OIV-MA-AS315-30 Determination of alkylphenols in wines by gas chromatography-mass spectrometry (GC-MS or GC-MS/MS)

Type IV method

1. Scope of application

The following method allows for the determination of the following molecules:

	Range studied
2-tert-butylphenol	1-100 µg/L
4-tert-butylphenol	1-100 µg/L
6-methyl-2-tert-butylphenol	1-100 µg/L
4-methyl-2-tert-butylphenol	1-100 µg/L
5-methyl-2-tert-butylphenol	1-100 µg/L
4,6-di-methyl-2-tert-butylphenol	1-100 µg/L
2,6-di-tert-butylphenol	1-100 µg/L
2,4-di-tert-butylphenol	1-100 µg/L

2. Standard references

- ISO 78-2: Chemistry – Layouts for standards,
- ISO 3696: Water for analytical laboratory use,
- Resolution OIV-OENO 418-2013.

3. Principle of the method

The method describes the analysis, on the one hand, by gas chromatography coupled with mass spectrometry (GC-MS), and on the other, by gas chromatography coupled with tandem mass spectrometry (GC-MS/MS). The sample is extracted in the

headspace using the solid-phase microextraction (SPME) technique.

4. Reagents and working solutions

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

4.1. Reagents

4.1.1. Quality I or II water for analytical usage (ISO 3696 standard)

4.1.2. Absolute ethanol (CAS No. 64-17-5)

4.1.3. Sodium chloride (CAS No. 7647-14-5)

4.1.4. 4-tert-butylphenol-d13 (CAS 225386-58-3)

4.1.5. 4-tert-butylphenol (CAS No. 98-54-4)

4.1.6. 2-tert-butylphenol (CAS No. 88-18-6)

4.1.7. 4-methyl-2-tert-butylphenol (CAS No. 2409-55-4)

4.1.8. 5-methyl-2-tert-butylphenol (CAS No. 88-60-8)

4.1.9. 6-methyl-2-tert-butylphenol (CAS No. 2219-82-1)

4.1.10. 4,6-di-methyl-2-tert-butylphenol (CAS No. 1879-09-0)

4.1.11. 2,4-di-tert-butylphenol (CAS No. 96-76-4)

4.1.12. 2,6-di-tert-butylphenol (CAS No. 128-39-2)

4.2. Stock solutions

Individual stock solutions at 1 g/L are prepared in ethanol for each alkylphenol as well as for the internal standard (e.g. 4-tert-butylphenol-d13).

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

4.3. Calibration solutions

In order to ensure the best possible traceability to the International System of Units (SI), the calibration range should be made up of solutions and powders with (a high grade of) purity of different alkylphenols, prepared by weight or volumetrically according to the SI.

The calibration range is carried out with 12% (v/v) ethanol (4.1.2), with the range of measurement (1-100 $\mu g \cdot L^{-1}$) covering 5 points, for example. These solutions are prepared at the time of analysis for immediate use after preparation (within a few hours).

The calibration equation obtained is generally a quadratic function.

5. Apparatus

5.1. GC-MS equipped with a “split-splitless” injector and mass-spectrometer detector or tandem mass spectrometer

5.2. Capillary column with apolar stationary phase, 5% phenylmethylpolysiloxane (e.g. 5MS, 30 m x 0.25 mm x 0.25 μm film) or equivalent

5.3. Calibrated 100- μL, 1-mL and 10-mL micropipettes

5.4. 20-mL SMPE vial, sealable by a perforated cap and Teflon seal

5.5. Solid-phase microextraction system (SPME) with polydimethylsiloxane-film-coated fibre of 100 μm in thickness, or equivalent

5.6. Balance

With traceability to the SI and 0.1 mg precision.

5.7. Measuring glassware

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

6. Preparation of samples

The internal standard 4-tert-butylphenol-d13 is used here by way of example; it is possible to use other internal standards.

A sample of 10 mL wine is placed in a 20-mL SPME glass vial (5.4) with roughly 2 g NaCl (4.1.3) and 50 μL 4-tert-butylphenol-d13 (internal standard) solution at 5 mg/L (4.1.4).

The vial is closed with a perforated cap and Teflon seal (5.4).

7. GC-MS Procedure

The procedure 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.

7.1. Extraction

The headspace SPME extraction is carried out for 20 minutes at 40 °C.

7.2. Injection

Desorption from the fibre is carried out for 10 minutes in the injector.

Injector at 260 °C in splitless mode

Helium flow rate: 1 mL/min

7.3. Gas chromatography parameters

Column: 5MS UI 30 m x 0.25 mm x 0.25 μm

Transfer line temperature: 300°C
Oven: 50°C
Then 10 °C/min up to 300 °C
Then 300 °C for 3 minutes
Run time: 28.0 minutes
Acquisition
Source temperature: 250 °C
Quad temperature: 150 °C
Acquisition: SIM

	Run time (min)	Ions (quantified)	Ions (qualified)
2-tert-butylphenol	8.9	135	107-150
4-tert-butylphenol-d13 (IS)	9.1	145	113-163
4-tert-butylphenol	9.2	135	107-150
6-methyl-2-tert-butylphenol	9.4	149	164-121
4-methyl-2-tert-butylphenol	10.0	149	164-121
5-methyl-2-tert-butylphenol	10.2	149	164-121
4,6-dimethyl-2-tert-butylphenol	10.5	163	135-178
2,6-di-tert-butylphenol	11.2	191	206-192
2,4-di-tert-butylphenol	12.0	191	206-192

Table 1: Ions used in mass spectrometry.

8. GC-MS/MS procedure

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

8.1. Extraction

The headspace SPME extraction is carried out for 5 minutes at 40 °C.

8.2. Injection

Desorption from the fibre is carried out for 8 minutes in the injector.

Injector at 250 °C in pulsed-split mode with a split ratio of 2:1

Helium flow rate: 2 mL/min

8.3. Gas chromatography parameters

Column: 5MS UI 30 mx0.25 mm x 0.25 μm or equivalent

Transfer line: 300 °C

Oven: 50 °C

Then 25 °C/min up to 130 °C

Then 10 °C/min up to 170 °C

Then 25 °C/min up to 300 °C

Then 300 °C for 3 minutes

Run time: 15.4 minutes

8.4. Acquisition

Source temperature: 250 °C

Quad temperature: 150 °C

Acquisition: MRM

	Run time (min)	Quantification transitions	Qualification transitions
2-tert-butylphenol	5.0	135>107	150>107 & 150>135
4-tert-butylphenol-d13 (IS)	5.1	145>113	163>113 & 163>145
4-tert-butylphenol	5.2	135>107	150>107 & 150>135
6-methyl-2-tert-butylphenol	5.3	149>121	164>121 & 164>149
4-methyl-2-tert-butylphenol	5.7	149>121	164>121 & 164>149
5-methyl-2-tert-butylphenol	5.8	149>121	164>121 & 164>149
4,6-dimethyl-2-tert-butylphenol	6.1	163>135	178>135 & 178>163

2,6-di-tert-butylphenol	6.6	206>191	191>163 & 191>57
2,4-di-tert-butylphenol	7.2	191>57	191>163 & 206>191

Table 2: Ions used in tandem mass spectrometry.

9. Expression of results

The results are expressed in µg/L.

10. Annex 1: Results of internal validation

The performance was measured using an intra-laboratory experimental approach: 5 materials covering the scope of application of the method (1; 5; 25; 50; 100 µg/L) were formulated, within a synthetic wine matrix (hydroalcoholic solution at 12% (v/v), 6 g/L tartaric acid, pH adjustment to 3.5 with 1M NaOH).

Each material was analysed 5 times under conditions of intermediate precision with 2 repetitions of each analysis. The analyses were performed in September and October 2018.

The calculations were made according to Resolution OIV-OENO 418-2013, “Practical Guide for the assessment, quality control, and uncertainty analysis of an oenological analysis method”.

GC-MS	% CV (k=2) Intermediate precision	CVr (%) Repeatability	Validated LOQ
2-tert-butylphenol	6.7%	4.3%	1 µg/L
4-tert-butylphenol	7.3%	5.1%	1 µg/L
6-methyl-2-tert-butylphenol	12.1%	10.2%	1 µg/L
4-methyl-2-tert-butylphenol	6.0%	4.6%	1 µg/L
5-methyl-2-tert-butylphenol	6.4%	4.9%	1 µg/L
4,6-dimethyl-2-tert-butylphenol	12.7%	10.5%	1 µg/L
2,6-di-tert-butylphenol	19.5%	14.6%	1 µg/L

2,4-di-tert-butylphenol	11.9%	9.9%	1 µg/L

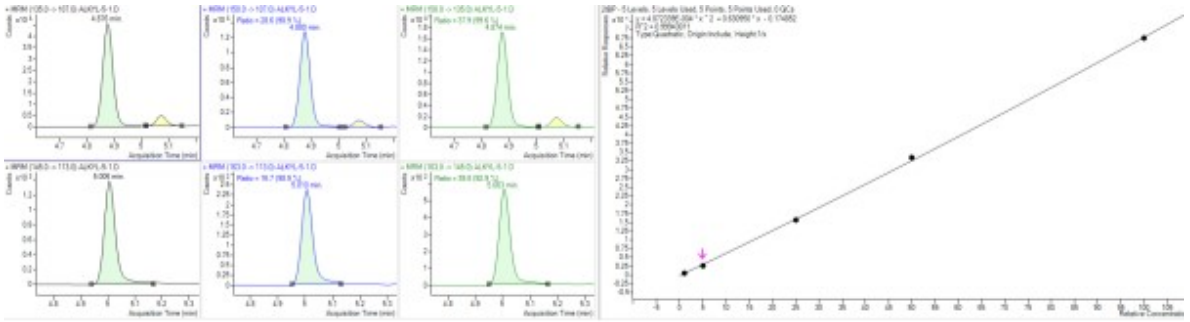
Table 3: Performance obtained with mass spectrometry.

GC-MS/MS	% CV (k=2) Intermediate precision	CVr (%) Repeatability	LOQ
2-tert-butylphenol	11.3%	10.1%	1 µg/L
4-tert-butylphenol	10.4%	11.0%	1 µg/L
6-methyl-2-tert-butylphenol	13.9%	13.5%	1 µg/L
4-methyl-2-tert-butylphenol	11.1%	9.6%	1 µg/L
5-methyl-2-tert-butylphenol	12.3%	10.3%	1 µg/L
4,6-dimethyl-2-tert-butylphenol	13.4%	12.6%	1 µg/L
2,6-di-tert-butylphenol	16.6%	16.8%	1 µg/L
2,4-di-tert-butylphenol	14.5%	12.4%	1 µg/L

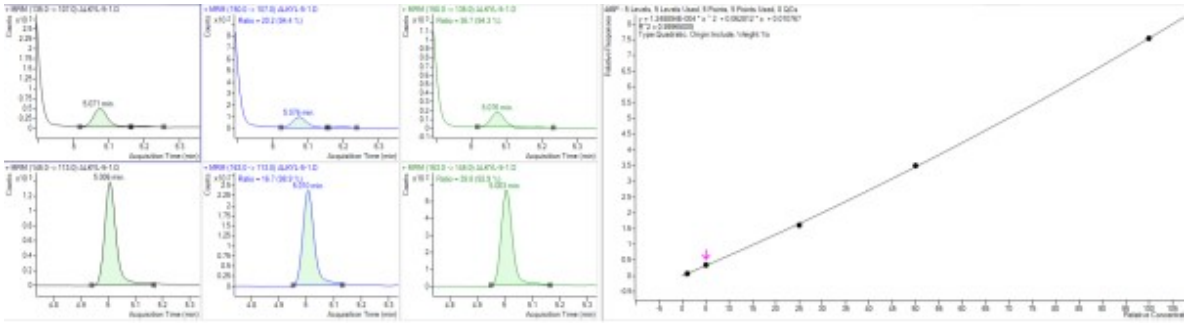
Table 4: Performance obtained with tandem mass spectrometry.

11. Annex 2: Example chromatograms and calibration curves

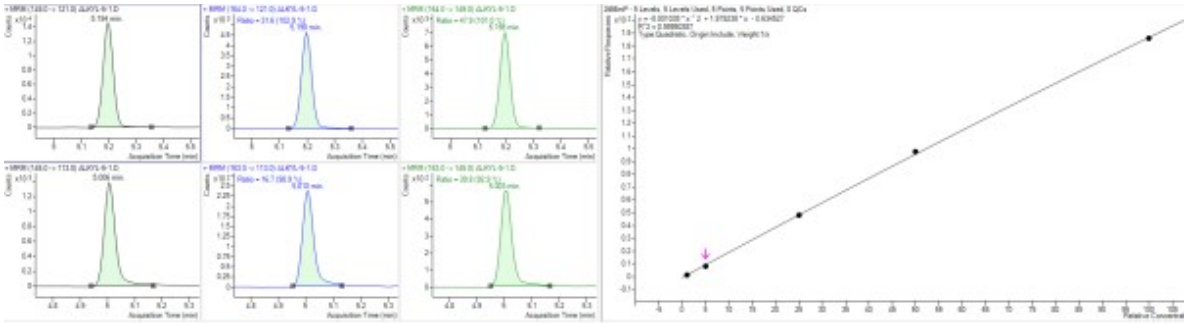
1. 2-tert-butylphenol



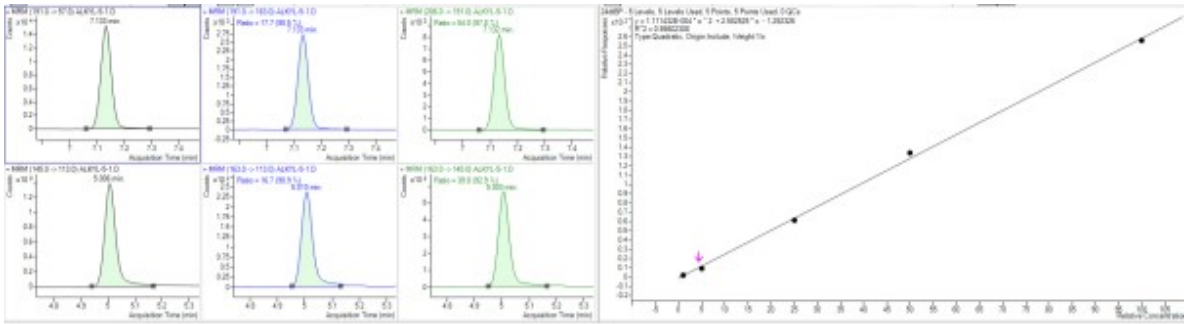
11.2. 4-tert-butylphenol



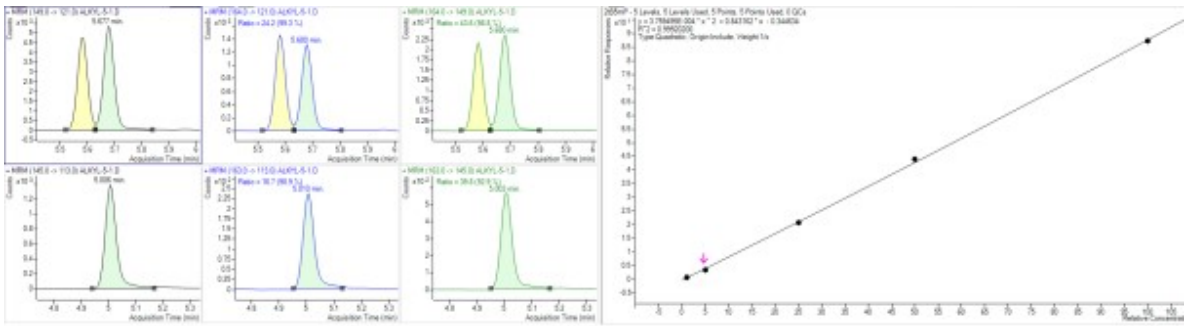
11.3. 6-methyl-2-tert-butylphenol



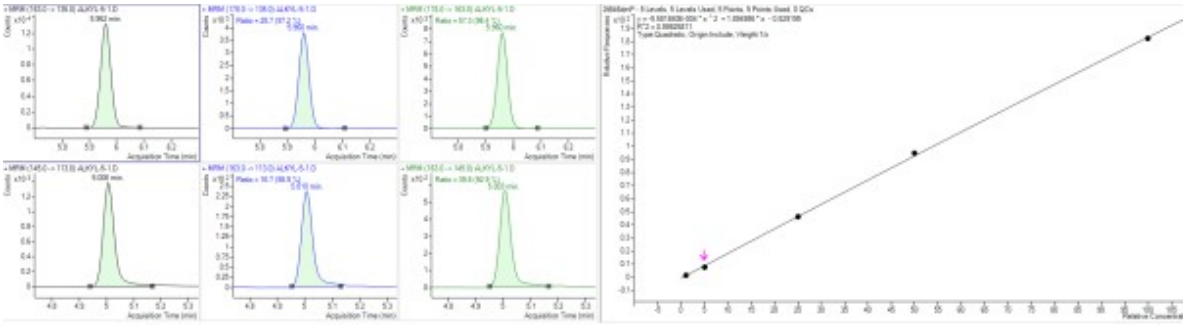
11.4. 4-methyl-2-tert-butylphenol



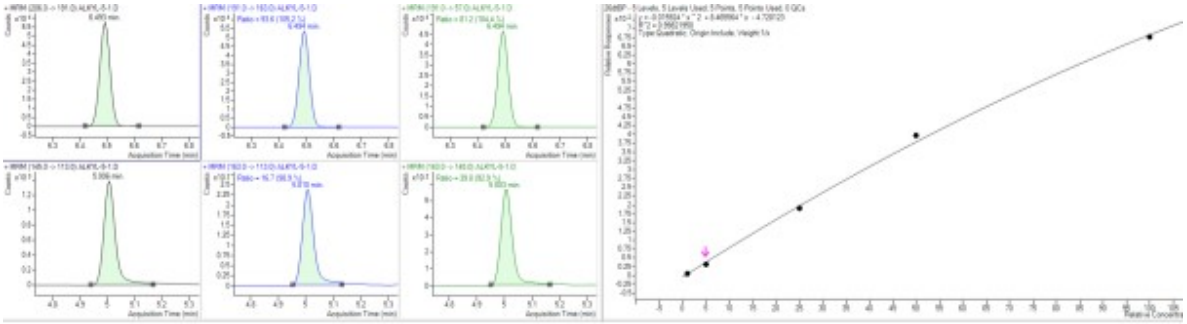
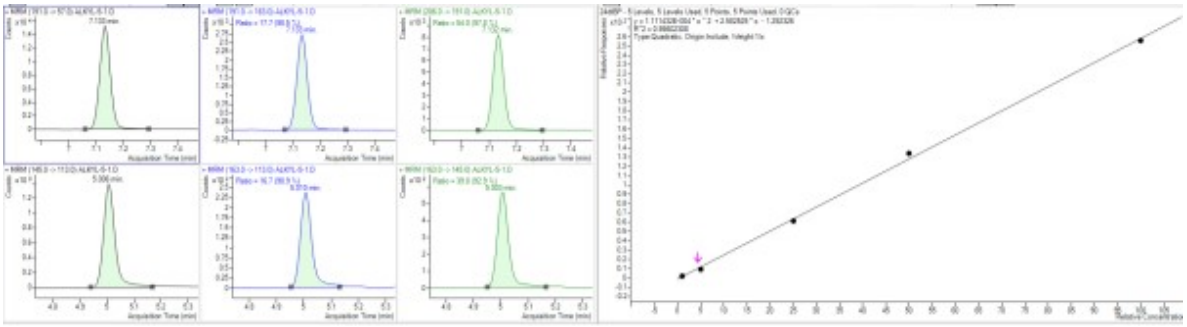
11.5. 5-methyl-2-tert-butylphenol



11.6. 4,6-dimethyl-2-tert-butylphenol



11.7. 2,6-di-tert-butylphenol



11.8. 2,4-di-tert-butylphenol