Artigo

PESTICIDES IN PROCESSED FOOD - MULTIRESIDUE PESTICIDES IN NATURAL GRAPE JUICES BY HIGH-RESOLUTION MASS SPECTROMETRY

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Grapes are among the most widely cultivated fruits in the world, with the majority of Brazilian production focusing on natural grape juice. In this work, a total of 40 samples of 2 types of natural grape juice were analyzed for the presence of 86 pesticide residues, with multiresidue extraction based on modified QuEChERS, by liquid chromatography with high-resolution mass spectrometry (LC-HRMS). Limits of detection and quantification were below 6 µg L^{-1} and 20 µg L^{-1}, RSD < 20%, R^2 > 0.99 and recovery ranged from 80 and 120%. Thus, the developed method was validated and good performance characteristics such as linearity, trueness, repeatability and inter-day precision were obtained. As results, 7 pesticides were detected in real samples (azoxystrobin, benalaxyl, carbendazim, tebuconazol, thiophanate-methyl, pyriproxiphen and pyrimethanil). The method was successfully validated for simultaneous evaluation of 86 multiclass pesticides residues levels, which was applied in 40 natural white and red grape juice market samples. The limits of quantification (LOQs) were low enough and the method was tested on samples showing its applicability, and therefore, this method can be used in routine analysis. Also, this work therefore also serves as an alert regarding the contamination of processed products.

Keywords: processed food; pesticide residue; validation; natural grape juice; LC-HRMS.

INTRODUCTION

Grape is the 5th most consumed fruit in the world, behind banana, watermelon, apple and orange. In Brazil their consumption and processing have increased by 134.3% and 236.9%, respectively, since 2016.[1] Natural grape juices possess the highest nutritional value of all processed grape products, acting as a mineral source.[2] As such juices are produced without fermentation or even sometimes dilution, the monitoring of levels of pesticide residue in these products is essential.[3,4]

For natural food, government agencies establish maximum residue limits (MRLs) that aim to ensure the safety of consumers;[5,6] however, for processed products MRL established for fruits are usually used. For grapes, these values are in the range of 0.5 to 6.0 mg kg^{-1}. In Brazil, these limits are set mainly by the National Health Surveillance Agency[7] and the Ministry of Agriculture, Livestock and Food Supply.[8]

Despite the wide use of pesticides in viticulture, the environmental concentrations can be very low, depending on the type of pesticide used, the degradation process and climatic conditions.[9] Accurate determination of pesticide residue levels thus requires the use of sensible analytical methods, most such procedures involve the use of extraction techniques, followed by purification, clean-up and chromatographic analysis. According to the literature, traditional methods of extraction, such as liquid-liquid, are giving way to solid-liquid extractions due to their simplicity and robustness.[10-13]

In relation to extraction methods, the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe), stands out as being quick and easy to prepare, essentially involving extraction, purification and clean-up.[14] The versatility and ease of adaptation of this methodology in relation to type of array has also contributed to its widespread use. In addition, preparation involving QuEChERS is often followed by chromatographic analysis. Another important step in the development of new methodologies is validation to ensure that the method is able to offer sensible results for the analysis in question.[15-16] This step focuses on determining parameters such as selectivity, linearity and limits of detection, as well as quantification, precision and accuracy.[17]

Chromatography coupled with mass spectrometry has proven to be one of the most important techniques employed for pesticide analysis in a wide range of matrices.[18-23] Liquid chromatography, however, is considered more comprehensive regarding the analysis of both matrices and analytes, and has been widely used coupled to mass spectrometry.[24-28] Indeed, chromatographic separation systems coupled to accurate high-resolution mass spectrometry (HRMS) are increasingly being incorporated into the routine work. Nevertheless, few studies have examined the performance of different analytical methods using HRMS for the determination of pesticides in natural grape juice. For instance, Munaretto *et al.*,[29] detected pesticide residues in apple, pear and grape juices using LC/Q-TOF.

In order to guarantee the quality of natural grape juices available in Brazil, the present work aimed to optimise and validate a procedure for the quantitative determination of 86 pesticide residues. This study was also designed to contribute to the confirmation of pesticide residue by LC-HRMS, a technique that has not yet been carefully studied regarding the determination of a wide range of pesticides. Selectivity and accuracy for this matrix were assured by method reproducibility confirmed based on the determination of validation parameters, using of HRMS in ESI ionization mode.

MATERIALS AND METHODS

Chemicals and reagents

Pesticide analytical standards of high purity (> 98%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany) and Sigma-Aldrich (Steinheim, Germany). Individual pesticide stock solutions of all compounds (1000 mg L^{-1}) were prepared in acetonitrile or methanol and stored at -5 ºC in the amber bottles. Acetic acid, formic acid and ammonium formiate were purchased from Sigma-Aldrich (Steinheim, Germany). HPLC-grade acetonitrile and methanol

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solvents were supplied by Merck (Darmstadt, Germany). Ultra-pure water obtained from Milli-Q-Plus® system (Millipore Reference Model, USA) was used throughout the study.

Sample preparation

Two different types of natural grape juice samples (pesticide-free - organic accreditation) were used for method development and validation, both produced in the southern brazilian state of Rio Grande do Sul. After validating the method, forty samples of varying brands and types of white and red natural grape juice, produced in the Rio Grande do Sul were analyzed, were acquired from supermarkets over a period of six months. All samples were stored at -5 ºC, in their original packaging under the recommended conditions until analysis.

Extraction procedure

Extraction was carried out based on Anastassiades *et al.*[14] with QuEChERS modified. A representative 10 mL aliquot of sample (previously homogenised) with 10 mL of acetonitrile with 0.1% acetic acid was placed in a disposable screw-capped polypropylene tube and shaken for 1 min manually. Thereafter, 4.0 g of magnesium sulfate and 1.7 g of sodium acetate were added, shaken 1 min and centrifuged at 4000 rpm for 8 min. 1 mL of the supernatant was removed and placed in a 15 mL falcon-type tube, added 100 mg of magnesium sulfate and 20 mg of primary secondary amine (PSA) and again centrifuged at 4000 rpm for 5 min. After the extraction process, a supernatant aliquot of approximately 1000 µL was filtered and diluted (50:50) in methanol for injection into the LC-HRMS system.

LC-HRMS analysis

Liquid chromatography tandem mass spectrometry of high resolution, in positive ESI(+) and negative ESI(–) ion mode was used to separate, identify and quantify the target compounds. LC separation was conducted using a Shimadzu (Kyoto, Japan) instrument equipped with LC 20AD and pumped with auto sampler SIL-20AXR, SPD-20 detector, form CTO-20. The analytical column employed was a RP-18 of 150 mm × 4.6 mm and 5 µm particle size (Agilent Technologies).

A binary gradient was used, started at 75% of the mobile phase A, constant for 2 min, followed by a linear increase of 5% A over 7.5 min, constant for 3.5 min and ending with 75% A constant for 7 min. This total 20 min run time. The mobile phase A contained pure water with 5 mmol L^{-1} ammonium formiate and 0.1% formic acid, and phase B contained pure water with 5 mmol L^{-1} ammonium formiate and methanol with 0.1% formic acid (1:9). The flow rate was set constant at 0.5 mL min^{-1} during the whole process and the injection volume was of 5 µL. For the mass spectrometric analysis, a quadrupole-time of flight system Bruker, Q-TOFII® (Billerica, USA) was used, set with the following parameters: electrospray ionization (ESI) voltage: 4500 eV, nebulisation gas flow: 3 L min^{-1}, drying gas flow: 15 L min^{-1}, interface temperature: 250 ºC. Nitrogen was used as the nebuliser and collision gas.

Validation parameters

Method accuracy and precision were evaluated by recovery studies using blank matrices of the studied two types grape juice samples spiked at three concentration levels, 10 µg L^{-1}, 100 µg L^{-1} and 200 µg L^{-1}. All experiments were tested with six replicates for each matrix, in accordance with SANTE/11813/2017[16] and INMETRO (National Institute of Metrology, Quality and Technology) DOQ-CGCRE-008.[15] Compounds quantitation in the spiked samples were carried out by comparing of the samples with those of matrix matched standard solutions (calibration curves). These, as well as the matrix-matched calibration curves, were prepared by spiking an aliquot of the blank extract with the desired amount of standard solution. LODs and LOQs were evaluated based on the analysis of low concentration standards, in methanol. LODs were established as the minimum concentration at which the signal was detected by the software (Compass DataAnalysis 4.3.110) for the characteristic ion ($[M + H]^+$ or $[M - H]^-$) with a mass error lower than 5 ppm.[16] LOQs were established as the lowest concentration tested providing acceptable recovery (between 70 and 110%) and precision (RSD lower than 20%).

Linearity was evaluated both in solvent and matrix, using matrix-matched calibration curves prepared in methanol in the concentration range of 0.5-200.0 µg L^{-1} (n = 7). The matrix effect was studied by comparing the slopes of the calibration curves in solvent and matrix. The repeatability of the instrumental method was estimated by determining the inter-day and intra-day relative standard deviation (RSD < 20%) by the repeated analysis of a spiked matrix extract at 10, 100 and 200 µg L^{-1} level, from run-to-run over one day and three days, respectively.

RESULTS AND DISCUSSION

Optimization of LC-HRMS methodology

For the analysis of target pesticides by HRMS, acquisition method was developed with monitoring exact mass. For that individual solutions of each analyte (0.5 mg L^{-1}) were injected by an external syringe pump using electrospray ionization in negative and positive mode.

Then, the chromatographic conditions were tested and first, two stationary phases were checked: C8 and C18. For the optimization of this stage, a mixed solution containing 100 µg L^{-1} of each compound was injected, using as mobile phase methanol and water as a gradient. Best results were obtained when C18 was used, as observed in the resolution and intensity of the peaks. After that, the composition of the mobile phase was optimized. A mobile phase containing 0.1% of formic acid in the aqueous solution and organic phase (methanol) was tested and it was observed that the peak shape improved. Then, as organic phase, methanol and acetonitrile were tested, being that the methanol showed better peak resolution. After that, several ionizers were added to the aqueous solution as acetic acid (0.1, 0.5 and 1%, v/v), or ammonium formate (5 mmol L^{-1}), using methanol as organic solvent. Suitable elution of the target compounds were achieved using water with 5 mmol L^{-1} ammonium formiate and 0.1% formic acid and methanol with 0.1% formic acid as mobile phase.

Optimization of sample extraction

The employed QuEChERS process has been successful in extracting multi residues from various different samples, including juices, in a number of previous studies.[14,24,30,31]

Extraction based on Anastassiades *et al.*[14] with a modified QuEChERS method was satisfactory for the pesticides residues and grape juice samples. The clean-up step, after the extraction, was important to later detection, since the compounds analyzed have different physicochemical characteristics.[32] For this purpose, a lower reagent concentration was used, resulting in a lower cost and reduced environmental impact. The disposal of all waste was carried out according to current environmental standards.

LC-HRMS parameters

A total of 86 pesticides were evaluated, with a recovery rate of between 80 and 120%, obtained from the natural grape juice samples. These results thus optimised the potential for ion input and collision energies for the verification of both positive ESI(+) as $[M + H]^+$ or in negative ESI(–) as $[M – H]^-$ ionization to obtain *m/z* values, as well as for the adducts $[M + Na]^+$, $[M + NH_4]^+$. The exact mass was used for compounds confirmations, with values of error below 5 ppm as expected for ESI-QTOF, as well as the isotope ratio, data shows in support information.[16] Figure 1 shows extracted ion chromatogram (EIC) corresponding to all transitions obtained at a concentration of 100 µg L^{-1} in grape juice extract.

Matrix effect

Method validation also included an evaluated of the matrix effect, as signal suppression or enhancement as a result of this matrix effect can severely compromise not only the quantitative analysis of compounds, but also method accuracy and reproducibility.[33]

Matrix effects depends on various factors, such as the nature of the pesticide, the type of matrix and the pesticide to matrix ratio, in addition to matrix components that can inhibit or enhance the analyte signal.[34] In the present work, two grape juices were selected for the evaluation of matrix effects, analysing standards of different concentrations both in pure solvents and in the selected matrices, and comparing the slopes of the calibration curves. Values ranging from -20% to 20% are in signal decrease/increase, from -50% to 20% and 20% to 50% an average effect, and values above 50% or below 50% a strong signal matrix effect.[35] Figure 2 shows the percentage of signal suppression or enhancement for the two juice types evaluated by LC-HRMS. It can be observed that only the pesticide 2.4-D, cymoxanil, dazomet, dimethoate, fenitrothion, parathion-methyl and thiabendazole pesticides had an average effect in relation to the matrix effect for red grape juice, and only dazomet showed an average effect when the matrix was white grape juice. White grape juice thus presented the lowest matrix effect, as 99% of compounds were unaffected. This results validated the dilution of the samples and the use of high sensitivity equipment. Thus, no significant difference between the different matrices was observed, suggesting that the use of matrix-matched calibration provides sensible quantitation for fruit juice pesticide analysis.

Validation

The parameters were evaluated in accordance with SANTE/11813/2017[16] and INMETRO[15] procedures for pesticide residue analysis in food. Performance characteristics of the optimised method were established by a validation procedure with spiked blank grape juice (as representative matrix), studying linearity, selectivity, accuracy (expressed as recovery), interday precision, LODs and LOQs. The linearity of the analytical response for all the compounds within the studied range was satisfactory, with correlation coefficients higher than 0.99 in all cases, as shown in Table 1.

Results of precision and accuracy indicate that good recoveries from juice samples were obtained throughout the proposed method. Mean recovery data and relative standard deviations (RSDs) were obtained by the precision/accuracy of the extraction method, as shown in Table 2. With intra-day RSDs ranged between 0.4% and 19.0%, and inter-day RSDs from 0.8% to 19.7%. These values demonstrate

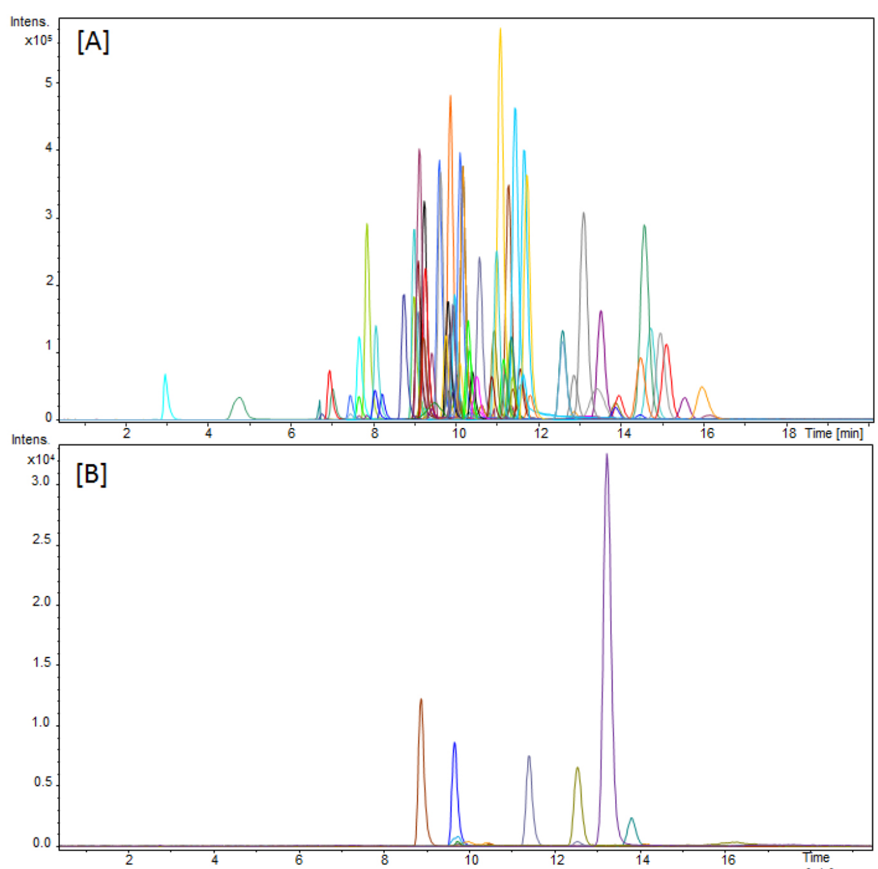

Figure 1. Extracted ion chromatogram corresponding to the analysis of a natural grape juice extract spiked at 100 µg L^{-1} in positive (A) and negative (B) ionization modes

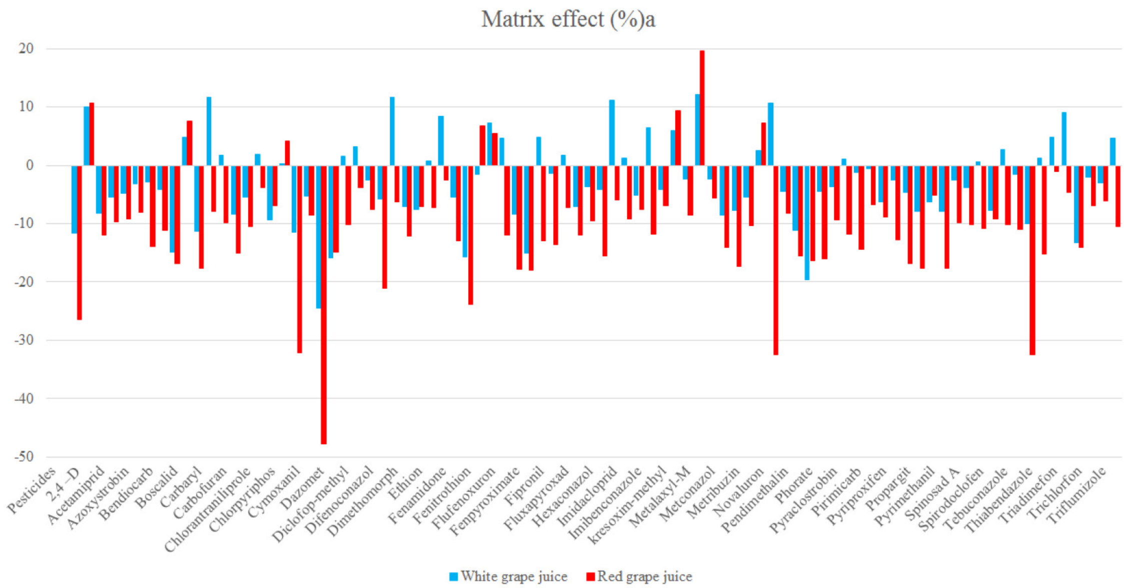

Figure 2. *Matrix effect for two different juice matrices and the 86 pesticides analyzed ([a]expressed as the percentage difference between the slopes of the corresponding calibration curves (solvent and matrix). Positive values indicate signal enhancement and negative values signal suppression)*

Table 1. Pesticide parameters

Pesticides	RT (min)	Ionization mode	Mass (*m/z*)	R^2	LOD (µg L^{-1})	LOQ (µg L^{-1})
2,4-D	9.7	–	218.9610	0.9991	5	10.0
Abamectin	9.7	+	890.5260	0.9993	2	5.0
Acetamiprid	7.8	+	223.0745	0.9995	1	5.0
Ametryn	9.9	+	228.1277	0.9996	5	10.0
Azoxystrobin	12.5	+	404.1240	0.9998	2	5.0
Benalaxyl	11.3	+	326.1750	0.9997	3	5.0
Bendiocarb	9.1	+	224.0917	0.9994	3	10.0
Bioallethrin	13.9	+	303.1954	0.9998	5	10.0
Boscalid	9.9	+	343.0399	0.9992	3	5.0
Bromoxynil	9.6	–	273.8497	0.9992	2	5.0
Carbaryl	9.2	+	202.0862	0.9987	5	10.0
Carbendazim	8.3	+	192.0767	0.9994	3	5.0
Carbofuran	9.1	+	222.1124	0.9995	3	5.0
Carboxine	9.2	+	236.0739	0.9987	2	5.0
Chlorantraniliprole	10.7	+	483.9757	0.9998	2	5.0
Cyproconazol	10.6	+	292.1211	0.9982	3	5.0
Chlorpyriphos	14.0	+	349.9335	0.9995	1	5.0
Clothianidin	7.7	+	250.0159	0.9996	1	5.0
Cymoxanil	8.2	+	221.0645	0.9992	2	5.0
Cyprodinil	11.1	+	226.1338	0.9996	5	10.0
Dazomet	11.1	+	163.0358	0.9998	2	5.0
Diazinon	11.4	+	305.1083	0.9994	4	10.0
Diclofop-methyl	12.8	+	358.0607	0.9988	3	5.0
Dichlorvos	9.1	+	220.9531	0.9971	3	5.0
Difenoconazol	11.6	+	406.0719	0.9996	2	5.0
Dimethoate	8.1	+	230.0068	0.9997	4	10.0
Dimethomorph	9.9	+	388.1310	0.9997	3	5.0
Diuron	9.9	+	233.0242	0.9990	5	10.0
Ethion	13.5	+	384.9948	0.9985	2	5.0
Etoxazole	14.6	+	330.1769	0.9992	5	10.0
Fenamidone	9.9	+	312.1165	0.9995	1	5.0
Fenarimol	10.2	+	331.0399	0.9993	2	5.0
Fenitrothion	10.4	+	278.0246	0.9978	3	5.0

Table 1. Pesticide parameters (cont.)

Pesticides	RT (min)	Ionization mode	Mass (*m/z*)	R^2	LOD (μg L^{-1})	LOQ (μg L^{-1})
Fluazinam	13.2	–	462.9430	0.9990	2	5.0
Flufenoxuron	13.8	–	489.0435	0.9996	1	5.0
Fluquinconazole	11.4	+	376.0162	0.9959	1	5.0
Fenpyroximate	15.8	+	422.2074	0.9995	6	10.0
Fenthion	11.2	+	279.0220	0.9984	3	10.0
Fipronil	10.6	+	453.9725	0.9995	1	5.0
Fluopicolide	10.1	+	382.9727	0.9996	1	5.0
Fluxapyroxad	10.0	+	382.0973	0.9994	1	5.0
Flutriafol	9.4	+	302.1099	0.9997	5	10.0
Hexaconazol	11.8	+	314.0821	0.9992	2	5.0
Imazalil	9.0	+	297.0555	0.9997	4	10.0
Imidacloprid	7.4	+	256.0595	0.9996	1	5.0
Iprovalicarb	10.6	+	321.2172	0.9994	1	5.0
Imibenconazole	13.7	+	412.9970	0.9972	2	5.0
Indoxacarb	11.4	+	528.0779	0.9993	1	5.0
Kresoxim-methyl	10.9	+	314.1386	0.9990	5	10.0
Lufenuron	12.5	–	508.9700	0.9991	5	10.0
Metalaxyl-M	9.6	+	280.1509	0.9994	5	10.0
Methamidophos	4.8	+	142.0086	0.9995	6	10.0
Metconazol	11.6	+	320.1524	0.9992	5	10.0
Methidathion	9.8	+	302.9691	0.9992	5	10.0
Metribuzin	9.3	+	215.0961	0.9992	5	10.0
Myclobutanil	10.3	+	289.1214	0.9991	5	10.0
Novaluron	11.4	–	491.0039	0.9994	6	10.0
Parathion methyl	10.0	+	264.0090	0.9973	5	10.0
Pendimethalin	14.5	+	282.1448	0.9973	4	10.0
Phosmet	9.7	+	318.0018	0.9993	5	10.0
Phorate	10.0	+	261.0201	0.9995	6	20.0
Phoxim	11.3	+	299.0613	0.9990	5	10.0
Pyraclostrobin	11.0	+	388.1058	0.9997	5	10.0
Pyrazophos	11.4	+	374.0934	0.9996	3	10.0
Pirimicarb	9.1	+	239.1502	0.9997	3	5.0
Pirimiphos-methyl	11.6	+	306.1035	0.9996	6	10.0
Pyriproxifen	13.1	+	322.1437	0.9991	2	5.0
Prochloraz	11.4	+	376.0380	0.9996	6	10.0
Propargit	14.5	+	368.1890	0.9989	3	5.0
Pyridabem	9.5	+	364.1468	0.9979	2	5.0
Pyrimethanil	10.1	+	200.1182	0.9996	3	5.0
Simazin	9.2	+	202.0854	0.9987	5	10.0
Spinosad A	13.7	+	732.4700	0.9996	6	10.0
Spinosad D	10.1	+	746.4800	0.9984	6	10.0
Spirodoclofen	14.9	+	411.1124	0.9990	2	5.0
Spiromesifen	14.9	+	371.2216	0.9989	1	5.0
Tebuconazole	11.2	+	308.1524	0.9994	1	5.0
Tetraconazole	10.4	+	372.0286	0.9981	2	5.0
Thiabendazole	7.7	+	202.0433	0.9997	3	5.0
Thiophanate-methyl	11.5	+	343.0529	0.9995	2	5.0
Triadimefon	10.3	+	294.1003	0.9997	1	5.0
Triadimenol	10.5	+	296.1160	0.9982	3	5.0
Trichlorfon	8.0	+	256.9298	0.9983	5	10.0
Trifloxystrobin	11.7	+	409.1369	0.9996	2	5.0
Triflumizole	11.8	+	346.0928	0.9997	5	10.0
Zoxamide	16.9	+	336.0319	0.9965	5	10.0

(RT): retention time. (R^2): coefficient of determination. (*m/z*): mass:charge ratio. (LOD): detection limit. (LOQ): limit of quantification.

Table 2. Recovery and analysis of 86 pesticides, with parameters (recovery and standard deviation) observed at three different concentrations of 10, 100 and 200 μg L^{-1}

Pesticides	Concentration (μg L^{-1})								
	10			100			200		
	Rec. (%)[a]	RSD (%)[a]	RSD_i (%)[a]	Rec. (%)[a]	RSD (%)[a]	RSD_i (%)[a]	Rec (%)[a]	RSD (%)[a]	RSD_i (%)[a]
2,4-D	83.7	19.0	10.5	101.9	6.5	5.7	87.7	8.2	12.2
Abamectin	103.0	5.1	7.2	98.6	2.2	3.0	93.5	5.0	4.6
Acetamiprid	92.1	1.2	15.5	84.7	1.1	5.6	80.5	2.0	4.9
Ametryn	98.9	4.4	5.1	97.9	1.6	1.7	94.1	3.5	5.0
Azoxystrobin	103.3	1.0	3.6	97.0	1.3	3.1	92.8	3.1	3.7
Benalaxyl	101.0	3.1	4.2	97.6	1.2	3.0	93.5	4.0	4.3
Bendiocarb	100.2	3.0	4.7	98.7	4.2	3.3	92.7	3.3	2.5
Bioallethrin	110.7	2.5	9.4	97.7	1.7	4.8	93.2	3.6	5.3
Boscalid	99.7	5.8	19.7	99.5	3.5	3.9	92.3	3.3	4.1
Bromoxynil	117.2	1.3	10.0	115.7	3.5	9.2	110.3	3.7	8.8
Carbaryl	100.0	6.7	11.5	97.8	1.2	2.2	91.0	2.1	3.8
Carbendazim	107.9	10.8	10.9	94.3	0.8	2.9	90.7	3.8	5.6
Carbofuran	101.0	7.0	12.6	97.3	0.4	2.0	91.5	2.5	2.0
Carboxine	96.0	2.7	6.4	100.5	1.2	2.3	93.7	2.3	3.6
Chlorantraniliprole	106.6	8.7	12.5	101.9	2.4	2.3	96.3	3.8	4.8
Cyproconazol	96.2	12.2	11.3	104.5	3.3	3.3	95.6	5.0	3.8
Chlorpyriphos	106.3	2.4	18.9	100.0	5.1	7.5	92.7	4.3	13.1
Clothianidin	85.3	12.4	11.5	80.1	4.8	5.1	81.6	4.5	4.1
Cymoxanil	84.0	3.2	3.7	82.0	3.6	12.2	87.2	3.0	9.3
Cyprodinil	100.6	3.0	4.2	98.6	2.0	2.9	93.6	3.1	2.9
Dazomet	89.3	5.5	13.3	83.0	6.3	7.8	86.8	4.1	13.0
Diazinon	92.1	3.2	6.3	96.7	1.2	4.1	92.7	3.4	16.4
Diclofop-methyl	89.8	11.6	12.0	96.2	1.1	4.8	93.0	3.1	8.0
Dichlorvos	100.9	10.1	12.1	103.0	6.3	5.7	97.7	5.1	4.7
Difenoconazol	102.4	10.6	17.2	98.8	2.9	3.6	92.9	3.8	5.6
Dimethoate	92.9	0.9	5.4	86.3	0.6	4.4	82.7	1.5	2.7
Dimethomorph	91.3	7.7	16.0	105.0	3.4	2.8	101.8	4.7	5.7
Diuron	106.3	2.6	5.4	97.3	2.1	5.5	92.6	3.9	4.0
Ethion	102.4	0.6	10.9	97.7	1.5	3.1	94.1	3.6	8.1
Etoxazole	103.7	2.0	12.8	98.1	1.1	6.8	93.9	3.8	15.1
Fenamidone	95.0	2.8	19.0	105.0	1.4	6.8	98.0	3.7	8.7
Fenarimol	107.7	0.8	8.4	100.3	1.0	4.1	94.4	2.5	2.4
Fenitrothion	108.3	2.4	11.5	97.7	4.9	6.7	86.1	3.8	7.1
Fluazinam	116.1	0.5	5.4	109.4	2.6	4.8	103.7	2.8	3.2
Flufenoxuron	88.8	17.1	18.3	110.5	2.1	9.8	118.2	11.1	14.6
Fluquinconazole	106.5	4.2	13.2	108.4	2.7	10.6	90.0	4.0	9.3
Fenpyroximate	99.8	2.3	19.2	99.9	1.2	14.2	93.5	3.6	18.4
Fenthion	99.6	6.7	10.1	93.7	1.1	4.2	88.2	3.0	8.6
Fipronil	88.1	14.9	18.6	104.6	7.2	4.3	97.3	3.3	3.8
Fluopicolide	106.7	1.1	9.8	101.3	0.4	1.6	95.3	2.9	3.1
Fluxapyroxad	97.7	1.6	2.9	101.3	2.0	2.0	94.8	3.3	2.6
Flutriafol	105.4	7.1	18.5	99.2	1.8	2.7	92.4	3.3	3.9
Hexaconazol	97.5	2.7	11.1	100.6	0.6	3.1	95.5	3.2	3.8
Imazalil	93.6	4.9	5.8	94.8	0.7	1.5	91.5	2.4	4.1
Imidacloprid	84.8	3.7	4.6	92.2	2.2	3.6	94.9	3.6	3.8
Iprovalicarb	98.8	11.0	11.1	99.0	1.7	1.7	93.4	2.6	2.8
Imibenconazole	82.8	13.7	16.6	92.9	3.0	3.3	94.2	6.4	6.6
Indoxacarb	93.4	14.9	15.9	107.1	5.6	5.2	98.6	2.6	2.6
Kresoxim-methyl	100.5	9.1	9.2	102.8	1.9	1.9	94.2	3.4	3.6
Lufenuron	108.6	17.3	15.4	119.8	2.9	10.2	118.6	1.8	12.2
Metalaxyl-M	99.8	3.3	3.4	98.9	4.4	4.5	92.0	3.4	3.7
Methamidophos	90.6	9.7	10.7	100.8	2.7	2.7	94.3	4.4	4.7
Metconazol	100.1	1.8	3.3	93.0	2.2	2.4	96.0	9.7	10.7
Methidathion	99.7	4.0	4.4	103.3	3.2	3.1	94.7	2.1	2.2
Metribuzin	93.1	14.0	15.5	93.5	1.1	1.9	88.0	2.3	3.1
Myclobutanil	106.6	8.5	8.6	106.8	1.8	3.5	98.5	2.2	2.9

Table 2. Recovery and analysis of 86 pesticides, with parameters (recovery and standard deviation) observed at three different concentrations of 10, 100 and 200 µg L^{-1} (cont.)

Pesticides	Concentration (µg L^{-1})								
	10			100			200		
	Rec. (%)[a]	RSD (%)[a]	RSD$_i$ (%)[a]	Rec. (%)[a]	RSD (%)[a]	RSD$_i$ (%)[a]	Rec (%)[a]	RSD (%)[a]	RSD$_i$ (%)[a]
Novaluron	100.5	11.8	11.7	111.1	1.4	9.4	97.0	1.9	11.3
Parathion methyl	90.9	3.4	3.5	94.3	5.2	7.7	83.3	4.5	5.4
Pendimethalin	96.4	17.7	18.3	98.0	8.5	8.7	85.6	5.2	6.1
Phosmet	116.8	3.0	3.5	104.3	2.0	2.9	95.2	2.0	2.9
Phorate	99.6	4.6	4.7	98.2	1.5	1.5	93.8	2.8	3.1
Phoxim	102.0	12.4	14.2	98.5	2.7	2.7	91.3	4.6	5.1
Pyraclostrobin	94.2	3.6	3.8	93.6	1.6	1.8	89.3	4.4	4.9
Pyrazophos	101.5	6.1	6.5	99.3	3.4	3.5	94.2	3.3	3.5
Pirimicarb	106.5	4.2	13.2	83.3	7.9	13.1	81.5	0.9	5.5
Pirimiphos-methyl	105.4	7.1	9.8	101.5	0.9	2.3	92.4	3.9	5.3
Pyriproxifen	87.7	7.5	8.5	94.3	5.0	5.3	87.2	10.2	11.7
Prochloraz	96.3	7.2	7.5	100.8	3.8	3.9	95.3	2.8	3.0
Propargit	87.5	10.9	12.5	97.6	3.2	3.3	91.4	6.6	7.2
Pyridabem	102.0	2.8	3.3	111.1	9.6	9.8	99.6	9.3	9.8
Pyrimethanil	99.9	5.6	5.6	100.8	1.7	1.7	95.8	2.1	2.2
Simazin	95.1	6.6	6.9	96.9	2.6	2.7	90.9	3.2	3.6
Spinosad A	107.5	6.7	7.2	100.1	3.3	3.3	94.2	4.5	4.7
Spinosad D	111.9	5.1	6.7	102.1	0.8	0.8	92.5	2.7	2.9
Spirodoclofen	97.5	4.7	4.8	95.1	5.1	5.3	87.0	4.6	5.2
Spiromesifen	90.6	11.1	12.1	97.2	2.8	2.9	90.9	5.6	6.2
Tebuconazole	97.0	4.4	4.5	99.2	2.3	2.3	93.7	3.8	4.1
Tetraconazole	103.8	6.1	6.4	110.1	2.2	2.4	100.8	3.3	3.3
Thiabendazole	89.0	10.0	12.7	89.8	2.0	2.5	85.8	3.7	4.8
Thiophanate-methyl	107.2	6.4	6.8	100.7	3.5	3.5	92.2	3.1	3.4
Triadimefon	102.4	8.2	8.4	96.0	9.7	10.1	94.2	7.5	8.0
Triadimenol	81.9	11.6	14.1	88.5	9.9	11.2	83.8	16.4	19.6
Trichlorfon	86.6	11.2	14.6	84.6	4.6	6.2	91.1	4.0	5.6
Trifloxystrobin	94.5	7.1	7.5	97.9	3.1	3.2	91.8	5.4	5.8
Triflumizole	97.9	5.8	6.0	99.3	0.9	0.9	91.8	7.4	8.1
Zoxamide	97.0	6.8	7.1	100.6	2.6	2.6	91.9	4.0	4.3

[a]Mean recovery and relative standard deviation from analysis of spiked samples (n = 6). (Rec.): recuperation. (RSD): relative standard deviation (intra-day). (RSD$_i$): relative standard deviation (inter-day).

the repeatability of the method and therefore its effectiveness for quantitative purposes.

Analysis of the obtained limits data shown in Table 1 reveals that good results were achieved, with LOD and LOQ values lower than 6 and 20 µg L^{-1}, respectively.

Survey of the studied pesticides in natural grape juice samples

The validated analytical methodology was then applied to analyse forty samples of natural grape juices obtained from local supermarkets in Caxias do Sul, southern Brazil, over a period of six months. Internal quality control was applied to every batch of samples, including matrix-matched calibration, a reagent blank, matrix blank and a spiked blank sample in order to evaluate the stability of the proposed method. In order to prove the effectiveness of the validated method and its suitability for routine analysis, it was applied to real samples. The results of grape juice analysis are displayed in Table 3.

Pesticides residues that were found in the white and red grape juice are described in Table 3. In Brazil, the limit of the compounds found varies between 50 and 6000 µg L^{-1} in samples grape analyzed.[7] The frequency of the residues' presence depends on origin where the product is harvested, since some farmers use same pesticides in different crops, regardless of whether they are allowed to or not. It should be emphasised that although pesticides were detected in the analyzed juice samples (Table 3), levels were lower than the respective MRLs established by the EU, CODEX and ANVISA for unprocessed fruit. As an example, Figure 3 shows a positive result for carbendazim in a red grape juice.

In this study, carbendazim was found always in higher proportions in juices analyzed compared to other compounds, followed by azoxystrobin, the two commonly used fungicides in the region. Tran *et al.*[36] similarly detected pesticide residues in juice samples, with carbendazim found in açaí juice, azoxystrobin and pyrimethanil found in mixed juice.

Although a number of researchers have analyzed pesticide residues in various fruits and fruit juices, studies investigating grape juice remain scarce. Mebdoua *et al.*[37] examined the presence of pesticides in vegetables and fruits, including grapes, obtaining positive results for 68.4% of samples. In the latter study, the pesticide benalaxyl was found in peach, apple, nectarine and plum samples, although metalaxyl was most commonly found in the tested grape samples. Elsewhere, Ferrer *et al.*[38] analyzed 53 pesticides in 13 types of fruit juices. The pesticide detected in the highest number of samples by these authors was carbendazim, a fungicide used widely in crop protection. Carbendazim was also detected most frequently by Radišic *et al.*,[39] who found it is almost 80% of investigated juice samples in Belgrade and Servia, and by

Table 3. Analytical results for real samples and maximum residue levels (MRL) assigned by different organizations

Pesticide	Concentration of pesticede residue ± sd (µg L^{-1})				MRL (µg L^{-1})		
	White juice	Positive findings	Red juice	Positive findings	ANVISA[7]/MAPA[8]	CODEX[5]	EU[6]
Azoxystrobin	24.79 ± 0.30	4	26.34 ± 0.10	2	500	2000	3000
Benalaxyl	22.20 ± 0.10	6	25.50 ± 0.40	2	100	300	300
Carbendazim	109.4 ± 0.31	11	148.9 ± 0.21	6	700	3000	300
Pyriproxifen	11.78 ± 0.17	3	ND	-	5000	-	5000
Pyrimethanil	15.87 ± 0.66	3	21.32 ± 1.10	3	5000	4000	50
Tebuconazole	14.11 ± 0.08	2	ND	-	2000	6000	500
Thiophanate-methyl	ND	-	10.70 ± 0.06	1	-	-	100

(MRL): maximum residue limit. (ND): not detected: (ANVISA): National Agency for Sanitary Surveillance. (MAPA): Ministry of Agriculture, Livestock and Food Supply. (EU): European Union. (CODEX): Alimentarius International Food Standards.

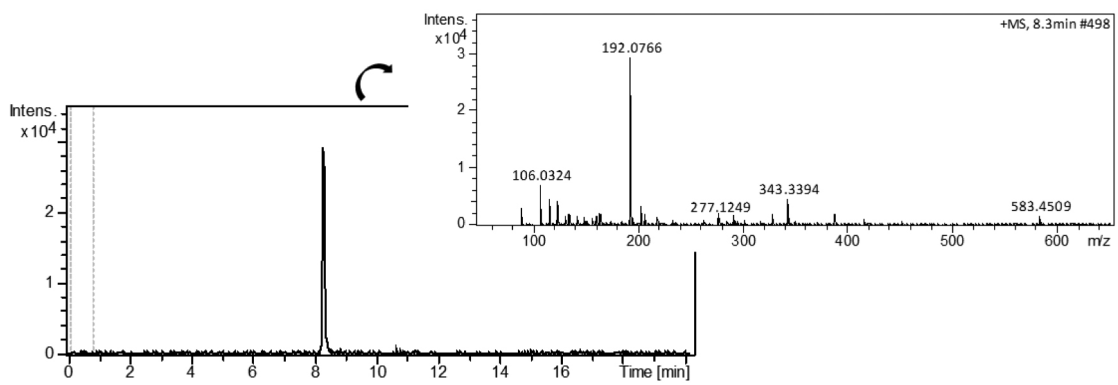

***Figure 3.** LC-HRMS chromatograms for white grape juice containing carbendazim at 109 µg L^{-1} in the positive sample*

Rizzetti *et al.*,[40] who found the fungicide in nearly every evaluated sample of orange juice in Santa Maria, Brazil. In a recent study, Souza *et al.*[30] detected in a single grape juice sample the pesticides azoxystrobin, metalaxyl and tebuconazole, at concentrations of 8, 5 and 10 µg L^{-1}, respectively. In contrast, Xu *et al.*[41] found no trace of fungicide residues in grape juice, while Farajzadeh *et al.*[42] similarly detected no residues of triazole pesticides in three samples of undiluted grape juice.

Picó and Kozmutza[43] investigated the presence of four pesticides in grape juice and determined whether they had any antioxidant degradation effects. Twelve of the 100 samples analyzed contained fenamiphos and methiocarb residues and their degradation products. The results seem to indicate that natural antioxidant compounds present in fruit juices can reduce the pesticide degradation rate through an oxidative mechanism, increasing the persistence. Pesticides detected in this study were also detected elsewhere in jellies via UFLC-TOFMS, including azoxystrobin in strawberry and raspberry jellies, carbendazim in plum, pyrimethanil in blackberry, thiophanate-methyl in strawberry and tebuconazole in peach (diet) and apricot, at concentrations ranging from 5.1 to 169.0 µg L^{-1}.[44]

CONCLUSIONS

In this study, a new multiresidue method was developed and validated for the simultaneous analysis of 79 pesticides in natural grape juice by LC-HRMS, using QuEChERS as the proposed extraction method. The developed method combines selectivity, high-resolution capability and LC-HRMS analysis with the advantages of QuEChERS, enabling the simple, fast and sensible analysis of pesticide residues in natural grape juices, a process which requires low consumption of organic solvents. This study has provided an important evaluation of the presence of potentially hazardous trace compounds in juices that are widely consumed, detecting a total of seven pesticide residues in real juice samples. Nevertheless, such monitoring is extremely important to ensure food safety standards.

SUPPLEMENTARY MATERIAL

The supplementary material is available at http://quimicanova.sbq.org.br in pdf format, with free access.

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