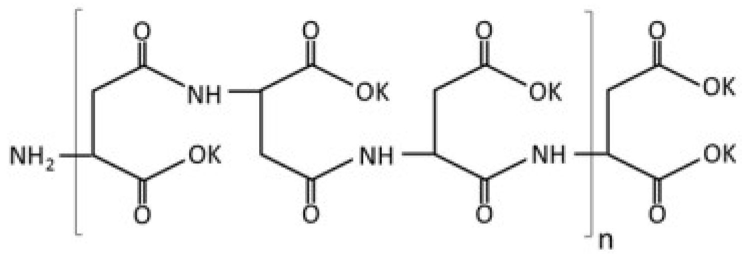
COEI-1-POTPOL Potassium polyaspartate

Chemical name	Homopolymer of potassium L-aspartate or potassium polyaspartate
Chemical formula	$[C_4H_5NO_3K]_n$
Topological formula	

where n ≈ 30

CAS No.: 64723-18 -8

1. **Object, origin and scop of application**

Oenological potassium polyaspartate is prepared exclusively from L-aspartic acid. The L-aspartic acid monomer used in the process is produced by fermentation. A thermal process converts the L-aspartic acid monomer into polysuccinimide, an insoluble compound. Polysuccinimide is then treated with potassium hydroxide under controlled conditions to obtain potassium polyaspartate. The potassium polyaspartate inhibits tartaric precipitation thanks to a 'colloid protector' effect. Potassium polyaspartate is effective for the tartaric stabilisation of wines.

2. **Synonyms**

Potassium polyaspartate, A-5D K/SD; A-5D K SD; A-5DK/SD; A-5DK; KPA.

3. **Labelling**

The following indications should appear on the packaging labelling:

- the name and sales denomination,
- the statement ‘Product for oenological use, limited use’,
- any additives,
- instructions for use,
- the batch number and potassium polyaspartate content (purity) as well as the expiry date and storage conditions (temperature, humidity and aeration),
- the name or company name and address of the manufacturer, packager or supplier,
- the net quantity,
- the indication that the aspartic acid is sourced from genetically-modified organisms and the modified characteristic where relevant.

4. Characterisation

4.1. Description

Light-brown, odourless powder containing 90% dry matter. It is entirely soluble in water (> 1000 g/L) yet insoluble in organic solvents (< 5 g/L), with a shelf life of 4 years at room temperature.

4.2. Chemical formula

Potassium polyaspartate is a polymer composed of aspartic acid units, with the following general formula: $[C_4H_5NO_3K]_n$, where n corresponds to the average degree of polymerisation ($n \approx 30$).

4.3. Degree of substitution

The degree of substitution of the potassium salt is at least 91.5% (in terms of anhydrous matter), in order to guarantee optimal solubility.

Assess the degree of substitution using the method described in Annex 1.

4.4. Molecular mass

Its average molecular mass, determined by gel permeation chromatography, is 5000 g/mol, which corresponds to the optimum efficiency of the product.

4.5. Composition

The purity of the product is verified by assaying the aspartic acid after total hydrolysis of the polymer and by comparing this value with the theoretical content of the monomer in the potassium polyaspartate according to its molecular formula. Refer to Annex 2 for the method description.

The content of anhydrous potassium polyaspartate matter should be at least 98%.

5. Trials

5.1. Free aspartic acid content in potassium polyaspartate

The free aspartic acid content should be $\leq$ 2.0%.

Carry out the determination according to the method described in Annex 3.

5.2. Humidity – Loss due to dehydration

Determine the loss in mass of a gram of dry product kept in an oven for 12-24 hours at 105 $\pm$ 2 °C. The mass should be constant and the loss in mass should be less than 10%.

5.3. Metal content

Before determining the metals, mineralise the sample by means of acid digestion (HNO_3, H_2O_2 and HCl). Conduct the mineralisation in a microwave oven. The sample should not be crushed or dehydrated before mineralisation.

The reagents used for mineralisation are as follows: HNO_3 (65%) (Suprapur or similar), HCl (37%) (Suprapur or similar) and H_2O_2 (35%).

Introduce the polyaspartate sample (between 0.5 and 2 g) into a 100-mL calibrated flask before adding 25 mL HNO_3, 2 mL HCl and 3 mL H_2O_2. At this stage, subject the mixture to digestion in a microwave oven with a maximum power of 1200 W: 60% power for 1 min, 30% for 10 min, 15% for 3 min and 40% for 15 min. Subsequently, make the calibrated flask up to volume with double-distilled water. The determination of the metals is practised on the solution thus obtained.

5.3.1. Iron

Determine the iron according to the method described in Chapter II of the *International Oenological Codex*. The iron content should be below 10mg/kg.

5.3.2. Arsenic

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

International Oenological Codex. The arsenic content should be below 3 mg/kg.

5.3.3. Lead

Determine the lead according to the method described in Chapter II of the *International Oenological Codex*. The lead content should be below 2 mg/kg.

5.3.4. Mercury

Determine the mercury according to the method described in Chapter II of the *International Oenological Codex*. The mercury content should be below 1 mg/kg.

5.3.5. Cadmium

Determine the cadmium according to the method described in Chapter II of the *International Oenological Codex*. The cadmium content should be below 1 mg/kg.

Annex 1: Determination of the degree of substitution

1. Principle

The degree of substitution of commercial potassium polyaspartate is determined by the analysis of the potassium content using the ICP-OES method.

The determination of potassium is conducted using a calibration curve obtained by injecting five different concentrations of a reference standard solution.

To calculate the degree of substitution, the potassium concentration measured is compared to the theoretical content at 100% substitution.

2. Equipment

- 100-mL Volumetric flasks (class A)
- Cyclonic atomisation chamber, standard quartz torch
- Ultrasonic bath
- Membrane filtration system with 0.45 m porosity

3. Reagents

- 65% Nitric acid (HNO_3)

- 10 000 mg/L Potassium (K) standard solution (potassium ICP/DCP standard solution with 10 000 µg/mL 5% HNO_3)
- Double-distilled water with superior resistivity of 10 MΩ.cm
- Aqueous solution acidified with 0.5% HNO_3 (calibration blank), to be used as a diluent for the preparation of the calibration solutions

Calibration solutions prepared by dilution of the stock solution (point 1.3.2); the reference values are indicated below:

	STD 1	STD 2	STD 3	STD 4	STD 5
Potassium (mg/L)	200	400	600	1000	2000

4. Procedure

The preparation to be analysed (KPA) is dissolved in double-distilled water.

5000 mg/L KPA solution (a): weigh around 500 g (note the exact weight) directly into a 100-mL calibrated flask, make up to volume with double-distilled water (1.3.3) and stir in an ultrasonic bath (1.2.3) for at least 10 minutes. Filter using membranes with 0.45 µm porosity.

Prepare the five-point calibration curve with the standard solutions as indicated in point 1.3.5.

The results should be calculated from the average of three measurements.

If the concentration lies outside the calibration curve, the sample should be diluted so that its concentration falls within the calibration curve.

To calculate the degree of substitution, compare the potassium concentration measured to the theoretical content established at 100% substitution (see point 1.5).

5. Calculations

The potassium content is calculated by the processor of the acquisition software. The calculation to be conducted is as follows:

$$A = A' \times n \qquad \text{(a)}$$

where:

- A: concentration of sample in mg/L

- A': concentration of diluted sample in mg/L
- n: dilution factor

The percentage of potassium in the KPA sample, expressed in dry weight, is calculated using formula (b):

$$\%K_{(\text{dry weight})} = A\,\frac{100}{W}\,\frac{100}{(100 - h\%)} \qquad \text{(b)}$$

where:

- A: result of equation (a)
- *w*: potassium polyaspartate in mg/L
- h%: humidity of the sample, as a percentage

The degree of substitution (DS) is calculated using equation (c):

$$\%DS_k = \frac{\&K_{(dry\ weight)}}{\frac{MA_K}{MM_{KPA\ monomer}}100} \qquad \text{(c)}$$

where:

- MA_K: atomic mass of potassium
- $MM_{KPAmonomer}$: calculated molecular mass of the polyaspartate monomer

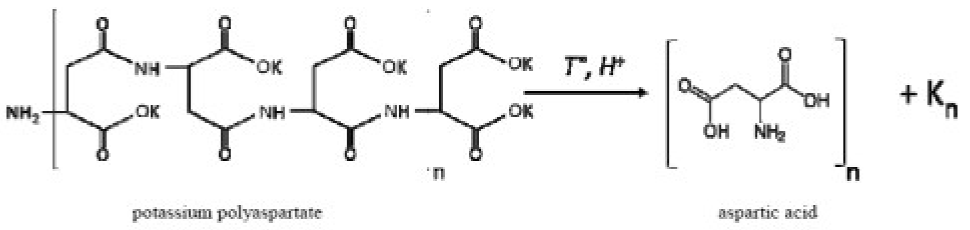
Annex 2: Determination of the purity of potassium polyaspartate

1. Principle

Analysis by HPLC-FLD of the free aspartic acid content after acid hydrolysis.

The principle consists of determining the free aspartic acid by HPLC after acid hydrolysis of the KPA. This acid hydrolysis takes place under conditions allowing for the complete depolymerisation of the KPA:

Potassium polyaspartate	Aspartic acid



2. Equipment/ Apparatus

1. Hot plate for acid hydrolysis
2. 4-mL Tinted-glass vials with screw cap
3. 0.1 mg Precision weighing balance
4. Calibrated flasks
5. HPLC system including a quarternary pump, an automatic sampler, a thermostat and a fluorometric detector (FLD)
6. C18 column (e.g. Syncronis aQ C18, 4.6 x 250 mm; 5 µm [Thermo])
7. Filtration system with membranes of 0.2 µm porosity

3. Reagents and sample preparation

1. For acid hydrolysis
    1. Potassium metabisulphite solution ($Na_2S_2O_2$) (CAS No. 16731-55-8) at a concentration of 10 g/L
    2. 6 M Hydrochloric acid (HCl)
    3. 5 M Sodium hydroxide (NaOH)
    4. Double-distilled water with superior resistivity of 10 m□.cm
    5. Potassium polyaspartate

3.2. For sample preparation

3.2.1. Aminocaproic acid ($C_6H_{13}NO_2$, CAS No.: 60-32-2)

4. Procedure

The procedure comprises three steps:

- hot acid hydrolysis of the potassium polyaspartate sample,
- preparation of the samples for analysis by HPLC-FLD of the standard solutions that will determine the aspartic acid concentration,
- analysis of the free aspartic acid after hydrolysis by HPLC (see Annex 3).

4.1. Phase 1: acid hydrolysis

4.1.1. Transfer into a 4–mL vial (2.2.2):

- 0.2 mL 10 g/L sodium metabisulphite solution (2.3.1),
- 0.5 g potassium polyaspartate weighed to the nearest mg,
- 2mL 6N HCl (2.3.2).

2. Heat to 108 ± 2°C for 72 hours (2.2.1).
3. Transfer to a 10-mL calibrated flask, add 2.4 mL 5 M NaOH (2.3.3) and make up to volume with double-distilled water (2.3.4).

2. Phase 2: preparation of the sample for HPLC analysis
    1. Microfilter 5 mL of medium (2.4.1.3) at 0.20 µm (2.2.7) in a 20-mL calibrated flask.
    2. Add 0.2 mL internal standard (aminocaproic acid) (2.3.6).
    3. Make up to volume with double-distilled water.

3. Phase 3: Analysis of samples by HPLC (see Annex 3)

5. Calculations

The polyaspartate concentration (KPA) is calculated as follows:

- KPA (mg/L) = (hydrolysed aspartic acid – free aspartic acid before hydrolysis) x f_{KPA}
- where f_{KPA} = 1.15, which is the conversion factor of KPA into aspartic acid, calculated based on the ratio between the molecular mass of the KPA monomer (average MM of KPA A5DK SD monomers=154) and the molecular mass of

aspartic acid (133.1), as per the equation:

$$f_{KPA} = \frac{MM_{KPA\ monomer}}{MM_{aspartic\ acid}} = 1.15$$

where the free aspartic acid is determined according to Annex 3.

Annex 3: Determination of free aspartic acid

1. **Principle**

The determination of aspartic acid in potassium polyaspartate as it was produced is carried out by HPLC coupled with fluorometric detection (FLD), after derivation of aspartic acid with ortho-phthalaldehyde (OPA). Potassium is determined using a calibration curve obtained by injecting the reference standard solutions.

2. **Equipment/ Apparatus**

- Calibrated flasks
- HPLC system including a quarternary pump, an automatic sampler, a thermostat and a fluorometric detector (FLD)
- C18 column, e.g. Syncronis aQ C18, 4.6 x 250 mm; 5 µm

3. **Reagents**

1. Aspartic acid (D,L-aspartic acid, $C_4H_7NO_4$ ≥ 99%, CAS No.: 617-45-8)
2. Solution 1: 8000 mg/L aspartic acid in double-distilled water
3. Solution 2: 200 mg/L aspartic acid in double-distilled water
4. Aminocaproic acid ($C_6H_{13}NO_2$, CAS No.: 60-32-2)

1000-mg/L aminocaproic acid stock solution in double-distilled water

Calibration solutions prepared by dilution of solution 1 (point 3.3.2) and solution 2 (3.3.3), whose reference values are indicated below:

	STD 1	STD 2	STD 3	STD 4	STD 5	STD 6
mL H_2O	18.8	19.0	15.0	19.750	19.375	18.750
mL Solution 1	-	-	-	0.250	0.625	1.250

mL Solution 2	0.2	1.0	5.0	-	-	-
Aspartic acid (mg/L)	**2**	**10**	**50**	**100**	**250**	**500**

3.5. Methanol for HPLC

3.6. Tetrahydrofuran for HPLC

3.7. Anhydrous sodium acetate (CAS No. 127-09-3)

3.8. Acetonitrile (CH_3CN) for HPLC

3.9. Sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$, CAS No. 1303-96-4)

O-phthalaldehyde (OPA): ($C_8H_6O_2$ ≥ 99%, CAS No.: 643-79-8)

3.10. Mercaptoethanol: (C_8H_6OS ≥ 99%, CAS No.: 60-24-2)

3.11. Double-distilled water with superior resistivity of 10 MΩ.cm

3.12. Derivation solution: in a 10-mL calibrated flask, introduce 100 mg OPA, 200 mL mercaptoethanol and 1 mL methanol, then make up to volume with a pH 10.5 buffer solution of 0.1 M sodium tetraborate decahydrate.

3.13. The solution should be prepared just before use since it degrades over the day following its preparation.

4. Mobile phases

[Channel A]: ultra-pure water

[Channel B]: 0.05 M sodium acetate buffer/tetrahydrofuran (96:4; v/v)

[Channel C]: methanol

Channel D]: acetonitrile

5. Procedure

The method consists of a reaction constituting the derivation of aspartic acid with the O-phtalaldehyde (OPA); the recovery rate for this process is 100%.

The instrumental parameters are as follows:

- temperature of the column: 40 °C,
- wavelength (λ): FLD Ex 340 nm, Em 450 nm,
- the separation is carried out in gradient mode (see point 3.4, Mobile phases):

Time (min)	% B	% C	% D	Flow (mL/min)

0.00	100.0	0.0	0.0	1.1
3.00	100.0	0.0	0.0	1.1
15.00	50.0	25.0	25.0	1.1
17.00	84.0	8.0	8.0	1.1
18.00	100.0	0.0	0.0	1.1
Run time: 21 min + 2 min downtime				

Prepare the calibration solutions by mixing 5.0 mL of the standard solution (3.3.6) and 0.2 mL of the internal standard solution (3.3.5) in a 20-mL calibrated flask, then make up to volume with double-distilled water and stir.

Dilute 5.0 µL of the sample (Annex 2, point 2.4.2) with 20 µL methanol, then derive with 0.5 µL OPA. Mix 10.0 µL of the thus-obtained solution 10 times in the injector, then inject after 0.5 min.

If the results exceed the upper limit of the calibration curve, dilute the sample and repeat the analytical procedure.

6. **Calculations**

The concentration of aspartic acid in the sample, expressed in mg/L, is obtained by applying the following formula:

$$Y = A.f.d$$

where:

- Y: concentration of aspartic acid in the sample, in mg/L
- A: peak area of the chromatogram
- f: response factor of the chromatogram peak
- d: dilution factor

Annex 4: Method of determination of the mean molecular mass of potassium polyaspartate

1. **Introduction**

The effectiveness of potassium polyaspartate used in tartaric stabilisation depends on the parameter of mean molecular mass, therefore it is necessary to have a method to determine the latter.

2. Objective

Parameter to be determined: mean molecular mass, expressed in g/mol.

3. Definitions

GPC/SEC: gel permeation chromatography.

4. Principle

The determination of the mean molecular mass requires the use of gel permeation chromatography (GPC/SEC). This is a type of molecular exclusion chromatography used to separate molecules according to their size.

5. Reagents and equipment

5.1. Double-distilled water with resistivity of > 10 MΩ.cm at 25°C
5.2. Anhydrous sodium sulphate (Na_2SO_4) with a purity of ≥ 99% (CAS No. 7757-82-6)
5.3. Anhydrous monobasic potassium phosphate (KH_2PO_4) with a purity of ≥ 99% (CAS No. 7787-77-0)
5.4. Sodium azide with a purity of ≥ 99% (CAS No. 26628-22-8). In using sodium azide, preventative measures should be taken to mitigate the risks of toxicity and instability (explosion)
5.5. Sodium polyacrylate salts with molecular masses of between 1000 and 1250 g/mol (CAS No. 9003-04-7)
5.6. L-aspartic acid with a purity of ≥ 98% (CAS No. 56-84-8)
5.7. Potassium polyaspartate with a purity of ≥ 98% (CAS No. 64723-18-8)

6. Apparatus

6.1. Filtration system with porosity of 0.22 µm
6.2. GPC column adapted to molecular mass intervals of between 500 and 10,000 g/mol
6.3. UV detector

7. Preparation of the sample

Prepare a volume of around 15 mL of 0.1% potassium polyaspartate solution (5.7) in the mobile phase (8.1) and filter it on a 0.22 µm filter (6.1). The polyaspartate solution in the buffer becomes unstable after 3 hours. Fresh solutions should therefore be prepared before each injection.

7.1. Calibration

Prepare 0.1% solutions for each standard (5.5 and 5.6) in the mobile phase (8.1).

Inject the standards to be used in order of decreasing molecular mass.

The calibration curve is obtained by representing the retention time (variable x) on a graph as a function of the logarithm of the mean molecular mass of the standards (5.5) (variable y) ($r^2 \geq 0.99$).

8. Procedure

8.1. Preparation of the mobile phase

The mobile phase is a buffer solution composed of 0.1 M Na_2SO_4 (14.2 g/L) (5.2), 0.01 M KH_2PO_4 (1.36 g/L) (5.3) and 20 mg/L sodium azide (5.4) in double-distilled water (5.1) filtered using a 0.22-µm filter. The buffer solution should be used within 4 days of preparation.

8.2. Chromatography conditions

Flow rate: 0.7 mL/min

Column: Ultrahydrogel™ Linear or similar, dimensions: 7.8 x 300 mm, filled with particles of an average diameter of 6 µm

Column temperature: 50°C

Run time: 40 min

Injection volume: 200 µL

UV detector: wavelength of 220 nm

9. Calculations

Compare the chromatographic profile corresponding to the sample with that of the standards (5.5). Calculate the molecular mass of polyaspartate, expressed in g/mol, according to the retention time of the sample and the calibration curve.