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

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 Ω.

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 µm). 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

- 5-methylfurfural 2 mg/L
- vanillin: 5 mg/L
- syringaldehyde: 10 mg/L
- coniferaldéhyde: 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

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

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 (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	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 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 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 (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	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

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 (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	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 (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	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

Bourbon	15	15	2.4	0.4	0.13	5.3	3.4	1.22	51	14	3.6
Cognac 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 (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	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 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 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 (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	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 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 2	16	15	4.8	0.3	0.11	2.3	1.9	0.67	14	13	1.1

7.7. Vanillin

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

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 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 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 (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	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 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 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 (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	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

Cognac 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 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 (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	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 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 2	7	6	36	1.0	0.34	1.0	40	14	40	9	4.3

8. Bibliography

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