COEI-1-STYDVB Adsorbent styrene divinylbenzene beads

CAS N°9003-69-4

1. Object, origin and scope of application

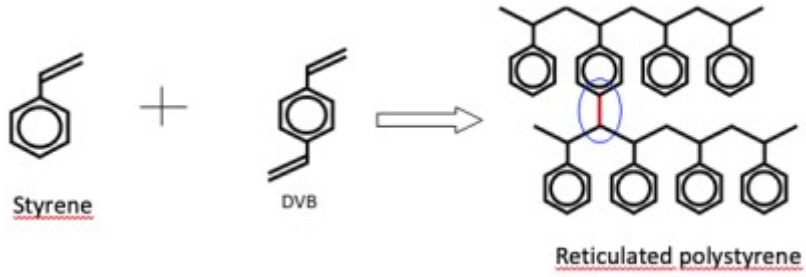
The adsorbent beads enable the reduction or elimination of organoleptic deviations characterised as “earthy-musty” by a physical process of adsorption.

The adsorbent beads are placed in columns complying with food contact standards to enable the percolation of the must or wine in accordance with the files in the OIV *International Code of Oenological Practices*. They adsorb the unpleasant-smelling molecules whose dimensions are smaller than the size of their pores. The desired effect is achieved through the combination of the volume of the styrene-divinylbenzene copolymer beads determined by the size of the pores, and the speed the must or wine passes through the beads. Contrary to treatments of fruit juices with these same beads, the flow rates for this application are extremely rapid.

2. Composition

Adsorbent styrene-divinylbenzene beads should be manufactured according to good manufacturing practices from the following substances: styrene or ethenylbenzene or vinylbenzene (CAS No. 100-42-5) and divinylbenzene or diethenylbenzene (CAS No. 1321-74-0), which are approved substances for use in materials and articles intended to come into contact with foodstuffs. See ‘1. References’.

They are produced by the polymerisation of divinylbenzene (DVB) in the presence of styrene (or vinylbenzene), which functions as a cross-linking agent; the initial concentration of styrene may vary from 0.5% to 40% maximum.



The cross-linked styrene-divinylbenzene copolymer is completely insoluble. In the majority of cases, it forms the structure of ion exchange resins or electrodialysis

membranes before their grafting. The adsorbent beads have a particle size of between 600 µm and 750 µm, and represent a specific surface area greater than or equal to 700 m^2/g, with pore diameters of between 1nm and 40 nm maximum. The inertia of the styrene-divinylbenzene copolymer beads should be satisfied.

Adsorbent beads should undergo pre-treatment or pre-conditioning with absolute ethanol (100% vol.) by the provider in order to eliminate residual monomers. They should be rinsed and conditioned before use, in accordance with the manufacturer's instructions.

3. Labelling

The main characteristics should be indicated on the label, including the batch number, the expiry date and the storage conditions.

4. Characteristics

They come in the form of odourless, porous, white beads. They are prepared and conditioned in wet form in 30-40% water, with the potential addition of sodium chloride, in order to prevent any drying out.

5. Limits and test methods

5.1. General statements

Adsorbent styrene-divinylbenzene copolymer beads or non-grafted resins, used in the treatment of foodstuffs, should comply with the following requirements:

- They should not transfer any of their components into foodstuffs in quantities that could put human health at risk, or lead to an unacceptable modification of the composition of foodstuffs or an alteration of their organoleptic characteristics.
- The determination of the release of organic substances (determination of total organic carbon: TOC) and migration tests for specific components are carried out by the manufacturer using "food simulants" under "conventional migration test conditions". These tests are compulsory in order to obtain any authorisation for the commercialisation of resins or adsorbent styrene-divinylbenzene copolymer beads, or any food contact material.
- The application of adsorbent styrene-divinylbenzene copolymer beads in must or

wine requires determining the migration of the specific components of the beads using 3 simulants. The determination of the migration of the specific components should be carried out using the following simulants: water, acetic acid at 3% (w/v) and ethanol at 20% vol. Additional concentrations may be tested for applications in specific products (e.g. liqueur wines).

- The contact time for the migration tests is 4 h, which is greatly superior to the contact time for the must or wine under treatment conditions, with this not exceeding several minutes.

5.2. Determination of Total Organic Carbon (TOC)

5.2.1. Control reagents

- Distilled and/or deionised test water with conductivity of less than 20 µS/cm at 25 °C and a TOC content of less than 0.2 mg/L.

2. Protocol

- Prepare 1 column and recover 100 mL test water from this column for a blank test (CB); the TOC content should be less than 0.5 mg/L,
- prepare 100 mL adsorbent beads, whose weight will have previously been determined,
- meanwhile, maintain the maximum temperature that can be reached during use (e.g. 20 °C),
- introduce 100 mL test water into the column, and progressively add the 100 mL of adsorbent beads that should be immersed; after sedimentation, use lateral tapping to create vibrations in the column and thus pack the resin in tightly to a constant volume,
- percolate 2 L test water through the resin, at a flow rate of 1000 mL per hour,
- maintain stagnation of the water for 24 hours,
- collect 5 successive fractions of 100 mL test water having undergone percolation through 100 mL adsorbent beads at a flow rate of 500 mL/h,
- analyse the TOC of the 5 fractions collected and of the blank test (CB) using an automatic TOC analyser.

5.2.3. Results

The sum of the results of the analyses of the TOC from each collection deducted from the value of the TOC from the blank test should not exceed 10 mg/L (acceptability criteria).

5.3. Migration testing of specific components: styrene and divinylbenzene

5.3.1. Objective

To verify that the profile of organic impurities or specific components of the adsorbent beads after pre-treatment by the manufacturer is compliant:

- a/ by estimating the total quantity of volatile organic impurities (styrene and divinylbenzene) present on the adsorbent beads,
- b/ by estimating the proportion of these impurities that may migrate into a solution with an extraction power (solvents or simulants) comparable to that of must and wine.

2. Solvents or simulants required

The following solvents are required:

- test water: distilled and/or deionised water with conductivity of less than 20 µS/cm at 25 °C and a TOC content of less than 0.2 mg/L,
- ethanol at 20% vol. obtained from absolute ethanol and distilled and/or deionised water,
- acetic acid at 3% made up of a mixture of acetic acid and distilled and/or deionised water at the ratio of 3:97 (w/w).

5.3.3. Protocol

- Prepare 1 column per simulant and sample 100 mL test water from this column for a blank test,
- prepare 100 mL adsorbent beads, whose weight will have previously been determined,
- meanwhile, maintain the maximum temperature that can be reached during use (e.g. 20°C),
- introduce 100 mL test water into each column, and progressively add the adsorbent beads that should be immersed up to a volume of 100 mL; after sedimentation, use lateral tapping to pack the resin in tightly to a constant

volume,

- percolate 2 L of test water and of each solvent or simulant through the resins, at a flow rate of 1000 mL per hour,
- maintain stagnation for 4 hours,
- collect 5 successive fractions of 100 mL simulant having undergone percolation through 100 mL adsorbent beads at a flow rate of 500 mL/h,
- analyse the specific components of the 5 fractions collected from each simulant and from the test water, and of the blank test, according to the method described in Annex 1.

5.3.4. Results

The specific migration limits (SML) are those of the analytical limit of detection, i.e. for divinylbenzene, the SML = not detected (ND), considering that the LOD = 0.02 mg/kg.

6. Usage limits

- The treatment should not change the organoleptic characteristics of the wine.
- The treatment should not visibly modify the colour of the wine.
- The treatment should not significantly reduce the concentration of metallic cations in the wine.
- The treatment should not significantly modify the pH of the must or wine.
- The resin should not release substances into the wine or must that could alter it.
- The reduction of the alcoholic strength of the wine should not exceed 0.1%.
- The operator may use conditioning and/or regenerating agents composed of water and inorganic acids, bases or salts, on the condition that the conditioned or regenerated resin is rinsed in water until the conditioning and regenerating agents are completely eliminated, before the introduction of the must or wine.

7. Determination of the volume of adsorbent beads (bed volume, BV, and of the flow rate of the must or wine to be treated (BV/H)

It is recommended that laboratory tests are performed to determine the quantities of beads and the flow rate to be applied and transposed to large-volume treatment.

7.1. Equipment

- Chromatography column, 10 mm in diameter and 250 mm in length, with 2 PTFE frits with a pore diameter of 50 µm at the ends,
- peristaltic pump,
- 5 L or 10 L must or wine contaminated with geosmin per test,
- adsorbent styrene-divinylbenzene copolymer beads.

7.2. Method

To determine the optimal flow rate for the elimination of geosmin (BV/h), apply the test on a volume of 5 or 10 mL beads (BV), which corresponds reciprocally to a 5- or 10-L volume of wine or must to be treated, so that the ratio of resin to the volume of wine or must to be treated is 1:1000. The optimal flow rate falls within the range of 150-250 BV/h.

The must to be treated should undergo prior degradation of its pectins and filtration, so that its turbidity is less than 10 NTU and the pores of the beads are thus not obstructed.

- Rinse the adsorbent beads well in water (osmosis water), then place in the column and tightly pack together by tapping on the column.
- Introduce the wine or must into the column using the peristaltic pump at a pre-determined flow rate. Check the output flow rate of the wine every 30 minutes using a graduated burette, to ensure there is no clogging of the resin. After treatment, check the free SO_2 and total SO_2, in order to readjust their content in the wine if needed.
- To verify that the treatment has no negative impact on the must or wine, carry out analyses on the 5 or 10 litres treated (Table 1).

Table 1: Analyses to be carried out before and after treatment

-	Reds	Whites
Alcoholic strength	X in the wine	X in the wine

Residual sugars	X in the wine	X in the wine
Total sugars	X in the must	X in the must
Volatile acidity	X	X
Total acidity	X	X
pH	X	X
Free and total SO_2	X	X
OD 280 nm	X	X
OD 320 nm	X	X
OD 420 nm or CIELAB	X	X
OD 520 nm or CIELAB	X	
OD 620 nm or CIELAB	X	

The quantity of adsorbent beads and the flow rate will be readjusted, either according to the rate of elimination of geosmin demonstrated by analysis (SPME-GC-MS method, which is the internal method of COFRAC-accredited laboratories), or, if the treatment is urgent, by a simple tasting confirmed later by analysis.

8. Regeneration

The adsorbent beads may be regenerated a maximum of 5 times, after the total or partial volume of must or wine has passed through.

Regeneration is carried out in the column by passing through a 4M sodium hydroxide solution with the slowest possible flow rate depending on the type of pump (e.g. 20 BV/h).

Rinsing is performed with drinking water of a known pH (initial pH), until the sodium hydroxide has been eliminated; this is controlled through measurement of the pH of the water used for rinsing, which should be identical to the initial pH.

9. Conditions of use

Storage, use and regeneration of the adsorbent beads, and their disposal as waste, should be carried out according to the techniques permitted for food contact materials. The manufacturer is required to provide all necessary information for their use and regeneration. According to the legislation in force, the adsorbent beads are to be disposed of in approved industrial waste treatment centres for recycling, specifically through depolymerisation.

10. References

- Regulation (EC) No. 1935/2004
- Regulation (EU) No. 10/2011, amended, Annex 1, Table 1
- FDA regulations as found in Title 21 of the Code of Federal Regulations (CFR), Part 173 – Secondary Direct Food Additives permitted in food for human consumption, §173.65

Annex 1: Determination of styrene and divinylbenzene in wines (Type IV Method)

1. Important notice

The user of this publication should be well aware of current laboratory practices. This publication is not intended to address any safety problems that may be related to its use. The user is responsible for establishing the appropriate health and safety practices, and ensuring respect for both the national regulations in force and the environment.

2. Scope of application

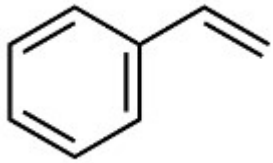
TESTING OF MIGRATION OF SPECIFIC COMPONENTS

3. Objective

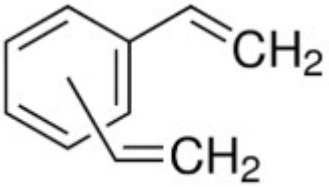
To verify that the profile of organic impurities or specific components of the adsorbent beads after pre-treatment by the manufacturer is compliant.

a/ By estimating the total quantity of volatile organic impurities present on the adsorbent beads;

b/ by estimating the proportion of these impurities that may migrate into a solution with an extraction power (solvents or simulants) comparable to that of must and wine.



Styrene



Divinylbenzene

Divinylbenzene exists in 3 forms: ortho, meta and para.

4. Standard references

ISO 78-2: Chemistry – Layouts for standards

5. Principle of the method

The method is gas chromatography coupled to mass spectrometry. The sample is extracted in the headspace using the solid-phase microextraction (SPME) technique. The wine/must sample is prepared by adding, to an SPME vial, roughly 2 g NaCl with 10 mL wine/must and 50 µL ethyl

heptanoate solution (internal standard) solution at 20 mg/L. The vial is sealed and stirred for 5 minutes. The internal standard used here is given by way of example; it is possible to use other internal standards.

For the measurement, six calibration points are used based on a stock solution containing all of the molecules to be studied.

6. Reagents and working solutions

During analysis – unless otherwise indicated – use only quality, recognised analytical reagents and distilled or demineralised water, or water of equivalent purity.

7. Reagents

- Type I or Type II water for analytical usage (ISO 3696 standard)
- Ethanol (CAS No. 64-17-5)
- Sodium chloride (CAS No. 7647-14-5)
- Ethyl heptanoate (CAS No. 106-30-9)
- Divinylbenzene (CAS No. 1321-74-0)

- Styrene (CAS No. 100-42-5)

8. Working solutions

Individual stock solutions at 1 g/L are prepared in ethanol for each molecule as well as for the internal standard (ethyl heptanoate).

Based on the individual stock solutions, working solutions are prepared with ethanol to the desired concentrations so as to cover the whole measurement range.

9. Calibration solutions

In order to ensure traceability to the International System of Units (SI), the calibration range should be made up of solutions with 12% (v/v) ethanol (5.1.2) covering 6 points of the range of measurement (1-100 $\mu g \cdot L^{-1}$). These solutions are prepared at the time of analysis for single use.

The calibration equation obtained is a second-degree equation.

10. Apparatus

The apparatus is given by way of example. The GC-MS technique used allows for the necessary variations or optimisations to be made according to the equipment configuration.

- GC-MS equipped with a “split-splitless” injector and mass-spectrometer detector
- Capillary column with apolar stationary phase, 5% phenylmethylpolysiloxane (e.g. HP-5MS, 30 m x 0.25 mm x 0.25 µm film) or equivalent
- Calibrated 100- µL, 1- µL and 10- µL microsyringes
- 20-mL SPME vial, sealable by a perforated capsule and Teflon®-faced cap
- Solid-phase microextraction system (SPME) with polydimethylsiloxane-film-coated fibre of 100 µm in thickness
- Balance
- This should have 0.1 mg precision.
- Measuring glassware

The measuring glassware for the preparation of reagents and calibration solutions is

class A.

11. Preparation of samples

1. Test samples

Place 10 mL wine/must in a 20-mL SPME glass vial (6.4) with roughly 2 g NaCl (5.1.3) and 50 µL ethyl heptanoate (internal standard) at 20 mg/L (5.1.4).

Seal the vial with a perforated cap and Teflon® seal (6.4).

11.2. GC-MS Procedure

11.2.1. Extraction

Carry out headspace SPME extraction for 25 minutes at room temperature.

11.2.2. **Injection**

Carry out desorption from the fibre for 10 minutes in the injector.

Injector at 260 °C in splitless mode

Helium flow rate: 2mL/min

11.2.3. **Gas chromatography parameters**

- Column: 5MS UI 30 m x 0.25 mm x 0.25 µm
- Transfer line: 300°C
- Oven: 45°C
- Then 2°C/min up to 80°C
- Then 3°C/min up to 92°C
- Then 40°C/min up to 300°C
- Then 300°C for 2 minutes
- Run time: 28.7 minutes

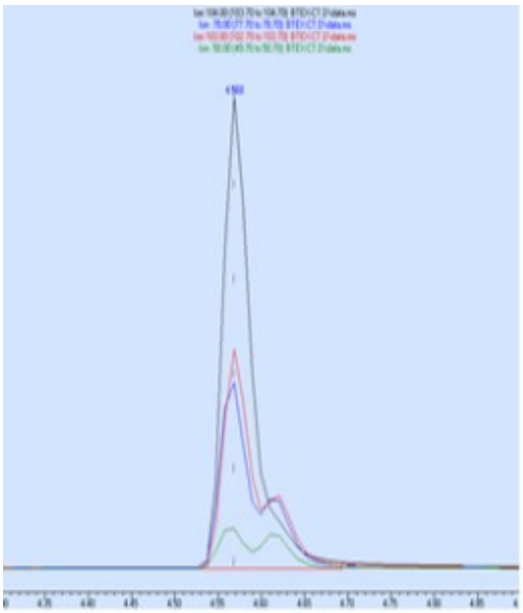
4. Acquisition

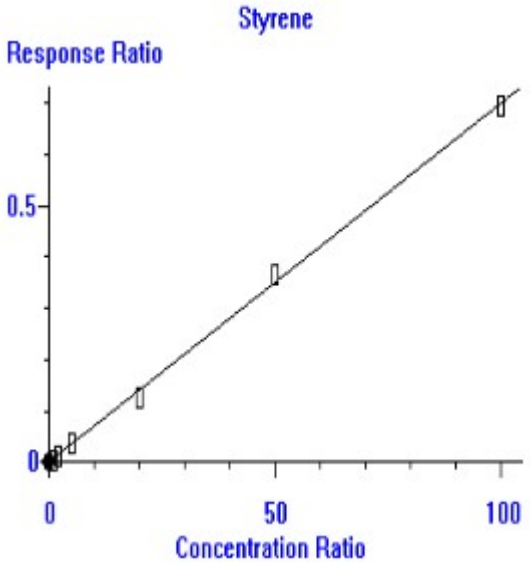
- Source temperature: 230°C
- Quad temperature: 150°C
- Acquisition: SIM

	Run time (min)	Ions (quantified)	Ions (qualified)
Ethyl heptanoate	15.0	88	113 / 101 / 158
Styrene	5.0	104	78 / 103 / 50
m,p-Divinylbenzene	15.4 & 16.1	130	128 / 115

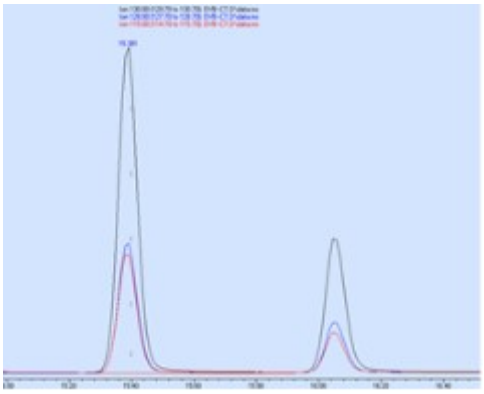
12. Results

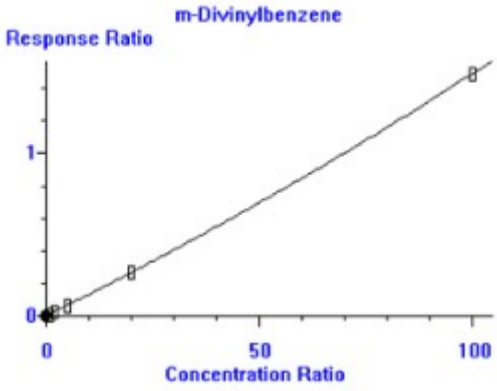
Example chromatogram and calibration curve for styrene





Example chromatogram and calibration curve for divinylbenzene





13. Expression of results

The results are expressed in µg/L.