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Assessing the feasibility of direct injection for pesticide residue analysis in grape juice by liquid chromatography/triple quadrupole mass spectrometry

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Abstract. In Brazil, the regulation of pesticide residues is guided by the National Health Surveillance Agency (ANVISA) and the Ministry of Agriculture and Livestock (MAPA), emphasizing the importance of monitoring pesticide levels in agricultural products to protect consumer health. High pesticide residue concentrations can compromise the organoleptic qualities and overall quality of the grape juice, in addition to being harmful to the consumer's health, making residue analysis crucial. Traditional methods for pesticide analysis in grape juice and wine, like Solid Phase Extraction (SPE) and Dispersive Solid-phase Extraction (dSPE), are time-consuming and costly. An alternative approach, the Dilute-and-Shoot (DnS) technique, has been explored using Liquid Chromatography-Mass Spectrometry (LC-MS), but its robustness and reliability have not been thoroughly assessed. In this study, 71 pesticides were analyzed in grape juice using Liquid Chromatography-Triple Quadrupole Mass Spectrometry (LC/MS-MS) with a direct injection method, including 450 injections over 9 days. The results showed that direct injection with only a 50% dilution and filtration was effective, with stable peak intensities up to 350 injections, indicating the feasibility of this method without complex sample preparation. The study suggests that simple procedures can improve injection throughput, although factors like pre-column saturation and column wear need further investigation for optimization.

1. Introduction

As highlighted by the FAO-OIV FOCUS (2016) [1], recent years have seen a significant rise in interest surrounding both fermented and non-fermented grape-based products. Grapes stand out among fruits for the considerable attention they have received in health-related scientific research. Grape juice, a grape by-product, is an intricate matrix primarily composed of water and a variety of metabolites, including sugars, organic acids, minerals, as well as phenolic and aromatic compounds [2]. It has been widely consumed across the globe for its distinct flavor and nutritional benefits [3], covering a wide range of consumers. The increase in the production, marketing and consumption of grape juices has been constant in recent years [4].

Pesticides are commonly used in agriculture due to their positive impact on crop yield [5]. Long-term exposure to these chemicals through consumption can lead to significant health risks. The established maximum residue limits (MRLs) are normally low concentrations. As a result, accurate and effective sample preparation methods are essential for detecting these trace pesticide levels in agricultural products [5].

High-performance liquid chromatography coupled with mass spectrometry (LC/MS-MS) is widely recognized as an effective tool for analyzing complex food matrices. Recent studies show that the multiple reaction monitoring (MRM) mode is particularly effective due to its high selectivity and sensitivity in detecting pesticide residues in food, especially in fruits and juices, as well as in the precise quantification of target compounds in complex food matrices [6].

Recent updates in sample preparation have been substantial. As a crucial first step in the analytical process,

it must be executed with precision, involving sometimes a detailed series of stages. Therefore, it represents a potential source of errors and ultimately determines whether the outcome of the analysis will be successful or fail. In this sense, direct filtration and injection without additional purification have proven to be effective, reducing the total analysis time [7].

This work highlights the importance of using modern analytical technologies and simplified approaches to ensure high-quality products that are safe for human consumption, in alignment with the principles of green chemistry. Therefore, the main objective was to explore the possibility to perform direct injection of grape juice, with only a previous dilution of 50% and filtration, for the analysis of pesticides residues.

2. Material and Methods

A commercial red grape juice was used for the tests. The sample was diluted twice with ultrapure water, it was spiked with 1 mg. L-1 of the mix of pesticides, transferred to a 2 ml with screw vial from Agilent Technologies, vortexed for 1 minute and, finally, filtered with PVDF membrane before the analysis. The compounds were determined according to Valentin et al. 2020 [8], Zou et al. 2020 [10] and Mastovska1 et al. 2017 [11]. The injection was performed using liquid injection configuration by LC/MS-MS (6470B Agilent Technologies). The equipment was configured with autosampler inlet and triple quadrupole MS 6470B with AJS (Agilent Technologies Jetstream) ESI (Eletrospray ionization) source in MRM (Multiple Reaction Monitoring) mode. The flow was 0.4 mL/min and the injection volume 3uL. The Figure 1 lists the instrument parameters used during the study.

For this methodology, 71 compounds were selected and can be found in Figure 2. Analytical standards for the compounds mix were purchased from CPA Chem, being all of them Certified Reference Material (ISO 9001, ISO 17025 and ISO 17034, traceable to NIST). The solvents Acetonitrile (ACN), Formic acid (CH_2O_2) and Ammonium Formate (NH4HCO2) were purchased from Merck.

Source parameters				
Parameter	**Value (+)**	**Value (-)**		
Gas Temp (°C)	250	250		
Gas Flow (l/min)	7	7		
Nebulizer (psi)	40	40		
SheathGasHeater	325	325		
SheathGasFlow	11	11		
Capillary (V)	3500	3500		
VCharging	0	1500		
Binary pump parameters				
Channel	**Solvent 1**	**Name 1**	**Solvent 2**	**Percent**
A	H20	0.1%ac form+10mM formiato amonio	ACN	95.0 %
B	ACN	95:5 ACN/H20+0.1%ac form+10mM formiato amonio	H20	5.0 %
Time	**A**	**B**		
10.00 min	5.0 %	95.0 %		
12.00 min	5.0 %	95.0 %		
12.01 min	95.0 %	5.0 %		
15.00 min	95.0 %	5.0 %		

Figure 1. Instrument parameters used for the determination of 71 pesticides in grape juice by LC/MS-MS.

Compound Name	Prec Ion	Prod Ion	Frag (V)	CE (V)	Ret Time (min)	Polarity
Abamectin	890.5	567.4	90	12	10.79	Positive
Abamectin	890.5	307.1	90	16	10.79	Positive
Abamectin	890.5	305.1	90	28	10.79	Positive
Acetamiprid	223.1	126	80	27	5.18	Positive
Acetamiprid	223.1	90	80	45	5.18	Positive
Ametoctradin	276.2	190.1	175	36	8.84	Positive
Ametoctradin	276.2	149.1	175	44	8.84	Positive
Ametryn	228.1	186.1	130	16	6.83	Positive
Ametryn	228.1	91.1	130	24	6.83	Positive
Azoxystrobin	404.1	372.1	110	8	8.03	Positive
Azoxystrobin	404.1	344.1	110	24	8.03	Positive
Azoxystrobin	404.1	329.1	110	32	8.03	Positive
Benalaxyl	326.2	294.1	90	4	8.83	Positive
Benalaxyl	326.2	208.1	90	21	8.83	Positive
Benalaxyl	326.2	148.1	90	27	8.83	Positive
Benthiavalicarb-isopropyl	382	180	140	20	7.64	Positive
Benthiavalicarb-isopropyl	382	116	140	20	7.64	Positive
Benthiavalicarb-isopropyl	382	72	140	20	7.64	Positive
Benzyladenine	226.11	91.2	140	20	5.06	Positive
Benzyladenine	226.11	65	140	40	5.06	Positive
Bixafene	414	394.1	140	16	8.62	Positive
Bixafene	414	265.9	140	28	8.62	Positive
Boscalid (Nicobifen)	343	307.1	145	16	8.08	Positive
Boscalid (Nicobifen)	343	272.1	145	32	8.08	Positive
Boscalid (Nicobifen)	343	271.2	145	32	8.08	Positive
Carbosulfan	381.2	160.2	105	12	11.69	Positive
Carbosulfan	381.2	118.1	105	36	11.69	Positive
Carbosulfan	381.2	76.1	105	36	11.69	Positive
Chinomethionat	235	207	105	12	9.07	Positive
Chinomethionat	235	207	104	15	9.07	Positive
Chinomethionat	235	163	105	28	9.07	Positive
Chlorantraniliprole	483.9	452.9	105	16	7.42	Positive
Chlorantraniliprole	483.9	285.9	105	8	7.42	Positive
Chloridrate Formetanate	222.1	165.1	120	12	1.66	Positive
Chloridrate Formetanate	222.1	46.2	120	28	1.66	Positive
Clethodim	360.1	268.1	100	8	9.77	Positive
Clethodim	360.1	164.1	100	16	9.77	Positive
Clothianidin	250.02	169	95	8	4.66	Positive
Clothianidin	250.02	131.9	95	8	4.66	Positive
Cyantraniliprole	484.2	453	140	26	5.97	Positive
Cyantraniliprole	484.2	286.1	140	19	5.97	Positive
Cyantraniliprole	475	443.9	140	16	5.97	Negative
Cyantraniliprole	475	285.9	140	14	5.97	Negative
Cyazofamid	325	261	90	4	8.83	Positive
Cyazofamid	325	108	90	8	8.83	Positive
Cymoxanil (Curzate)	199.1	128	50	4	5.27	Positive
Cymoxanil (Curzate)	199.1	110.9	50	12	5.27	Positive
Cyproconazole	292.1	125	100	32	7.7	Positive
Cyproconazole	292.1	70	100	16	7.7	Positive
Cyprodinil	226.1	91.1	140	36	8.26	Positive
Cyprodinil	226.1	76.9	140	50	8.26	Positive
Deltamethrin	523	506	100	8	6.7	Positive
Deltamethrin	523	281	100	12	6.7	Positive
Difenconazole	406.1	337	120	10	9.05	Positive
Difenconazole	406.1	251	120	20	9.05	Positive
Dimethomorph(E)	388.1	301.1	145	20	7.62	Positive
Dimethomorph(E)	388.1	165.1	145	32	7.62	Positive
Dithianon	296	264	50	20	8.29	Negative
Dithianon	296	238	50	20	8.29	Negative
Dithianon	296	164	50	20	8.29	Negative
Diuron	235	72	110	20	6.86	Positive
Diuron	233.03	72.1	110	20	6.86	Positive
Emamectin Benzoate	1008.57	158	150	40	9.98	Positive
Emamectin Benzoate	1008.57	126	150	40	9.98	Positive
Emamectinbenzoate	886.4	158.3	50	15	9.98	Positive
Emamectinbenzoate	886.4	82.7	50	27	9.98	Positive
Ethofenprox	394.24	359	100	5	11.52	Positive
Ethofenprox	394.24	177	100	5	11.52	Positive
Etofenprox	394.2	177.3	90	8	11.52	Positive
Etofenprox	394.2	107.1	90	40	11.52	Positive
Etoxazole	360.2	141	120	26	10.47	Positive
Etoxazole	360.2	113	120	58	10.47	Positive
Famoxadon	392.1	330.9	85	4	9.19	Positive
Famoxadon	392.1	238	85	12	9.19	Positive
Fenamidone	312	236.1	100	8	7.97	Positive
Fenamidone	312	92.2	100	28	7.97	Positive
Fenamidone	312	65.1	100	56	7.97	Positive
Fenarimol	331	268	130	20	7.99	Positive
Fenarimol	331	81	130	28	7.99	Positive
Fenthion	279	247.1	90	8	8.98	Positive
Fenthion	279	169.1	90	12	8.98	Positive
Fludioxonil	247	169	95	32	7.77	Negative
Fludioxonil	247	126	95	32	7.77	Negative
Fluopicolid	382.9	172.9	110	20	8.19	Positive
Fluopicolid	382.9	144.9	110	56	8.19	Positive
Flupyradifurone	289	127	140	24	5.47	Positive
Flupyradifurone	289	90	140	42	5.47	Positive
Fluxapyroxad	382.1	362.1	120	20	8.01	Positive
Fluxapyroxad	382.1	234.1	120	20	8.01	Positive
Haloxyfop-R-methyl	376.1	316	90	16	9.45	Positive
Haloxyfop-R-methyl	376.1	90.9	90	40	9.45	Positive
Imibenconazole	411	171	120	20	9.62	Positive
Imibenconazole	411	125.02	120	40	9.62	Positive
Imidacloprid	256	208.9	80	12	4.84	Positive
Imidacloprid	256	175	80	12	4.84	Positive
Indaziflam	302.3	158.1	103	13	7.66	Positive
Indaziflam	302.3	138	103	25	7.66	Positive
Indoxacarb	528.1	203	110	45	9.59	Positive
Indoxacarb	528.1	150	110	20	9.59	Positive
Iprovalicarb	321.2	202.9	80	0	7.87	Positive
Iprovalicarb	321.2	119	80	16	7.87	Positive
Isofetamide	360.2	210	50	20	8.75	Positive
Isofetamide	360.1	125	50	20	8.75	Positive
Kresoxim methyl	314.1	267	85	0	8.84	Positive
Kresoxim methyl	314.1	222.1	85	10	8.84	Positive
Lufenuron	509	325.5	138	18	9.96	Negative
Lufenuron	509	174.7	138	37	9.96	Negative
Mandipropamid	412.13	356.1	110	4	8.14	Positive
Mandipropamid	412.13	328.1	110	8	8.14	Positive
Metaflumizone	507.1	287.1	150	24	9.99	Positive
Metaflumizone	507.1	178	150	28	9.99	Positive
Metaflumizone	507.1	116	150	48	9.99	Positive
Metalaxyl-M	280.2	220	90	12	6.89	Positive
Metalaxyl-M	280.2	160.2	90	24	6.89	Positive
Metconazole	320.1	125	130	48	8.47	Positive
Metconazole	320.1	70.1	130	24	8.47	Positive
Mifentrifoconazole	400	70	50	20	8.44	Positive
Mifentrifoconazole	398	70	50	20	8.44	Positive
Myclobutanil	289.1	125.1	110	32	7.96	Positive
Myclobutanil	289.1	70.1	110	16	7.96	Positive
Oryzalin	347.1	288	120	20	8.45	Positive
Oryzalin	347.1	198	120	35	8.45	Positive
Oxatiapipoline	540	500	50	23	8.51	Positive
Oxatiapipoline	540	167	50	30	8.51	Positive
Prohexadione	211	167	70	20	5.15	Negative
Prohexadione	211	123	70	14	5.15	Negative
Propargite	368.1	231.2	80	0	10.57	Positive
Propargite	368.1	175.2	80	8	10.57	Positive
Pydiflumetofen	426	194	120	20	9.34	Positive
Pydiflumetofen	426	170.9	120	50	9.34	Positive
Pyraclostrobin	388.11	193.8	95	8	9.23	Positive
Pyraclostrobin	388.11	163.1	95	20	9.23	Positive
Pyrazophos	374.1	222.1	115	16	9.12	Positive
Pyrazophos	374.1	194.1	115	32	9.12	Positive
Pyridaben	365.1	309.1	80	4	10.94	Positive
Pyridaben	365.1	147.2	80	20	10.94	Positive
Pyrimethanil	200.1	106.9	120	20	7.14	Positive
Pyrimethanil	200.1	82	120	25	7.14	Positive
Pyriproxyfen	322.2	185	110	20	10.09	Positive
Pyriproxyfen	322.2	96	110	12	10.09	Positive
Quinomethionate	235	207	104	15	9.07	Positive

Figure 2. Pesticides analyzed in this study, with their precursor ion, product ion, fragmentation, collision energy, retention time and polarity.

3. Results and Discussion

This study evaluated the use of liquid chromatography coupled with tandem mass spectrometry (LC/MS-MS) with direct injection of red grape juice without sample preparation.

For this evaluation, batches of 50 samples were injected, and the only procedure performed was cleaning the accumulated dirt from the ESI ion source. Other procedures normally evaluated in an analytical routine, such as column exchange, pre-column, sampler valve rotor, nebulizer needle, HPLC tubing, or filters, etc., were not performed since the goal was to assess the robustness of the method.

The literature shows that most studies use LC-MS/MS to determine compounds in wines or grape derivatives with

sample preparation through solid-phase extraction, solid-phase microextraction, liquid-liquid partition or dispersive solid-phase extraction (QuEChERS) [9,11,12]. Being the QuEChERS (Quick Easy Cheap Effective Rugged and Safe) the most popular sample preparation methods [13]. The technique uses liquid-liquid partitioning with acetonitrile, followed by purification of the extract through dispersive solid-phase extraction (d-SPE). Initially developed for analyzing pesticide residues in high-moisture fruits and vegetables, the QuEChERS method has recently gained widespread use for detecting a broad range of analytes in a diverse array of sample types [13].

In this sense, for the determination of pesticide residues in musts and wines, the OIV recommends sample preparation using the method OIV-MA-AS323-08 [14]. This method defines the steps involved in extraction using the QuEChERS method and the analysis of the obtained extracts by GC/MS and/or LC/MS-MS.

However, several studies use direct injection of wines in LC-MS or the dilution and shoot option [15, 16, 17, 18, 19, 20], but none of these studies observe a thorough evaluation of the method's robustness using this type of technique. LC-MS with direct injection often proves to be efficient in a short time frame since the presence of contaminants or compounds that are not of interest but are in the matrix causes an effect known as ion suppression [20]. The ion suppression effect causes the equipment to lose sensitivity considerably in a short period, necessitating the cleaning or replacement of components such as nebulizers, capillaries, optical parts, or even the first quadrupole. For the use of direct injection methodology, it is crucial to assess the effects that the matrix will have on the equipment in order to evaluate the feasibility of using this technique.

For this evaluation, it is essential to use the more complex matrix for the study, making as many injections as possible before the equipment loses signal partially or completely. The option to maintain ESI source cleaning was made because it is a routine procedure recommended by manufacturers, also necessary for sample preparation injections with the techniques mentioned. For other consumable items or procedures considered non-routine, it was decided to keep them as is until partial or complete signal loss occurred. It is important to remember that regardless of whether or not sample preparation is used, some HPLC and MS components must be checked or replaced after each batch of samples analyzed.

Some examples of components that must be observed or replaced are pre-columns and analytical columns due to the accumulation of dirt and consequently the loss of efficiency in the separation of compounds. The sampler valve rotor, for instance, has a maximum number of rotations, and this must be considered regardless of the type of extract. The injector needle seat is another item that must be replaced after a specific number of injections, along with the injection needle. For the MS, the nebulizer must be cleaned weekly in an ultrasonic bath with a solvent compatible with the type of extract being analyzed, and the capillary and optical parts, including the octapole and lenses, follow the same principle.

After the application and evaluation of the methodology, batch determinations were carried out. Fifty injections were made in each of the batches. After each batch, the source was opened, photographed, and cleaned, and then the next batch was processed. All results were evaluated using the MassHunter Qualitative software, which uses the Find by MRM algorithm.

With these results, after the application of Find by MRM, the repeatability of the compounds was assessed by checking performance after this number of injections. Once it was confirmed that the results were within the expected range (up to 5% response variation), the second batch was processed, and so on until the ninth batch. The variation was assessed by plotting the chromatograms across the batches (Figure 1). As a result, it was possible to perform 450 injections with only routine cleaning of the ion source, without changing any consumables or cleaning/performing procedures on other parts of the equipment. After 350 analyses, a small loss of intensity was observed in the peaks, therefore, a maximum of 350 injections were estimated with the cleaning procedure adopted in this study.

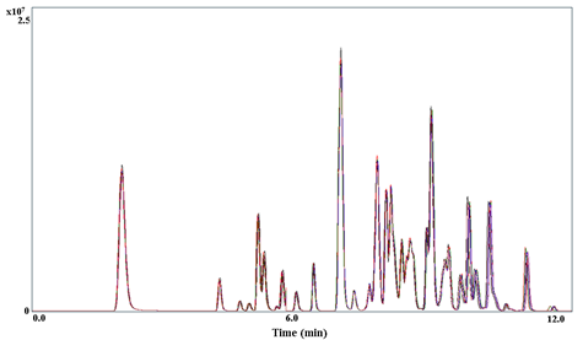

Figure 3. Overlay of chromatograms from nine batches of 50 grape juice samples each.

To obtain an accurate count of the number of samples that can be analyzed using this technique, it is necessary to evaluate the maximum number of injections that the column, pre-column, needle seat, valve rotor, and nebulizer needle can withstand before determining the exact number the MS can perform.

Although robustness information for other items was unavailable, it was determined that the system is robust even with a simple intervention on the ion source. With this information, we could then compare the real gain in terms of time and cost between sample preparation with the most commonly used techniques and the dilute-and-shoot technique.

4. Conclusion

The study shows that using direct injection with minimal sample preparation (just 50% dilution and filtration) is an effective and efficient method for detecting pesticides in grape juice, allowing up to 350 consistent injections. While further research is needed to refine certain variables, the approach marks a significant step forward in analytical methods for beverage safety, particularly in the wine industry.

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