RESOLUTION OIV-OENO 262-2014

PVI/PVP ADSORBENT COPOLYMERS – CODEX

THE GENERAL ASSEMBLY,

CONSIDERING article 2 paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

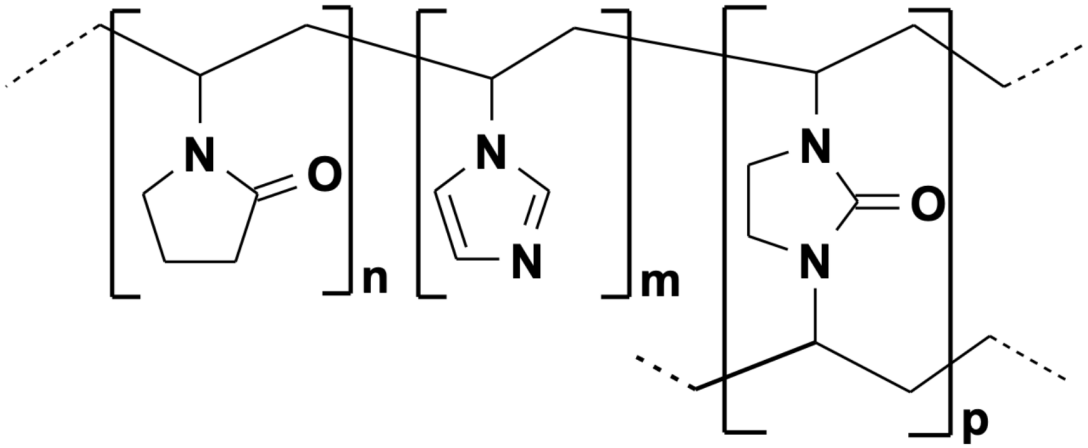
FURTHER to the proposal of the Sub-commission of Methods of Analysis of Wine, and the "Specifications of Oenological Products" group of experts;

CONSIDERING the work of the "Food Safety" group of experts and the favourable opinion given by the group of experts “Food Safety” in its 21th Session on 27 March 2014,

CONSIDERING OENO resolutions 1/2007 and 2/2007 concerning oenological practices

DECIDES to supplement the International Oenological Codex with the following monograph and to modify the corresponding sheets of the International Code of Oenological Practices accordingly:

ADSORBENT COPOLYMERS OF POLYVINYLIMIDAZOLE /POLYVINYLPYRROLIDONE (PVI/PVP)



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(C6H9NO)n(C5H6N2)m(C7H10N2O)p

CAS No. 87865-40-5

1. OBJECT, ORIGIN AND SCOPE OF APPLICATION

Adsorbent copolymers of PVI/PVP are insoluble and slightly-hygroscopic powders. They are manufactured by "popcorn" polymerization of N-vinylimidazole (CAS no. 1072-63-5,) and N-vinyl-2-pyrrolidone (CAS no. 88-12-0,), with a ratio of 9:1. N,N´-divinylimidazolidin-2-one (CAS no. 13811-50,) is used as crosslinking agent at a level of less than 2% by weight of the total amount of the monomers.

Adsorbent copolymers of PVI/PVP are added to must or wine in accordance with the files described in the Code of Oenological Practices of the OIV in amounts of less than 500 mg/l.

Adsorbent copolymers of PVI/PVP can be added to must or wine in order to prevent the defects caused by excessive metal contents or to reduce undesirably-high metal concentrations.

The must or wine must be filtered through a filter media with pores whose diameter is no greater than 3 microns and with a filtration pressure no greater than 0.8 bars.

2. SYNONYMS

Terpolymer of 1-vinylimidazole, 1-vinylpyrrolidone, and 1,3-divinylimidazolidinone.

Cross-linked copolymer of vinylimidazole/vinylpyrrolidone.

3. LABELLING

The labelling must indicate that the PVI/PVP adsorbing copolymer is for oenological use. The storage and safety conditions must also be indicated.

The label must mention a 3-year use-by date.

4. CHARACTERS

Powder with a white to yellowish colour.

PVI/PVP adsorbing copolymers are insoluble in practically all current solvents. It is therefore impossible to measure the molecular weight.

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5. TESTS

5.1. Loss on desiccation

Tare a metal capsule 50 mm in diameter. Place in the recipient between 0.8 and 1.4 g of PVI/PVP adsorbent copolymer, homogenised beforehand and weighed preciselyin a closed balance. Dry in a drying oven at 140°C ± 5°C for 1 hour. Allow to cool in a desiccator. Weigh again.

The loss on desiccation must be less than 5 %.

5.2. Ash

Heat a porcelain crucible until it is dark red; allow to cool in a desiccator and weigh. Place 1.5 g of PVI/PVP adsorbent copolymer in the crucible and incinerate at a constant weight in a muffle furnace at 800°C ± 25°C, allowing the crucible to cool in a desiccator after each incineration, the duration of the first incineration being 6 hours. If necessary, pre-incinerate the sample.

The weight of the ash must be less than 0.02 %.

5.3. Preparation of the solution for tests:

After weighing the ash, dissolve it in 1 ml of concentrated hydrochloric acid (R) and 10 ml of distilled water. Heat to activate dissolution. Make up to 20 ml with distilled water. 1 ml of this solution contains the mineral matter of 0.075 g of PVI/PVP adsorbent copolymer.

5.4. Zinc

Using the solution for tests prepared as in point 5.3, measure zinc according to the method described in Chapter II.

The zinc content must be less than 1 mg/kg.

5.5. Iron

Using the solution for tests prepared as in point 5.3, measure iron according to the method described in Chapter II.

The iron content must be less than 1 mg/kg.

5.6. Copper

Using the solution for tests prepared as in point 5.3, measure copper according to the

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method described in Chapter II.

The copper content must be less than 1 mg/kg.

5.7. Lead

Using the solution for tests prepared as in point 5.3, measure lead according to the method described in Chapter II.

The lead content must be less than 2 mg/kg.

5.8. Cadmium

Using the solution for tests prepared as in point 5.3, measure cadmium according to the method described in Chapter II.

The cadmium content must be less than 1 mg/kg.

5.9. Arsenic

Do not use the solution for tests prepared as in point 5.3.

Determine the arsenic according to the method described in Chapter II.

The arsenic content must be less than 2 mg/kg.

5.10. Mercury

Do not use the solution for tests prepared as in point 5.3.

Determine the mercury according to the method described in Chapter II.

The mercury content must be less than 1 mg/kg.

5.11. Organic impurities

Determine the organic impurities according to the method described in Appendix 1.

The limits of organic impurities must be as follows:

- The vinylpyrrolidone content must be less than 5 mg/kg
- The vinylimidazole content must be less than 10 mg/kg
- The divinylimidazolidinone content must be less than 2 mg/kg
- The pyrrolidone content must be less than 50 mg/kg
- The imidazole content must be less than 50 mg/kg

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5.12. Measurement of total nitrogen

Place approximately 450 mg of PVI/PVP adsorbing copolymer (test portion m mg) in a mineralisation flask, add 10 g of Missouri Catalyst[1] , and 3 glass beads. Wash all the particles that adhere to the neck of the flask with a small quantity of sulphuric acid (R). Add in total 20 ml of sulphuric acid (R), running it along the walls of the flask, and mix the contents by rotation. Continue the analysis according to the method described in Chapter II.

The total nitrogen content must lie between 26.0 and 29.0% with respect to the dry weight.

5.13. Solubility in an aqueous medium

Place 10 g of PVI/PVP adsorbent copolymer in a graduated 200-ml flask containing 100 ml of water. Shake the bottle and allow the contents to rest for 24 hours. Filter on a filter membrane with 2.5 µm diameter pores, and then on a filter membrane with 0.8 µm diameter pores. The dry residue remaining after evaporation of the filtrate on a water bath must be less than 0.5%.

5.14. Solubility in acid and alcohol

Introduce 1 g of PVI/PVP adsorbent copolymer into a bottle containing 500 ml of the following mixture:

Acetic acid	3 g
Ethanol	10 ml
Water	100 ml

Allow to rest for 24 hours. Filter on a filter membrane with 2.5 µm diameter pores, then on a filter membrane with 0.8 µm diameter pores. Concentrate the filtrate on a water bath. Finish the evaporation on a water bath in a calibrated silica capsule 70mm in diameter. The dry residue remaining after evaporation must be less than 1%, taking into account all the residue of the evaporation of the 500 ml of the mixture of acetic acid, ethanol and water.

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5.15. Determination and content of monomers in musts and wines

5.15.1. Analytical method

Proceed with the determination according to the analytical method in Appendix 2

5.15.2. Limits of monomers in musts and wines[2]

The vinylpyrrolidone content must be less than 10 µg/l
The vinylimidazole content must be less than 10 µg/l
The pyrrolidone content must be less than 25 µg/l
The imidazole content must be less than 150 µg/l

6. STORAGE

The PVI/PVP adsorbing copolymer must be kept in a cool place. The recipients must be dry and hermetically sealed.

Appendix 1

Determination by gas chromatography of the constitutive monomers and/or impurities liable to be found in copolymers of vinylpyrrolidone-vinylimidazole (vinylimidazole, vinylpyrrolidone, pyrrolidone, divinylethyleneurea and imidazole)

1. Principle

Detection and determination of the constitutive monomers and/or impurities liable to be found in copolymers of vinylpyrrolidone-vinylimidazole (vinylimidazole, vinylpyrrolidone, pyrrolidone, divinylethyleneurea and imidazole).

The analysis is carried out by capillary gas chromatography using a nitrogen specific detector (NSD). The substances to be analysed are extracted beforehand from the polymer by acetone.

2. Range of contents to be determined

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Vinylimidazole	2-55 µg/g
Vinylpyrrolidone:	2-50 µg/g
Pyrrolidone:	2-70 µg/g
Divinylethyleneurea:	2-33 µg/g
Imidazole:	2-50 µg/g

3. Reagents and reference material

3.1. Vinylpyrrolidone-vinylimidazole copolymers;
3.2. Vinylimidazole, M(C5H6N2) = 94.12 g/mol
purity > 99% (GC), e.g. Fluka, item no. 95005
(R: 22-34, S: 26-36/37/39-45)
3.3. Vinylpyrrolidone (1-vinyl-2-pyrrolidone), M(C_6H_9NO) = 111.14 g/mol
purity = 99.8% (GC), e.g. Fluka, item no. 95060
(R: 20/21/22-36/37/38-40, S: 26-36/37/39)
3.4. Pyrrolidone, (2-pyrrolidone), M(C_4H_7NO) = 85.11 g/mol
purity > 99% (GC), e.g. Fluka, item no. 83300
(R: 36/37/38, S: 26-36)
3.5. Divinylethyleneurea (N,N-divinylimidazolidone), M($C_7H_{10}N_2O$) = 138.17 g/mol
purity ≥ 99% (GC), BASF reference material
(R: 36/38-40, S: 26-36/37)
3.6. Imidazole, ($C_3H_4N_2$) = 68.08 g/mol
purity > 99.5% (GC), e.g. Fluka, item no. 56748
(R: 22-34, S: 26-36/37/39-45)
3.7. Benzonitrile,
purity > 99% (G), e.g. Merck-Schuchardt, item no. 801800
(R: 10-35, S: 23-26-45)
3.8. Acetone,
purity ≥ 99% (GC), e.g. Fluka, item no. 00585
(R: 11, S: 9-16-23-33)

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4. Apparatus

4.1. Capillary gas chromatograph with an automatic sampler, split injector, nitrogen specific detector (NSD).

4.2. Fused silica capillary column, with a polyethylene glycol film, (e.g. DB-Wax, J&W Scientific)

- Length: 30 m
- Internal diameter: 0.25 mm
- Film thickness: 0.5 µm

4.3. Data acquisition and processing system

4.4. Analytical balance accurate to 0.1 mg

4.5. Laboratory glassware and standard apparatus

4.6. Rotary mixer capable of housing small-capacity flasks, e.g. 50 ml.

5. Solutions

5.1. Internal standard solution

Benzonitrile, 250 µg/ml in acetone (3.8)

5.2. Stock calibration solution

Prepare a stock calibration solution of different concentrations in acetone (3.8) containing vinylimidazole, vinylpyrrolidone, pyrrolidone, divinylethyleneurea and imidazole with amounts ranging from 250 mg/l to 1000 mg/l.

5.3. Calibration solutions

Prepare at least two calibration solutions with different concentrations in acetone (3.8). Each solution must contain a suitable quantity of the internal standard as well as vinylimidazole, vinylpyrrolidone, pyrrolidone, divinylethyleneurea and imidazole so that the calibration points include the values currently being measured.

Example: 4 µl-200 µl of stock solution (5.2) + 24 ml of acetone (3.8) + 1 ml of internal standard solution (5.1).

6. Example of chromatographic conditions

Temperatures:

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Injector: 220°C
Oven: 160°C

- then programmed at a rate of 5°C/min up to 210°C

Final isothermal period: 210°C, 7 min
Detector (NSD): 250°C
Carrier gas: helium
Column head pressure: 140 kPa (1.4 bar)
Split flow: 10 ml/min
Septum purge: 5 ml/min
Volume injected: 1.0 µl

7. Preliminary check of the analytical system

7.1. Resolution

Prepare a solution of benzonitrile and vinylimidazole (10 and 2 µg/ml in acetone).

Inject this solution into the chromatograph under the conditions described in 6.

The analysis is considered satisfactory when the resolution of the two chromatographic peaks is at least 1.5 (R > 1.5), with a return to the baseline between the two peaks.

7.2. Sensitivity

To check the sensitivity:

1. Carry out a preliminary analysis of a sample (8.1) under the conditions described in section 6.
2. Add to the sample 2 µg/g divinylethyleneurea then repeat the analysis under the conditions described in section 6.

If the sample does not contain divinylethyleneurea the system is suitable when the peak of added divinylethyleneurea presents a signal-to-noise ratio of at least 10.

If the sample contained divinylethyleneurea a clear increase in the signal should be observed.

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8. Procedure

8.1. Preparation of the samples

Weigh about 2g of sample, accurate to 0.1 mg, then mix it with 1 ml of internal standard solution (5.1) and 24 ml of acetone (3.8). Extract the sample for 4 h on the rotary mixer (4.6) then analyse the supernatant solution under the conditions described in point 6.

For routine determinations, analyse each sample twice.

8.2. Chromatograms

Extracted by acetone from a copolymer (fig. 1)

Extracted by acetone from a copolymer supplemented with analytes (fig. 2)

9. Calculation

9.1. Calibration factor

Chromatographic calibration factor f(i):

$$f(i) = \frac{A(i)_0 \times m(I.S.)_0}{m(i)_0 \times A(I.S)._0}$$

where:

$A(i)_0$= peak area of analyte i in the chromatogram for the calibration solution (mVs)

$m(i)_0$= initial weight of reference product i in the calibration solution [mg]

$A(I.S)._0$= peak area of the internal standard in the chromatogram of the calibration solution (mVs)

$m(I.S.)_0$= initial weight of the internal standard in the calibration solution [mg]

The weight ratio w(i) of analyte i is calculated in the following way:

$$w(i) = \frac{A(i) \times m(I.S.)}{A(I.S.) \times m(s) \times f'(i)}$$

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where:

w(i) = weight ratio of the analyte i [µg/g]

A(i) = peak area of the analyte I in the chromatogram of the sample solution (mVs)

A(I.S.) = peak area of the internal standard in the chromatogram of the sample solution (mVs)

m(I.S.) = initial weight of the internal standard added to the sample [µg]

m(s) = initial weight of sample [g]

f'(i) = average chromatographic calibration factor

For routine determinations, the result is expressed as a whole number.

10. Characteristics of the method

10.1. Specificity, selectivity

In the chromatogram, the peaks are identified according to their retention time in comparison with the retention time of the solutions of pure analytes (3.2 to 3.6) injected under the same conditions.

Check that the components of the sample have a retention time different from that of the internal standard and that the resolution between peaks is always greater than 1.5.

10.2. Linearity

During calibration, the calibration factors were determined at 6 levels of concentration for each analyte. The calibration curves are straight lines (cf. fig. 3-7) with the following coefficients of determination:

Vinylimidazole	$R^2 = 0.9987$
Vinylpyrrolidone	$R^2 = 0.9999$
Pyrrolidone	$R^2 = 0.9956$
Divinylethyleneurea	$R^2 = 0.9937$
Imidazole	$R^2 = 0.9982$

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10.3. Limit of quantification

The calibration measurements were used to determine the following limits of quantification:

Vinylimidazole	2 µg/g
Vinylpyrrolidone	2 µg/g
Pyrrolidone	2 µg/g
Divinylethyleneurea	2 µg/g
Imidazole	2 µg/g

10.4. Precision

To determine the precision under repeatability conditions, a copolymer sample was analysed 6 times: (Table 1)

Table 1

		Vinylimidazole	Vinylpyrrolidone	Pyrrolidone	Divinylethyleneurea	Imidazole
1. Determination	[µg/g]	nq*	nd**	4.1	nd	10.7
2. Determination	[µg/g]	nq	nd	4.3	nd	10.8
3. Determination	[µg/g]	nq	nd	4.2	nd	11.5
4. Determination	[µg/g]	nq	nd	4.3	nd	11.8
5. Determination	[µg/g]	nq	nd	3.9	nd	10.2
6. Determination	[µg/g]	nq	nd	3.9	nd	10.8
Average	[µg/g]	nq	nd	4.1	nd	11.0

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Standard deviation	[µg/g]			0.2		0.6
Coeff. of variation	%			4.8		5.1
Measurement uncertainty	[µg/g]			0.6		1.7
Relative measurement uncertainty	%			14		15

*nq = not quantifiable

**nd= not detectable

In the sample, the vinylpyrrolidone and divinylethyleneurea could not be detected and the vinylimidazole could not be quantified.

10.4.1. Repeatability

The copolymer sample was supplemented with all the analytes then analysed 6 times. The accuracy under repeatability conditions can be deduced from the repeatability for vinylpyrrolidone, divinylethyleneurea and vinylimidazole. (Table 2)

Table 2

		Vinylimidazole	Vinylpyrrolidone	Pyrrolidone	Divinylethyleneurea	Imidazole
1. Determination	[%]	102.3	112.4	97.0	103.3	90.7
2. Determination	[%]	98.5	101.9	89.6	102.1	91.7
3. Determination	[%]	111.8*	111.5	105.7	111.1	112.6*
4. Determination	[%]	102.7	103.3	91.9	104.8	94.5
5. Determination	[%]	104.2	101.0	89.3	102.7	97.0
6. Determination	[%]	100.4	104.9	90.4	110.3	95.4
Average	[%]	101.6	105.8	94.0	105.7	93.9

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Standard deviation	[%]	2.2	4.9	6.4	3.9	2.6
Coeff. of variation	[%]	2.2	4.7	6.8	3.7	2.8
Measurement uncertainty	[%]	6.6	14.8	19.2	11.8	7.8
Relative measurement uncertainty	[%]	7	14	20	11	8

* = outlier value according to the Dixon test

10.5. Addition recovery

The recovery can be calculated from table 2.

Vinylimidazole:	101.6%
Vinylpyrrolidone:	105.8%
Pyrrolidone:	94.0%
Divinylethyleneurea:	105.7%
Imidazole:	93.9%

Note

Applicability to other copolymers of vinylpyrrolidone-vinylimidazole

The method was validated for Divergan HM. In principle, we can consider the determination is also valid for other copolymers of vinylpyrrolidone-vinylimidazole.

Appendices

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Appendices

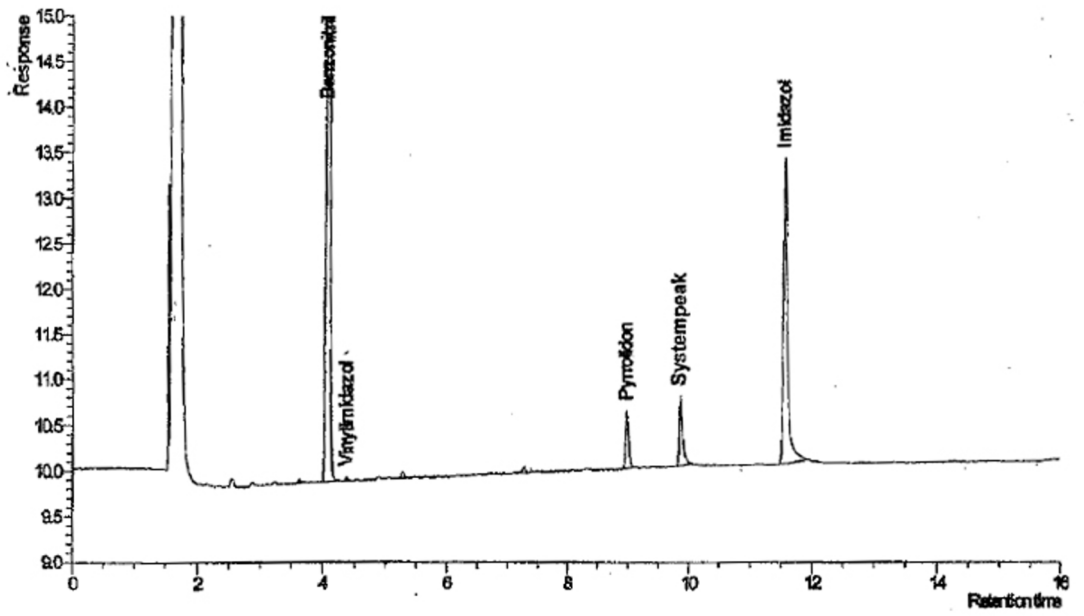


Fig. 1: Chromatogram of the copolymer extract (with internal standard)

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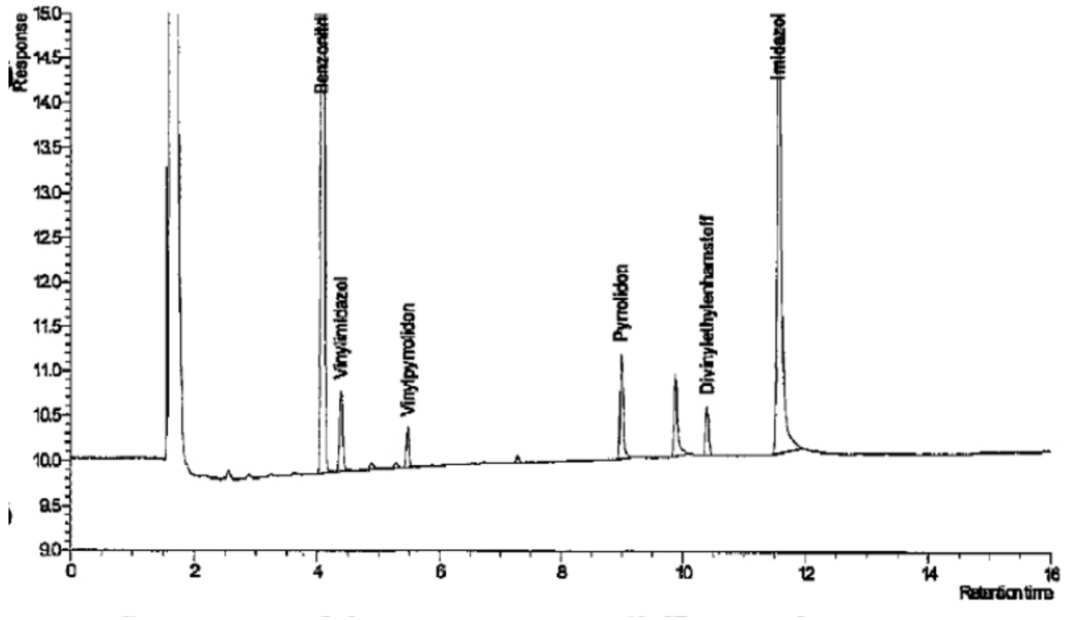


Fig. 2 Chromatogram of the copolymer extract (with internal standard), supplemented by 2.1 µg/g of vinylimidazole, 2.1 µg/g of vinylpyrrolidone, 3.9 µg/g of pyrrolidone, 2.1 µg/g of divinylethyleneurea, and 12.7 µg/g of imidazole.

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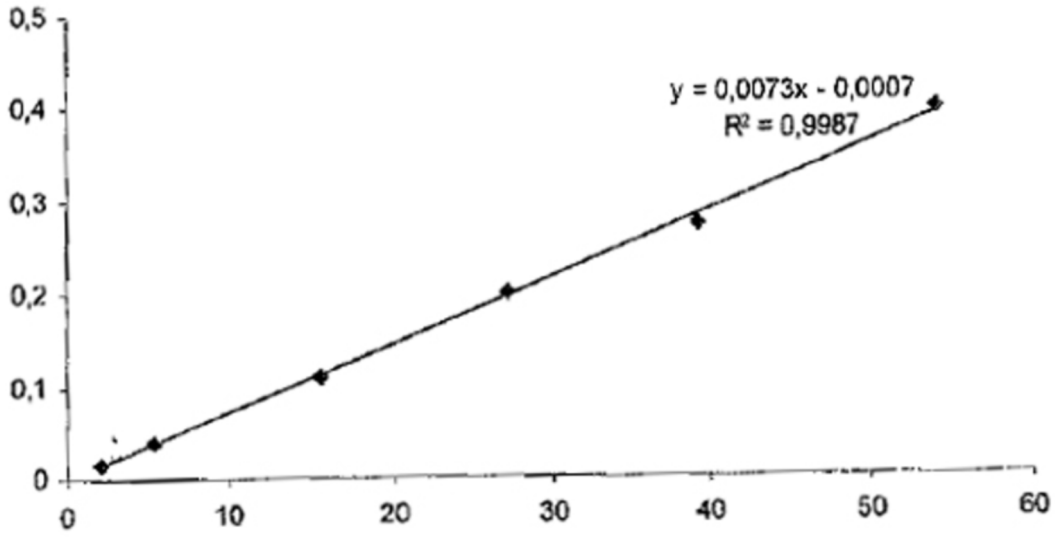
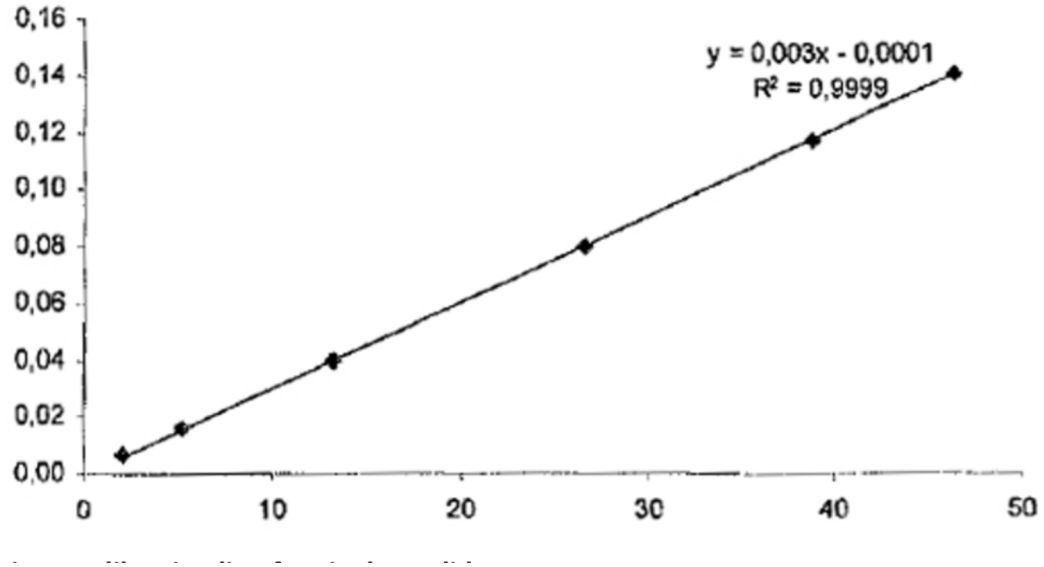


Fig. 3: calibration line for vinylimidazole

Analyte peak area*test sample (int. std.)
Peak area (int. std.) [mg]
Analyte test sample related to standard test sample [µg/g]



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Fig. 4: *calibration line for vinylpyrrolidone*

Analyte peak area*test sample (int. std.)
Peak area (int. std.) [mg]
Analyte test sample related to standard test sample [µg/g]

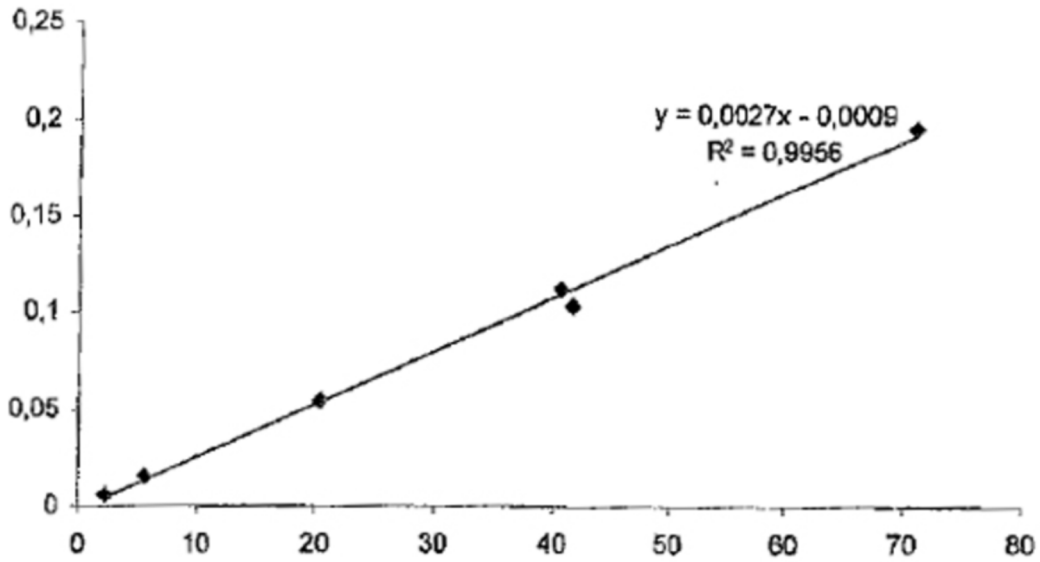


Fig. 5: calibration line for pyrrolidone

Analyte peak area*test sample (int. std.)
Peak area (int. std.) [mg]
Analyte test sample related to standard test sample [µg/g]

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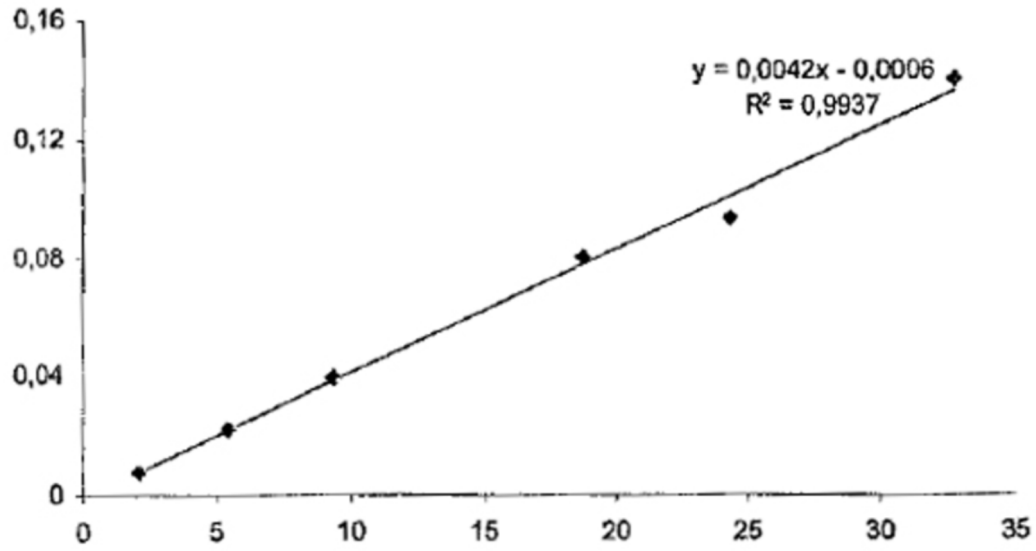


Fig. 6: calibration line for divinylethyleneurea

Analyte peak area*test sample (int. std.)
Peak area (int. std.) [mg]
Analyte test sample related to standard test sample [µg/g]

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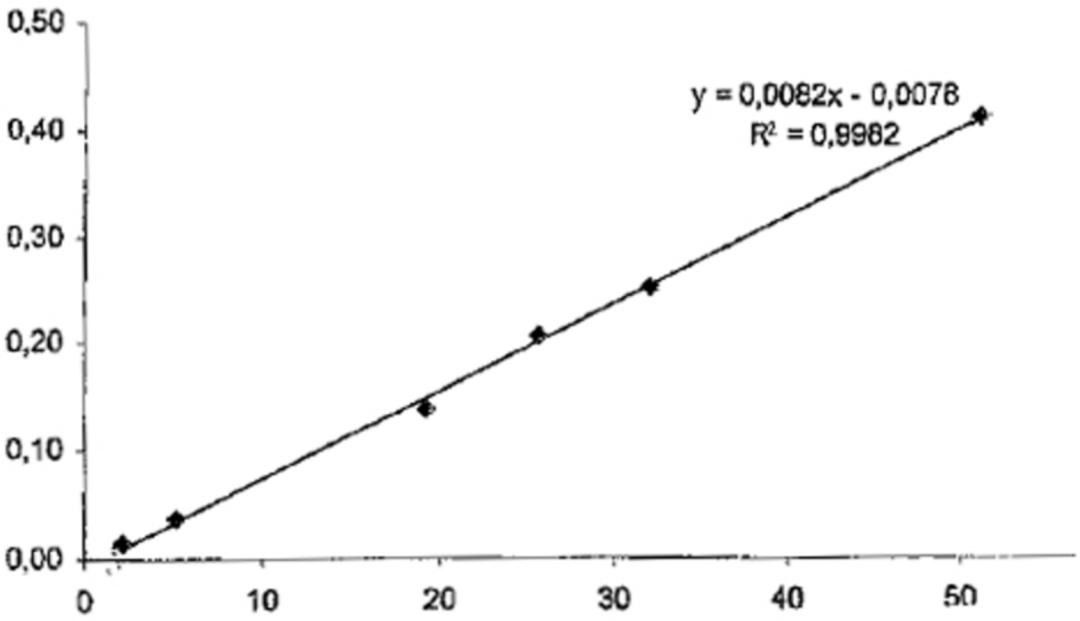


Fig. 7: calibration line for imidazole

Analyte peak area*test sample (int. std.)

Peak area (int. std.) [mg]

Analyte test sample related to standard test sample [µg/g]

Appendix 2

Analytical method for the detection of Imidazole, Pyrrolidone and residual monomers (Vinylpyrrolidone, Vinylimidazole, Divinylimidazolidinone) in wines and musts

1. Scope

The method described here is suitable for the determination of Imidazole, Pyrrolidone, Vinylimidazole and Vinylpyrrolidone in white, red, sweet and dry wines, and must.

Divinylimidazolidinone has a half-life of 3.75 min at pH-value of 3.7. Thus determination is not appropriate in wine and must.

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The study described covers the concentration ranges of 5 to 125 µg/l for Imidazole, 25 to 250 µg/l for Pyrrolidone, 2 to 25 µg/l for Vinylimidazole and 2 to 12.5 µg/l for Vinylpyrrolidone.

2. Definitions

HPLC :High performance liquid chromatography

LC-MS: Liquid Chromatography – Mass spectrometry

MRM: multiple-reaction monitoring

3. Principle

Samples are analyzed directly by LC-MS on a reversed-phase column (C18). Detection is then carried out in multiple-reaction monitoring mode.

4. Reagents and Materials

4.1. Chemicals

4.1.1. Methanol (LiChrosolov) (CAS: 67-56-1) quality for CL-SM

4.1.2. Bidistilled water

4.1.3. Heptafluorobutyric acid, puriss., ≥99,5% (CAS: 375-22-4)

4.2. Preparation of eluents

4.2.1. Solvent A:

Pipette 0.6 ml of heptafluorobutyric acid (4.1.3) into 1000 ml bidistilled water (4.1.2), shake and degas.

4.2.2. Solvent B:

Add 300 ml of bidistilled water (4.1.2) to 700 ml of methanol (4.1.1) and shake. Pipette 0.6 ml of heptafluorobutyric acid (4.1.3) into this solution, shake and degas.

4.3. Standards

4.3.1. Imidazole, ≥99,5 % (CAS: 288-32-4)

4.3.2. Pyrrolidone, ≥99 % (CAS: 616-45-5)

4.3.3. Vinylimidazole, ≥99 % (CAS: 1072-63-5)

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4.3.4. Vinylpyrrolidone, =99,8 % (CAS: 88-12-0)

4.4. Preparation of standard solutions

4.4.1. Preparation of the stock standard solutions (1,00 g/l):

Weigh exactly 100 mg of standards (4.3.1-4.3.4), transfer them without loss into a 100 ml volumetric flask, fill with bidistilled water (4.1.2) to about 90 ml, shake and adjust to 100 ml.

4.4.2. Preparation of the mixed standard solution (Imidazole: 62.5 mg/l; Pyrrolidone: 62.5 mg/l; Vinylimidazole: 12.5 mg/l; Vinylpyrrolidone: 6.25 mg/l):

Pipette 6.25 ml of the Imidazole stock solution (4.4.1), 6.25 ml of the Pyrrolidone stock solution (4.4.1), 1.25 ml of the Vinylimidazole stock solution (4.4.1) and 0.625 ml of the Vinylpyrrolidone stock solution (4.4.1) to a 100 ml volumetric flask, fill with bidistilled water (4.1.2) to about 90 ml, shake and adjust to 100 ml.

4.4.3. Preparation of the working standard solution:

Pipette 40 µl mixed standard solution (4.4.2) to a 25-ml volumetric flask, fill with bidistilled water to 25 ml and shake.

4.5. Preparation of the matrix calibration curve

Matrix-matched calibration solutions are prepared in an uncontaminated wine or must. Dilute the mixed standard solution (4.4.2) appropriately with the sample to give five working standards.

Calibration standards must be prepared just before measurement!

end volume	mixed standard	Imidazole	Pyrrolidone	Vinylimidazole	Vinylpyrrolidone
25 ml	0 µl	0 µg/l	0 µg/l	0 µg/l	0 µg/l
25 ml	10 µl	25 µg/l	25 µg/l	5 µg/l	2.5 µg/l
25 ml	20 µl	50 µg/l	50 µg/l	10 µg/l	5 µg/l
25 ml	30 µl	75 µg/l	75 µg/l	15 µg/l	7.5 µg/l
25 ml	40 µl	100 µg/l	100 µg/l	20 µg/l	10 µg/l
25 ml	50 µl	125 µg/l	125 µg/l	25 µg/l	12.5 µg/l

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5. Apparatus

5.1. Analytical balance accurate to 0.1mg

5.2. Assorted precision pipettes and volumetric flasks

5.3. HPLC vials (4 ml)

5.4. High-performance liquid chromatograph with mass spectrometric detector (Applied Biosystems API 4000 or equivalent)

5.5. Knauer Eurospher 100-5 C18 column with an integrated pre-column or equivalent

Internal diameter: 4.6 mm

Length: 250 mm

Stationary Phase: C18, pore size: 100 Å, particle size: 5 µm, end-capped

6. Sample preparation

6.1. Model wine Solution

The model wine solution is prepared according to Martínez-Rodríguez and Polo, 2000 (Characterization of the Nitrogen Compounds Released during Yeast Autolysis in a Model Wine System).

Four grams of tartaric acid, 0.1 g of acetic acid, and 120 mL of ethanol are dissolved in 800 mL of water (bidistilled). After adjustment of the pH value to 3.2 with 2N sodium hydroxide, the solution is made up to 1000 mL. The model wine solution is brought to temperature at 20°C.

6.2. Sample preparation for migration analysis

The amount of 0.5 grams Divergan HM are added to 1 litre of model wine solution and stirred at 20°C for 48 hours (at approximately 150 rpm).

Prior to analysis the sample is centrifuged (approximately 3 min, 4500 rpm) and filtered through a 0.45 µm membrane filter.

6.3. Other samples (e.g. musts and wines)

Clear samples are filled directly into sample vials and ready for chromatography without any sample preparation. Cloudy wine samples are filtered through a 0.45 µm membrane filter before injection, and the first fractions of filtrate are discarded.

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7. LC-MS Analysis

7.1. Operating conditions for HPLC:

- Injection volume: 10 µl
- Flow rate: 1 ml/min
- Gradient:

$$85:15\,(A:B) \xrightarrow{10\text{ min}} 85:15 \xrightarrow{5\text{ min}} 0:100 \xrightarrow{10\text{ min}} 0:100 \xrightarrow{5\text{ min}} 85:15 \xrightarrow{15\text{ min}} 85:15$$

- Column heater: 25 °C
- Run time: 45 min

7.2. MS conditions:

- Mass spectrometer: Applied Biosystems API 4000 or equivalent
- Scan Type: MRM
- Polarity: Positive
- Ion Source: Turbo Spray
- Duration: 20,005 min; 1364 Cycles
- Curtain Gas: 40 psi
- Ionspray Voltage: 2500 V
- Temperature: 550 °C
- Ion Source Gas 1: 60 psi
- Ion Source Gas 2: 60 psi
- Collision Gas: Medium

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- Entrance Potential: 10 V
- Collar 2: 0

compound	Q1 Mass (amu)	Q3 mass (amu)	Dwell (msec)	Parameter	Start	Stop
Imidazole	69.08	42.20	75.00	DP	81.00	81.00
				CE	31.00	31.00
				CXP	2.00	2.00
Pyrrolidone	86.10	44.10	75.00	DP	66.00	66.00
				CE	31.00	31.00
				CXP	6.00	6.00
	86.10	69.00	75.00	DP	66.00	66.00
				CE	23.00	23.00
				CXP	4.00	4.00
Vinylimidazole	95.09	41.10	75.00	DP	71.00	71.00
				CE	33.00	33.00
				CXP	0.00	0.00
	95.09	69.20	75.00	DP	71.00	71.00
				CE	29.00	29.00
				CXP	12.00	12.00

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Vinylpyrrolidone	112.08	69.20	75.00	DP	51.00	51.00
				CE	21.00	21.00
				CXP	4.00	4.00
	112.08	84.00	75.00	DP	51.00	51.00
				CE	17.00	17.00
				CXP	14.00	14.00

DP: Declustering Potential (in volts)
CE: Collision Energy (in volts)
CXP: Collision Cell Exit Potential (in volts)

8. Evaluation

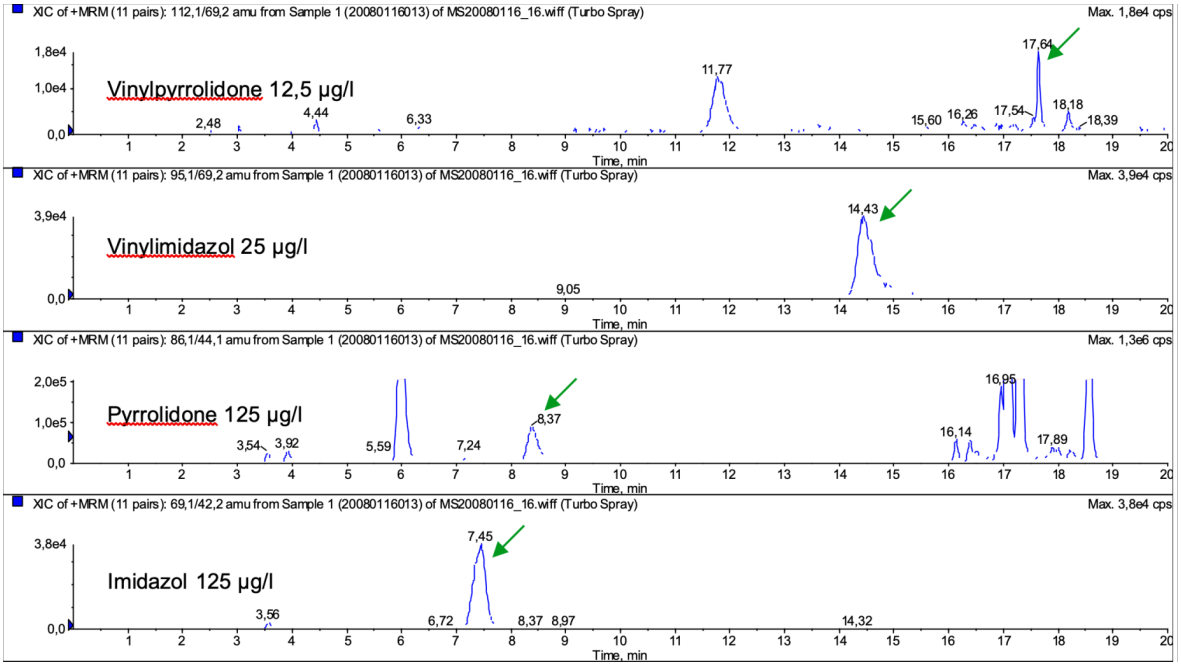
8.1. Identification:

Inject 10 µl of working standard solution (4.4.3) to ascertain the retention times. Approximate retention times are:

compound	retention time
Imidazole	7.45 min
Pyrrolidone	8.37 min
Vinylimidazole	14.43 min
Vinylpyrrolidone	17.64 min

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Chromatogram of a matrix calibration standard in white wine:

8.2. Quantification:

Mass transfers for quantification:

compound	mass transfer
Imidazole	69.1 □ 42.2
Pyrrolidone	86.1 □ 44.1
Vinylimidazole	95.1 □ 69.2
Vinylpyrrolidone	112.1 □ 69.2

Use the standard addition method for quantification.

8.3. Expression of results

Results should be expressed in µg/l for Imidazole, Pyrrolidone, Vinylimidazole and

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Vinylpyrrolidone with no decimals (e.g. 3 µg/l).

8.4. Limit of detection and limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) depend on the individual measurement conditions of the chemical analysis and are to be determined by the user of the method.

The limit of detection (LOD) and the limit of quantification were estimated using the instrumentation and conditions mentioned as an example above (section 7) following the instructions in the resolution OENO 7-2000 (E-AS1-10-LIMDET) “Estimation of the Detection and Quantification Limits of a Method of Analysis“. Following the "Logic Diagram for Decision-Making" in point 3 the "graph" approach should be applied following paragraph 4.2.1. For this purpose a window is drawn on the multiple reaction monitoring chromatogram, enclosing the range of a tenfold peak width at mid-height (w½) either side at the retention time of an analyte peak in the relevant part of the chromatogram. Two parallel lines are then drawn which just enclose the maximum amplitude of the signal window. The separation between these two lines gives hmax, expressed in abundance units, which is multiplied by 3 for LOD, by 10 for LOQ, and finally converted into concentration units by implementing the individual response factor.

compound	limit of detection (LOD)	limit of quantification (LOQ)
Imidazole	5 µg/l	12 µg/l
Pyrrolidone	25 µg/l	83 µg/l
Vinylimidazole	2 µg/l	6 µg/l
Vinylpyrrolidone	2 µg/l	6 µg/l

9. Precision and trueness

As matrices three different wines (dry white wine, dry red wine and sweet red wine) and grape juice were used. Within-laboratory reproducibility, repeatability and recovery were calculated based on matrix calibration and three spikes (Imidazole: 40/60/80 µg/l; 2-Pyrrolidone: 40/60/80 µg/l; Vinylimidazole: 8/12/16 µg/l;

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Vinylpyrrolidone: 4/6/8 µg/l).

9.1. Imidazole

	fortification	mean of series	standard deviation	corresponding CV	Horwitz RSD %
within-laboratory reproducibility (SDwlR):	40 µg/l	41	2	5 %	26
	60 µg/l	61	3	5 %	24
	80 µg/l	80	5	6 %	23
repeatability (SDr):	40 µg/l	41	1	2 %	
	60 µg/l	61	2	3 %	
	80 µg/l	80	4	5 %	
recovery (WDF):	40 µg/l	102 %			
	60 µg/l	101 %			
	80 µg/l	101 %			
	0	101 %			

9.2. Pyrrolidone

	fortification	mean of series	standard deviation	corresponding CV	Horwitz RSD %
within-laboratory reproducibility (SDwlR):	40 µg/l	42	9	22 %	26
	60 µg/l	60	9	15 %	24
	80 µg/l	81	9	11 %	23

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repeatability (SDr):	40 µg/l	42		5	12	%
	60 µg/l	60		4	7	%
	80 µg/l	81		8	9	%
recovery (WDF):	40 µg/l	105	%			
	60 µg/l	100	%			
	80 µg/l	101	%			
	0	102	%			

9.3. Vinylimidazole

	fortification	mean of series		standard deviation	corresponding CV		Horwitz RSD %
within-laboratory reproducibility (SDwlR):	8 µg/l	8		0	4	%	33
	12 µg/l	12		1	5	%	31
	16 µg/l	16		1	4	%	30
repeatability (SDr):	8 µg/l	8		0	4	%	
	12 µg/l	12		0	3	%	
	16 µg/l	16		0	3	%	
recovery (WDF):	8 µg/l	101	%				
	12 µg/l	102	%				
	16 µg/l	102	%				
	0	102	%				

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9.4. Vinylpyrrolidone

	fortification	mean of series	standard deviation	corresponding CV	Horwitz RSD %
within-laboratory reproducibility (SDwlR):	4 µg/l	3	1	31 %	37
	6 µg/l	4	1	26 %	35
	8 µg/l	5	2	29 %	33
repeatability (SDr):	4 µg/l	3	1	25 %	
	6 µg/l	4	1	22 %	
	8 µg/l	5	1	26 %	
recovery (WDF):	4 µg/l	66 %			
	6 µg/l	63 %			
	8 µg/l	66 %			
	0	65 %			

fortification mean of series standard deviation corresponding CV Horwitz RSD %
within-laboratory reproducibility (SDwlR): 4 µg/l 3 1 31 % 37
6 µg/l 4 1 26 % 35
8 µg/l 5 2 29 % 33
repeatability (SDr): 4 µg/l 3 1 25 %
6 µg/l 4 1 22 %
8 µg/l 5 1 26 %
recovery (WDF): 4 µg/l 66 %
6 µg/l 63 %
8 µg/l 66 %
0 65 %

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[1] Missouri Catalyst (= 49.9% K_2SO_4 + 49.8% Na_2SO_4 + 0.3% $CuSO_4$), Merck, Darmstadt or the equivalent

[2] The calculation of the upper limits was based on the results obtained from the migration tests with the recommended dosage of 0,5 g/l, the maximum application time of 48 hours, and a treatment temperature of 20 °C, multiplied by a factor of 2.

Under acidic conditions (at lower pH-values) divinylimidazolidinone (divinylethylene-urea) is not stable and hence degrades to imidazolidinone and vinyl alcohol. Furthermore imidazolidinone degrades to urea and ethylene glycol. Vinyl alcohol is in chemical equilibrium with acetaldehyde.

Imidazolidinone was included in the toxicological assessment as well as acetaldehyde, urea and ethylene glycol.

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