BIO Web of Conferences **7**, 04004 (2016) DOI: 10.1051/bioconf/20160704004
39th World Congress of Vine and Wine

Pesticide residues screening in wine by mass spectrometry

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Abstract. Recently, a study (from PAN Europe) covered 40 bottles of wine – 34 conventional and six organic ones – purchased inside the EU. According to the results, the 34 bottles of conventional wine together contained 148 pesticide residues. All 34 bottles contained from one to ten pesticides, bringing the average per bottle to more than four. Of the six bottles of organic wine tested, one sample contained a low concentration of a possibly carcinogenic pesticide. According to PAN Europe, the "contamination of wines is a direct result of over-reliance on pesticides in grape production". This study, between others, to prove the importance of develop methods sensivity and confident for pesticide detection in wine. A multi-residue method was developed for the determination ca of 250 pesticide residues in wine using Quechers extraction, gas chromatography-tandem mass spectrometry (GC-MS-MS) and liquid chromatography-tandem mass spectrometry (LC-MS-MS). The method was validated with the evaluation of follow parameters: Linearity, Precision, Accuracy, Matrix effect, Limit of detection and Limit of Quantification. The method was approved and was able to quantify pesticide residues in more than 60 samples of wine.

1. Introduction

In this paper, we report a rapid modified QuEChERS method for multiresidue analysis for ca of 300 pesticides in wine with good selectivity, sensitivity, and cost effectiveness. In order to demonstrate the suitability of the method for routine regulatory purposes, the method was validated and the statistical parameters are discussed.

2. Experimental

2.1. Reagents and standards

The certificated analytical standards were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Alemanha). All the solvents and chemicals used in the study were of HPLC grade.

2.2. Instrumentation

Ultra high-performance liquid chromatography UHPLC-MS/MS (liquid chromatography, Shimadzu and MSMS, API 4000 Applied Biosystems) equipped with Phenomenex Kinetex column (50 mm × 2.1 mm, 2.6 μm) was used for quantification of pesticides. The flow rate was 250 μL/min, the column temperature 30 °C, and the injection volume 5 μL. A binary gradient of 0.1% HCOOH and HCOONH 5 mM in water (A) and 0.1% HCOOH and HCOONH 5 mM in CHOH (B) was employed. The mobile-phase gradient was programmed as follows: 0 min, 20% B; 10 min, 80% B; 10.01 min, 90% B; 13 min, 90% B; 13.01 min, 20% B; and 16 min, 20% B. Mass spectral analyses were operated in the positive ion mode using a ESI interface. The electrospray ionization (ESI) needle spray voltage was 5000. The heated capillary was 500 °C. Collision Gas Pressure was 5. The pesticides were detected in MS/MS conditions, programming the chromatographic run in MRM mode (multiple reaction monitoring).

2.3. Method validation

2.3.1. Specificity

The specificity of the analytical method for pesticides detection was confirmed by obtaining positive results from honey containing the analyte, coupled with negative results from samples which do not contain it (negative controls). The matrix effect was assessed by preparing pesticide standards in blank matrix extracted from untreated honey. The matrix extracts were analyzed before spiking to confirm the absence of the test pesticides in them.

2.3.2. Linearity

The quantification of pesticide was based on a six-point matrix-matched calibration graph by plotting the detector response against concentration of the calibration standards within the range 2.5–50 μg/L making three replicates for each concentration. A linear regression of six calibration points for each component was used to determine the relationship with the analyte concentrations calculated for each component on the basis of their occurrence in the reference material.

2.3.3. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were determined by signal-to-noise approach.

2.3.4. Method Accuracy (Recovery) and Precision (Repeatability)

Method recovery studies were performed at two spiking concentration levels (10 μg/kg and 50 μg/kg).

2.3.5. Determination of uncertainties

Combined uncertainty in estimation was determined for all the pesticides at the two fortification levels studied (10 and 50 μg/kg) as the statistical procedure of the EURACHEM/CITAC Guide CG 4.

3. Results and discussion

3.1. Method validation

3.1.1. Specificity

The specificity of the method toward the studied analytes was good. No interferences due to matrixes were found. Hence, no further time-consuming concentration/cleanup pretreatments were required.

3.1.2. Linearity

The analytes showed linear behavior in the studied concentration range of 2,5–50 μg/L. The correlation coefficient (r^2) was found to be ≥ 0.99 for all pesticides.

3.1.3. LOD and LOQ

LOD and LOQ were estimated as the lowest concentrations of pesticide injected that yielded a signal/noise ratio of 3 and 10, respectively. The LOQs attained in the proposed method fit with maximum residue limits (MRLs) for grape. The limits were reported in Table 1.

3.1.4. Recovery and precision

The single-step extraction method adopted for wine samples provided satisfactory recovery. These data are in agreement with the criteria of document no. SANCO/12495/2011, that recommend general recovery limits of 70–120% within laboratory repeatability $\leq$ 20% [18]. Therefore, the method could be considered sufficiently accurate and precise for the purpose.

3.1.5. Uncertainty of measurement

The study of uncertainty was performed at 2 concentration levels (10 and 50 μg/kg), identifying and studying the most important parameters that determined the uncertainty of the analytical method. The parameters selected were point calibration, standard solution, weigh, volume, and precision; their contributions to method uncertainty were calculated as indicated in the experimental section. The different contributions of uncertainty for each concentration level, together with the relative combined standard uncertainty, are shown in Table 1.

3.2. Wine samples analysis

Sixty wine samples were analyzed between 2012 and 2015. The samples were from different wineries in the state of Rio Grande do Sul, Brazil. About 300 pesticides were analyzed and 30 were detected. 40 samples were determined at least one pesticide.

4. Conclusion

Based on these results, the methodology for the determination of pesticides in wine, described in this report, are validated and approved for execution.

Table 1. Results for LOQ, Recovery, Precision and Uncertainty of Measurement.

Pesticide	LOQ	Recovery	SD%	UM
3OHCarbofurano	0.01	76.62	3.41	0.0006
Acefato	0.01	82.82	6.78	0.0014
Acetamiprid	0.01	79.63	3.84	0.0008
Acrinatrin	0.01	86.37	7.00	0.0015
Aldicarb	0.01	84.07	5.43	0.001
AldicarbSulfone	0.01	78.10	4.83	0.0009
AldicarbSulfoxide	0.01	98.26	3.56	0.0009
Aletrina	0.01	73.38	4.31	0.0008
Ametrina	0.01	76.66	4.10	0.0008
Amicarbazone	0.01	86.39	6.91	0.0015
Azaconazole	0.01	96.63	4.33	0.0010
AzinfosEtil	0.01	84.89	6.80	0.001
AzinfosMetil	0.01	86.38	6.58	0.0014
Azoxistrobin	0.02	84.91	11.80	0.0025
Benalaxil	0.01	85.57	6.10	0.0013
Benfluralina	0.24	93.80	18.62	0.0249
Benfurocarb	0.02	97.09	8.73	0.0021
Bifentrina	0.01	81.91	3.68	0.0007
Bioaletrina	0.02	84.25	10.30	0.0022
Bitertanol	0.01	97.89	5.95	0.0014
Boscalide	0.02	87.39	9.69	0.0021
Bupirimato	0.01	91.68	5.13	0.0012
Buprofezin	0.01	85.86	6.13	0.0013
Cadusafos	0.01	82.20	3.55	0.0007
Carbaril	0.01	82.87	7.27	0.0015
Carbendazim	0.01	111.80	4.04	0.0011
Carbofenotion	0.01	94.27	5.32	0.0012
Carbofurano	0.01	85.51	5.19	0.0011
Carboxim	0.01	82.59	5.15	0.0011
Carpropamida	0.01	86.65	6.33	0.0014
CialofopPbutil	0.01	87.27	6.67	0.0014
Cianofenfos	0.01	81.22	6.47	0.0013
Cifenotrina	0.04	85.22	3.81	0.0040
Cimoxamil	0.01	88.61	5.28	0.0012
Cipermetrina	0.05	86.90	5.24	0.0056
Ciproconazol	0.03	85.41	13.68	0.0029
Ciprodinil	0.01	86.62	4.24	0.0009
Cletodim	0.01	102.43	5.64	0.0014
Clorfentezine	0.01	88.26	6.35	0.0014
Clorfenvinfos	0.01	85.74	7.27	0.0015
Clorfluazuron	0.01	92.12	5.56	0.0013
ClorimuromEtil	0.01	94.75	6.04	0.0014
Cloroprofan	0.01	88.52	6.92	0.0015
Clorpirifos	0.01	106.75	3.73	0.0010
ClorpirifosMetil	0.01	91.66	5.27	0.0012
Clortiofos	0.01	90.46	6.04	0.0014
Clotianidina	0.01	76.64	5.96	0.0011
Coumofos	0.01	90.80	5.01	0.0011
Cyazofamide	0.01	92.42	3.79	0.0009
Cyflutrina	0.25	94.80	15.75	0.0254
Cyromazine	0.01	79.56	5.54	0.0011

Table 1. Continued.

Pesticide	LOQ	Recovery	SD%	UM
Dazomete	0.01	91.45	5.28	0.0012
Deltametrina	0.06	87.60	5.28	0.0057
DemetonSmetil	0.01	88.41	4.28	0.0009
Diazinon	0.01	84.40	6.56	0.0014
Diclofluanid	0.01	88.32	5.89	0.0013
DiclofopMetil	0.01	80.41	5.56	0.0011
Dicloran	0.10	85.80	10.16	0.0108
Diclorvos	0.01	102.42	4.25	0.0011
Dicrotofos	0.01	74.14	4.38	0.0008
Difenoconazol	0.01	80.32	3.25	0.0006
Diflubenzuron	0.01	88.69	6.60	0.0015
Dimetoato	0.01	95.07	4.37	0.0010
Dimetomorf	0.02	97.24	8.48	0.0020
Diniconazol	0.01	92.81	5.23	0.0012
Dinocap	0.01	75.90	5.70	0.0011
Dinoterb	0.01	93.88	2.56	0.0006
DisulfotonSulfone	0.01	89.91	5.39	0.0012
DisulfotonSulfoxide	0.01	80.64	6.05	0.0012
Diuron	0.02	88.79	7.18	0.0016
Dodemorf	0.01	93.90	4.83	0.0011
EPN	0.01	89.27	3.35	0.0007
Epoxiconazol	0.01	87.79	5.77	0.0013
Etiofencarb	0.01	89.59	4.81	0.0011
EtiofencarbSulfone	0.01	88.13	5.54	0.0012
EtiofencarbSulfoxide	0.01	89.07	6.95	0.0015
Etion	0.01	81.64	5.31	0.0011
Etiprole	0.01	87.00	4.29	0.0009
Etofenprox	0.01	78.08	7.43	0.0014
Etoprofos	0.01	79.45	6.05	0.0012
Etoxisulfuron	0.01	77.72	7.66	0.0015
Etrinfos	0.01	89.74	6.84	0.0015
Famoxadone	0.02	91.24	7.93	0.0018
Fenamidone	0.01	92.50	6.59	0.0015
Fenamifos	0.01	87.67	6.66	0.0014
FenamifosSulfone	0.01	96.77	3.17	0.0008
FenamifosSulfoxide	0.01	87.90	7.01	0.0015
Fenarimol	0.02	88.04	9.60	0.0021
Fenazequin	0.01	92.67	5.14	0.0012
Fenbuconazol	0.01	91.22	4.37	0.0010
fenhexamid	0.01	87.00	5.23	0.0011
FenoxapopPetil	0.01	100.12	3.14	0.0008
Fenpiroximate	0.01	87.49	5.25	0.0011
Fenpoprimorf	0.01	80.88	3.21	0.0006
Fenpropatrina	0.04	87.74	3.68	0.0040
Fention	0.01	80.57	7.30	0.0015
FentionSulfone	0.01	88.90	5.67	0.0012
FentionSulfoxide	0.01	92.70	4.48	0.0010
Fenvalerato	0.02	74.26	2.46	0.0023
Fipronil	0.01	82.35	6.81	0.0014
FipronilSulfone	0.01	103.10	4.31	0.0011
Flazasulfuron	0.01	95.33	2.75	0.0006
Fluazifop	0.01	82.32	6.58	0.0013

Table 1. Continued.

Pesticide	LOQ	Recovery	SD%	UM
Fludioxonil	0.01	87.36	6.50	0.0014
Flufenacet	0.01	85.77	6.10	0.0013
Flufenoxuron	0.01	91.17	6.37	0.0014
Fluquinconazol	0.01	96.14	4.23	0.0010
Flusilazole	0.01	87.85	6.01	0.0013
Flutriafol	0.01	88.88	5.57	0.0012
Folpete	0.02	89.51	7.82	0.0017
Fostiazate	0.01	78.64	5.09	0.0010
Furatiocarb	0.01	82.58	5.54	0.0011
heptenofos	0.01	85.87	5.55	0.0012
Hexaconazol	0.01	80.70	5.64	0.0011
Hexazinona	0.01	80.06	3.16	0.0006
Hexitiazox	0.01	83.57	5.86	0.0012
Imazalil	0.01	84.93	5.82	0.0012
Imazapir	0.01	106.60	3.98	0.0011
Imibenconazole	0.01	90.69	5.74	0.0013
Imidacloprid	0.02	86.34	8.95	0.0019
Indoxacarb	0.02	88.54	9.08	0.0020
Iprovaliocarb	0.01	84.64	6.24	0.0013
Isocarbofos	0.00	94.30	1.63	0.0004
Isoxaflutole	0.01	84.04	7.09	0.0015
KresoximMetil	0.02	83.10	7.85	0.0016
LambdaCialotrina	0.04	84.32	3.69	0.0039
Linuron	0.01	88.29	4.74	0.0010
Lufenuron	0.01	84.25	6.33	0.0013
Malaoxon	0.01	81.94	4.80	0.0010
Malation	0.01	93.48	6.24	0.0014
Mepanipirim	0.01	88.54	6.30	0.0014
Mesotrione	0.02	71.01	10.86	0.0019
Metalaxil	0.02	87.78	11.02	0.0024
Metamidofos	0.01	74.31	4.31	0.0008
Meticonazol	0.01	79.50	6.44	0.0013
Metidation	0.01	89.60	6.81	0.0015
Metiocarb	0.01	86.63	6.96	0.0015
MetiocarbSulfone	0.01	91.07	3.77	0.0009
MetiocarbSulfoxide	0.002	96.04	0.97	0.0002
Metobromuron	0.01	90.39	5.71	0.0013
Metomil	0.01	79.63	5.63	0.0011
Metoxifenozide	0.02	78.95	8.88	0.0017
Metribuzim	0.02	87.65	7.51	0.0016
MetsulfuronMetil	0.01	82.32	6.16	0.0013
Mevinfos	0.01	76.26	4.34	0.0008
Miclobutanil	0.02	90.17	10.15	0.0023
Molinato	0.01	95.76	5.22	0.0012
Monocrotofos	0.01	74.38	6.73	0.0012
Monuron	0.01	83.13	3.87	0.0008
Napronamide	0.01	87.56	6.98	0.0015
Nitenpiram	0.01	103.73	3.80	0.0010
Nuarimol	0.01	88.85	6.78	0.0015
Ometoato	0.01	109.00	4.35	0.0012
Oxadixyl	0.01	109.70	4.12	0.0011
Oxamil	0.01	77.37	3.83	0.0007

Table 1. Continued.

Pesticide	LOQ	Recovery	SD%	UM
Oxasulfuron	0.01	87.33	6.58	0.0014
Oxicarboxin	0.01	78.08	4.12	0.0008
Paclobutrazol	0.01	90.82	6.36	0.0014
ParaoxonEtil	0.01	90.64	4.04	0.0009
ParaoxonMetil	0.01	92.17	4.84	0.0011
Penconazol	0.01	89.72	6.82	0.0015
Pencycuron	0.01	85.63	6.18	0.0013
pendimentalina	0.01	92.84	4.33	0.0010
Permetrina	0.03	75.66	3.12	0.0029
Phentoato	0.01	90.41	6.27	0.0014
Phorato	0.01	94.47	5.85	0.0014
Phosalone	0.01	81.96	6.90	0.0014
Phosfamidon	0.01	91.08	4.64	0.0010
Phosmet	0.01	93.78	4.62	0.0011
Picoxistrobin	0.01	85.13	3.83	0.0008
PiperonilButoxide	0.02	90.05	7.92	0.0018
Piraclostrobin	0.02	86.11	10.11	0.0022
Pirazofos	0.02	87.70	7.59	0.0016
Piridabem	0.01	104.04	4.42	0.0011
Piridafention	0.01	80.66	7.66	0.0015
pirimetanil	0.01	115.30	4.55	0.0013
Pirimicarb	0.01	91.07	4.64	0.0010
PirimifosEtil	0.01	87.26	7.04	0.0015
PrimifosMetil	0.01	86.15	5.75	0.0012
procloraz	0.01	82.78	7.32	0.0015
Profenofos	0.01	82.44	5.69	0.0012
promecarb	0.01	92.82	3.69	0.0008
prometrin	0.01	81.61	5.75	0.0012
Propargito	0.01	86.03	5.95	0.0013
Propiconazol	0.01	95.31	3.01	0.0007
propoxur	0.01	86.93	5.26	0.0011
Protiofos	0.01	88.49	5.95	0.0013
Pyretrins	0.01	90.07	4.57	0.0010
Pyrifenox	0.01	87.96	7.02	0.0015
Pyriproxifen	0.01	77.62	3.18	0.0006
Simazina	0.01	93.11	4.35	0.0010
Spirodiclofeno	0.01	89.87	6.08	0.0014
Spiroxamine	0.01	87.50	7.10	0.0015
Spnosad	0.01	80.82	4.15	0.0008
SulfometuronMetil	0.01	89.85	5.64	0.0013
Sulfotep	0.01	93.42	6.11	0.0014
Sulprofos	0.01	86.28	6.26	0.0013
tebuconazol	0.02	102.06	9.66	0.0024
Tebufenozide	0.01	81.97	6.96	0.0014
Tebufenpirad	0.01	80.02	7.32	0.0015
Terbufos	0.01	87.97	6.49	0.0014
Tetraconazol	0.02	93.36	8.76	0.0020
Tetradifon	0.01	84.54	3.65	0.0008
Thiacloprid	0.01	85.31	6.54	0.0014
Thiometon	0.01	86.53	7.04	0.0015
Thionazin	0.01	98.07	4.77	0.0012
Tiabendazol	0.01	81.40	6.62	0.0013

Table 1. Continued.

Pesticide	LOQ	Recovery	SD%	UM
Tiametoxam	0.01	84.07	4.75	0.0010
Tiobencarb	0.01	90.90	6.02	0.0014
Tiobencarb	0.01	90.90	6.02	0.0014
Tiodicarb	0.01	78.12	3.77	0.0007
TiofanatoMetil	0.02	97.39	7.13	0.0017
Tolclofosmetil	0.01	90.08	6.25	0.0014
Tolifluanid	0.01	85.61	6.14	0.0013
triadimefon	0.01	89.35	6.98	0.0015
Triadimenol	0.02	92.27	7.17	0.0016
Triazofos	0.01	86.20	6.17	0.0013
Triclorfon	0.01	83.62	5.52	0.0011
Tricyclazol	0.01	80.28	3.20	0.0006
Tridemorf	0.01	87.59	7.07	0.0015
Triflimizole	0.01	87.35	6.86	0.0015
Trifloxitrobim	0.01	86.30	7.15	0.0015
Triflumuron	0.01	89.89	4.15	0.0009
Triticonazol	0.01	90.50	6.72	0.0015
Vamidation	0.00	73.20	2.21	0.0004

References

[1] European Commission Regulation (EC), "No. 396/2005 of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC," Official Journal of the European Union, vol. L70, pp. 1–16

[2] Document No. SANCO/12495/2011, Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed, `http://ec.europa.eu/food/plant/protection/pesticides/docs/qualcontrol_en.pdf`