Method OIV-MA-AS323-08 Method type II

Assay of pesticide residues in wine following extraction using the Quechers method

(Résolution Oeno 436/2012)

1. INTRODUCTION

Several reference documents were used in the preparation of this analysis method, which has been validated by a laboratory [1] [2].

2 –SCOPE

This method defines the steps involved in extracting pesticide residues in wine using the QuEChERS method *(Quick Easy Cheap Effective Rugged and Safe)* and the analysis of the extracts obtained by GC/MS and/or LC/MS-MS.

3 -PRINCIPLE

The sample is extracted using acetonitrile, followed by liquid-liquid partitioning induced by adding magnesium sulphate and sodium chloride and buffering with citrate salts. The extract is then purified using an amino adsorbent (dispersive SPE with APS and magnesium sulphate). To improve their stability during storage, the extracts are acidified by adding a small quantity of formic acid. The final extract may be used directly in the determination by GC/MS and LC/MS-MS.
For analyses by LC/MS-MS only, the dispersive SPE is not essential.

4. REAGENTS AND MATERIALS

4.1. General points and safety issues

Pesticides are potentially toxic, and safe handling practices must be implemented to protect the analysts, notably when preparing the stock solutions from commercially-available active ingredients.

Take all necessary precautions to prevent pesticide contamination of water, solvents, and other products.

Unless otherwise specified, the reagents used shall be of recognised analytical quality.

4.2 Water, HPLC quality

4.3 Acetonitrile [75-05-8] HPLC quality

4.4 Methanol [67-56-1], HPLC quality

4.5 Magnesium sulphate anhydrous [7487-88-9], particles

4.6 Magnesium sulphate, anhydrous [7487-88-9], fine powder

4.7 Sodium chloride [7647-14-5]

4.8 Disodium hydrogen citrate, sesquihydrate [6132-05-4]

4.9 Trisodium citrate dihydrate [6132-04-3]

4.10 Mixture of buffer salts for the extraction step:

Weigh 4 g of anhydrous magnesium sulphate in particle form, 1 g of sodium chloride, 1 g of trisodium citrate dihydrate and 0.5 g of disodium hydrogen citrate sesquihydrate and place these reagents in a flask. The prior mixing of salts avoids the formation of crystals.

4.11 Formic acid solution in acetonitrile

Dilute 0.5 mL of formic acid up to a volume of 10 mL with acetonitrile.

4.12 Primary and secondary amine (PSA) adsorbent

For exemple, Bondesil-PSA® 40 µm Varian N° 12213023 1

4.13 Internal standard solutions and quality-control standard solutions

Several compounds may be used as internal standards: for example triphenylphosphate [115-86-6] and triphenylmethane [519-73-3].

Use a quality control standard to indicate the extraction efficiency of the residues from the samples: for example tris(1,3dichloroisopropyl)phosphate or TCPP.

Solutions of a suitable concentration should be prepared.

Example: preparing the TCPP solution at 10mg/L

Place 1 mL of stock solution containing 500 mg/L of tris(1,3-dichloroisopropyl)phosphate in a 50 mL volumetric flask and make up to volume with acetonitrile.

4.14 Calibration ranges; standard solutions containing different active ingredients

4.14.1 Standard stock solutions

Prepare stock solutions with a concentration of active ingredients of 500 mg/l in a suitable solvent (acetone for example).
Store at -18°C.

4.14.2 Surrogate solutions

A mixture of active ingredients selected to suit the equipment used (GC or LC)

and to satisfy calibration range restrictions.

4.14.3 Calibration range

Standard solutions in acetonitrile

A calibration range is prepared from surrogate solutions with the objective of obtaining a calibration line from 20 to 500 µg/l.

Standard solutions in a wine matrix

From a wine that does not contain any active ingredients, a blank matrix is prepared in accordance with protocol 6.1.1, then supplemented with increasing quantities of active ingredients in order to produce a calibration line from 20 to 500 µg/l.

5. Equipment

5.2. Glassware and volumetric laboratory equipment:

5.2.1. 100 mL stoppered flasks

5.2.2. 50 mL and 12 mL single-use centrifuge tubes with screw-on stoppers
5.2.3. 10 mL class A graduated test tubes
5.2.4. 10 mL, 50 mL and 100 mL class A volumetric flasks
5.2.5. Piston-operated volumetric apparatus with delivered volumes ranging from 30 µl to 1,000 µl, checked in accordance with ISO 8655-6
5.2.6. 2 mL sampling syringes

5.3. Nylon microfilters with a pore size of 0.45 µm

5.4. Analytical balance

5.5. High-speed mixing device (such as a Vortex mixer)

5.6. Centrifuge for 50 mL and 12 mL tubes, capable of generating 3,000 g.

5.7. LC/MS-MS system, with an electrospray ionisation interface.

5.8. GC/MS system, fitted with suitable injection and detection devices (for example ion trap or triple quadrupole).

6. PROCEDURE

6.1. Preparing the samples.

6.1.1. Extraction using the QuEchERS method

Place 10 g or measure 10 mL of the sample (wine) into a centrifuge tube and add 10 mL of acetonitrile and 100 µL of a 10 mg/L solution of tris(1,3-dichloroisopropyl)phosphate. Shake vigorously for 1 minute. Pour the mixture of salts (4.10) into the centrifuge tube containing the liquid mixture.
Shake vigorously for 1 minute. Centrifuge for 5 minutes at 3,000 g.

Filter approximately 1 mL of the solution through Nylon 25 mm/45 µm filters in preparation for the LC-MS analysis.

6.1.2. Purifying the extract using an amino adsorbent ("dispersive SPE" with APS)

Place 6 mL of the acetonitrile phase described in 6.1.1. in a centrifuge tube containing 900 mg of magnesium sulphate in the form of a fine powder (4.6) and 150 mg of APS (4.12). Stopper the tube and shake vigorously for 30 s, then

centrifuge for 5 min at 3,000 g. Without pausing, isolate and acidify the extract purified in this way by adding 50 µl of formic acid solution (4.11).
The GC-MS analysis can then be performed.

NOTE: to minimise matrix effects, a solution of "protectant" agents can be added to the sample extracts and the calibration solutions [3]

To prepare a 10 mL solution of "protectant" agents:
Weigh out 15 mg of sorbitol, 300 mg of ethylglycerol and 100 mg of gluconolactone,
Add 2mL of water
Make up to 10 mL with acetonitrile.

20 µl of this solution is added to each flask containing the calibration solutions (1 mL) and sample extracts (1 mL).

6.2. Results and Calculations

6.2.1. Identification of the residues

The residues are identified by considering certain parameters:

- their retention time
- their mass spectrum
- the relative abundance of the ion fragments (it is advisable to operate with 1 or 2 MS/MS transitions and 2 or 3 ions in MS).

6.2.2. Quantification

The extracts obtained in 6.1.1 and 6.1.2 can be analysed using various instruments, parameters and columns. However, the conditions should be adapted for each compound depending on the instruments used in order to obtain the best sensitivities.

Use the standard solutions to prepare a 5-point calibration range to check the linearity for each active ingredient.

The concentration in mg/kg (or mg/L) for each substance identified is obtained directly from the calibration line.

6.2.3. Extraction efficiency

The extraction efficiency can be checked by adding a quality-control standard to the samples, TCPP for example (see 6.1.1).

The efficiency must be between 70 and 120%.

The efficiency results are not taken into consideration when correcting the levels of residues in wines, but do allow validation of the procedure.

7. RELIABILITY OF THE METHOD

The results of the validation carried out in accordance with MA-F-AS1-08-FIDMET [4] and MA-F-AS1-09-PROPER [5], are indicated in the table below.
The average recovery rates are between 70% and 120% (the spiking levels covered a concentration range from 0.020 mg/L to 0.200 mg/L)

7.1 Repeatability (expressed in CVr%)
Repeatability (expressed as CVr%) is on average equal to 10%.

7.2 Reproducibility (expressed in CV_R%)
Reproducibility (expressed as CV_R%) is on average equal to 30%.

	Recovery rate %.	CV_r%	CV_R%	HorRat
Metalaxyl	89	7	26	1.1
Chlorpyrifos ethyl	81	13	23	1.0
Tebuconazole	99	9	32	1.3
Cyprodinil	93	9	29	1.1
Tebufenozide	102	11	28	1.2
Fludioxonil	101	7	40	1.4
Benalaxyl	98	9	29	1.1
Cyproconazole	92	11	31	1.3
Tebufenpyrad	95	10	31	1.2
Pyraclostrobin	116	6	29	1.2
Vinclozolin	84	9	28	1.1
Mepanipyrim	82	11	30	1.1
Boscalid	95	7	28	1.1
Iprovalicarb	106	7	33	1.2
Iprodione	108	10	27	1.1
Procymidone	100	11	34	1.2
Pyrimethanil	75	12	27	1.0
Carbendazim	113	11	41	1.6
Fenbuconazole	94	6	48	2.0
Fenitrothion	90	13	36	0.7
Metrafenone	93	8	19	0.7
Penconazole	109	8	35	1.1
Flusilazole	93	8	37	1.3
Oxadixyl	86	8	37	1.3
Azoxystrobin	84	8	30	1.2
Dimethomorph	90	9	36	1.4
Fenhexamid	87	8	22	0.8

All results from inter-laboratory tests that have been conducted on reliability data are presented in **Appendix A**

8. BIBLIOGRAPHY

[1] P. Paya. J. Oliva. A. Barba. M. Anastassiades. D. Mack. I. Sigalova. B. Tasdelen; "Analysis of pesticides residues using the Quick Easy Cheap Affective Rugged and Safe (QuEChERS) pesticide multiresidue method in combination with gas and liquid chromatography and tandem mass spectroscopy detection". *Anal Bioanal Chem.* 2007.

[2] EN 15662: 2008 – Foods of plant origin - Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE – QuEChERS method; January 2009; AFNOR

[3]K. Mastovska. Steven J. Lehotay. and M. Anastassiades; "Combination of analyte protectants to overcome matrix effects in routine GC analysis of pesticides residues in food matrixes". *Anal. Chem.* 2005. 77. 8129-8137.

[4]MA-F-AS1-08-FIDMET. OIV: Reliability of Analytical Methods (resolution oeno 5/99).

[5]MA-F-AS1-09-PROPER. OIV: Protocol for the planning. performance and interpretation of performance studies pertaining to methods of analysis (resolution 6/2000).

FV 1410: Results of the inter-laboratory study

APPENDIX A

RESULTS OF THE RELIABILITY STUDY

This document presents the results of the validation study on the method of assay of pesticide residues in wine following extraction using QuEChERS (FV 1340).

The study was performed in accordance with the OIV documents MA-F-AS1-08-FIDMET and MA-F-AS1-09-PROPER

1 - Participating laboratories

Sixteen laboratories took part in the study:

LABORATOIRE INTER RHONE	France
INSTITUT FUR HYGIENE UND UMWELT	Germany
LABORATORIO AGROENOLÓGICO UNIVERSIDAD CATÓLICA DEL MAULE	Chile
AGRICULTURAL OFFICE OF BORSOD-ABAUJ-ZEMPLEN COUNTY	Hungary
PESTICIDE RESIDUE ANALYTICAL LABORATORY	Hungary
AUSTRIAN AGENCY FOR HEALTH AND FOOD SAFETY	Austria
COMPETENCE CENTER FOR PLANT PROTECTION PRODUCTS	Austria
LABORATOIRE DEPARTEMENTAL DE LA SARTHE	France
LABORATOIRE PHYTOCONTROL	France
BENAKI PHYTOPATHOLOGICAL INST. PESTICIDES RESIDUES LAB.	Greece
LABORATOIRE DUBERNET OENOLOGIE	France
ARPAL DIPARTIMENTO LA SPEZIA	Italy
ARPA VENETO – SERVIZIO LABORATORI VERONA	Italy
ARPALAZIO – SEZIONE DI LATINA	Italy
ANALAB CHILE S.A.	Chile
LABORATORIO REGIONAL DE LA CCAA DE LA RIOJA	Spain
SCL LABORATOIRE DE BORDEAUX	France
ARPA – FVG DIP. DI PORDENONE	Italy

2 - Samples - Active ingredients analysed

For this study. 12 samples were proposed:

- four red wines: A. B. G. H
- four white wines: C. D. I. J
- two port wines: E. K
- two muscat wines: F. L

The 27 active substances were determined across the 12 samples covering a concentration range of 0.015 mg/L to 0.200 mg/L (see table below).

	A – G *mg/L*	**B – H** *mg/L*	**C – I** *mg/L*	**D – J** *mg/L*	**E – K** *mg/L*	**F – L** *mg/L*
Metalaxyl	0.050	0.040	0.100	0.020		
Chlorpyrifos ethyl	0.100	0.040	0.200	0.020		
Tebuconazole	0.025	0.080	0.050	0.040		
Cyprodinil	0.050	0.040	0.100	0.020		
Tebufenozide	0.050		0.100			
Fludioxonil	0.025		0.050			
Benalaxyl	0.052	0.041	0.104	0.021		
Cyproconazole	0.054	0.086	0.108	0.043		
Tebufenpyrad	0.050	0.040	0.100	0.020		
Pyraclostrobin	0.050		0.100			
Vinclozolin		0.040		0.020	0.050	0.100
Mepanipyrim		0.080		0.040	0.025	0.050
Boscalid		0.080		0.040	0.100	0.200
Iprovalicarb					0.050	0.100
Iprodione		0.076		0.038	0.047	0.094
Procymidone		0.020		0.010		
Pyrimethanil		0.040		0.020		
Carbendazim				0.054		0.027
Fenbuconazole		0.080		0.040		

Fenitrothion		0.040		0.020		
Metrafenone		0.040		0.020		
Penconazole		0.016		0.008		
Flusilazole		0.040		0.020		
Oxadixyl	0.050				0.025	
Azoxystrobin	0.100				0.050	
Dimethomorph	0.100				0.050	
Fenhexamid	0.100				0.050	

4 - Statistical assessment

All raw results are presented in FV 1410.
In each table. eliminated or nonsensical values appear in a different font.

4.1 Eliminated values

Some values are eliminated before evaluation in the following cases:

- to evaluate the repeatability of the method we have used the principle of double-blind samples: some laboratories only gave a single result on paired samples. this value was eliminated (noted in the tables as "**xxx**")

- when the results are expressed in the format "less than" (noted in the tables as "**xxx**")

The COCHRAN and GRUBBS tests were successively applied to paired samples to eliminate abnormal variances on the one hand and abnormal extreme average values on the other. The values eliminated by both these tests appear in the tables as "**xxx**".

4.2 Repeatability - Reproducibility

The repeatability and reproducibility parameters are grouped in **Table 1.**

In this table the following items are indicated for each substance:

- n: number of tests selected
- average: results average

- TR%: average recovery rate
- CVr%: repeatability as a % of the average
- CV_R%: reproducibility as a % of the average
- PR CV_R%: reproducibility as a % calculated using the Horwitz equation (PR $CV\% = 2C^{-0.1505}$)
- HoR : HorRaT value (CV_R% / PR CV_R%)

The evaluation criteria selected are:

- recovery rate between 70% and 120%
- the results obtained under reproducibility conditions are compared to those predicted according to the Horwitz model using the HorRat value. Reproducibility values are deemed satisfactory when this ratio is less than or equal to 2
- repeatability is considered satisfactory when it does not exceed the value of 0.66 x the Horwitz reproducibility

TABLE 2: Reliability values

		Red wine 1	Red wine 2	White wine 1	White wine 2	Port	Muscat
metalaxyl	n	12	13	13	11	8	
	Average	0.051	0.041	0.105	0.033	0.014	
	TR%	102	103	82	69		
	CV_r%	6	8	6	9	5	
	CV_R%	26	26	17	26	33	
	$PRCV_R$%	25	26	22	27	30	
	HoR	1.1	1	0.9	0.6	1.1	
chlorphyriphos	n	9	12	11	11		
	Average	0.073	0.031	0.166	0.018		
	TR%	73	78	83	90		
	CV_r%	11	16	11	15		
	CV_R%	30	27	18	18		
	$PRCV_R$%	24	27	21	29		
	HoR	1.3	1	0.9	0.6		
tebuconazole	n	12	14	15	14		
	Average	0.025	0.078	0.05	0.04		
	TR%	100	98	100	100		
	CV_r%	6	10	10	9		
	CV_R%	37	30	30	31		
	$PRCV_R$%	28	23	25	26		
	HoR	1.3	1. 3	1.2	1.2		
cyprodinIl	n	15	14	13	14		
	Average	0.045	0.036	0.098	0.023		
	TR%	90	90	94	96		
	CV_r%	19	6	3	3		
	CV_R%	36	34	13	31		
	$PRCV_R$%	26	26	23	28		
	HoR	1.4	1.3	0.6	1.1		
tebufenozide	n	10		11			
	Average	0.049		0.106			
	TR%	98		106			
	CV_r%	16		6			
	CV_R%	25		30			
	$PRCV_R$%	25		22			
	HoR	1		1.3			

TABLE 2 (continued): Reliability values

		Red wine 1	Red wine 2	White wine 1	White wine 2	Port	Muscat
fludioxonil	n	10		11	10		
	Average	0.026		0.064	0.015		
	TR%	104		98	100		
	CV_r%	4		8	10		
	CV_R%	47		30	43		
	$PRCV_R$%	28		24	30		
	HoR	1.7		1.2	1.4		
benalxyl	n	12	12	12	12		
	Average	0.046	0.04	0.099	0.023		
	TR%	88	98	95	110		
	CV_r%	8	7	7	14		
	CV_R%	37	32	25	21		
	$PRCV_R$%	25	26	23	28		
	HoR	1.4	1.2	1.1	0.8		
cyproconazote	n	14	15	14	14		
	Average	0.049	0.08	0.095	0.042		
	TR%	91	93	95	98		
	CV_r%	23	7	7	7		
	CV_R%	36	32	25	33		
	$PRCV_R$%	25	23	23	26		
	HoR	1.4	1.4	1.1	1.3		
tebufenpyrad	n	15	14	14	12		
	Average	0.042	0.038	0.094	0.021		
	TR%	84	95	94	105		
	CV_r%	21	6	5	6		
	CV_R%	33	31	26	32		
	$PRCV_R$%	26	31	26	32		
	HoR	1.3	1.2	1.1	1.1		
Pyraclostrobin	n	8		9			
	Average	0.055		0.121			
	TR%	110		121			
	CV_r%	6		5			
	CV_R%	31		26			
	$PRCV_R$%	25		22			
	HoR	1.2		1.2			

TABLE 2 (continued): Reliability values

		Red wine 1	Red wine 2	White wine 1	White wine 2	Port	Muscat
Vinclozolin	n		10		9	11	11
	Average		0.031		0.020	0.039	0.08
	TR%		78		100	78	80
	CV_r%		8		10	14	4
	CV_R%		35		26	27	22
	$PRCV_R$%		24		29	26	23
	HoR		1.4		0.9	1	0.9
Mepanipyrin	n		12		13	10	11
	Average		0.063		0.028	0.022	0.046
	TR%		79		70	88	92
	CV_r%		8		24	5	7
	CV_R%		35		36	20	28
	$PRCV_R$%		24		27	29	25
	HoR		1.4		1.3	0.7	1.1
Boscalid	n	11	12		11	12	11
	Average	0.022	0.097		0.034	0.083	0.174
	TR%	105	121		85	83	87
	CV_r%	12	7		6	6	4
	CV_R%	45	30		26	16	17
	$PRCV_R$%	28	23		27	23	21
	HoR	1.6	1.3		1	0.7	0.8
Iprovalicarb	n	11	12			13	13
	Average	0.016	0.016			0.052	0 ?1
	TR%	107	114			104	100
	CV_r%	9	8			5	6
	CV_R%	39	38			28	27
	$PRCV_R$%	30	30			25	23
	HoR	1.3	1.3			1.1	1.2
Iprodione	n		10		10	10	8
	Average		0.079		0.039	0.053	0.101
	TR%		104		103	113	107
	CV_r%		10		7	10	13
	CV_R%		35		24	25	17
	$PRCV_R$%		23		26	25	22
	HoR		1.5		0.9	1	0.8

TABLE 2 (continued): Reliability values

		Red wine1	Red wine 2	White wine 1	White wine 2	Port	Muscat
Procymidone	n		11		11		
	Average		0.018		0.011		
	TR%		90		110		
	CV_r%		12		10		
	CV_R%		34		34		
	$PRCV_R$%		29		31		
	HoR		1.2		1.1		
Pyrimethanil	n		15	10	14		
	Average		0.036	0.011	0.027		
	TR%		60	46	120		
	CV_r%		9	20	7		
	CV_R%		26	31	25		
	$PRCV_R$%		26	31	28		
	HoR		1	1	0.9		
Carbendazim	n				8		9
	Average				0.057		0.033
	TR%				106		120
	CV_r%				11		10
	CV_R%				36		45
	$PRCV_R$%				25		27
	HoR				1.5		1.7
Fenbuconazole	n		8		7		
	Average		0.067		0.042		
	TR%		84		105		
	CV_r%		6		5		
	CV_R%		45		50		
	$PRCV_R$%		24		26		
	HoR		1.9		2		
Fenitrothion	n		11		10		
	Average		0.034		0.019		
	TR%		85		95		
	CV_r%		16		10		
	CV_R%		31		40		
	$PRCV_R$%		27		29		
	HoR		1.2		1.4		

TABLE 2 (continued): Reliability values

		Red wine 1	Red wine 2	White wine 1	White wine 2	Port	Muscat
Metrafenone	n		7		7		
	Average		0.038		0.018		
	TR%		95		90		
	CV_r%		8		7		
	CV_R%		18		19		
	$PRCV_R$%		26		29		
	HoR		0.7		0.6		
Penconazole	n		14		13		
	Average		0.017		0.009		
	TR%		106		113		
	CV_r%		8		8		
	CV_R%		31		38		
	$PRCV_R$%		30		33		
	HoR		1		1.2		
Flusilazole	n		13		13		
	Average		0.035		0.019		
	TR%		88		95		
	CV_r%		6		9		
	CV_R%		37		36		
	$PRCV_R$%		26		29		
	HoR		1.4		1.2		
Oxadixyl	N	7				10	
	Average	0.04				0.023	
	TR%	80				92	
	CV_r%	10				5	
	CV_R%	18				31	
	$PRCV_R$%	26				28	
	HoR	0.7				1.1	
Azoxystrobine	N	12				13	
	Average	0.078				0.045	
	TR%	78				90	
	CV_r%	10				6	
	CV_R%	29				31	
	$PRCV_R$%	23				26	
	HoR	1.2				1.2	

TABLE 2 (continued): Reliability values

		Red wine 1	Red wine 2	White wine 1	White wine 2	Port	Muscat
Dimethomorph	N	12		9	9	13	
	Average	0.086		0.019	0.019	0.047	
	TR%	86				94	
	CV_r%	6		8	14	8	
	CV_R%	30		41	44	29	
	$PRCV_R$%			29	29	25	
	HoR			1.4	1.5	1.2	
Fenhexamid	N	11		11	10	11	
	Average	0.083		0.026	0.025	0.039	
	TR%	83		96	93	78	
	CV_r%	7		9	10	7	
	CV_R%	31		18	19	18	
	$PRCV_R$%	23		28	28	26	
	HoR	1.3		0.6	0.7	0.7	