Determination of 111 Pesticide Residues in Wines by Ultra High Performance Liquid Chromatography and Quadrupole Orbitrap High Resolution Mass Spectrometer

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Overview

Purpose: To demonstrate simultaneous screening and quantification of multi pesticide residues in different wine matrixes by utilizing the Thermo Scientific™ Q Exactive™ quadrupole-Orbitrap mass spectrometer.

Methods: The wine samples were extracted by acetonitrile (containing 0.1 % acetic acid) and purified by QuEChERS method. Then MS and MS^2 data was acquired with high resolution and accurate mass for qualitative and quantitative multi pesticide residues from many different matrixes using ultra-high performance liquid chromatography (UPLC) coupled to the Q Exactive mass spectrometer.

Results: 111 pesticide residues in different wine were subjected to simultaneous screening and quantification. The linearity of all the 111 pesticides ranged from 1 ng / mL to 100 ng / mL with correlation coefficients greater than 0.99. By detecting spiked samples, the limit of quantification of the method was 5 μg/kg for all the pesticides residues and the recovery was in the range of 63.3 % ~123.7 % with the RSD 3.2 % ~ 18.8 %.

Introduction

Wine as a kind of health-benefit alcohol drinking which has been accepted by more and more Chinese consumers. A large number of wines are imported into China from France, Italy, Spain, and other countries every year. It is laborious and time-consuming work for entry-exit inspection departments to quantify multi pesticide residues in wines by triple quadrupole mass spectrometer system according regulatory requirements. The Q Exactive instrument, a true high resolution and accurate mass (HR/AM) mass spectrometer, has been used for simultaneous screening and quantification of multi-pesticide residues and multi veterinary residues in various matrixes. Herein in this study, a fast and simple method for simultaneous screening and quantification of 111 pesticide residues in wines was developed for routine tests through the combination QuEChERS technology and UHPLC Q Exactive system.

Methods

Sample preparation

All pesticide standards (Table 1) were purchased from Sigma-Aldrich(Taufkirchen, Germany) and Dr. Ehrenstorfer(Augsburg, Germany). The wine samples were extracted by acetonitrile (containing 0.1 % acetic acid), and salted out by anhydrous magnesium sulphate and anhydrous sodium acetate. After concentration, the analyst was dissolved by methanol and water, then cleaned up by disperse solid-phase extraction (d-SPE) to reduce matrix interference.

Liquid Chromatography and Mass Spectrometry

A Thermo Scientific™ UltiMate 3000 UHPLC and Q Exactive mass spectrometer were used for data acquisition. Chromatography analysis was carried out by a C18 column (Phenomenex, 100 mm× 3.0mm, 2.6 μm) with H2O containing 5 mmol/L ammonium acetate solution (A) and acetonitrile (B) as the mobile phase in gradient elution program (Table 2). The flow rate was set at 0.40 mL /min. The injection volume was set at 10 μL.

Table 2 Gradient elution program for multi pesticide residues analysis

Time/min	A phase	B phase
0	90%	10%
2	90%	10%
5	10%	90%
10	10%	90%
11	90%	10%
13	90%	10%

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A H-ESI II source was coupled to a Q Exactive quadrupole Orbitrap mass spectrometer. Source conditions were set as: positive mode; spray voltage: 3000 V; sheath Gas: 35 arb; AUX gas: 10 arb; evaporator temperature: 300 ; capillary temperature: 350 ; scan range: 100-1000 Da. The experimental method was Full scan-ddMS2 mode. Resolving power was set at 70 k (FWHM at m/z 200) for MS1 scan while 17.5k for MS2 scan. The others parameters were set as: AGC target, 5E5(MS1), 5E5(MS2); MAX injection time: 100ms (MS1); 50ms (MS2); dynamic excluded 5s.

Data Analysis

Thermo Scientific™ Xcalibur™ platform was used for data analysis. Thermo Scientific™ QualBrowser™ software was used for initial spectra viewing while Thermo Scientific™ QuanBrowser™ for quan-process.

Table 1 Information table of 111 pesticides

No.	Compound	Molecular formula	Accurate mass, m/z	Precursor ion	RT, min
1	Methomyl	$C_5H_{10}N_2O_2S$	163.05357	$[M+H]^+$	3.82
2	Aldicarb sulfone	$C_7H_{14}N_2O_4S$	223.07525	$[M+H]^+$	4.87
3	Carbendiazim	$C_9H_9N_3O_2$	192.0773	$[M+H]^+$	4.75
4	Isoprocarb	$C_{11}H_{15}NO_2$	194.1181	$[M+H]^+$	5.77
5	Carbaryl	$C_{12}H_{11}NO_2$	202.0868	$[M+H]^+$	5.59
6	Fenobucarb	$C_{12}H_{17}NO_2$	208.13376	$[M+H]^+$	6.08
7	Aldicarb	$C_7H_{14}N_2O_2S$	213.2898	$[M+Na]^+$	5.18
8	Carbofuran	$C_{12}H_{15}NO_3$	222.11302	$[M+H]^+$	5.5
9	Pirimicarb	$C_{11}H_{18}N_4O_2$	239.1508	$[M+H]^+$	5.61
10	Imidacloprid	$C_9H_{10}ClN_5O_2$	256.06013	$[M+H]^+$	4.77
11	Diethofencarb	$C_{14}H_{21}NO_4$	268.15489	$[M+H]^+$	6.03
12	Phoxim	$C_{12}H_{15}N_2O_3PS$	299.06193	$[M+H]^+$	6.28
13	Thiophanate-methyl	$C_{12}H_{14}N_4O_4S_2$	343.05348	$[M+H]^+$	5.35
14	Tebufenozide	$C_{22}H_{28}N_2O_2$	353.22291	$[M+H]^+$	5.18
15	Methoxyfenozide	$C_{22}H_{28}N_2O_3$	369.21782	$[M+H]^+$	6.2
16	Indoxacarb	$C_{22}H_{17}ClF_3N_3O_7$	528.07857	$[M+H]^+$	6.66
17	Fluazuron	$C_{20}H_{10}Cl_2F_5N_3O_3$	506.00978	$[M+H]^+$	6.86
18	Diflubenzuron	$C_{14}H_9ClF_2N_2O_2$	311.0399	$[M+H]^+$	6.21
19	Chlorfluazuron	$C_{20}H_9Cl_3F_5N_3O_3$	539.97078	$[M+H]^+$	7.14
20	Diafenthiuron	$C_{23}H_{32}N_2OS$	385.23137	$[M+H]^+$	7.42
21	Chlortoluron	$C_{10}H_{13}ClN_2O$	213.07947	$[M+H]^+$	5.56
22	Thiobencarb	$C_{12}H_{16}ClNOS$	258.07194	$[M+H]^+$	6.73
23	Fenothiocarb	$C_{13}H_{19}NO_2S$	254.12147	$[M+H]^+$	6.39
24	Fenoxycarb	$C_{17}H_{19}NO_4$	302.13923	$[M+H]^+$	6.29
25	Pyributicarb	$C_{18}H_{22}N_2O_2S$	331.14802	$[M+H]^+$	7.18
27	Acetamiprid	$C_{10}H_{11}ClN_4$	223.07506	$[M+H]^+$	4.87
28	Triallate	$C_{10}H_{16}Cl_3NOS$	304.00966	$[M+H]^+$	7.47
29	Acephate	$C_4H_{10}NO_3PS$	184.01973	$[M+H]^+$	1.62
30	Diphenylamine	$C_{12}H_{11}N$	170.09697	$[M+H]^+$	6.34
27	Acetamiprid	$C_{10}H_{11}ClN_4$	223.07506	$[M+H]^+$	4.87
31	Carboxine	$C_{12}H_{13}NO_2S$	236.07452	$[M+H]^+$	5.67
32	Rotenone	$C_{23}H_{22}O_6$	395.14946	$[M+H]^+$	6.31
33	Pyrimethanil	$C_{12}H_{13}N_3$	200.11877	$[M+H]^+$	6.08
34	Flusilazole	$C_{16}H_{15}F_2N_3Si$	316.10817	$[M+H]^+$	6.19
35	Paclobutrazol	$C_{15}H_{20}ClN_3O$	294.13731	$[M+H]^+$	5.91
36	Buprofezin	$C_{16}H_{23}N_3OS$	306.16401	$[M+H]^+$	7.34
37	Metalaxyl	$C_{15}H_{21}NO_4$	280.15489	$[M+H]^+$	5.64
38	Benalaxyl	$C_{20}H_{23}NO_3$	326.17561	$[M+H]^+$	6.51
39	Furathiocarb	$C_{18}H_{26}N_2O_5S$	383.16408	$[M+H]^+$	7.01
40	Quinoclamine	$C_{10}H_6ClNO_2$	208.01654	$[M+H]^+$	5.38
41	Diniconazole	$C_{15}H_{17}Cl_2N_3O$	326.08269	$[M+H]^+$	6.39
42	Flonicamid	$C_9H_6F_3N_3O$	230.05412	$[M+H]^+$	4.44
43	Thiabendazole	$C_{10}H_7N_3S$	202.0439	$[M+H]^+$	5.01
44	Difenzoquatmethylsulfate	$C_{18}H_{20}N_2O_4S$	361.12221	$[M+H]^+$	3.37
45	Promecarb	$C_{12}H_{17}NO_2$	208.13376	$[M+H]^+$	6
46	Oxamyl	$C_7H_{13}N_3O_3S$	220.07559	$[M+H]^+$	4.68
47	Diphenamid	$C_{16}H_{17}NO$	240.13884	$[M+H]^+$	5.85
48	Tebufenpyrad	$C_{18}H_{24}ClN_3O$	334.16862	$[M+H]^+$	6.93
49	Mepronil	$C_{17}H_{19}NO_2$	270.1494	$[M+H]^+$	6.26
50	Chlorsulfuron	$C_{12}H_{12}ClN_5O_4$	358.03767	$[M+H]^+$	4.21
51	Allidochlor	$C_8H_{12}ClNO$	174.06857	$[M+H]^+$	5.31
52	Brodifacoum	$C_{31}H_{23}BrO_3$	523.09089	$[M+H]^+$	5.9
53	Pencycuron	$C_{19}H_{21}ClN_2O$	329.14207	$[M+H]^+$	6.68
54	Napropamide	$C_{17}H_{21}NO_2$	272.16505	$[M+H]^+$	6.26
55	Imazalil	$C_{14}H_{14}Cl_2N_2O$	297.05613	$[M+H]^+$	6.28
56	Hexythiazox	$C_{17}H_{21}ClN_2O_2S$	353.10905	$[M+H]^+$	7.23
57	Thiamethoxam	$C_8H_{10}ClN_5O_3S$	292.02711	$[M+H]^+$	4.41
58	Bensulfuron-methyl	$C_{16}H_{18}N_4O_7S$	411.09746	$[M+H]^+$	5.26
59	Chlorantraniliprole	$C_{18}H_{14}BrCl_2N_5O_2$	481.97862	$[M+H]^+$	5.84
60	Propamocarb	$C_9H_{21}N_2O_2Cl$	189.16031	$[M+H]^+$	2.81
61	Ethiofencarb	$C_{11}H_{15}NO_2S$	226.09018	$[M+H]^+$	5.67
62	Methiocarb	$C_{11}H_{15}NO_2S$	226.09018	$[M+H]^+$	6
63	Aldicarb sulfoxide	$C_7H_{14}N_2O_3S$	207.08034	$[M+H]^+$	2.32
64	Monolinuron	$C_9H_{11}ClN_2O_2$	215.05873	$[M+H]^+$	5.7
65	Linuron	$C_9H_{10}Cl_2N_2O_2$	249.01976	$[M+H]^+$	6.06
66	Isouron	$C_{10}H_{17}N_3O_2$	212.13991	$[M+H]^+$	5.28
67	Tebuthiuron	$C_9H_{16}N_4OS$	229.1123	$[M+H]^+$	5.08
68	Molinex	$C_9H_{17}NOS$	188.11091	$[M+H]^+$	6.26
69	3-Hydroxycarbofuran3-	$C_{12}H_{15}NO_4$	238.10794	$[M+H]^+$	4.68
70	Thiacloprid	$C_{10}H_9ClN_4S$	253.03148	$[M+H]^+$	5.12
71	Pyraclostrobine	$C_{19}H_{18}ClN_3O_4$	388.1064	$[M+H]^+$	6.6
72	6-benzylaminopurine6-	$C_{12}H_{11}N_5$	226.10927	$[M+H]^+$	4.83
73	Fenpyroximate	$C_{24}H_{27}N_3O_4$	422.20784	$[M+H]^+$	7.34
74	Metosulam	$C_{14}H_{13}Cl_2N_5O_4S$	418.01436	$[M+H]^+$	4.88
75	Metominostrobin	$C_{16}H_{16}N_2O_3$	285.12391	$[M+H]^+$	5.82
76	Desmedipham	$C_{16}H_{16}N_2O_4$	301.11882	$[M+H]^+$	5.92
77	Tebupirimfos	$C_{13}H_{23}N_2O_3PS$	319.12452	$[M+H]^+$	7.22
78	Norflurazon	$C_{12}H_9ClF_3N_3O$	304.04646	$[M+H]^+$	5.71
79	Bitertanol	$C_{20}H_{23}N_3O_2$	338.18684	$[M+H]^+$	6.24
80	Pymetrozine	$C_{10}H_{11}N_5O$	218.10382	$[M+H]^+$	3.23

81	Pyraclostrobine	$C_{19}H_{18}ClN_3O_4$	388.1064	$[M+H]^+$	6.6
82	Pyrazoxyfen	$C_{20}H_{16}Cl_2N_2O_3$	403.06163	$[M+H]^+$	6.42
83	Pyrazosulfuron-ethyl	$C_{14}H_{18}N_6O_7S$	415.10359	$[M+H]^+$	4.6
84	Fenamidone	$C_{17}H_{17}N_3OS$	312.11705	$[M+H]^+$	6.09
85	Flazasulfuron	$C_{13}H_{12}F_3N_5O_5S$	408.05894	$[M+H]^+$	6.42
86	Florasulam	$C_{12}H_8F_3N_5O_3S$	360.03782	$[M+H]^+$	4.47
87	Penoxsulam	$C_{16}H_{14}F_5N_5O_5S$	484.07139	$[M+H]^+$	4.82
88	Boscalid	$C_{18}H_{12}Cl_2N_2O$	343.04048	$[M+H]^+$	6.14
89	Metconazole	$C_{17}H_{22}ClN_3O$	320.15297	$[M+H]^+$	6.31
90	Cyclohexanecarboxamide	$C_{14}H_{17}Cl_2NO_2$	302.07145	$[M+H]^+$	6.12
91	Propaquizafop	$C_{22}H_{22}ClN_3O_5$	444.13261	$[M+H]^+$	6.88
92	Iprovalicarb	$C_{18}H_{28}N_2O_3$	321.21782	$[M+H]^+$	6.03
93	Amisulbrom	$C_{13}H_{13}BrFN_5O_4S_2$	465.96547	$[M+H]^+$	6.7
94	Cyazofamid	$C_{13}H_{13}ClN_4O_2S$	325.05259	$[M+H]^+$	6.41
95	Chloridazon	$C_{14}H_8Cl_2N_4$	222.04341	$[M+H]^+$	4.75
96	Fludioxonil	$C_{12}H_6F_2N_2O_2$	249.04756	$[M+H]^+$	7.23
97	Metrafenone	$C_{19}H_{21}BrO_5$	409.06483	$[M+H]^+$	6.83
98	Oxadixyl	$C_{14}H_{18}N_2O_4$	279.13447	$[M+H]^+$	6.96
99	Probenazole	$C_{10}H_9NO_3S$	224.03814	$[M+H]^+$	4.75
100	Simeconazole	$C_{14}H_{20}FN_3OSi$	294.14378	$[M+H]^+$	6.04
101	Thiodicarb	$C_{10}H_{18}N_4O_4S_3$	355.05684	$[M+H]^+$	5.37
102	Tricyclazole	$C_9H_7N_3S$	190.0439	$[M+H]^+$	4.9
103	Fenazaquin	$C_{20}H_{22}N_2O$	307.18104	$[M+H]^+$	7.58
104	Piperonyl butoxide	$C_{12}H_{15}O_3$	208.11083	$[M+H]^+$	3.82
105	Mandipropamid	$C_{23}H_{22}ClNO_4$	412.13157	$[M+H]^+$	6.05
106	Ivermectine	$C_{48}H_{74}O_{14}$	897.49708	$[M+Na]^+$	9.06
107	Abamectin	$C_{48}H_{72}O_{14}$	895.48143	$[M+Na]^+$	7.6
108	Eprinomectin	$C_{50}H_{75}NO_{14}$	936.50798	$[M+Na]^+$	7.16
109	Doramectin	$C_{50}H_{74}O_{14}$	921.49708	$[M+Na]^+$	8.12
110	Flumioxazin	$C_{19}H_{15}FN_2O_4$	355.10942	$[M+H]^+$	7.23
111	Spinosad	$C_{41}H_{65}NO_{10}$	732.4687	$[M+H]^+$	9.25

Results and Discussions

Sample Pretreatment

Due to simultaneous extraction of 111 pesticides including organophosphate, carbamate, benzoyl-urea, sulfonylurea, neonicotinoids and so on, it is necessary to consider the choice of extraction solvent, salts and dispersive solid phase extraction powder for QuEChERS process. After optimization experiments, the final choice was showed as following: 1) acetonitrile containing 0.1% acetic acid; 2) 0.8 g of magnesium sulfate and 0.5 g of sodium acetate used for removing water and salting out; 3) 20 mg of PSA as a dispersive SPE purification materials.

Data Acquisition

The full scan-ddMS2 scan mode was used for data acquisition. A selected mass range m/z 100-1000 was set up to obtain accurate mass for all pesticides while MS2 spectra of pesticides were trigged if the intensity of a certain pesticide in the inclusion list met or exceeded the threshold. The accurate mass of the precursor ion from the MS1 spectrum and that of fragment ions from MS2 spectrum could meet regulatory requirements for qualitative and quantitative purpose. Representative spectra are depicted in Fig 1.

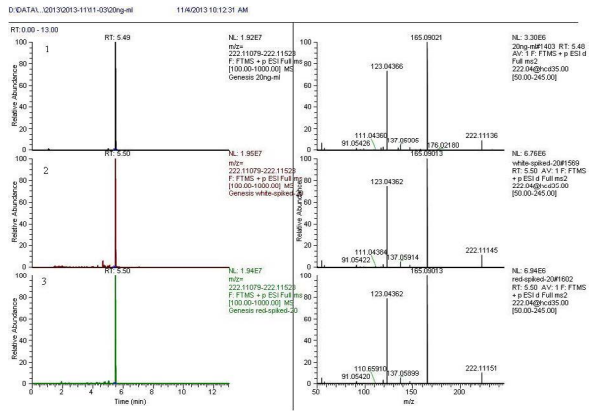

Fig.1 the chromatogram and two-stage full mass scan spectrum of carbofuran in standard solution and spiked samples of white wine and red wine（1: 20 ng/mL standard solution; 2: spiked sample of white wine, 20 μg/kg; 3: spiked sample of red wine, 20 μg/kg）

The linear range, limit of quantification, recovery and precision

Pesticide residues in wines were confirmed according to four criteria established by the pesticide standard: RTs, accurate m/z, fragments, and MS2 spectra. The linearity of all the 111 pesticides ranges from 1 ng/mL to 100 ng/mL with correlation coefficients greater than 0.99. By detecting spiked samples, the limit of quantification of the method was 5 μg/kg for all pesticide residues and the recovery was in the range of 63.3 % ~123.7 % with the RSD 3.2 % ~ 18.8 %.

Routing Sample testing

The established method was used for 50 wine samples routing analysis. Those samples include different color (white wine, red wine), different sweetness (dry wine, semi-dry wine, semi-sweet wine, sweet wine), different states (calm wine and sparkling wine), different origin (France, Portugal, the United States, Italy, Germany, Australia, Spain, China, Argentina, Chile, etc.) Six pesticides were detected from eight samples which was showed in Table 3.

Table 3 the pesticide residues detected in wines (μg/kg)

No.	type of wine	Origin	Carbendi azim	Thiophana te-methyl	pyrime thanil	Meta laxyl	boscalid	Iprova licarb
1	red wine	France	-	-	37.0	-	-	-
2	white wine	Italy	-	-	-	8.1	-	11.2
3	white wine	France	6.3	35.4	-	-	22.5	-
4	red wine	Italy	-	-	-	7.6	-	14.2
5	pink wine	Italy	-	-	-	11.8	-	-
6	red wine	Spain	-	-	-	14.3	-	-
7	sparkling white wine	France	-	-	-	9.0	-	-
8	sparkling pink wine	Germany	-	-	-	7.3	-	-

Conclusion

1) 111 pesticide residues in wines were simultaneously screened and quantified.
2) The linearity of all the 111 pesticides ranges from 1 ng/mL to 100 ng/mL with correlation coefficients greater than 0.99.
3) By detecting spiked samples, the limit of quantification of the method was 5 μg/kg for all the pesticides residues and the recovery was in the range of 63.3 % ~123.7 % with the RSD 3.2 % ~ 18.8 %.
4) This method can be used for high throughput screening and confirmation of multiple pesticide residues in wines.

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