocarb	0.005–0.03	0.995	0.005	84.7	9.5	0.0008	15.3	0.0009	18.2	61
pyraclostrobin	0.005–0.03	0.978	0.005	95.0	14.6	0.0007	14.1	0.0016	32.1	11
pyrimethanil	0.005–0.03	0.998	0.005	86.7	9.3	0.0009	18.2	0.0010	20.8	44
pyriproxyfen	0.005–0.03	0.997	0.005	83.4	9.0	0.0007	13.8	0.0009	17.2	106
tebuconazole	0.005–0.03	0.995	0.005	90.8	9.6	0.0008	15.2	0.0010	19.8	119
tebufenpyrad	0.005–0.03	0.998	0.005	89.9	11.0	0.0010	19.8	0.0011	22.6	65
tefluthrin	0.005–0.03	0.991	0.005	80.0	13.5	0.0004	7.8	0.0013	25.0	51
tetraconazole	0.005–0.03	0.999	0.005	89.1	9.9	0.0010	20.0	0.0010	20.8	27
trifloxystrobin	0.005–0.03	0.992	0.005	86.7	12.8	0.0006	11.9	0.0013	25.7	161

[a] RSD was obtained during recovery analyses.
[b,c] U_r = uncertainty of repeatability.
[d,e] U_R = uncertainty of reproducibility.
[f] ME = matrix effect.

conclusions are of limited reliability. Regarding origin, 2 out of 5 samples from Austria (40%) were positive. Fifteen out of 35 samples from Slovenia (42.9%) were positive. The ratio of positive samples is comparable for Slovenia and Austria, but because of the small set of samples from Austria, this ratio is of limited reliability.

Calculation was performed from residues in juice to residues in fruit using fruit portion and processing factors (Zincke et al., 2024). Processing factors used are presented in Supplement 3 A. For active substances without an available processing factor, a processing factor of 1 was used. This approach is the same as that used by EFSA in annual reports on the monitoring of pesticide residues (EFSA, 2026). It is also used by laboratories conducting official control of food on the market. We assumed the worst-case scenario: 100% of residues were attributed to each fruit or vegetable type in the juice. Calculations were conducted with formula:

C (fruit) in mg kg^{-1} = C (juice) in mg L^{-1} × 100%/(fruit portion in % × processing factor)

For example, for a juice sample containing 57% apples and 43% pears, with a measured residue of 0.022 mg L^{-1} for pyrimethanil, residues were calculated as 0.076 mg kg^{-1} for apple (0.022 × 100 / (57 × PF = 0.51)) and 0.051 mg kg^{-1} for pear (0.022 × 100 / 43). Calculated residues in fruit were compared to valid MRLs. Three samples exceeded the MRLs (7.5%): boscalid in apple, fludioxonil in apple, and fluopyram in table grapes. For all three samples, available processing factors were used. Other fruit contained residues below valid MRLs. For calculations of residues in fruit from concentrations in juices where a processing factor of 1 was used, the calculated residues may be underestimated. Processing factors for juices are usually less than 1, meaning that not whole of the residue is transferred to the juice. However, a greater error could occur if an inappropriate processing factor is used, such as one from a different type of juice. This approach affects the underestimation of MRL exceedances and risk assessment. However, at present, this is the best available method for calculating residues in fruit.

Our results were compared with those from other scientific papers. Ferrer et al. (2011) found tebuconazole in peach juice samples. The proportion of samples containing tebuconazole was 5.7%, which is lower than in our observation (3 out of 34 fruit juice samples: 8.8%). Wołejko et al. (2014) analysed 10 fruit juice samples, which, among the active substances sought in our laboratory, contained boscalid (in 40.0% of samples), cyprodinil, fludioxonil, pyrimethanil, and trifloxystrobin. The same active substances were present in our juices, with boscalid also being the most frequently found (in 23.5% of fruit juice samples). Torović et al. (2021) analysed 139 samples of fruit juice, of which 71.2% were positive. This proportion is higher than in our laboratory, where 16 of 34 juice samples were positive (47.1%). Torović et al. (2021) also observed that almost 60% of positive samples contained multiple residues, up to 6, which is higher than in our research, where 7 out of 16 positive fruit juice samples (43.8%) contained multiple residues, up to 5. On the other hand, Torović et al. (2021) observed only 2.2% of samples not compliant with valid MRLs. This proportion is lower than in our case (3 out of 34 samples: 8.8%). Torović et al. (2021) found, among the active substances analysed in our laboratory, azoxystrobin, myclobutanil, and pyriproxyfen, which were not found in our research, and cyprodinil, pyrimethanil, and trifloxystrobin, which were also found in our research. Among the latter, the maximum concentration was 0.029 mg kg^{-1}. Our maximum concentration was higher at 0.044 mg L^{-1}.

Table 7
Results of juice and wine analyses and calculated concentrations in fruit and vegetables.

	Juice (mg L^{-1})	Wine (mg L^{-1})	Apple (mg kg^{-1})	Apricot (mg kg^{-1})	Blueberry (mg kg^{-1})	Carrot (mg kg^{-1})	Orange (mg kg^{-1})	Peach (mg kg^{-1})	Pear (mg kg^{-1})	Strawberry (mg kg^{-1})	Table grapes (mg kg^{-1})	Wine grapes (mg kg^{-1})
Boscalid												
MRL			2	5		2	2	5				5
min	0.005	0.006	0.118	0.024		0.104	0.116	0.021				0.015
max	0.044	0.034	2.242	0.177		0.104	0.116	0.088				0.146
median	0.013	0.015	0.803	0.042		0.104	0.116	0.048				0.058
Cyprodinil												
MRL			2					2	2			3
min	0.008	0.007	0.015					0.030	0.020			0.712
max	0.013	0.037	0.023					0.030	0.020			3.686
median	0.011	0.015	0.019					0.030	0.020			1.509
Fludioxonil												
MRL			5	5				10		4	5	4
min	0.006	0.005	0.756	0.689				1.002		1.018	0.229	0.042
max	0.044	0.018	8.549	0.689				4.404		1.018	0.229	0.139
median	0.011	0.009	2.182	0.689				2.703		1.018	0.229	0.070
Fluopyram												
MRL			0.8		7			1.5		2	2	1,5
min	0.005	0.005	0.081		0.107			0.036		0.030	3.213	0.007
max	0.015	0.035	0.356		0.107			0.036		0.067	3.213	0.174
median	0.009	0.012	0.189		0.107			0.036		0.044	3.213	0.058
Iprovalicarb												
MRL												2
min		0.006										0.009
max		0.017										0.026
median		0.011										0.016
Pyraclostrobin												
MRL				1								
min	0.014			0.055								
max	0.014			0.055								
median	0.014			0.055								
Pyrimethanil												
MRL			15				8		15			5
min	0.022	0.006	0.076				0.044		0.051			0.004
max	0.022	0.062	0.076				0.044		0.051			0.087
median	0.022	0.016	0.076				0.044		0.051			0.018
Tebuconazole												
MRL			0,3					0,6	0,3			1
min	0.006	0.005	0.041					0.016	0.018			0.028
max	0.014	0.024	0.074					0.032	0.018			0.152
median	0.008	0.006	0.057					0.024	0.018			0.035
Trifloxystrobin												
MRL			0.7							1	3	
min	0.005		0.478							0.017	0.124	
max	0.008		0.563							0.037	0.124	
median	0.007		0.520							0.027	0.124	

3.3. Survey of pesticide residues in wine

Survey results are presented in Table 7. In 11 of 41 samples (26.8%), no residues were found. Thirty samples (73.2%) tested positive. Seven active substances were identified, with concentrations ranging from 0.005 to 0.062 mg L^{-1}. Boscalid was the most frequently found, present in 18 of 41 samples (43.9%). The highest number of active substances in a single sample was five, observed in two red wine samples. The number of samples containing multiple residues was 23 (56.1%). Ecological wine was pesticides free.

Concentrations of boscalid in wine were 0.006–0.034 mg L^{-1}. Using the uncertainty of reproducibility for boscalid from this study (20.1%), the confidence interval is 0.005–0.041 mg L^{-1}. Concentrations of cyprodinil in wine were 0.007–0.037 mg L^{-1}. Using the uncertainty of reproducibility for cyprodinil from this study (19.6%), the confidence interval is 0.006–0.044 mg L^{-1}. Concentrations of fludioxonil in wine were 0.006–0.044 mg L^{-1}. Using the uncertainty of reproducibility for fludioxonil from this study (16.9%), the confidence interval is 0.005–0.051 mg L^{-1}. Concentrations of fluopyram in wine were 0.005–0.015 mg L^{-1}. Using the uncertainty of reproducibility for fluopyram from this study (13.0%), the confidence interval is 0.004–0.017 mg L^{-1}. Concentrations of iprovalicarb in wine were 0.006–0.017 mg L^{-1}. Using the uncertainty of reproducibility for iprovalicarb from this study (27.3%), the confidence interval is 0.004–0.022 mg L^{-1}.

Concentrations of pyrimethanil in wine were 0.006–0.062 mg L^{-1}. Using the uncertainty of reproducibility for pyrimethanil from this study (20.8%), the confidence interval is 0.005–0.075 mg L^{-1}. Concentrations of tebuconazole in wine were 0.005–0.024 mg L^{-1}. Using the uncertainty of reproducibility for tebuconazole from this study (19.8%), the confidence interval is 0.004–0.029 mg L^{-1}. In conclusion, using the uncertainty of reproducibility from this study to calculate the confidence interval, the concentration range of residues in wine was 0.004–0.075 mg L^{-1}.

Considering the type of wine, 17 out of 20 red wines (85.0%) and 13 out of 21 white wines (61.9%) were positive. The proportion of positive red wines was slightly higher than that of white wines. Considering origin, 5 out of 10 samples from Podravje (50.0%), 9 out of 10 samples from Posavje (90.0%), 9 out of 12 samples from Primorska (75.0%), and 7 out of 9 samples from other countries (77.8%) were positive. In total, 27 out of 32 samples produced in Slovenia (84.4%) were positive. The proportion of positive samples in Slovenia is slightly higher than that for other countries. Since the set of wines from other countries is rather small (9 compared to 32 from Slovenia), this comparison is of limited reliability.

Residue levels in wine were converted to residue levels in wine grapes using processing factors (Zincke et al., 2024), which were available for both white and red wine for all active substances found. Processing factors used are presented in Supplement 3B. Calculations were conducted with formula:

$$C \text{ (fruit) in mg kg}^{-1} = C \text{ (wine) in mg L}^{-1}/\text{processing factor}$$

An example of calculating residues in wine grapes from residues in wine is cyprodinil, with a measured residue of 0.03686 mg L^{-1} in red wine. Residues in wine grapes were calculated as 3.686 mg kg^{-1} in fruit (0.03686 / PF = 0.01). Calculated residues in wine grapes were compared with valid MRLs. One sample exceeded the MRL for cyprodinil (2.4%). The other wine grape samples contained residues below the valid MRLs.

Our results were compared with those from other scientific papers. González et al. (2022) found in wines from the Canary Islands 11 active substances analysed in our laboratory: azoxystrobin, kresoxim-methyl, myclobutanil, penconazole, tetraconazole and trifloxystrobin, which were not determined by our laboratory, and boscalid, fluopyram, iprovalicarb, pyrimethanil and tebuconazole, which were determined in our laboratory as well. 78.6% of wines from the Canary Islands were positive for pesticide residues. In our case, the proportion was similar (73.2%). González et al. (2022) observed the highest number of active substances per sample as 6, while we had one substance fewer. The most common residues found by González et al. (2022) were tebuconazole, fluopyram and boscalid. The last two were also the most frequently found in our wines. The highest concentration reported by González et al. (2022) was 0.027 mg kg^{-1}. Sykalia et al. (2024) found lambda-cyhalothrin in their wine samples, which was sought by our laboratory but not found, and iprovalicarb, which was also found in our laboratory. Kosma et al. (2021) determined cyprodinil in Greek wines among eight active substances sought in our laboratory as well. Pinheiro et al. (2025) observed tebuconazole in wines from South America with a maximum concentration of 0.034 mg L^{-1}, which is higher than our maximum concentration for this active substance (0.024 mg L^{-1}). Pérez-Mayán et al. (2021) analysed Spanish wines and identified, among 12 active substances sought by our laboratory, fluopicolid and myclobutanil, which were not found in our research, and boscalid, cyprodinil, fludioxonil, iprovalicarb, pyrimethanil and tebuconazole, which were also found in our research. Santana-Mayor et al. (2023) determined 25 different active substances in 152 Spanish wine samples, of which 5.3% exceeded MRLs when residues in wine grapes were calculated. This is higher than in our study. The proportion of positive samples observed by Santana-Mayor et al. (2023) was 90.8%, which is also higher than in our case (73.2%). Twelve active substances were also sought by our laboratory: azoxystrobin, kresoxim-methyl, myclobutanil, penconazole, pyraclostrobin and tetraconazole, which were not found in our study, and boscalid, cyprodinil, fludioxonil, iprovalicarb, pyrimethanil and tebuconazole, which were also found in our study. The highest concentration in wine observed by Santana-Mayor et al. (2023) was 1.47 mg kg^{-1} for pyrimethanil, which is almost 24 times higher than in our case. Fernández-Fernández et al. (2025) positively identified 13 active substances sought by our laboratory: azoxystrobin, fluopicolide, kresoxim-methyl, myclobutanil, tetraconazole and trifloxystrobin, which were not found in our research, and boscalid, cyprodinil, fludioxonil, fluopyram, iprovalicarb, pyrimethanil and tebuconazole, which were also found in our research. The highest concentration was observed for pyrimethanil: 0.158 mg L^{-1}, which is 2.5-times higher than our maximum concentration.

3.4. Risk assessment

A consumer risk assessment was conducted for active substances found in juice and wine samples. Input values were calculated as contents in fruit and vegetables. For boscalid, cyprodinil, fludioxonil, iprovalicarb and pyrimethanil, no ARfD was set. In such cases, the ADI was used as a worst-case scenario instead. Results are presented in Table 8. Chronic exposure calculations are in detail presented in Supplement 4. Chronic exposure is acceptable for all identified compounds. For boscalid, fludioxonil, pyraclostrobin, pyrimethanil, tebuconazole and trifloxystrobin, the highest chronic exposure was observed for the DE child diet. For cyprodinil and iprovalicarb, the highest chronic exposure was observed for the PT general diet. For fluopyram, the highest chronic exposure was observed for the NL toddler diet. Acute exposure is acceptable for fluopyram, iprovalicarb, pyraclostrobin, pyrimethanil, tebuconazole and trifloxystrobin. Boscalid, cyprodinil and fludioxonil are not acutely toxic, but if the ADI is used instead of the ARfD for acute exposure calculation, short-term exposure is exceeded for boscalid in apples consumed by children, for cyprodinil in wine grapes consumed by adults, and for fludioxonil in apples and peaches consumed by children. The EFSA PRIMo model rev. 3.1 automatically uses the ADI as input value for calculations of acute exposure when the ARfD is not set. This is because the ARfD value is always higher than, or in the worst case equal to, the ADI. However, during the authorisation of PPPs and active substances in the EU, acute exposure is not relevant when the ARfD is not allocated and calculations are not required. In such cases, the active substance is considered not to be acutely toxic. Therefore, any possible acute risk observed in this study is questionable. It should also be noted that during the authorisation process, MRLs are set or checked, and residues are always equal to or lower than the MRLs. When a sample exceeding the MRL is found during routine food control on the market, an acute risk assessment is required to determine whether the product can remain on the market or must be removed. MRLs are trading standards and not toxicological limits, but they must be toxicologically acceptable. Exceeded MRLs do not necessarily represent a risk to consumers.

Table 8
Short-term (IESTI) and long-term (TMDI) risk assessment of analysed samples.

Active substance	ADI (mg kg bw^{-1} d^{-1})	ARfD (mg kg bw^{-1})	TMDI (% ADI)	IESTI (% ARfD)
boscalid	0.04	/	27	604
cyprodinil	0.03	/	13	291
fludioxonil	0.37	/	8	249
fluopyram	0.012	0.5	59	47
iprovalicarb	0.015	/	0,3	4
pyraclostrobin	0.03	0.03	0,1	6
pyrimethanil	0.17	/	0,7	5
tebuconazole	0.03	0.03	2	26
trifloxystrobin	0.1	0.5	7	12

4. Conclusions

A multiresidual method for the determination of 32 active substances in fruit juices, vegetable juices, and wine was introduced and validated. LOQs for all substances were 0.005 mg L^{-1}. R^2 values for calibration curves were ≥ 0.99. Recoveries during validation ranged from 72.2% to 104.2%, with RSDs from 6.3% to 18.0%. Measurement uncertainties of reproducibility ranged from 12.7% to 36.0%. The method complies with current guidelines. Its robustness was verified through the analysis of 81 samples: 34 fruit juice, 6 vegetable juice, and 41 wine samples. Nine active substances were found, with a maximum of five per sample. Among them were three fluorinated pesticides: fludioxonil, fluopyram and trifloxystrobin. Pesticide residues were present in 58.0% of samples. After calculating concentrations in raw fruit or vegetables, four samples (3.7%) exceeded MRLs. Chronic consumer exposure was acceptable, but acute exposure could be of concern for boscalid in apples consumed by children, for cyprodinil in wine grapes consumed by adults, and for fludioxonil in apples and peaches consumed by children. Comparison with the literature showed that the active substances found by our laboratory were also found in other laboratories.

Acknowledgements

The author expresses thanks to Janja Debevc for her help with the preparation of the extracts. For financial support author expresses thanks to the Slovenian Research and Innovation Agency (ARIS) research core funding No. P4-0133 and through the METROFOOD-SI project Ref. No. I0-E006. Author also acknowledges the financial support of the Ministry of the Republic of Slovenia for Agriculture, Forestry and Food (MAFF), Administration of the Republic of Slovenia for Food Safety, Veterinary and Plant Protection (AFSVPP).

CRediT authorship contribution statement

Helena Baša Česnik: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The author declare the following financial interests/personal relationships which may be considered as potential competing interests: Helena Baša Česnik reports financial support was provided by Slovenian Research and Innovation Agency. If there is only one authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2026.150618.

Data availability

Data will be made available on request.

References

Alder, L., Hill, A., Holland, P. T., Lantos, J., Lee, S. M., MacNeil, J. D., … Ambrus, A. (2000). Guidelines for single-laboratory validation of analytical methods for trace-level concentrations of organic chemicals. In A. Fajgelj, & A. Ambrus (Eds.), *Principles and practices of method validation* (pp. 179–252). The Royal Society of Chemistry.

Bakirci, G. T., Acay, D. B. Y., Bakirci, F., & Ötleş, S. (2014). Pesticide residues in fruits and vegetables from the Aegean region, Turkey. *Food Chemistry, 160*, 379–392. https://doi.org/10.1016/j.foodchem.2014.02.051

Baša Česnik, H., Gregorčič, A., Velikonja Bolta, Š., & Kmecl, V. (2006). Monitoring of pesticide residues in apples, lettuce and potato of the Slovene origin, 2001-04. *Food Additives and Contaminants, 23*, 164–173.

Baša Česnik, H., Velikonja Bolta, Š., Gregorčič, A. (2011). Pesticide residues in agricultural products of Slovene origin found in 2001-2009. V: STOYTCHEVA, Margarita (ed.). Pesticides - formulations, effects, fate. Rijeka: InTech, pp. 199–223.

Concha-meyer, A., Grandon, S., Sepúlveda, G., Diaz, R., Yuri, J. A., & Torres, C. (2019). Pesticide residues quantification in frozen fruit and vegetables in Chilean domestic market using QuEChERS extraction with ultra-high-performance liquid chromatography electrospray ionization Orbitrap mass spectrometry. *Food Chemistry, 295*, 64–71. https://doi.org/10.1016/j.foodchem.2019.05.046

Čuš, F., Baša Česnik, H., & Velikonja Bolta, V. (2022). Pesticide residues, copper and biogenic amines in conventional and organic wines. *Food Control, 132*, Article 108534. https://doi.org/10.1016/j.foodcont.2021.108534

EFSA, 2021. The 2019 European Union report on pesticide residues in food, EFSA Journal 2021;19(4):6491, doi:https://doi.org/10.2903/j.efsa.2021.6491.

EFSA. (2025). The 2023 European Union report on pesticide residues in food. EFSA Journal. 2025;23:e9398. doi:https://doi.org/10.2903/j.efsa.2025.9398.

EFSA (2026). The 2024 European Union report on pesticide residues in food. EFSA Journal. 2026;24:e10054. doi:https://doi.org/10.2903/j.efsa.2026.10054.

Farajzedah, M. A., Barazandeh, S., & Mogaddam, M. R. A. (2025). Introducing a new sorbent based on hydrogen-bonded organic framework for the extraction of some pesticides from different grape juice samples. *Analytica Chimica Acta, 1371*, Article 344410. https://doi.org/10.1016/j.aca.2025.344410

Fernández-Fernández, V., Ramil, M., Blanco, P., Andradres, M. S., & Rodríguez, I. (2025). Pesticides in wines from two major production regions in the north of Spain. *Journal of Food Composition and Analysis, 142*, Article 107525. https://doi.org/10.1016/j.jfca.2025.107525

Ferrer, C., Martínez-Bueno, M. J., Lozano, A., & Fernández-Alba, A. R. (2011). Pesticide residue analysis of fruit juices by LC–MS/MS direct injection. One year pilot survey. *Talanta, 83*, 1552–1561. https://doi.org/10.1016/j.talanta.2010.11.061

Gao, Q., Wang, Y., Li, Y., Yang, W., Jiang, W., Liang, Y., & Zhang, Z. (2024). Residue behaviors of six pesticides during apple juice production and storage. *Food Research International, 177*, Article 113894. https://doi.org/10.1016/j.foodres.2023.113894

González, P. A., Dans, E. P., Dacal, A. C. A., Peña, M. Z., & Luzardo, O. P. (2022). Differences in the levels of sulphites and pesticide residues in soils and wines and under organic and conventional production methods. *Journal of Food Composition and Analysis, 112*, Article 104714. https://doi.org/10.1016/j.jfca.2022.104714

ISO. (2019). *Accuracy (trueness and precision) of measurement methods and results - Part2: Basic method for the determination of repeatability and reproducibility of a standard measurement method (ISO 5725-2-2019)*. Genewa: International Organization for Standardization.

Jankowska, M., Kaczy'nski, P., Hrynko, I., Rutkowska, E., Iwaniuk, P., Ilyasova, G., & Łozowicka, B. (2024). *Chemosphere, 369*, Article 143858. https://doi.org/10.1016/j.chemosphere.2024.143858

Kosma, C. I., Koloka, O. L., Albanis, T. A., & Konstantinou, I. K. (2021). Accurate mass screening of pesticide residues in wine by modified QuEChERS and LC-hybrid LTQ/Orbitrap-MS. *Food Chemistry, 360*, Article 130008. https://doi.org/10.1016/j.foodchem.2021.130008

Kumari, D., & John, S. (2019). Health risk assessment of pesticide residues in fruits and vegetables from farms and markets of Western Indian Himalayan region. *Chemosphere, 224*, 162–167. https://doi.org/10.1016/j.chemosphere.2019.02.091

Lehotay, S. J. (2007). Determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate: Collaborative study. *Journal of AOAC International, 90*, 485–520.

Loughlin, T. M. M., Peluso, L., Etchegoyen, A., Alonso, L. L., de Castro, C., Percudani, C., & Marino, D. J. G. (2018). Pesticide residues in fruits and vegetables of the argentine domestic market: Occurrence and quality. *Food Control, 93*, 129–138. https://doi.org/10.1016/j.foodcont.2018.05.041

Pérez-Mayán, L., Ramil, M., Cela, R., & Rodríguez, I. (2021). Determination of pesticide residues in wine by solid-phase extraction on-line combined with liquid chromatography tandem mass spectrometry. *Journal of Food Composition and Analysis, 104*, Article 104184. https://doi.org/10.1016/j.jfca.2021.104184

Philippe, V., Neveen, A., Marwa, A., & Basel, A.-Y. A. (2021). Occurrence of pesticide residues in fruits and vegetables for the eastern Mediterranean region and potential impact on public health. *Food Control, 119*, Article 107457. https://doi.org/10.1016/j.foodcont.2020.107457

Pinheiro, D.F., Maciel, G.M., Lima, N.P., Liviz, C.A.M., Lima, N.F., Haminiuk, C.W.I. (2025). Assessment of pesticides concentration in south American wines by liquid chromatography-tandem mass spectrometry. Journal of Food Composition and Analysis, 140, 107225. doi:https://doi.org/10.1016/j.jfca.2025.107225.

Qin, G., Chen, Y., He, F., Yang, B., Zou, K., Shen, N., Zuo, B., Liu, R., Zhang, W., & Li, Y. (2021). Risk assessment of fungicide pesticide residues in vegetables and fruits in the mid-western region of China. *Journal of Food Composition and Analysis, 95*, Article 103663. https://doi.org/10.1016/j.jfca.2020.103663

Santana-Mayor, A., Rodríguez-Ramos, R., Herrera-Herrera, A. V., Conde-González, J. E., & Socas-Rodríguez, B. (2023). *Food Control, 153*, Article 109957. https://doi.org/10.1016/j.foodcont.2023.109957

SANTE. (2020). *Guidance document on pesticide analytical methods for risk assessment and post-approval control and monitoring purposes*. . Sante 12830/2020. DG SANTE, European Comission

SANTE. (2021). *Analytical quality control and method validation procedures for pesticide residues analysis in food and feed.* . Sante 11312/2021. DG SANTE, European Comission

Shamsipur, M., Yazdanfar, N., & Ghambarian, M. (2016). Combination of solid-phase extraction with dispersive liquid–liquid microextraction followed by GC–MS for determination of pesticide residues from water, milk, honey and fruit juice. *Food Chemistry, 204*, 289–297. https://doi.org/10.1016/j.foodchem.2016.02.090

Skubic, M., Baša Česnik, H., Velikonja Bolta, Š., Rusjan, D., & Šircelj, H. (2025). Is common dandelion (Taraxacum officinale agg.) foraged for food in vineyards pesticide residues free? *Foods, 14*, 1–17. https://doi.org/10.3390/foods14040684

Sykalia, D. L., Trantopoulos, E. P., Tsoutsi, C. S., & Albanis, T. A. (2024). Optimization and validation of analytical methodology for determination of pesticides in grape, must and wine samples with QuEChERS extraction and gas chromatography–mass spectrometry. *Beverages, 10*, Article 53. https://doi.org/10.3390/beverages10030053

Szpyrka, E., Kurdziel, A., Matyaszek, A., Podbielska, M., Rupar, J., & Słowik-Borowiec, M. (2015). Evaluation of pesticide residues in fruits and vegetables from the region of South-Eastern Poland. *Food Control, 48*, 137–142. https://doi.org/10.1016/j.foodcont.2014.05.039

Torović, L., Vuković, G., & Dimitrov, N. (2021). Pesticide residues in fruit juice in Serbia: Occurrence and health risk estimates. *Journal of Food Composition and Analysis, 99*, Article 103889. https://doi.org/10.1016/j.jfca.2021.103889

Wołejko, E., Łozowicka, B., & Kaczyński, P. (2014). Pesticide residues in berries fruits and juices and the potential risk for consumers. *Desalination and Water Treatment, 52*, 3804–3818. https://doi.org/10.1080/19443994.2014.883793

Zhang, Q., Zhao, J., Xie, R., Xiao, W., Mao, X., Yuan, C., Wang, Y., & Wan, Y. (2023). A simple and efficient method for determining the pyrethroid pesticide residues in freshly squeezed fruit juices using a water stable metal–organic framework. *Microchemical Journal, 187*, Article 108392. https://doi.org/10.1016/j.microc.2023.108392

Zhao, H., Li, R., & Hu, J. (2023). Frequently used pesticides and their metabolites residues in apple and apple juice from markets across China: Occurrence and health risk assessment. *LWT, 178*, Article 114610. https://doi.org/10.1016/j.lwt.2023.114610

Zincke, F., Kittelmann, A., Scholz, R., Michalski, B., & Tietz, E. (2024). *European database of processing factors for pesticides residues in food (version 4)*. Zenodo. https://doi.org/10.5281/zenodo.12685883