RESOLUTION OENO 11/2002

POLYVINYLPOLYPYRROLIDONE P.V.P.P.

THE GENERAL ASSEMBLY,

HAVING CONSIDERED Article 5 of the October 13, 1954 International Convention on Unification of the Methods of Analysis and Appraisal of Wines,

WITH THE PROPOSAL of the Sub Commission on Methods of Analysis and Appraisal of Wines,

DECIDES to replace the existing monograph by the following monograph in the aforementioned International Oenology Codex:

POLYVINYLPOLYPYRROLIDONE

POVIDONE

(PVPP)

(C_6H_9NO)n = (111,1)n

N° SIN: 1202

1. OBJET, ORIGIN AND FIELD OF APPLICATION

Insoluble polyvinylpolypyrrolidone is a polymer poly[1-(2-oxo-1-pyrrolidinylethylene] reticulated to render it insoluble. It is made by polymerisation of N-vinyl-2-pyrrolidone in the presence of different catalysers (for example sodium hydroxide) or in the presence of N'N-divinylimidazolidone.

PVPP fixes the polyphenols in wines; this adsorption depends on the rate of polymerisation. Its application rate is limited.

2. SYNONYMS

- poly(1-ethenylpyrrolidin-2-one)
- Crospovidone (nomenclature of pharmacope)
- Reticulated polyvidone
- Reticulated homopolymer of 1-ethenyl-2-pyrrolidone

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- Reticulated insoluble polymer of N-vinyl-2-pyrrolidone
- P.V.P. insoluble
- Polyvinylpolypyrrolidone (PVPP).

3. LABELLING

The label must indicate that PVPP is for oenological usage, minimum guaranteed efficiency vis-à-vis safety test and storage conditions.

4. CHARACTERISTICS

Light powder, white and creamy white.

Insoluble in water and in organic solvents.

Insoluble in strong acid minerals and in alkaly.

5. TEST TRIALS

5.1. Loss through drying

Place 2 g of PVPP in a 70 mm diameter silica capsule; dry in an incubator at 100-105° C for 6 hours. Let cool in the desiccators. Weigh. Weight loss must be less than 5%.

It is also possible to carry this out more quickly by titration with the Karl-Fischer. (F.V. 1076) procedure.

Note: All limits set above refer to the dried product.

5.2. Ashes

Incinerate the residue left over in test trial 5.1 progressively without going over 600° C. (Ash mass should be less than 0.5%).

5.3. Preparation for test trial solution

After weighing the ashes, dissolve 1 ml of concentrated hydrochloric acid (R) and 10 ml of distilled water. Heat to activate the solution. Bring up to 20 ml with distilled water. 1 ml of this solution contains 0,10 g of PVPP mineral matter.

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5.4. Heavy metals

10 ml of solution prepared according to point 5.3 is put in a test tube with 2 ml of a pH 3.5 (R) buffer solution and 1.2 ml of reactive thioacetamide (R). There should be no precipitation. If a brown colour appears, it should be inferior to the test sample as indicated in Chapter II (Heavy metal content, expressed in lead, must be less than 10 mg/kg).

5.5. Lead

Using the solution prepared idem, determine the lead, following the procedure in Chapter II or by atomic absorption spectrophotometer procedure. Lead content must be below 5 mg/kg.

5.6. Mercury

Determine the mercury, following the procedure in Chapter II. Mercury content must be below 1 mg/kg.

5.7. Zinc

Determine the zinc, following the procedure described in Chapter II. Zinc content must be below 5 mg/kg.

5.8. Arsenic

Determine the arsenic, following the procedure in Chapter II. Arsenic content must be below 3 mg/kg.

5.9. Cadmium

Determine the cadmium using the method described in Chapter II of the International oenological Codex by atomic absorption spectrophotometer procedure.

Cadmium content must be below 1 mg/kg.

5.10. Sulphates

Determine the sulphates, following the procedure in Chapter II. Sulphate content must be below 1 mg/kg.

5.11. Determining total nitrogen

Introduce approximately 0.20 g of PVPP weighed precisely in a 300 ml flask with 15 ml concentrated sulphuric acid (R) and 2 g of mineralisation catalyst (R) and continue the

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operation as indicated in Chapter II. (Total nitrogen content must be between 11 and 12.8%).

5.12. Solubility in a water medium

Introduce 10 g of PVPP in a 200 ml flask containing 100 ml of distilled water. Mix and leave for 24 hours. Filter through a gauze screen with a porosity of 2.5 μm and then through a gauze screen with a porosity of 0.8 μm. The residue left from the evaporation of dried filtrate over 100°C hot water, must be less than 50 mg (solubility in water must be less than 0.5%).

5.13. Solubility in acid and alcohol.

Introduce 1 g of PVPP in a flask containing 500 ml of the following mixture

Acetic acid	3 g
Ethanol	10 ml
Water	100 ml

Let sit 24 hours. Filter through a gauze screen with a porosity of 2.5 □m and then through a gauze screen with a porosity of 0.8 □m. Concentrate the filtrate over 100°C hot water. Stop evaporation over a 100° C hot water in a 70 mm diameter previously weighed silica capsule. The residue left by dry evaporation must be less than 10 mg, taking into account the residue left by evaporation of 500 ml of a mixture of acetic-acid ethanol (solubility in acetic acid and alcohol medium must be less than 1%).

6. PVPP EFFICIENCY WITH REGARDS TO ADSORPTION OF POLYPHENOLIC . COMPOUNDS

6.1. Salicylic acid essay

6.1.1. Reagents:

- 0.1 M sodium hydroxide solution
- 0.1M salicylic acid solution (13.81 g salicylic acid are dissolved in 500 ml of methanol and diluted with 1l of water).

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6.1.2. Operating mode:

- Weigh 2-3 grams of PVPP in a 250 ml conical flask and write down the MASS M at $\pm$ 0.001 g.
- Calculate the dry extract of the sample (solid weight percentage) as P in % to the nearest decimal.
- Add the 0.1M salicylic acid solution according to the following formula:
- 43. M . P = ml to be added
- Close the flask and shake for 5 minutes.
- Pour the 25°C mixture on a filter over a Büchner funnel connected to a 250 ml tube; empty it until there is at least 50 ml of filtrate (the filtrate must be clear).
- Use a pipette take 50 ml of the filtrate and put it in a 250 ml conical flask.
- Determine the neutralisation point of phenolphthalein and write down the volume V_s with a 0.1M sodium hydroxide solution.
- Titrate 50 ml of the salicycal acid solution (sample test) in the same manner and write down the volume V_b.

6.1.3. Calculation:

$$\%\ activity = \frac{V_b - V_s}{V_b} .100$$

The percentage of activity must be equal or greater to 30%.

6.2. Determining the adsorption capacity of oenocyanine (30% minimum)

6.2.1. Principle

A small amount of PVPP is put in contact with a oenocyanine solution for 5 minutes. Adsorption at 280 nm of treated oenocyanine solution is compared to a standard solution and a blank solution made up of only solvent. The decrease of adsorption to 280 nm is used as a relative measurement of PVPP capacity to adsorb oenocyanine.

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6.2.2. Reagents

- oenocyanine (hydrate of)
- Ethanol (absolute)
- Distilled water.

6.2.3. Material

- Spectrophotometer, UV visible.
- Quartz cuvettes, 1 cm of optical path.
- Beakers, 150 ml.
- Graduated flask, 1 litre.
- Teflon stirring rods and magnetic mixer.
- Syringes.
- Filters for syringes, (0,45 µm porosity).

6.2.4. Methods

- Solution E. Dissolve 80 mg of oenocyanine hydrate in 50 ml of ethanol. Quantitatively transfer to a one litre graduated flask (with distilled water) and dilute to volume indicated with the distilled water. Label this solution E, and keep in an amber coloured tube. This is the standard solution.
- Solution R. Prepare the reference solution by diluting 50 ml of ethanol in 1 litre of distilled water. This is the reference solution.
- Weigh 3 volumes of, 50 mg □ 0,1 mg of samples in 150 ml beakers. Add the Teflon mixing rods and put under the magnetic mixer.

NOTE: The contact time between the sample and the solution is critical. In the following steps, the addition of the solution to the samples will be in increments in order to foresee exactly 5 minutes between the introduction of the solution and the filtration of each sample.

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- Using a pipette, add 100 ml of sample solution E, to 2 of the solutions and add 100 ml of solution R to the third sample. Put the timer on, once the 100 ml has been added.
- Shake for 5 minutes 5 seconds.
- With the aid of syringe and a filter with pores measuring 0.45 µm in diameter, withdraw a part of the solution immediately and filter in a clean flask. The filtered solutions can be stored in a cool and dark place for maximum 1 hour before measuring UV absorbency.
- Set up the UV spectrophotometer in compliance with manufacturers' instructions in order to measure absorbency at 280 nm. Put the machine at zero on 280 nm and use the R solution as a blank.
- Measure the degree of absorbency of each filtered extract at 280 nm compared to solution R by using quartz cuvettes with 1 cm optical path.

6.2.5. Calculations

$$Absorbency\ capactiy = \frac{A_o - (A_T - A_B) \times 100}{A_B}$$

Given that:

- A_o= Solution E absorbency
- A_T= Sample solution absorbency
- A_B= Blank solution absorbency (PVPP without oenocyanine)

Calculate the average for the two sample solutions.

7. DETERMINING OF THE N-VINYL-2-PYRROLIDONE MONOMER IN PVPP WITH THE AID OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH UV DETECTION.

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7.1. Principle

The N-vinyl-2-pyrrolidone monomer is the extract PVP polymer with methanol. The methanol solution is analysed by HPLC by using C8 type deactivated reversed phase column. This quantification is carried out by UV detection at 235 nm. Soluble PVP is eliminated when entering the column by a back flush technique.

This method can be applied to samples of which the monomer concentration is between 0.4 and 100 mg/l. The content of N-vinyl–2–pyrrolidone in PVPP should not exceed 10 mg per kg.

7.2. Reagents

- Methanol, HPLC grade.
- water, micro filtered rest > 18 M□.
- N-vinyl-2-pyrrolidone

7.3. Equipment

7.3.1. Glassware

- Assembling HPLC to filter solvents; entirely in glass.
- Filters for mobile phases, nylon 0.45 μm.
- Graduated pipettes (10, 20 and 100 ml).
- Volumetric flasks (100 and 1000 ml).
- 7.5 ml polyethylene pipettes
- Spatulas used for the handling of powder grams.
- Small flasks with polyethylene stoppers.
- Filters with porosity 0.45 □m in glass microfibers.

7.3.2. Instruments

- Scale, which can measure to the nearest 0.1 mg.

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- Magnetic mixer
- HPLC system with type C8 column and UV-Visible detector.

7.4. Procedure

7.4.1. Preparation of the mobile phase

- Using a pipette, introduce 200 ml of HPLC grade methanol in a 1000 ml flask. Dilute as needed, with HPLC grade water and mix.
- Filter/degasify the mobile phase and then transfer to the solvent reservoir to pump HPLC.

7.4.2. Preparation of reference solution

- VP 1000 mg/l reference solution

Weigh about 100 mg of N-vinyl-2-pyrrolidone to the nearest 0.1 mg in a 100 ml volumetric flask. Dilute the volume as needed with the mobile phase

- VP 100 mg/l reference solution

Dilute 10 ml of the solution at 1000 mg/l to the needed volume, with the mobile phase, in a 100 ml volumetric flask.

- VP 10 mg/l reference solution

Dilute 10 ml of the solution at 100 mg/l to the needed volume, with the mobile phase, in a 100 ml volumetric flask.

- VP 1 mg/l reference solution

Dilute 10 ml of the solution at 10 mg/l to the needed volume, with the mobile phase, in a 100 ml volumetric flask.

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7.4.3. Preparation of the sample

- In a small flask, weigh about 2.0 g of PVPP ± 0.1 mg.
- Using a pipette, introduce 20 ml of HPLC grade methanol in the flask containing the sample.
- Close the flask vacuum tight and put it under an automatic mixer. Extract for 1 hour at a speed of 130 rotations per minute.
- After one hour, remove the flask from the mixer. Filter the supernatant with a filter with a porosity of 0.45 □m in glass micro fibres.

7.4.4. Analysis by HPLC

- Install the HPLC equipment in compliance with the manufacturers' instructions and balance the column and the detector with the mobile phase for at least one hour before analysing the reference test specimen and the samples.

HPLC conditions (as an example)
Vol. injection 20 micro litres
Solvent flow 1 ml/minute
Detection 235 nm
Duration 10 minutes for reference solutions without back flushing (60 minutes for samples with back flushing of columns) of which 10 minutes for back flushing and 50 minutes for the reconditioning of the column.

- Inject a reference specimen of 10 mg/l de N-vinyl-2-pyrrolidone (absolute concentration) three times every 6 to 10 samples to control the performance of the system.

7.4.5. Calculations

$$mg/l\ of\ VP = \frac{20 \times (peak\ surface\ area\ of\ the\ sample) \times (response\ factor)}{sample\ in\ gram}$$

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$$with\ responsivity = \frac{(concentration\ of\ reference\ solution\ in\ mg/l)}{(peak\ surface\ area\ of\ the\ reference\ solution)}$$

Comment

- Detection limit and minimum quantifiable quantity

Detection limit (signal/noise = 3 for PVPP sample with a content of 0.27 mg/l in N-vinyl-2-pyrrolidone) is ~ 0.10 mg/l with a minimum quantifiable (signal/noise = 10) of 0.33 mg/l.

- Recovery

During a laboratory test, the N-vinyl-2-pyrrolidone, overloaded with PVPP with 1.10 and 100 mg/l of VP, was respectively recovered at 108%, 99.0% and 102%.

- Retention time

The average length of peak retention of N-vinyl-2-pyrrolidone (at a rate of 10 mg/l) is 6.34 ± 0.08 minutes, for a column system + 13 cm long precolumn.

- Interferences

The appropriate duration for back flushing will be set for each system, otherwise a rigorous blocking of the column will take place.

8. DETERMINING THE FREE N,N'- DIVINYLIMIDAZOLIDONE IN THE PVPP BY GAS CHROMATOGRAPHY.

This must be determined when the PVPP preparation technique N,N'-divinylimidazolidone.

the free N,N'- divinylimidazolidone in PVPP must not exceed 2 mg per kg.

8.1. Principle

Measuring by gas chromatography on a capillary column of free N',N-divinylimidazolidone in a solvent (acetone) from non-soluble PVPP. Detection limit is 1

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mg/kg.

8.2. Internal test specimen solution:

Dissolve 100 mg ± 0.1 mg, of heptanoic acid in 500 ml of acetone.

8.3. Preparation of the sample

Weigh 2 to 2.5 g ± 0.2 mg of polymer and pour into a 50 ml conical flask. Using a pipette, add 5 ml of internal standard solution, then 20 ml of acetone. Shake the mixture for 4 hours. Leave for 15 hours to stabilize and analyse the supernatant by gas chromagraphy.

8.4. Calibration solution

Weigh 25 mg ± 0.2 mg of N,N'- divinylimidazolidone and pour into a volumetric flask; add acetone up to 100 ml. Using a pipette, transfer 2.0 ml of this solution in a 50 ml volumetric flask and add acetone up to 50ml. Transfer 2 ml of this solution to a 25 ml volumetric flask, add 5 ml of internal standard solution (see above) and adjust the volume with acetone.

8.5. Gas chromatography conditions (as an example):

Column (fused silica) capillary (cross linked carbowax - 20 M), length 30 m, inner diameter 0.25 mm, film thickness 0.5 µm.

Programmed column temperature	140°C to 240°C, 4°C/ minute.
Injector	split injector, 220°C
	Flow rate 30 ml/min.
Detector	Thermionic detector (optimised in compliance with manufacturer's' instructions), 250°C
Carrier gas	Helium, 1 bar (suppression).
Volume injected	1 µl of sample floating to the up solution or reference test sample solution.

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8.6. Procedure

Validation of response factor for specific conditions of analysis is possible thanks to repeated injections of calibration solutions.

Analyse the sample. The N,N'- divinylimidazolidone content in non-soluble PVPP must not exceed 0.1%.

8.7. Calculation of response factor:

$$f = \frac{Wd \times Ase}{Wse \times Ad}$$

Wd - quantity of N,N'-divinylimidazolidone used (mg)

Wse – quantity of internal standard used (mg)

Ase - peak area of standard solution

Ad - peak area of N,N'- divinylimidazolidone .

8.8. Calculation of N,N'-divinylimidazolidone content:

$$C_D = \frac{1000f.Ad.Wse}{Ase.Ws}\ (mg/kg)$$

C_D = concentration of N,N'-divinylimidazolidone (mg/kg)

f = response factor

Ad = peak area of N,N'-divinylimidazolidone

Wse = quantity of internal standard added to the sample (mg)

Ase = peak area of internal standard solution

Ws = quantity of sample used (g)

9. STORING CONDITIONS

PVPP must be kept in a ventilated place in vacuum packed containers away from volatile elements that is might adsorb.

Declaration of Danemark

"When differences in specifications of purity, definitions and analytical methods exist

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between OIV and other competent intergovernmental organizations, such as Codex Alimentarius and European Union, Denmark believes that every possible effort must be done to identify why these differences exist and to reconcile them as far as possible, in order to avoid the existence of different international regulations on the same subject."

ANNEX Karl-Fischer procedure

1. FIELD OF APPLICATION

This method is used to determine the water content in a transverse link PVP. Vinylpyrrolidone residue does not interfere with the usual rate present (0.1%). This method is able to detect water with concentrations above 0.05% (m/m).

2. PRINCIPLE

The sample is dissolved in anhydrous methanol and titrated using a Karl-Fischer reagent (KF) without pyridine. Water reacts to the titrating solution in the following way:

$$2RN + H_2O + I_2 + (RNH)SO_3CH_3 \rightarrow (RNH)SO_4CH_3 + 2(RNH)I$$

The final point (excess 12) is determined by controlling the change in current between two micro-electrodes and the polarized platinum. The typical KF titration is completely automated and directly produces the calculated water levels.

3. REAGENTS

1. Karl Fischer reagent without pyridine (example by AQUASTAR AXI698A or the equivalent)
2. anhydrous methanol
3. Silica gel with humidity indicator for desiccation of the tube in the cell.
4. The analytic al standard can be obtained from specialized laboratories (actually : BASF, D-67056 Ludwigshafen)

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4. APPARATUS

Karl Fischer Titrimeter

5. METHOD

1. Fill the titration recipient with 50 ml of anhydrous methanol or an amount sufficient to cover the electrodes. Fill the desiccation tubes above the cell of the fresh silica gel.

2. Calibrate the titration solution by using distilled water as a specimen. Record the weight of the sample and the tare, as indicated in the instrument instruction booklet. The apparatus will automatically calculate the average titer and will store the figure for three testings. (the assay solution H20/ml in grams). If an analytical balance is available for reporting the sample weight, follow instructions in the manual.
3. Add 0.075 g to 0.150 g of sample (to the nearest 0.1 mg) in a reaction recipient and mix for 2 minutes. Report the weight of the sample and the tare. The apparatus will measure the assay and automatically determine the % water content.
4. Carry out analysis in duplicate

6. CALCULATION

1. Titrate the assay solution KF, T

$$\frac{water\ specimen\ in\ mg}{assay\ solution\ used\ in\ ml}$$

2. % of water in the sample

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$$\frac{0.1\ TV}{S}$$

Where

V = ml of assay solution used

S = weight of samples in grams

7. INTERFERENCES

High concentrations of vinylpyrrolidone (>0.5%) residues react with iodine and produce very imprecise results.

(A 1% vinylpyrrolidone residual rate corresponds to a H20 rate taken from 0.16% (m/m).

An excess base in the sample risks changing the solution pH and can produce low level results. Samples with pH levels >8 should be buffered with 5 g benzoïc acid for 50 ml.

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