

# COMPENDIUM OF INTERNATIONAL METHODS OF ANALYSIS FOR SPIRITUOUS BEVERAGES AND ALCOHOLS

## DETERMINATION OF THE PRINCIPAL COMPOUNDS EXTRACTED FROM WOOD DURING

AGEING OF SPIRIT DRINKS OF VITIVINICULTURAL ORIGIN (Type II)

### **OIV-MA-BS-16 Determination of the principal compounds extracted from wood during ageing of spirit drinks of viti- vinicultural origin**

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Type II method

#### **1. Purpose and applicability**

The present method pertains to the determination of furfural, 5-hydroxymethylfurfural, 5-methylfurfural, vanillin, syringaldehyde, coniferaldehyde, sinapaldehyde, gallic, ellagic, vanillic, and syringic acids, and scopoletin, by high-performance liquid chromatography.

#### **2. Principle**

Determination by high-performance liquid chromatography (HPLC), with detection by ultraviolet spectrophotometry at several wavelengths, and by spectrofluorimetry.

#### **3. Reagents**

The reagents must be of analytical quality. The water used must be distilled water or water of at least equivalent purity. It is preferable to use microfiltered water with a resistivity of 18.2 M  $\Omega$ .

3.1. 96% vol. alcohol.

3.2. HPLC-quality methanol (Solvent B).

3.3. Acetic acid diluted to 0.5% vol. (Solvent A).

3.4. Mobile phases: (given only an example).

Solvent A (0.5% acetic acid) and solvent B (pure methanol). Filter through a membrane (porosity 0.45  $\mu\text{m}$ ). Degas in an ultrasonic bath, if necessary.

3.5. Reference standards of 99% minimum purity: furfural, 5-hydroxymethyl furfural, 5-methylfurfural, vanillin, syringaldehyde, coniferaldehyde, sinapaldehyde, gallic, ellagic, vanillic, and syringic acids, and scopoletin.

3.6. Reference solution: the standard substances are dissolved in a 50% vol. aqueous-alcoholic solution. The final concentrations in the reference solution should be of the order of:

- furfural: 5 mg/L
- 5-hydroxymethyl furfural: 10 mg/L

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- 5-methylfurfural 2 mg/L
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- vanillin: 5 mg/L
  - syringaldehyde: 10 mg/L
  - coniferaldehyde: 5 mg/L
  - sinapaldehyde: 5 mg/L
  - gallic acid: 10 mg/L
  - ellagic acid: 10 mg/L
  - vanillic acid: 5 mg/L
  - syringic acid: 5 mg/L
  - scopoletin: 0.5 mg/L.

### 4. Apparatus

Standard laboratory apparatus

- 4.1. A high-performance liquid chromatograph capable of functioning in binary gradient mode and equipped with:
  - 4.1.1. A spectrophotometric detector capable of measuring at wavelengths from 280 to 313 nm. It is however preferable to work with a multiple wavelength detector with a diode array or similar, in order to confirm the purity of the peaks.
  - 4.1.2. A spectrofluorimetric detector – excitation wavelength: 354 nm, emission wavelength: 446 nm (for the trace determination of scopoletin; which is also detectable at 313 nm by spectrophotometry).
  - 4.1.3. 4.1.3 An injection device capable of introducing 10 or 20 µL (for example) of the test sample.
  - 4.1.4. A high-performance liquid chromatography column, RP C18 type, 5 µm maximum particle size.
- 4.2. Syringes for HPLC.
- 4.3. Device for membrane-filtration of small volumes.
- 4.4. Integrator-computer or recorder with performance compatible with the entire apparatus, and in particular, it must have several acquisition channels.

### 5. Procedure

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### 5.1. Preparation of the injection

The reference solution and the spirit drink are filtered if necessary through a membrane with a maximum pore diameter of 0.45 µm.

5.2. Chromatographic operating conditions: Carry out the analysis at ambient temperature under the conditions defined in 4.1 using the mobile phases (3.4) with a flow of approximately 0.6 ml per minute following the gradient below (given as an example only)

| Time                   | 0 min | 50 min | 70 min | 90 min |
|------------------------|-------|--------|--------|--------|
| solvent A (water acid) | 100%  | 60%    | 100%   | 100%   |
| solvent B (methanol)   | 0%    | 40%    | 0%     | 0%     |

Note that in certain cases this gradient should be modified to avoid co-elutions.

### 5.3. Determination

5.3.1. Inject the reference standards separately, then mixed.

Adapt the operating conditions so that the resolution factors of the peaks of all the compounds are equal to at least 1.

5.3.2. Inject the sample as prepared in 5.1, after filtering it through a membrane.

5.3.3. Measure the area of the peaks in the reference solution and the spirit drink and calculate the concentrations.

## 6. Expression of results

Express the concentration of each constituent in mg/l.

## 7. Performance characteristics of the method (precision)

The following data were obtained in 2009 from an international method-performance study on a variety of spirit drinks, carried out following internationally-agreed procedures.

Key to the tables below:

|     |                                                         |
|-----|---------------------------------------------------------|
| nLT | Number of participating laboratories                    |
| nL  | Number of laboratories used to calculate precision data |
| r   | repeatability limit                                     |

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|       |                                                               |
|-------|---------------------------------------------------------------|
| Sr    | repeatability standard deviation                              |
| RSDr  | repeatability standard deviation expressed as % of the mean   |
| R     | reproducibility limit                                         |
| SR    | reproducibility standard deviation                            |
| RSDR  | reproducibility standard deviation expressed as % of the mean |
| PRSDR | RSDR predicted with the Horwitz formula (%)                   |
| HoR   | HorRat value = RSDR / PRSDR                                   |

### 7.1. Gallic acid

|             | nLT | nL | Mean<br>(mg/L) | r<br>(mg/L) | Sr<br>(mg/L) | RSDr<br>(%) | R<br>(mg/L) | SR<br>(mg/L) | RSDR<br>(%) | PRSDR<br>(%) | HoR |
|-------------|-----|----|----------------|-------------|--------------|-------------|-------------|--------------|-------------|--------------|-----|
| Whisky      | 16  | 15 | 1.2            | 0.2         | 0.07         | 6.1         | 1.2         | 0.43         | 36          | 16           | 2.3 |
| Brandy      | 15  | 14 | 0.4            | 0.1         | 0.04         | 8.1         | 0.6         | 0.20         | 47          | 18           | 2.6 |
| Rum         | 16  | 16 | 2.0            | 0.2         | 0.06         | 2.9         | 1.7         | 0.62         | 31          | 14           | 2.1 |
| Cognac<br>1 | 16  | 16 | 6.1            | 0.5         | 0.18         | 3.0         | 9.1         | 3.3          | 53          | 12           | 4.4 |
| Bourbon     | 16  | 16 | 7.3            | 0.5         | 0.18         | 2.4         | 6.2         | 2.2          | 30          | 12           | 2.6 |
| Cognac<br>2 | 16  | 16 | 21.8           | 1.7         | 0.60         | 2.8         | 21.7        | 7.7          | 35          | 10           | 3.5 |

### 7.2. 5-Hydroxymethylfurfural

|        | nLT | nL | Mean<br>(mg/L) | r<br>(mg/L) | Sr<br>(mg/L) | RSDr<br>(%) | R<br>(mg/L) | SR<br>(mg/L) | RSDR<br>(%) | PRSDR<br>(%) | HoR |
|--------|-----|----|----------------|-------------|--------------|-------------|-------------|--------------|-------------|--------------|-----|
| Whisky | 16  | 14 | 5.0            | 0.2         | 0.09         | 1.7         | 1.1         | 0.39         | 8           | 13           | 0.6 |
| Brandy | 16  | 14 | 11.1           | 0.3         | 0.09         | 0.8         | 2.8         | 1.01         | 9           | 11           | 0.8 |

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|          |    |    |      |     |      |     |      |      |    |    |     |
|----------|----|----|------|-----|------|-----|------|------|----|----|-----|
| Rum      | 16 | 14 | 9.4  | 0.3 | 0.09 | 1.0 | 1.4  | 0.50 | 5  | 11 | 0.5 |
| Cognac 1 | 16 | 14 | 33.7 | 1.2 | 0.42 | 1.3 | 12.5 | 4.5  | 13 | 9  | 1.4 |
| Bourbon  | 16 | 14 | 5.8  | 0.2 | 0.07 | 1.2 | 1.1  | 0.4  | 7  | 12 | 0.6 |
| Cognac 2 | 16 | 14 | 17.5 | 0.4 | 0.13 | 0.8 | 4.6  | 1.6  | 9  | 10 | 0.9 |

### 7.3. Furfural

|          | nLT | nL | Mean<br>(mg/L) | r<br>(mg/L) | Sr<br>(mg/L) | RSDr<br>(%) | R<br>(mg/L) | SR<br>(mg/L) | RSDR<br>(%) | PRSDR<br>(%) | HoR |
|----------|-----|----|----------------|-------------|--------------|-------------|-------------|--------------|-------------|--------------|-----|
| Whisky   | 15  | 14 | 2.9            | 0.1         | 0.04         | 1.4         | 0.7         | 0.24         | 8           | 14           | 0.6 |
| Brandy   | 15  | 12 | 1.2            | 0.2         | 0.05         | 4.5         | 0.5         | 0.18         | 15          | 16           | 0.9 |
| Rum      | 15  | 13 | 1.7            | 0.1         | 0.04         | 2.3         | 0.3         | 0.09         | 5           | 15           | 0.4 |
| Cognac 1 | 15  | 14 | 10.6           | 0.5         | 0.18         | 1.7         | 3.8         | 1.4          | 13          | 11           | 1.1 |
| Bourbon  | 15  | 13 | 15.3           | 0.6         | 0.23         | 1.5         | 1.4         | 0.49         | 3           | 11           | 0.3 |
| Cognac 2 | 15  | 13 | 13.9           | 0.6         | 0.20         | 1.5         | 1.9         | 0.69         | 5           | 11           | 0.5 |

### 7.4. Vanillic acid

|          | nLT | nL | Mean<br>(mg/L) | r<br>(mg/L) | Sr<br>(mg/L) | RSDr<br>(%) | R<br>(mg/L) | SR<br>(mg/L) | RSDR<br>(%) | PRSDR<br>(%) | HoR |
|----------|-----|----|----------------|-------------|--------------|-------------|-------------|--------------|-------------|--------------|-----|
| Whisky   | 15  | 12 | 0.2            | 0.1         | 0.03         | 14.2        | 0.2         | 0.06         | 28          | 20           | 1.4 |
| Brandy   | 15  | 11 | 0.2            | 0.1         | 0.04         | 16.5        | 0.1         | 0.05         | 20          | 20           | 1.0 |
| Rum      | 15  | 14 | 1.5            | 0.1         | 0.03         | 2.3         | 1.4         | 0.51         | 35          | 15           | 2.3 |
| Cognac 1 | 15  | 14 | 0.8            | 0.3         | 0.10         | 12.6        | 0.7         | 0.2          | 31          | 17           | 1.9 |

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|             |    |    |     |     |      |     |     |      |    |    |     |
|-------------|----|----|-----|-----|------|-----|-----|------|----|----|-----|
| Bourbon     | 15 | 15 | 2.4 | 0.4 | 0.13 | 5.3 | 3.4 | 1.22 | 51 | 14 | 3.6 |
| Cognac<br>2 | 15 | 14 | 2.7 | 0.6 | 0.21 | 7.7 | 2.0 | 0.70 | 26 | 14 | 1.9 |

### 7.5. 5-Methylfurfural

|             | nLT | nL | Mean<br>(mg/L) | r<br>(mg/L) | Sr<br>(mg/L) | RSDr<br>(%) | R<br>(mg/L) | SR<br>(mg/L) | RSDR<br>(%) | PRSDR<br>(%) | HoR |
|-------------|-----|----|----------------|-------------|--------------|-------------|-------------|--------------|-------------|--------------|-----|
| Whisky      | 11  | 11 | 0.1            | 0.0         | 0.01         | 10.7        | 0.1         | 0.03         | 35          | 24           | 1.5 |
| Brandy      | 11  | 11 | 0.2            | 0.0         | 0.01         | 6.1         | 0.1         | 0.04         | 18          | 20           | 0.9 |
| Rum         | 11  | 8  | 0.1            | 0.1         | 0.02         | 13.6        | 0.1         | 0.03         | 22          | 22           | 1.0 |
| Cognac<br>1 | 11  | 11 | 0.5            | 0.1         | 0.02         | 4.7         | 0.5         | 0.18         | 39          | 18           | 2.2 |
| Bourbon     | 11  | 10 | 1.7            | 0.1         | 0.03         | 2.0         | 0.6         | 0.20         | 12          | 15           | 0.8 |
| Cognac<br>2 | 11  | 11 | 0.8            | 0.2         | 0.07         | 10.0        | 0.7         | 0.26         | 35          | 17           | 2.1 |

### 7.6. Syringic acid

|             | nLT | nL | Mean<br>(mg/L) | r<br>(mg/L) | Sr<br>(mg/L) | RSDr<br>(%) | R<br>(mg/L) | SR<br>(mg/L) | RSDR<br>(%) | PRSDR<br>(%) | HoR |
|-------------|-----|----|----------------|-------------|--------------|-------------|-------------|--------------|-------------|--------------|-----|
| Whisky      | 16  | 16 | 0.4            | 0.1         | 0.03         | 6.7         | 0.2         | 0.08         | 19          | 18           | 1.0 |
| Brandy      | 15  | 15 | 0.2            | 0.1         | 0.02         | 12.6        | 0.1         | 0.05         | 29          | 21           | 1.4 |
| Rum         | 16  | 15 | 2.5            | 0.2         | 0.06         | 2.3         | 0.8         | 0.29         | 11          | 14           | 0.8 |
| Cognac<br>1 | 16  | 15 | 1.4            | 0.4         | 0.13         | 9.0         | 0.7         | 0.26         | 18          | 15           | 1.2 |
| Bourbon     | 16  | 16 | 3.4            | 0.2         | 0.08         | 2.3         | 1.2         | 0.43         | 13          | 13           | 0.9 |
| Cognac<br>2 | 16  | 15 | 4.8            | 0.3         | 0.11         | 2.3         | 1.9         | 0.67         | 14          | 13           | 1.1 |

### 7.7. Vanillin

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|             | nLT | nL | Mean<br>(mg/L) | r<br>(mg/L) | Sr<br>(mg/L) | RSDr<br>(%) | R<br>(mg/L) | SR<br>(mg/L) | RSDR<br>(%) | PRSDR<br>(%) | HoR |
|-------------|-----|----|----------------|-------------|--------------|-------------|-------------|--------------|-------------|--------------|-----|
| Whisky      | 16  | 16 | 0.5            | 0.1         | 0.03         | 6.8         | 0.3         | 0.09         | 19          | 18           | 1.1 |
| Brandy      | 15  | 15 | 0.2            | 0.1         | 0.02         | 9.6         | 0.2         | 0.06         | 25          | 20           | 1.2 |
| Rum         | 16  | 16 | 1.2            | 0.2         | 0.06         | 4.6         | 0.5         | 0.18         | 15          | 16           | 1.0 |
| Cognac<br>1 | 16  | 16 | 1.2            | 0.3         | 0.11         | 8.9         | 0.8         | 0.27         | 22          | 16           | 1.4 |
| Bourbon     | 16  | 16 | 3.2            | 0.3         | 0.11         | 3.5         | 1.2         | 0.41         | 13          | 13           | 0.9 |
| Cognac<br>2 | 16  | 16 | 3.9            | 0.3         | 0.09         | 2.3         | 1.7         | 0.62         | 16          | 13           | 1.2 |

### 7.8. Syringaldehyde

|             | nLT | nL | Mean<br>(mg/L) | r<br>(mg/L) | Sr<br>(mg/L) | RSDr<br>(%) | R<br>(mg/L) | SR<br>(mg/L) | RSDR<br>(%) | PRSDR<br>(%) | HoR |
|-------------|-----|----|----------------|-------------|--------------|-------------|-------------|--------------|-------------|--------------|-----|
| Whisky      | 16  | 13 | 1.0            | 0.1         | 0.03         | 2.6         | 0.2         | 0.08         | 8           | 16           | 0.5 |
| Brandy      | 15  | 13 | 0.2            | 0.1         | 0.02         | 8.1         | 0.2         | 0.07         | 33          | 20           | 1.6 |
| Rum         | 16  | 13 | 4.8            | 0.1         | 0.04         | 0.8         | 0.7         | 0.23         | 5           | 13           | 0.4 |
| Cognac<br>1 | 16  | 12 | 3.2            | 0.2         | 0.08         | 2.6         | 0.5         | 0.19         | 6           | 14           | 0.4 |
| Bourbon     | 16  | 14 | 10.5           | 0.3         | 0.10         | 0.9         | 1.1         | 0.39         | 4           | 11           | 0.3 |
| Cognac<br>2 | 16  | 13 | 9.7            | 0.3         | 0.09         | 0.9         | 1.2         | 0.43         | 4           | 11           | 0.4 |

### 7.9. Scopoletin

|        | nLT | nL | Mean<br>(mg/L) | r<br>(mg/L) | Sr<br>(mg/L) | RSDr<br>(%) | R<br>(mg/L) | SR<br>(mg/L) | RSDR<br>(%) | PRSDR<br>(%) | HoR |
|--------|-----|----|----------------|-------------|--------------|-------------|-------------|--------------|-------------|--------------|-----|
| Whisky | 10  | 9  | 0.09           | 0.007       | 0.0024       | 2.6         | 0.04        | 0.01         | 15          | 23           | 0.6 |

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|             |    |   |      |       |        |     |      |      |    |    |     |
|-------------|----|---|------|-------|--------|-----|------|------|----|----|-----|
| Brandy      | 10 | 8 | 0.04 | 0.002 | 0.0008 | 2.2 | 0.02 | 0.01 | 16 | 26 | 0.6 |
| Rum         | 10 | 9 | 0.11 | 0.005 | 0.0018 | 1.6 | 0.07 | 0.03 | 23 | 22 | 1.0 |
| Cognac<br>1 | 10 | 8 | 0.04 | 0.004 | 0.0014 | 3.3 | 0.02 | 0.01 | 17 | 26 | 0.7 |
| Bourbon     | 10 | 8 | 0.65 | 0.015 | 0.0054 | 0.8 | 0.26 | 0.09 | 15 | 17 | 0.8 |
| Cognac<br>2 | 10 | 8 | 0.15 | 0.011 | 0.0040 | 2.7 | 0.06 | 0.02 | 15 | 21 | 0.7 |

### 7.10. Coniferaldéhyde

|             | nLT | nL | Mean<br>(mg/L) | r<br>(mg/L) | Sr<br>(mg/L) | RSDr<br>(%) | R<br>(mg/L) | SR<br>(mg/L) | RSDR<br>(%) | PRSDR<br>(%) | HoR |
|-------------|-----|----|----------------|-------------|--------------|-------------|-------------|--------------|-------------|--------------|-----|
| Whisky      | 13  | 12 | 0.2            | 0.04        | 0.02         | 9.2         | 0.1         | 0.04         | 23          | 21           | 1.1 |
| Brandy      | 12  | 12 | 0.2            | 0.04        | 0.02         | 9.8         | 0.1         | 0.04         | 27          | 21           | 1.3 |
| Rum         | 13  | 13 | 0.6            | 0.07        | 0.03         | 4.6         | 0.3         | 0.11         | 21          | 18           | 1.2 |
| Cognac<br>1 | 12  | 12 | 0.8            | 0.09        | 0.03         | 4.3         | 0.5         | 0.18         | 23          | 17           | 1.4 |
| Bourbon     | 13  | 13 | 4.6            | 0.24        | 0.09         | 1.9         | 1.1         | 0.38         | 8           | 13           | 0.6 |
| Cognac<br>2 | 13  | 13 | 1.3            | 0.16        | 0.06         | 4.5         | 0.7         | 0.25         | 19          | 15           | 1.2 |

### 7.11. Sinapaldehyde

|        | nLT | nL | Mean<br>(mg/L) | r<br>(mg/L) | Sr<br>(mg/L) | RSDr<br>(%) | R<br>(mg/L) | SR<br>(mg/L) | RSDR<br>(%) | PRSDR<br>(%) | HoR |
|--------|-----|----|----------------|-------------|--------------|-------------|-------------|--------------|-------------|--------------|-----|
| Whisky | 14  | 14 | 0.3            | 0.06        | 0.02         | 7.5         | 0.2         | 0.09         | 31          | 19           | 1.6 |
| Brandy | 14  | 13 | 0.2            | 0.03        | 0.01         | 4.6         | 0.2         | 0.05         | 27          | 20           | 1.3 |
| Rum    | 14  | 12 | 0.2            | 0.06        | 0.02         | 11.2        | 0.2         | 0.08         | 46          | 21           | 2.2 |



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|             |    |    |     |      |      |      |     |      |    |    |     |
|-------------|----|----|-----|------|------|------|-----|------|----|----|-----|
| Cognac<br>1 | 14 | 13 | 1.6 | 0.17 | 0.06 | 3.7  | 0.6 | 0.20 | 13 | 15 | 0.8 |
| Bourbon     | 15 | 13 | 8.3 | 0.38 | 0.14 | 1.6  | 2.3 | 0.81 | 10 | 12 | 0.8 |
| Cognac<br>2 | 14 | 12 | 0.3 | 0.08 | 0.03 | 11.4 | 0.5 | 0.18 | 73 | 20 | 3.7 |

### 7.12. Ellagic acid

|             | nLT | nL | Mean<br>(mg/L) | r<br>(mg/L) | Sr<br>(mg/L) | RSDr<br>(%) | R<br>(mg/L) | SR<br>(mg/L) | RSDR<br>(%) | PRSDR<br>(%) | HoR |
|-------------|-----|----|----------------|-------------|--------------|-------------|-------------|--------------|-------------|--------------|-----|
| Whisky      | 7   | 7  | 3.2            | 0.6         | 0.20         | 6.3         | 4.0         | 1.41         | 44          | 13           | 3.2 |
| Brandy      | 7   | 7  | 1.0            | 0.4         | 0.16         | 16          | 1.2         | 0.42         | 43          | 16           | 2.7 |
| Rum         | 7   | 7  | 9.5            | 0.9         | 0.30         | 3.2         | 11          | 4.0          | 42          | 11           | 3.7 |
| Cognac<br>1 | 7   | 7  | 13             | 1.1         | 0.41         | 3.2         | 14          | 5.0          | 39          | 11           | 3.6 |
| Bourbon     | 7   | 7  | 13             | 2.7         | 0.95         | 7.4         | 14          | 4.9          | 39          | 11           | 3.5 |
| Cognac<br>2 | 7   | 6  | 36             | 1.0         | 0.34         | 1.0         | 40          | 14           | 40          | 9            | 4.3 |

## 8. Bibliography

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