

## Determination of Pesticides in Grape Juices by QuEChERS and Liquid Chromatography-Tandem Mass Spectrometry

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In this study, we describe a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for quantifying 25 pesticides in grape juice. This method was optimized and validated according with INMETRO (Instituto Nacional de Metrologia Qualidade e Tecnologia) for the simultaneous determination of pesticide, which are widely used in grape culture in Brazil. The method was based on the QuEChERS (quick, easy, cheap, effective, rugged and safe) methodology. We used AOAC Official Method 2007.01 and Standard Method EN 15662 for sample preparation, both methods presented statistically equivalent recoveries and coefficients of variation. At 0.005 and 2 mg L<sup>-1</sup>, fortified blank grape juices had average recoveries of 101 and 112% and average coefficients of variation of 16 and 11%, respectively. The repeatability was tested in three concentrations 0.005, 0.1 and 2 mg L<sup>-1</sup>, affording coefficients of variation 16.4, 20.7 and 10.8%, respectively.

**Keywords:** pesticide residue, grape juice, QuEChERS, sample preparation, LC-MS/MS

### Introduction

During production, processing, storage, and transport of food a variety of residues and contaminants may enter the food chain. Crops are treated with pesticides and against pests and may leave residues in products of plant.<sup>1</sup> Thus, determination of pesticide residues in food matrices has become a necessity in view of the toxicity and stability of these xenobiotics.<sup>2</sup>

Due to the low detection levels required by the regulatory bodies and the complex nature of the matrices, which the target compounds are present, the trace level detection and identification with prior efficient sample preparation are important aspects in the analytical method.<sup>3</sup>

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the most powerful techniques for the analysis of pesticides in a variety of complex matrices.<sup>3</sup> Nowadays, liquid chromatography-mass spectrometry

(LC-MS) is preferred over gas chromatography (GC) because currently used pesticides are quite polar, thermally labile or not easily vaporized.<sup>4</sup> Therefore, there are several LC-MS/MS methods for multi pesticide analyses.<sup>3,5</sup>

Despite of multiresidue analysis of these compounds at trace levels has been carried out since the 70s, analysis of pesticides still remains a challenge because different chemical classes are present at low concentrations in complex matrices. Thus, it is necessary to continue developing multi-residue analytical methods with higher recoveries and lower limits of detection as well as incorporating the ultimate innovations to them.<sup>6</sup>

Sensitive multiresidue analytical methods are required to satisfy the demand for monitoring pesticide residues at low concentration levels (e.g., at the micrograms *per* kilogram level) in various agricultural crops, such as fruits and vegetables. Thus, LC-MS/MS with electrospray ion (ESI) source, operated in multiple reaction monitoring (MRM) mode has become the predominant analysis technique in the detection of multiresidue pesticides due to its outstanding selectivity and high sensitivity.<sup>7-10</sup>

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The determination of pesticide residues in food matrices is a formidable challenge mainly because of the small quantities of analytes and large amounts of interfering substances which can be co-extracted with analytes and, in most cases, adversely affect the results of an analysis. However, safety concerns require that pesticides of the wide range of chemical properties (including acidic, basic and neutral) should be monitored.

Extraction and purification of samples is required prior to residue determination.<sup>3</sup> Because of the wide variety of food matrices, the sample must initially be cleaned up before final analysis. That is why the analytical chemist is faced with the need to develop new methodologies for determining such residues in a single analytical run. To accomplish the goal, “quick, easy, cheap, effective, rugged and safe” QuEChERS methodology has been developed. It is a streamlined and effective extraction and cleanup approach for the analysis of diverse analyte residues in food matrices.<sup>2</sup> Thus, it was used in this study.

It is widely recognized that the QuEChERS method is relevant in pesticide residue analysis. Many official laboratories around the globe are routinely using it due to the advantages encapsulated in its name. However, the frontiers of the application of QuEChERS are not yet established. González-Curbelo *et al.*<sup>9</sup> had shown that this method is effective for the analysis of other groups of compounds, including pharmaceuticals, mycotoxins, and polycyclic aromatic hydrocarbons, in a wide variety of complex matrices.

The LC-MS/MS in MRM mode has been widely used for quantitative and qualitative pesticide analysis.<sup>10-12</sup> Dynamic multiple reaction monitoring (DMRM) is an alternative to the use of MRM; this mode avoids the need to define segments of timeframe for the selected group of transitions, based on retention time, number of target species, and dwell time. In fact, “virtual” time segments are automatically constructed by the software during the analysis (as a timeframe of continuous movement during the analysis time), improving the shape of the peak and increasing the sensitivity.<sup>10</sup>

Brazil is the third largest fruit producer worldwide and an important exporter of tropical and subtropical fresh and processed fruits, mainly to European countries and the United States.<sup>11</sup>

Results from Brazilian pesticide monitoring programs have shown that almost half of the 13,556 samples of 22 different crops analyzed tested positive for at least one pesticide.<sup>12,13</sup> Thus, pesticides analysis for food security is need to assure population health.

There are several multi-residue methods, however, there were few methods developed for pesticides widely used in

specific culture and region. For example, Rebelo *et al.*<sup>14,15</sup> developed a LC-MS/MS multi-pesticide method, for pesticides which were widely used for rice culture in Brazil. Thus, there is a need for methods which could be applied for specific situations.

For those reasons the aim of the present study was to develop a multi-residue method for pesticides, which were widely used in grape culture in Brazil. In addition, this method is full validated according with INMETRO (Instituto Nacional de Metrologia Qualidade e Tecnologia) that is the institution responsible for quality assurance in Brazil. Thus, the present method could be direct used to assure the security of Brazilian grape juice.

## Experimental

### Reagents and chemicals

A mix with all pesticides (Table 1) was purchased from SPEX CertiPrep Group (Metuchen, NJ, USA), this mix had the pesticides at 1000 mg L<sup>-1</sup> concentration. Formic acid (≥ 99.8%) was purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, USA). Methanol HPLC grade was purchased from JT Baker® (Phillipsburg, NJ, USA). Kit QuEChERS AOAC-2007.01 and EN 15662:2008 were purchased from Agilent (Agilent Technologies, Santa Clara, CA, USA).

There are two tubes in QuEChERS kits, tube 1, with 50 mL of volume, used for extraction and tube 2, with 2 mL of volume, used for cleanup.

In AOAC-2007.01 QuEChERS kits, tube 1 had 6 g anhydrous magnesium sulfate (MgSO<sub>4</sub>) and 1.5 g anhydrous sodium acetate (50 mL of volume, tube 1), and tube 2 had 150 mg of MgSO<sub>4</sub> and 50 mg of PSA (primary secondary amine). It was purchased from Agilent Technologies (Santa Clara, CA, USA).

In EN 15662:2008 QuEChERS kits, tube 1 had 4 g MgSO<sub>4</sub>, 1 g NaCl, 1 g citrate, 0.5 g disodiumcitrate sesquihydrate and tube 2 had 150 mg of MgSO<sub>4</sub> and 25 mg of PSA. It was purchased from Agilent Technologies (Santa Clara, CA, USA).

Stock solutions were prepared by dissolving the standards in methanol at a concentration of 100 mg L<sup>-1</sup>. Two intermediate mixture solution C and D at concentrations of 5 and 1 mg L<sup>-1</sup>, respectively. Calibration solutions were prepared via the dilution of solutions C and D in methanol at 0.0025, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 2, e 5 mg L<sup>-1</sup> concentration levels.

The blank samples were from Santa Catarina middle east (Brazilian state), Brazil, in the region of Videira city.

**Table 1.** Molecular weight, precursor ion, quantifier and qualifier. Average retention times ( $t_r$ ), fragmentation energy, collision energy (CE) of quantifier (CE1) and qualifier transitions (CE2) for detected pesticide residues in grape juices

Pesticide	Molecular weight	Precursor ion	Quantifier ion	Qualifier ion	$t_r$ / min	Fragmentation energy / V	CE1 / V	CE2 / V	
Acephate	183.17	[M + H] <sup>+</sup>	184	143	95	1.11	50	25	0
Azoxystrobin	403.39	[M + H] <sup>+</sup>	404	372	344	7.72	80	25	10
Boscalid	343.21	[M + H] <sup>+</sup>	343	307	271	7.97	100	35	20
Carbendazim	191.19	[M + H] <sup>+</sup>	192	160	132	1.67	90	35	15
Carbofuran	221.25	[M + H] <sup>+</sup>	222	123	165	6.25	80	5	20
Carbosulfan	380.54	[M + H] <sup>+</sup>	381	118	160	12	90	10	20
Cymoxanil	198.18	[M + H] <sup>+</sup>	199	128	110	4.06	50	15	5
Ciproconazol	291.78	[M + H] <sup>+</sup>	292	70	125	8.32	100	20	30
Chlorpyrifos	350.59	[M + H] <sup>+</sup>	349	198	293	10.3	90	10	19
Clothianidin	249.68	[M + H] <sup>+</sup>	250	131	169	2.59	70	5	10
Difenoconazol	406.26	[M + H] <sup>+</sup>	406	251	337	9.32	130	15	25
Dimethoate	229.26	[M + H] <sup>+</sup>	230	198	171	3.2	70	10	5
Dimetomorfe	387.86	[M + H] <sup>+</sup>	388	301	165	8.08	130	35	20
Diuron	233.09	[M + H] <sup>+</sup>	233	72.1	159	7.32	90	30	15
Fenamidone	311.4	[M + H] <sup>+</sup>	312	92	236	7.87	80	10	30
Fenarimol	331.2	[M + H] <sup>+</sup>	331	268	81	8.4	130	35	25
Fenpyroximate	421.49	[M + H] <sup>+</sup>	422	366	135	10.8	110	35	15
Imidacloprid	255.66	[M + H] <sup>+</sup>	256	208	175	2.42	80	15	15
Indoxacarb	527.83	[M + H] <sup>+</sup>	528	150	203	9.33	100	25	40
Iprovalicarb	320.43	[M + H] <sup>+</sup>	321	119	202	8.29	80	5	15
Metalaxmetalaxyl	279.33	[M + H] <sup>+</sup>	280	220	160	7.26	80	20	10
Tebuconazole	307.82	[M + H] <sup>+</sup>	308	70	124	8.88	80	40	15
Tetraconazole	372.15	[M + H] <sup>+</sup>	372	159	70	8.4	110	20	45
Thiametoxam	291.71	[M + H] <sup>+</sup>	292	211	181	1.7	100	20	10
Triadimefon	293.75	[M + H] <sup>+</sup>	294	197	225	8.13	90	10	10
Triadimenol	295.76	[M + H] <sup>+</sup>	296	70	99	8.29	80	20	5
Trifloxystrobin	408.37	[M + H] <sup>+</sup>	409	186	145	9.37	100	50	15

### LC-MS/MS analysis

The analyses were carried out in the LC-MS/MS system: it consist of a LC system 1260 Infinity Quaternary LC coupled to a triple quadrupole mass spectrometer (QqQ) 6430 detector. Both were from Agilent Technologies (Santa Clara, CA, USA). The chromatographic separations were carried out using a poroshell 120 EC-C18, 100 × 2.1 mm internal diameter (i.d.) and 2.7 μm particle size (Agilent Technologies, Santa Clara, CA, USA). Before the separation column, a pre-column was installed, guard 3PK-poroshell 120 EC-C18, 5 × 2.1 mm i.d. and 2.7 μm particle size (Agilent Technologies, Santa Clara, CA, USA). Chromatographic analysis was carried out using gradient elution and mobile phase consisted of formic acid 0.1%/methanol (95:5, v/v) (A), and formic acid 0.1%/methanol (5:95, v/v). The ultrapure water used to prepared formic acid solution was obtained from a Milli-Q® water system (Millipore; Bedford, USA). The gradient program started at 30% B, with linear gradient until 95% B in 7.5 min and, then, linear gradient until 100% in 15 min

and constant for 2 min. The re-equilibrium time (post time) was 4.5 min. The flow remained constant at 0.3 mL min<sup>-1</sup>, the column temperature was fixed at 40 °C, and the injection volume was 1 μL.

All pesticides were analyzed in the positive ionization mode, parent ions were detected as [M + H].

It was used a Quadruple Triple 6430 mass spectrometer as detector. The optimized conditions were gas flow of 11 L min<sup>-1</sup>; gas nebulizer at 15 psi, gas temperature at 300 °C, and capillary voltage of 4000 V. Nitrogen 99.99% was used as nebulizer and 99.9999% as collision gas. For data acquisition, the software Agilent Mass Hunter was used. For the detection in the MS/MS, we used the DMRM mode.

For the selection of the precursor and product ion, 2 μL of the standard solution of pesticide (2 mg L<sup>-1</sup>) was injected in the LC-MS/MS. Different fragmentation energies (20-160 V), different collision energies (0-60 V) and acceleration energies (1-7 V) were investigated for each compound.

The most intense transition was used as the quantifying ion and the second most intense transition used as the qualifying ion for confirmation of the analysis.

#### Extraction

The sample preparation method of QuEChERS AOAC-2007.01 and EN 15662:2008 were used for samples of grape juice. 15 mL of grape juice was transferred to tube 1 (kit QuEChERS). Then, we added 1% acetic acid in acetonitrile and the samples were shaken vigorously (by hand) for 1 min. Next, the extract was centrifuged at 3500 rpm for 5 min in a Fanem® Excelsa 2206 (Fanem® Ltda). We removed 1 mL of the supernatant and transferred it to tube two (kit QuEChERS). The extract was shaken manually for 30 seconds and centrifuged again for 5 min at 3500 rpm. In EN 15662:2008, 5 µL of 5% formic acid was added in tube 2, after centrifugation. Prior to injection, samples were filtered with 0.45 µm nylon syringe filter (Millex-HN, Millipore, Bedford, MA, USA). Matrix-matched standards were prepared using blank samples that were extracted according to the sample preparation procedure mentioned above.

## Results and Discussion

#### Optimization of LC-MS/MS analysis

All pesticides were analyzed in the positive ionization mode, precursor ions were detected as  $[M + H]^+$ .

The procedure used for the identification of pesticide residues included retention time, two transitions, and the dynamic monitoring reaction monitoring, DMRM. The monitored ions for each compound are listed in Table 1.

The software tool Mass Hunter, used in this study, constructs automatically Table 1, based on retention times for the analytes in a detection window (Delta RT) to avoid loss of the analytes due to the peak dislocation, and a constant cycle of digitalization period (to provide enough number of data points in all peaks detected, i.e.,  $> 10$ ). In this study, the Delta RT was fixed at 1 min.<sup>10</sup>

Table 2 shows peak height before and after optimization. At one hand, some pesticides had a slight increase in peak height, for example, clothianidin and cymoxanil had shown a 0.42 and 1.99% increase in peak height. At the other hand, some pesticides had brutal increase in peak height, for example, triadimefom had a 3399% increase in peak height after LC-MS/MS optimization.

#### Chromatographic conditions

Increasing the sample throughput of the ever-growing

**Table 2.** Increase of peak height after LC-MS/MS optimization

Pesticide	Peak height		Peak height increase / %
	Before	After	
Acephate	2951	6884	133
Azoxystrobin	54069	114315	111
Boscalid	1469	9095	519
Carbendazim	20573	26134	27
Carbofuran	37447	111298	197
Carbosulfan	15767	52560	233
Cymoxanil	8430	8598	2
Ciproconazol	8028	16412	104
Chlorpyrifos	3444	9750	183
Clothianidin	2837	2849	0
Difenoconazol	4952	24486	394
Dimethoate	10118	16784	66
Dimetomorfe	2945	18827	539
Diuron	4988	23025	362
Fenamidone	2264	2504	11
Fenarimol	1560	3072	97
Fenpyroximate	26935	53935	100
Imidacloprid	3048	4033	32
Indoxacarb	654	2716	315
Iprovalicarb	12410	114989	827
Metalaxyl	28500	77995	174
Tebuconazole	3709	23280	528
Tetraconazole	397	15500	3804
Thiametoxam	3440	8877	158
Triadimefon	275	9623	3399
Triadimenol	852	10039	1078
Trifloxystrobin	2859	59443	1979

number of analyses (routine) has become obligatory in method development.<sup>16</sup> High throughput analysis in LC can be achieved with sub-3 µm core-shell technology. Core-shell columns are basically packed with 2.6 or 2.7 µm particles, including a 1.7 or 1.9 µm solid inner core surrounded by a thin 0.35 or 0.5 µm porous layer. An efficiency of ca. 80% of the one achieved with fully porous sub-2 µm particles has been reported with sub-3 µm core-shell particles but with a 2 to 3-times lower backpressure compared to sub 2 µm totally porous particles.<sup>16-19</sup>

To achieve high throughput analysis, we used a 100 × 2.1 mm i.d. column packed with 2.7 µm core-shell particles. This columns afford comparable efficiency and lower backpressure than columns packed with sub 2 µm totally porous particles. Using this column, we obtained low run time (17 min), low re-equilibration time (4.5 min), low mobile phase consumption (0.30 mL min<sup>-1</sup>). In addition, chromatographic analysis was carried out at 306 bar.

## Comparison between AOAC-2007.01 and EN 15662:2008

Anastassiades *et al.*<sup>20</sup> described the QuEChERS method for the multiclass, multiresidue analysis of pesticides in fruits and vegetables. Lehotay *et al.*<sup>21</sup> modified the method to use relatively strong acetate buffering conditions and Anastassiades *et al.*<sup>22</sup> chose to use weaker citrate buffering conditions in terms of ionic strength. In 2010, Lehotay *et al.*<sup>23</sup> compared AOAC Official Method 2007.01, which uses acetate buffering, with Standard Method EN 15662, which calls for citrate buffering. They found that both methods afford excellent and comparable results for 34 representative pesticides in apple-blueberry sauce.

In this study, we compared AOAC-2007.01 and EN 15662:2008 for extraction and clean-up of 25 pesticides in grape juice. Comparisons were carried out with blank samples fortified at 0.3 mg L<sup>-1</sup> and the results were shown in Table 3.

As shown in Table 3, AOAC-2007.01 and EN 15662:2008 afford similar coefficient of variation (CV) and recuperations. Excepted by carbosulfan that afforded

recuperation lower than the accepted limit, 70-120%, for EN 15662:2008 with 19.34% recuperation.<sup>24</sup>

The F-test tells us whether two variances are “significantly” different from each other. Considering the F-test at a confidence level of 95% [ $p$  (0.05)], CV obtained in AOAC-2007.01 and EN 15662:2008 were not significantly different. Thus, there were no statistic difference between CVs obtained with both method for the 25 pesticides studied.<sup>25,26</sup>

We use a *t*-test to compare averages, to decide whether or not they are “equal”. Statisticians say that the null hypothesis is tested, which states that the average values from two populations (AOAC-2007.01 and EN 15662:2008) are not different. The F-test indicates that the CVs are statistically equivalent at a 95% confidence interval. Thus, *t*-test is carried out assuming equivalent variances.

The *t*-test, assuming similar CVs, showed that carbosulfan and imidacloprid average concentrations in AOAC-2007.01 were different than concentrations in EN 15662:2008, while there were no evidence that the

**Table 3.** Comparison between recoveries obtained with AOAC-2007.01 and EN 15662:2008. Three blank grape juices were fortified at 0.3 mg L<sup>-1</sup> for both QuEChERS methods. *t* critical value was 2.77 and F critical value was 19. In the table were given calculated values for F-test and *t*-test

Pesticide	AOAC-2007.01			EN 15662:2008			<i>t</i> calculated	F calculated
	Average	Recovery / %	Coefficient of variation / %	Average	Coefficient of variation / %	Recovery / %		
Acephate	0.2893	96.42	3.40	0.3008	2.97	100.26	1.39	1.44
Azoxystrobin	0.2996	99.87	4.61	0.3027	4.33	100.89	0.24	1.2
Bifenthrin	0.236	78.66	7.16	0.1868	5.06	62.25	3.91	3.49
Boscalid	0.3126	104.21	3.74	0.3129	4.25	104.31	0.02	1.38
Carbofuran	0.31	103.34	4.13	0.3235	4.64	107.83	1.04	1.37
Carbosulfan	0.2368	78.94	7.26	0.058	12.72	19.34	14.45	5.54
Cymoxanil	0.3152	105.05	4.19	0.3215	4.23	107.18	0.51	1.03
Chlorpyrifos	0.256	85.33	4.61	0.2415	4.76	80.50	1.33	1.11
Clothianidin	0.2855	95.18	33.27	0.3315	4.59	110.49	1.2	15
Difenoconazol	0.2839	94.64	5.04	0.2858	3.74	95.26	0.15	1.93
Dimethoate	0.3353	111.76	3.58	0.3435	4.25	114.50	0.68	1.34
Dimetomorfe	0.2972	99.05	3.40	0.2979	1.83	99.30	0.19	1.27
Diuron	0.3205	106.82	3.96	0.3261	4.26	108.70	0.45	1.18
Fenamidone	0.2924	97.46	3.93	0.2923	4.28	97.42	0.01	1.17
Fenarimol	0.2453	81.77	5.67	0.25	5.44	83.35	0.42	1.68
Fenpyroximate	0.3034	101.12	4.32	0.3011	3.59	100.35	0.92	1.34
Imidacloprid	0.3539	117.96	2.20	0.3119	3.91	103.97	5.19	1.01
Indoxacarb	0.2902	96.73	4.69	0.2933	3.34	97.76	0.28	2.2
Iprovalicarb	0.3114	103.80	3.85	0.313	4.89	104.35	1.6	0.62
Metalaxyl	0.3048	101.60	4.30	0.3063	4.24	102.10	0.12	1.29
Tebuconazole	0.2881	96.04	5.03	0.289	4.12	96.33	0.07	1.65
Tetraconazole	0.2811	93.70	5.16	0.2849	4.04	94.97	0.32	1.77
Thiametoxam	0.2972	99.06	2.65	0.3002	1.69	100.07	0.65	1.82
Triadimefon	0.2927	97.57	3.42	0.2921	4.42	97.36	0.05	1.42
Triadimenol	0.2704	90.12	4.99	0.2742	4.38	91.41	0.34	1.51
Trifloxystrobin	0.3139	104.63	4.08	0.3145	4.17	104.83	0.05	1.02

remaining pesticides concentrations in both tests were different.

The AOAC-2007.01 method afforded better results for carbosulfan and imidacloprid than EN 15662:2008, thus, it was used as standard QuEChERS method in our laboratory.

#### Matrix effects

LC-MS/MS is susceptible to matrix effects which can adversely affect quantification depending on the analyte, matrix, sample preparation, instrumentation, and operating conditions. Among the approaches that reduce matrix effects, the most common in pesticide residue applications is matrix-matched calibration because it is relatively inexpensive and simple. In addition, it has been shown to work well during method validation when fortified samples are exactly matched with samples used for calibration.<sup>27-29</sup>

In 2012, Kwon *et al.*<sup>29</sup> measured the variability of matrix effects for 38 representative pesticides in 20 samples each (including different varieties) of rice, orange, apple, and spinach extracted using the QuEChERS method for analysis by LC-MS/MS. They found that only oranges gave > 20% matrix effects for a few pesticides. In this study,

we compared responses from fortified blank samples and standard solution.

Table 4 had shown the average values and CVs of fortified blank grape juice at 2 mg L<sup>-1</sup> and standard solutions at 2 mg L<sup>-1</sup>. Each experiment was carried out in triplicate. Calculated F and *t* values for fortified blank grape juice and standard solution are also given in Table 4.

The F-test, at a confidence level of 95% [*p* (0.05)], had shown that variances of the 25 pesticides for fortified blank grape juice and standard solution were not significantly different.

The *t*-test, at a confidence level of 95% [*p* (0.05)], assuming similar variances, had shown that concentrations obtained in fortified blank grape juice and standard solution were not significantly different for the 25 pesticides studied.

Chromatograms obtained for fortified samples and standard solutions were overlapped, which meant that matrix effect is negligible in this study.

#### Analytical methods validation

In Brazil, the National Agency for Sanitary Surveillance-ANVISA establishes maximum residue limits (MRLs) for

**Table 4.** Matrix effect study. Three blank grape juices and three fortified at 2 mg L<sup>-1</sup> and three standard solutions were prepared at 2 mg L<sup>-1</sup> level; *t* critical value was 2.77 and F critical value was 19. In the table were given calculated values for F-test and *t*-test

Pesticide	Average <sup>a</sup>	Coefficient of variation <sup>a</sup> (CV) / %	Average <sup>b</sup>	Coefficient of variation <sup>b</sup> (CV) / %	F calculated	<i>t</i> calculated
Acephate	1.71	5.1	1.65	7.8	2.19	0.7
Azoxystrobin	1.62	4.1	1.70	7.6	3.74	0.99
Boscalid	1.72	2.0	1.82	7.4	15.52	1.17
Carbendazim	1.51	3.1	1.57	6.9	5.46	0.92
Carbofuran	1.74	4.4	1.64	3.7	1.56	1.81
Carbosulfan	1.75	3.7	1.85	7.1	4.01	1.21
Chlorpyrifos	1.72	3.8	1.81	8.1	5.05	0.98
Clothianidin	1.63	2.9	1.76	6.7	6.27	1.87
Difenoconazol	1.74	3.5	1.83	7.5	5.08	1.02
Dimethoate	1.51	2.6	1.56	7.5	8.97	0.71
Diuron	1.52	4.0	1.58	7.0	3.34	0.86
Fenamidone	1.61	2.8	1.71	6.9	6.51	1.3
Fenarimol	1.63	4.0	1.71	7.7	4.15	0.96
Fenpyroximate	1.78	4.0	1.90	7.6	3.99	1.31
Imidacloprid	1.65	2.7	1.76	7.5	8.8	1.36
Indoxacarb	1.60	4.2	1.69	7.5	3.51	1.09
Iprovalicarb	1.74	2.6	1.82	6.8	7.45	1.07
Metalaxyl	1.52	3.0	1.58	6.9	5.79	0.9
Tebuconazole	1.66	4.0	1.75	7.7	4.22	0.97
Tetraconazole	1.60	4.2	1.69	7.6	3.53	1.05
Thiametoxam	1.57	4.2	1.70	7.3	3.58	1.61
Triadimefon	1.67	2.5	1.74	6.7	7.47	1.06
Triadimenol	1.57	3.7	1.64	7.5	4.65	0.91
Trifloxystrobin	1.66	3.7	1.74	6.9	3.87	1.11

<sup>a</sup>Standard solutions; <sup>b</sup>fortified blank solutions.

various types of food. Table 5 shows the MRLs of pesticides and calibrations ranges in study.<sup>30</sup>

Recently, some pesticides have been banned after reevaluation process. The frequent changes in the manufacture of pesticides and the discovery of new active ingredients of different classes make it difficult to monitor and control such residues, requiring constant improvement of the analytical methods.<sup>31,32</sup> Thus, it is necessary to develop new and effective methods. In addition, this method was focused in pesticides, which were widely used in grape juice culture in Brazil, and the method is validated according INMETRO (Instituto Nacional de Metrologia Qualidade e Tecnologia), which is the Brazilian organization responsible for quality assurance.<sup>33</sup>

The concentration range (Table 6) presented linearity with the analytical signal, indicated by the values of determination coefficient ( $r^2$ ) greater than 0.99 for all compounds in solvent. The method presented sensitivity, once the angular coefficients of straights showed elevated values.<sup>31,34,35</sup>

Method precision was evaluated using CVs.<sup>31-35</sup> Seven blank grape juice samples were independently fortified at 0.005, 0.1 and 2 mg L<sup>-1</sup> and prepared by two technicians (technician 1 and technician 2). Then, CVs for 25 pesticides were evaluated by each technician are shown in Table 6.

**Table 5.** Calibration range and MRL

Pesticide	Calibration range	MRL <sup>a,b</sup>
Acephate	0.0025-1	Na
Azoxystrobin	0.005-2	0.5
Boscalid	0.01-5	3
Carbofuran	0.0025-1	Na
Carbosulfan	0.0025-1	Na
Cymoxanil	0.0025-1	0.1
Chlorpyrifos	0.005-2	Na
Clothianidin	0.0025-1	0.01
Difenoconazol	0.01-5	0.2
Dimethoate	0.0025-1	Na
Dimethoate	0.01-5	2
Diuron	0.0025-1	0.01
Fenamidone	0.01-5	0.2
Fenarimol	0.0025-2	0.05
Fenpyroximate	0.0025-1	Na
Imidacloprid	0.0025-1	Na
Indoxacarb	0.005-2	Na
Iprovalicarb	0.0025-1	Na
Metalaxmetalaxyl	0.005-2	1
Tebuconazole	0.01-5	0.5
Tetraconazole	0.005-2	0.2
Thiametoxam	0.005-2	0.2
Triadimefon	0.01-5	Na
Triadimenol	0.0025-1	Na
Trifloxystrobin	0.0025-1	Na

<sup>a</sup>MRL: maximum residue limits; <sup>b</sup>it is the lowest MRL value between Brazil, Japan and USA; Na: not authorized.

**Table 6.** CVs obtained for two technicians (technician 1 and technician 2) for blank grape juice fortified at 0.005, 1 and 2 mg L<sup>-1</sup>

Technician	0.005 mg L <sup>-1</sup>		1 mg L <sup>-1</sup>		2 mg L <sup>-1</sup>	
	1	2	1	2	1	2
Acephate	16.3	14.4	13	13.8	8.5	13
Azoxystrobin	15.6	11.2	26.3	23.3	10.4	9.7
Boscalid	19.1	11.9	19	24.3	9.5	13.3
Carbofuran	15.2	18.4	21.1	17.5	9	6.7
Carbosulfan	27.7	17.6	26.6	29.6	13.9	13.4
Chlorpyrifos	18.8	14.1	26.1	23.2	9.1	9.1
Clothianidin	17.2	13	17.6	16.9	13	10.5
Difenoconazol	23.7	26.7	16	20.6	17.3	17.3
Dimethoate	15.2	13.5	20.6	24.4	11.8	13.5
Dimethoate	8.1	11.5	19.5	17.5	8.3	10.9
Diuron	13.6	11.5	12.1	15.3	10.9	12.1
Fenamidone	14.7	12.1	23.9	22.8	12.2	10.1
Fenarimol	21.2	20.3	29.1	29.5	8.2	11.3
Fenpyroximate	20.4	17.5	17	21.2	13.9	12.9
Imidacloprid	10.7	11.8	20.6	21.7	11.7	10.5
Indoxacarb	14.3	15	25.9	24.7	12.9	11.2
Iprovalicarb	18.8	13.9	14.6	13.4	11.1	10.3
Metalaxyl	16.5	13.8	19.7	17.4	7.4	9.9
Tebuconazole	13	15	20.7	16.6	13.3	12
Tetraconazole	14.3	13.8	23.9	21.6	9.9	12.6
Thiametoxam	11	14.1	22.5	17.8	10.5	8
Triadimefon	16.9	14.4	25.3	25.6	8.6	7.4
Triadimenol	19.1	18.8	22.5	22.3	11.8	10.7
Trifloxystrobin	13.2	10.9	17	16.2	6.6	7.6

The 25 pesticides investigated showed CVs within the recommended range given by SANCO, 2011 (< 20%) (Table 6). Thus, we concluded that the presented method is precise.<sup>24</sup>

Recovery was evaluated at 0.005 and 2 mg L<sup>-1</sup> for three independent samples (Table 7).<sup>35</sup> Pesticides investigated showed recovery values within the recommended range (70-120%). However fenpyroximate had shown recovery higher than 120%, while carbosulfan had shown recoveries lower than 70%.

Limits of detection (LOD) were defined as  $LOD = X + s \times t$ . We measured the signal from 7 blanks (containing no analyte) where  $X$  is the average value calculated from 7 replicate samples and  $s$  is the standard deviation calculated from these 7 replicate samples;  $t$  is the Student's value for  $p = 0.05$  and 6 degrees of freedom, 2.36. Limits of quantification (LOQ) were defined as the lower calibration levels. Recovery and CV were evaluated at the lowest calibration level to verify acceptances criteria (Table 8).

In the lowest calibration level, investigated pesticides showed recovery values and CV within the recommended range (70-120%, < 20%). However, carbosulfan had shown recovery slight lower than 70% (66%) and tebuconazole had shown recovery slight higher than 120% (122%).

In addition, ten grape juice brands from Santa Catarina middle east, Brazil, were analyzed to evaluate the method

**Table 7.** Recoveries and average concentrations for three independent fortified black samples at 0.005 and 2 mg L<sup>-1</sup>

Pesticide	Concentration / %	
	0.005 mg L <sup>-1</sup>	2 mg L <sup>-1</sup>
Acephate	100.86	90.75
Azoxystrobin	114.02	110.19
Boscalid	119.00	118.61
Carbofuran	96.89	106.52
Carbosulfan	30.31	68.78
Chlorpyrifos	108.06	119.72
Chlorpyrifos	112.74	117.61
Clothianidin	102.93	117.77
Difenoconazol	107.30	113.97
Dimethoate	118.49	109.18
Diuron	96.89	110.92
Fenamidone	101.45	113.93
Fenarimol	103.72	111.58
Fenpyroximate	106.81	130.39
Imidacloprid	102.99	113.57
Indoxacarb	103.25	114.15
Iprovalicarb	104.76	115.17
Metalaxmetalaxyl	96.55	118.09
Tebuconazole	99.84	115.87
Tetraconazole	105.77	113.93
Thiametoxam	99.53	118.43
Triadimefon	110.95	111.56
Triadimenol	72.27	99.67
Trifloxystrobin	96.96	116.38

**Table 8.** Limits of detection (LOD), limits of quantification (LOQ), recovery and CV at the lowest calibration level

Pesticide	LOD <sup>a</sup> / (mg L <sup>-1</sup> )	LOQ <sup>b</sup> / (mg L <sup>-1</sup> )	CV <sup>c</sup> / %	Recovery / %
Acephate	0.0021	0.0025	10.5	87.6
Azoxystrobin	0.0007	0.005	10.3	96.7
Boscalid	0.0093	0.01	18.1	116.8
Carbofuran	0.0021	0.0025	9.2	84.5
Carbosulfan	0.0017	0.0025	12.5	66.0
Chlorpyrifos	0.0012	0.0025	15.5	85.1
Chlorpyrifos	0.004	0.005	11.6	94.4
Clothianidin	0.0019	0.0025	6.3	85.2
Difenoconazol	0.0077	0.01	18.0	102.0
Dimethoate	0.0023	0.0025	14.6	86.1
Diuron	0.0022	0.0025	6.5	86.7
Fenamidone	0.0054	0.01	20.0	116.7
Fenarimol	0.0017	0.0025	6.1	87.7
Fenpyroximate	0.0009	0.0025	24.3	88.2
Imidacloprid	0.0018	0.0025	7.1	91.8
Indoxacarb	0.0013	0.005	8.1	92.7
Iprovalicarb	0.0019	0.0025	13.4	84.9
Metalaxyl	0.0038	0.005	7.5	95.4
Tebuconazole	0.0079	0.01	16.6	122.8
Tetraconazole	0.0037	0.005	13.3	95.3
Thiametoxam	0.0012	0.005	11.0	91.8
Triadimefon	0.0055	0.01	15.1	101.7
Triadimenol	0.0012	0.0025	10.1	88.6
Trifloxystrobin	0.0005	0.0025	10.9	84.1

<sup>a</sup>LOD: limits of detection; <sup>b</sup>LOQ: limits of quantification; <sup>c</sup>CV: coefficient of variation.



efficiency. The 25 pesticides were not found in nine of ten samples (concentration lower than LOD). However, one of the samples had shown presence of azoxystrobin, metalaxyl, tebuconazole in the concentrations of 8, 5 and 10  $\mu\text{g L}^{-1}$ , respectively. These concentrations are lower than the MRL established in Brazil, USA and Japan.

## Conclusions

The validation of the presented method was carried in accordance with INMETRO, obtained results had shown that our method is linear, selective, accurate, and exact. The LOD and LOQ were adequate for pesticide analysis in grape juice, since these parameters were lower than established MRLs. The matrix effect was not found in the pesticides evaluated and the calibration curve was built in solvent.

The main objective of this study was develop an inexpensive and efficient method for the determination of pesticide residues in grape juice. Thus, our objectives were achieved, since the presented method was validated and it is cheap and fast.

## Supplementary Information

Supplementary data are available free of charge at <http://jbcs.sbq.org.br> as PDF file.

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