OIV-MA-AS315-31 Qualitative determination of sweeteners in wine by liquid chromatography coupled with mass spectrometry (LC-MS)

Type IV method

1. Scope

This method is suitable for the determination of presence of five artificial sweeteners (aspartame, potassium acesulfame, sodium cyclamate, saccharin and sucralose) as well as the natural sweetener stevioside in white, rosé and red wine.

2. Definitions

ESI – Electrospray Ionisation

LC – Liquid chromatography

LC-MS – Liquid chromatography coupled with mass spectrometry

m/z – Mass to charge ratio

MS – Mass spectrometry

MS/MS – Mass spectrometry acquisition mode measuring product ions

QTOF – Quadrupole time-of-flight mass spectrometry

RP – Reverse phase

RT – Retention time

UHPLC – Ultra-high-performance liquid chromatography

3. Principle

Wine is analysed directly using a liquid chromatography coupled with mass spectrometry system (LC-MS). In liquid chromatography (LC), separation is performed using a reverse phase (RP) column and detection is accomplished by mass spectrometry (MS) according to the compounds' mass to charge ratio (m/z). The MS data combined with the retention time (RT) are used for the identification and quantitation of sweeteners.

4. Reagents and materials

4.1. Reagents:

4.1.1. Acetonitrile, purity $\geq$ 99.95 % (CAS Number 75-05-8)

4.1.2. Purified water: 18 MΩ.cm, TOC $\leq$ 5 µg/L

4.1.3. Formic Acid, purity $\geq$ 98 % (CAS Number 64-18-6)

4.1.4. Aspartame, purity ≥ 99.0 % (CAS Number 22839-47-0)

4.1.5. Acesulfame K, purity ≥ 99.9 % (CAS Number 55589-62-3)

4.1.6. Cyclamate, Sodium, purity ≥ 99.8 % (CAS Number 139-05-9)

4.1.7. Saccharin, purity ≥ 99 % (CAS Number 81-07-2)

4.1.8. Sucralose, purity ≥ 98.0 % (CAS Number 56038-13-2)

4.1.9. Stevioside, purity ≥ 95.0 % (CAS Number 57817-89-7)

4.1.10. Wines representative of the working matrices and previously verified to be absent of any sweeteners in order to be used for the preparation of calibration solutions and standards.

4.2. Solution preparation (as an example)

Standards and calibration solutions are kept in the fridge at approximately 6 °C. Aspartame solutions are unstable in acid media. Therefore, they must be prepared fresh each time the standard is analysed.

4.2.1. Standard solutions

Individual standard solutions at 1 g/L are prepared, e.g., by dissolving 10.0 mg of each sweetener in 10 mL volumetric flasks and filling up to the mark with water (4.1.2) or with ethanol solution at 12% V/V.

4.2.2. Calibration standards

Calibration standards are prepared and analysed by LC-MS as any other sample (see 6).

The calibration standards are prepared in wine (4.1.10) by diluting the appropriate amount of standard solution (4.2.1) to obtain the concentrations 50 µg/L, 100 µg/L, 500 µg/L and 1000 µg/L of each sweetener.

If better method performance is needed it is recommended to perform calibration with the same matrix being evaluated.

5. Apparatus

1. Syringe filters: 0.2 µm polypropylene membrane, 25 mm diameter.
2. Laboratory glassware, namely class A volumetric flasks.
3. Analytical balance with a resolution of ± 0.0001 g
4. Micropipettes for volumes from 5 µL to 1000 µL.
5. High Performance Liquid Chromatography instrument coupled with mass spectrometer.
    1. Standard HPLC and UPLC systems are possible given that the chromatographic separation is adjusted accordingly.
    2. Several MS system configurations are possible such as quadrupole, ion trap, time-of-flight and also hybrid systems.

6. Sampling

Each wine sample is prepared by filtration with a syringe filter (5.1) prior to injection.

If necessary, samples are degassed beforehand using, for example, an ultrasound bath or nitrogen bubbling. If concentrations fall outside the calibration range, samples should be diluted.

Better performance may also be achieved with additional sample preparation steps such as dilution (relying on the instrument sensitivity), sample cleanup and extraction.

7. Procedure

The following description, given as an example, refers to a UHPLC-QTOF instrument equipped with an ESI source. Modifications may occur according to the type of equipment or manufacturer's instructions.

7.1. LC analysis:

- Mobile phase A: purified water (4.1.2) with 0.1 % formic acid (4.1.3
- Mobile phase B: acetonitrile (4.1.1) with 0.1 % formic acid (4.1.3)
- Injection volume: 2 µL
- Sampler temperature: 10 °C Column: RP C8 2.1 mm x 100 mm, 1.9 µm
- Column Oven: 30 °C

Gradient:

Time Min	Flow mL/min	% A	% B
0	0.4	90	10
3	0.4	60	40
3	0.4	1	99
4	0.4	1	99
4	0.8	1	99
5.5	0.8	1	99
5.5	0.5	90	10

9.5	0.5	90	10
9.5	0.4	90	10

7.2. Mass Spectrometer parameters:

- ESI: negative ionisation
- Source Temp: 200 °C
- Capillary Voltage: 3000 V
- Acquisition Mode: broadband collision-induced dissociation (bbCID)
- Consists of alternating acquisition of spectra of Full Scan and MS/MS modes (acquisition of precursor and product ions respectively)
- Collision Energy: 30 eV
- Acquisition spectra rate: 2.0 Hz
- Dry Gas Flow: 8 L/min;
- Nebuliser pressure: 2.0x10^5 Pa (2.0 bar)

8. Identification

Sweetener identification is confirmed using a standard for each compound (4.1.4, 4.1.5, 4.1.6, 4.1.7, 4.1.8 and 4.1.9). The data gathered for peak confirmation is the RT for guidance (these may vary depending on the chromatographic parameters) and *m/z* of the precursor and product ions (Table 1).

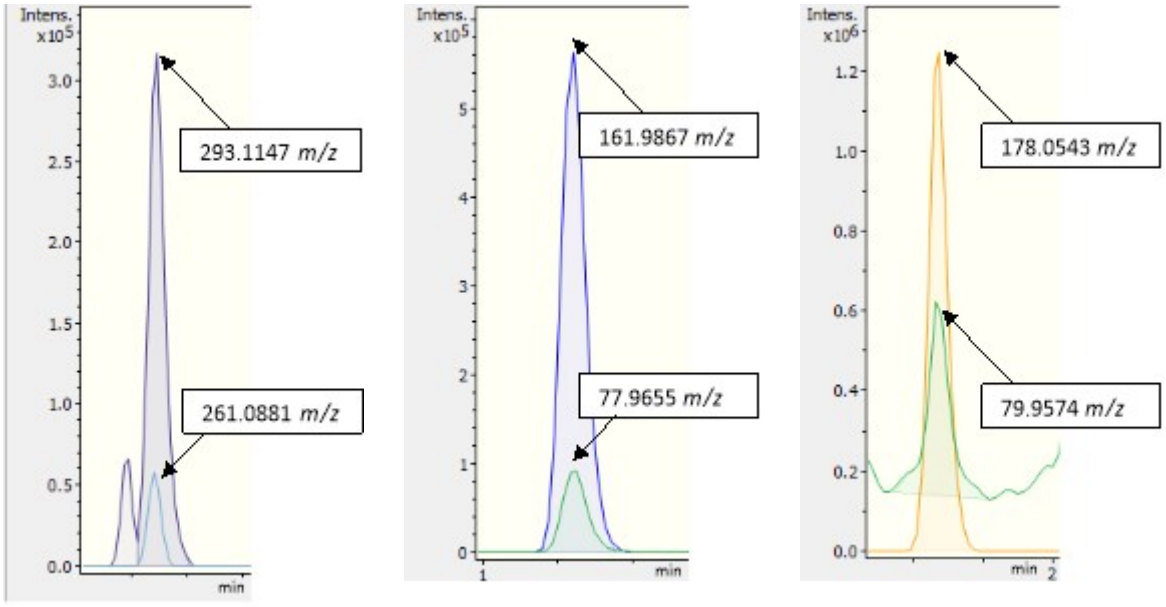
Table 1 – Sweeteners identification data: RT, precursor m/z and product m/z

Sweetener	RT min	Ion	Precursor *m/z*	Product *m/z*
Acesulfame K	1.24	$[M]^-$	161.9867	77.9655
Aspartame	2.30	$[M-H]^-$	293.1143	261.0881
Cyclamate Na	1.66	$[M]^-$	178.0543	79.9574
Saccharin	1.55	$[M-H]^-$	181.9917	41.9985
Sucralose	2.14	$[M-H]^-$	395.0073	359.0306
Stevioside	3.63	$[M-H]^-$	803.3707	641.3026

Note: The ions used for quantitation are underlined in Table 1.

Ion signals are monitored with extracted ion chromatograms with ± 3 mDa tolerance (Figure 1).

Aspartame $[M-H]^-$ Acesulfame K $[M]^-$ Cyclamate Na $[M]^-$



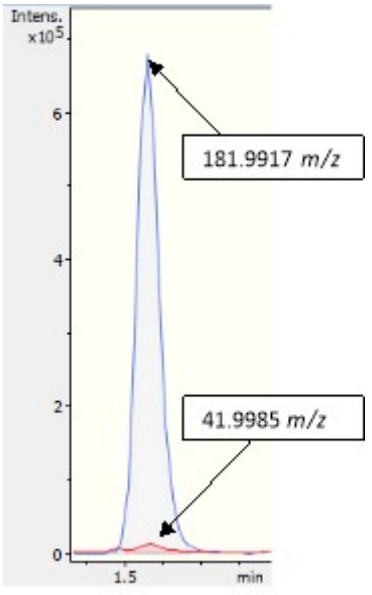
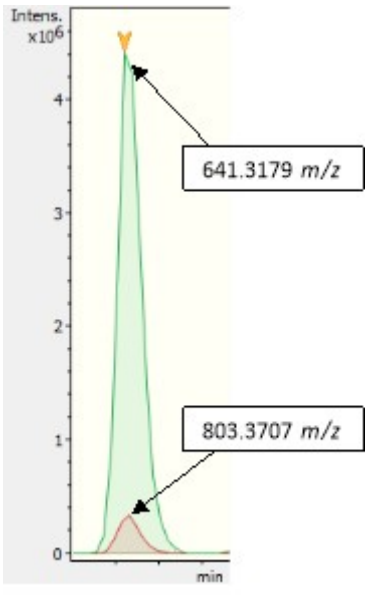
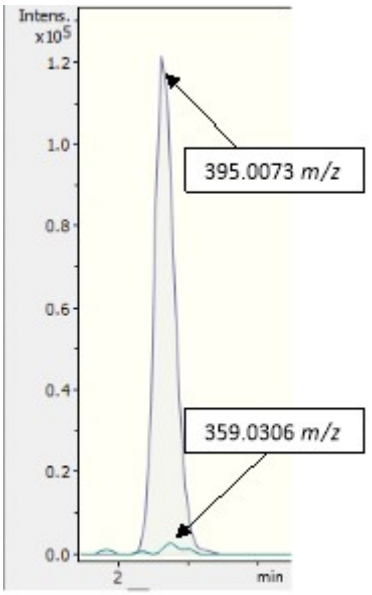
Saccharin $[M-H]^-$	Stevioside $[M-H]^-$	Sucralose $[M-H]^-$
		

Figure 1 – PFigure 1-Precursor and product peak confirmation for 250 µg/L standard

Note: An example of low standard sensitivity and additional transitions are given in appendix

9. Calculus

Results are calculated from the calibration curve which is obtained with the amount (µg/L) vs the peak area of each sweetener:

$$C = \frac{A_S - Int}{S}$$

Where C is the sweetener concentration (µg/L), A_S is the sample peak area, Int is the calibration curve Y-axis interception point and S is the calibration curve slope.

10. Results

Concentrations are expressed in µg/L without decimals.

11. Internal validation

1. Matrices

Validation was performed using a total of 43 different wines: 20 red wines, 10 rosé wines and 13 white wines. These wines were selected from several regions with the aim of obtaining great variability of characteristics in order to make a comprehensive approach. Bellow there is a table summarizing the major characteristics of the wines.

Table 2 – Matrices main characteristics

	Red wine (R)		Rosé wine (Ro)		White wine (W)	
Regions	Alentejo	4	Douro	3	Açores	1
	Bairrada	1	Vinho Verde	1	Alentejo	2
	Dão	3	Other[1]	6	Dão	1
	Douro	4			Douro	1
	Lisboa	1			Lisboa	1
	Valladolid	1			Vinho Verde	4
	Other[1]	6			Other[1]	3
Alcoholic Strength by Volume % v/v	12.1 – 17.2		9.8 – 12.6		8.7 – 13.6	
Sugar content g/L (glucose + fructose)	0.5 – 108.0		0.7 – 28.8		0.2 – 17.1	
Total Acidity g/L (tartaric acid)	4.6 – 6.4		4.7 – 6.0		5.2 – 7.1	
pH	3.5 – 3.8		3.2 – 3.5		3.2 – 3.4	
Intensity	2.4 – 16.2		0.1 – 0.5		0.03 – 0.29[2]	

[1] Without geographical indication

(2) Absorbance at 420 nm instead of intensity

11.2. Linearity

The method proved to be linear within a range of concentrations between 50 µg/L and 1000 µg/L

11.3. Calibration

A total of 14 independent calibrations were made counting 6 red wines, 4 rosé wines and 4 white wines. Then, for each compound, calibrations were made considering 3 different approaches:

- One unified calibration for all the matrices
- 2 groups of matrices consisting in one group for white wines and another group with the remaining wines (red wines and rosé wines)
- 3 groups of matrices consisting of white wines, rosé wines and red wines

Herein presented are the optimized results of the validation study. According to the selected calibration conditions, for acesulfame, saccharin and sucralose calibration functions and subsequent calculations were preformed considering one group for white wines and a second group with the remaining matrices, red wines

and rosé wines. For aspartame, cyclamate and stevioside three groups of matrices were considered: red wines, rosé wines and white wines.

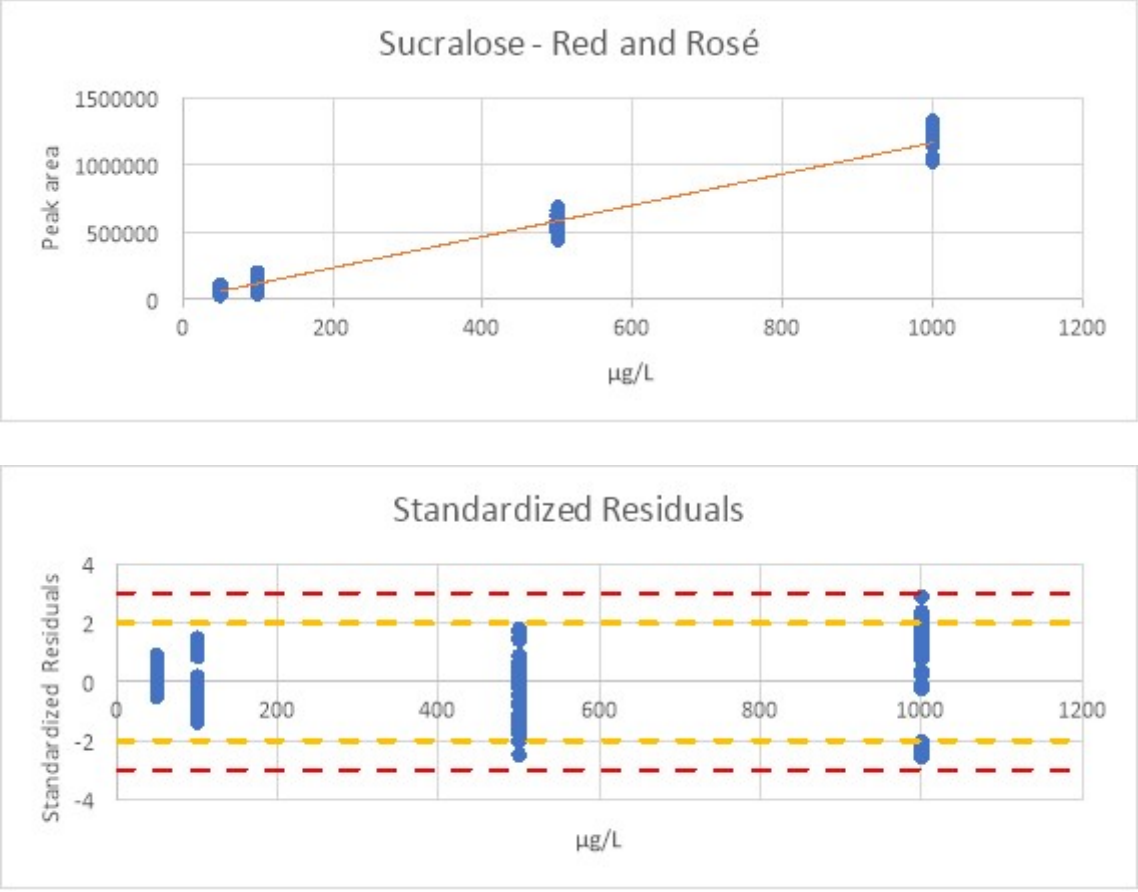
Table 3 – Calibration scheme for each compound

Calibrations	Individual			Combined
Matrices	White wine	Rosé wine	Red wine	Red wines + Rosé wines
Acesulfame	X			X
Aspartame	X	X	X	
Cyclamate	X	X	X	
Saccharin	X			X
Stevioside	X	X	X	
Sucralose	X			X

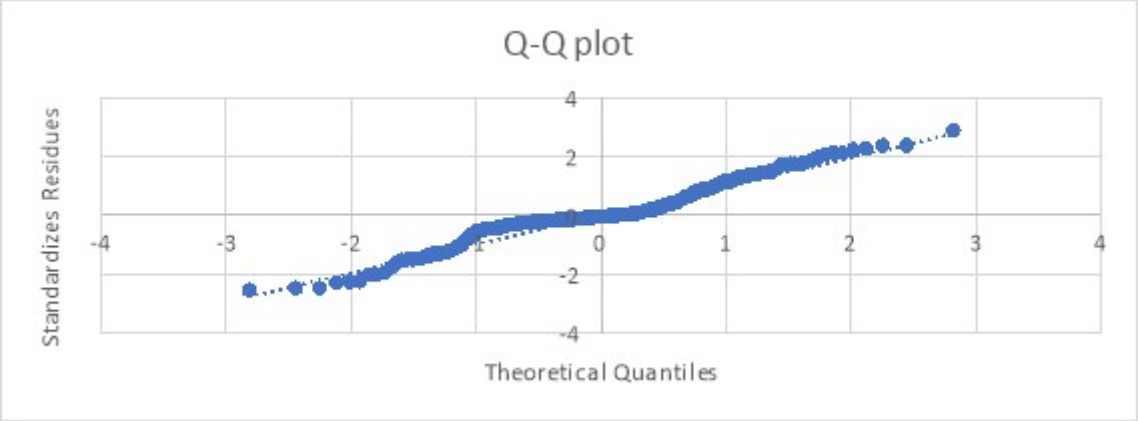
Given the heteroskedasticity and normal distribution of the residuals, the regression model employed was the weighted least square regression.

As an example, sucralose for the group of red and rosé wines at a concentration range 50 µg/L to 1000 µg/L is presented below.

Figure 2 – Calibration curve, standardized residuals and Q-Q plot for the combined red and rosé wines calibration for sucralose



Yellow	2x standard deviation
red	3x standard deviation



11.4. Limits of detection and limits of quantitation

The limits of quantitation were obtained through calculation from the calibration curves

Table 4 – LOD and LOQ values obtained for each compound

	LOD (mg/L)			LOQ (mg/L)		
	White wine	Rosé wine	Red wine	White wine	Rosé wine	Red wine
Acesulfame K	0.003	0.003		0.011	0.011	
Aspartame	0.004	0.006	0.004	0.014	0.019	0.014
Cyclamate Na	0.002	0.005	0.004	0.006	0.015	0.014
Saccharin	0.002	0.005		0.006	0.016	
Stevioside	0.002	0.002	0.005	0.005	0.005	0.016
Sucralose	0.014	0.007		0.048	0.022	

11.5. Repeatability

Repeatability was assessed at three spiking levels: 50 µg/L corresponding to the reporting limit, 250 µg/L and 1000 µg/L. This evaluation is based on 8 replicate injections at each spiking level and for each matrix.

In the following tables the repeatability values obtained for each sweetener are

presented including the mean concentration measured in each sample, the standard deviation (Std. Dev.), the percentual relative standard deviation for repeatability (RSDr %) and the Horwitz Ratio for repeatability (HorRat (r)).

Table 5 – Repeatability values for potassium acesulfame at 3 spiking levels

Acesulfame	White wine (W)								
Sample	W1	W2	W3	W4	W5	W6	W7	W8	W9
Mean µg/L	45	42	49	233	207	240	1036	926	1060
Std. Dev.	1.4	2.1	0.8	3.3	5.2	2.6	13.2	13.7	15.8
Recovery %	89 %	84 %	98 %	93 %	83 %	96 %	104 %	93 %	106 %
RSDr %	3.2 %	5.0 %	1.6 %	1.4 %	2.5 %	1.1 %	1.3 %	1.5 %	1.5 %
HorRat (r)	0.13	0.20	0.06	0.07	0.13	0.05	0.08	0.09	0.09
Acesulfame	Rosé wine (Ro)								
Sample	Ro1	Ro2	Ro3	Ro4	Ro5	Ro6	Ro7	Ro8	Ro9
Mean µg/L	49	52	53	248	248	247	1063	1091	1097
Std. Dev.	2.0	1.2	1.4	2.9	3.5	3.9	14.1	13.2	15.5
Recovery %	98 %	104 %	107 %	99 %	99 %	99 %	106 %	109 %	110 %
RSDr %	4.1 %	2.3 %	2.6 %	1.2 %	1.4 %	1.6 %	1.3 %	1.2 %	1.4 %
HorRat (r)	0.17	0.09	0.10	0.06	0.07	0.08	0.08	0.08	0.09

Acesulfame	Red wine (R)								
Sample	R1	R2	R3	R4	R5	R6	R7	R8	R9
Mean µg/L	56	50	57	275	241	260	1195	1064	1160
Std. Dev.	1.2	2.0	1.4	3.2	5.1	4.3	13.8	14.5	10.0
Recovery %	112 %	101 %	115 %	110 %	96 %	104 %	120 %	106 %	116 %

RSDr %	2.1 %	3.9 %	2.4 %	1.2 %	2.1 %	1.6 %	1.2 %	1.4 %	0.9 %
HorRat (r)	0.08	0.16	0.10	0.06	0.11	0.08	0.07	0.09	0.05

Table 6 - Repeatability values for aspartame at 3 spiking levels

Aspartame	White wine (W)								
Sample	W1	W2	W3	W4	W5	W6	W7	W8	W9
Mean µg/L	34	51	45	237	231	235	981	973	982
Std. Dev.	7.3	4.2	6.7	27.5	7.6	10.9	29.0	18.0	23.2
Recovery %	68 %	101 %	91 %	95 %	92 %	94 %	98 %	97 %	98 %
RSDr %	21.6 %	8.3 %	14.7 %	11.6 %	3.3 %	4.6 %	3.0 %	1.8 %	2.4 %
HorRat (r)	0.87	0.33	0.59	0.59	0.17	0.24	0.19	0.12	0.15

Aspartame	Rosé wine (Ro)								
Sample	Ro1	Ro2	Ro3	Ro4	Ro5	Ro6	Ro7	Ro8	Ro9
Mean µg/L	38	42	41	200	211	210	833	905	916
Std. Dev.	3.0	2.9	4.3	6.8	5.2	5.9	20.9	34.0	22.5
Recovery %	75 %	85 %	82 %	80 %	84 %	84 %	83 %	90 %	92 %
RSDr %	8.0 %	6.9 %	10.6 %	3.4 %	2.5 %	2.8 %	2.5 %	3.8 %	2.5 %
HorRat (r)	0.32	0.28	0.43	0.17	0.13	0.14	0.16	0.24	0.15
Aspartame	Red wine (R)								
Sample	R1	R2	R3	R4	R5	R6	R7	R8	R9

Mean µg/L	46	51	50	227	254	230	956	1099	1013
Std. Dev.	8.6	3.2	8.1	16.9	10.4	7.3	21.8	39.0	20.2
Recovery %	92 %	103 %	100 %	91 %	102 %	92 %	96 %	110 %	101 %
RSDr %	18.5 %	6.3 %	16.2 %	7.4 %	4.1 %	3.2 %	2.3 %	3.5 %	2.0 %
HorRat (r)	0.74	0.25	0.65	0.38	0.21	0.16	0.14	0.22	0.13

Table 7 - Repeatability values for sodium cyclamate at 3 spiking levels

Cyclamate	White wine (W)								
Sample	W1	W2	W3	W4	W5	W6	W7	W8	W9
Mean µ g/L	51	50	50	261	247	246	1092	1040	1045
Std. Dev.	1.0	1.4	1.4	2.8	4.2	3.5	12.2	17.7	14.4
Recovery %	103 %	100 %	101 %	104 %	99 %	99 %	109 %	104 %	105 %
RSDr %	1.9 %	2.9 %	2.9 %	1.1 %	1.7 %	1.4 %	1.1 %	1.7 %	1.4 %
HorRat (r)	0.08	0.12	0.11	0.05	0.09	0.07	0.07	0.11	0.09
Cyclamate	Rosé wine (Ro)								
Sample	Ro1	Ro2	Ro3	Ro4	Ro5	Ro6	Ro7	Ro8	Ro9
Mean µg/L	42	42	44	232	228	233	982	992	1002
Std. Dev.	1.6	1.3	0.8	2.8	4.4	4.5	14.9	6.0	12.9
Recovery %	84 %	85 %	88 %	93 %	91 %	93 %	98 %	99 %	100 %
RSDr %	3.9 %	3.0 %	1.7 %	1.2 %	2.0 %	1.9 %	1.5 %	0.6 %	1.3 %
HorRat (r)	0.16	0.12	0.07	0.06	0.10	0.10	0.10	0.04	0.08

Cyclamate	Red wine (R)								
Sample	R1	R2	R3	R4	R5	R6	R7	R8	R9
Mean µg/L	51	55	54	250	265	243	1069	1160	1086
Std. Dev.	1.2	1.3	1.4	5.5	5.2	4.2	27.4	13.9	18.4
Recovery %	103 %	110 %	108 %	100 %	106 %	97 %	107 %	116 %	109 %
RSDr %	2.4 %	2.4 %	2.6 %	2.2 %	2.0 %	1.7 %	2.6 %	1.2 %	1.7 %
HorRat (r)	0.10	0.10	0.10	0.11	0.10	0.09	0.16	0.08	0.11

Table 8 - Repeatability values for saccharin at 3 spiking levels

Saccharin	White wine (W)								
Sample	W1	W2	W3	W4	W5	W6	W7	W8	W9
Mean µg/L	45	45	59	216	214	252	920	909	1055
Std. Dev.	1.5	1.4	1.4	5.1	5.1	3.7	21.3	23.7	21.5
Recovery %	89 %	91 %	119 %	86 %	86 %	101 %	92 %	91 %	105 %
RSDr %	3.3 %	3.0 %	2.4 %	2.4 %	2.4 %	1.5 %	2.3 %	2.6 %	2.0 %
HorRat (r)	0.13	0.12	0.10	0.12	0.12	0.08	0.15	0.16	0.13
Saccharin	Rosé wine (Ro)								
Sample	Ro1	Ro2	Ro3	Ro4	Ro5	Ro6	Ro7	Ro8	Ro9
Mean µg/L	58	56	56	303	276	278	1263	1190	1204
Std. Dev.	1.4	2.0	0.6	5.5	3.5	4.8	28.8	24.8	25.2
Recovery %	116 %	112 %	112 %	121 %	110 %	111 %	126 %	119 %	120 %
RSDr %	2.4 %	3.5 %	1.1 %	1.8 %	1.3 %	1.7 %	2.3 %	2.1 %	2.1 %

HorRat (r)	0.10	0.14	0.04	0.09	0.07	0.09	0.14	0.13	0.13
Saccharin	Red wine (R)								
Sample	R1	R2	R3	R4	R5	R6	R7	R8	R9
Mean µg/L	47	44	46	224	203	199	955	906	885
Std. Dev.	1.4	0.5	1.5	4.4	2.2	2.9	20.6	20.1	25.8
Recovery %	94 %	88 %	92 %	89 %	81 %	80 %	95 %	91 %	88 %
RSDr %	3.0 %	1.1 %	3.2 %	2.0 %	1.1 %	1.5 %	2.2 %	2.2 %	2.9 %
HorRat (r)	0.12	0.04	0.13	0.10	0.06	0.07	0.14	0.14	0.18

Table 9 - Repeatability values for stevioside at 3 spiking levels

Stevioside	White wine (W)								
Sample	W1	W2	W3	W4	W5	W6	W7	W8	W9
Mean µg/L	41	43	30	262	265	204	1094	1116	860
Std. Dev.	0.4	0.4	0.7	2.0	31.2	1.9	13.6	12.9	6.6
Recovery %	83 %	86 %	60 %	105 %	106 %	81 %	109 %	112 %	86 %
RSDr %	1.0 %	1.0 %	2.2 %	0.8 %	11.8 %	0.9 %	1.2 %	1.2 %	0.8 %
HorRat (r)	0.04	0.04	0.09	0.04	0.60	0.05	0.08	0.07	0.05
Stevioside	Rosé wine (Ro)								
Sample	Ro1	Ro2	Ro3	Ro4	Ro5	Ro6	Ro7	Ro8	Ro9
Mean µg/L	50	39	41	237	254	286	935	1104	1109
Std. Dev.	0.8	1.3	0.9	2.6	5.3	7.1	10.5	10.2	18.3

Recovery %	99 %	77 %	81 %	95 %	102 %	114 %	93 %	110 %	111 %
RSDr %	1.7 %	3.4 %	2.2 %	1.1 %	2.1 %	2.5 %	1.1 %	0.9 %	1.6 %
HorRat (r)	0.07	0.14	0.09	0.06	0.11	0.13	0.07	0.06	0.10

Stevioside	Red wine (R)								
Sample	R1	R2	R3	R4	R5	R6	R7	R8	R9
Mean µg/L	60	40	43	262	211	210	1048	904	921
Std. Dev.	0.9	0.7	0.4	4.0	4.0	2.6	18.0	18.4	11.9
Recovery %	120 %	80 %	86 %	105 %	85 %	84 %	105 %	90 %	92 %
RSDr %	1.5 %	1.8 %	1.0 %	1.5 %	1.9 %	1.3 %	1.7 %	2.0 %	1.3 %
HorRat (r)	0.06	0.07	0.04	0.08	0.10	0.06	0.11	0.13	0.08

Table 10 - Repeatability values for sucralose at 3 spiking levels

Sucralose	White wine (W)								
Sample	W1	W2	W3	W4	W5	W6	W7	W8	W9
Mean µg/L	53	52	53	221	225	223	986	973	1021
Std. Dev.	5.3	7.8	8.1	10.8	27.5	6.5	29.8	43.9	31.5
Recovery %	106 %	103 %	105 %	88 %	90 %	89 %	99 %	97 %	102 %
RSDr %	10.0 %	15.1 %	15.4 %	4.9 %	12.2 %	2.9 %	3.0 %	4.5 %	3.1 %
HorRat (r)	0.40	0.61	0.62	0.25	0.63	0.15	0.19	0.28	0.19
Sucralose	Rosé wine (Ro)								
Sample	Ro1	Ro2	Ro3	Ro4	Ro5	Ro6	Ro7	Ro8	Ro9
Mean µg/L	35	43	36	215	236	194	944	1075	905

Std. Dev.	4.1	2.1	2.2	7.2	7.4	7.7	21.3	27.5	19.3
Recovery %	70 %	86 %	71 %	86 %	94 %	78 %	94 %	108 %	91 %
RSDr %	11.7 %	5.0 %	6.2 %	3.3 %	3.1 %	4.0 %	2.3 %	2.6 %	2.1 %
HorRat (r)	0.47	0.20	0.25	0.17	0.16	0.20	0.14	0.16	0.13

Sucralose	Red wine (R)								
Sample	R1	R2	R3	R4	R5	R6	R7	R8	R9
Mean µg/L	50	46	48	236	255	228	1017	1194	1041
Std. Dev.	7.7	3.1	6.8	11.5	9.2	8.4	16.9	27.5	23.0
Recovery %	100 %	92 %	96 %	94 %	102 %	91 %	102 %	119 %	104 %
RSDr %	15.3 %	6.9 %	14.1 %	4.9 %	3.6 %	3.7 %	1.7 %	2.3 %	2.2 %
HorRat (r)	0.61	0.28	0.57	0.25	0.18	0.19	0.10	0.15	0.14

Table 11 – Repeatability summary table

Compound	Recovery	RSDr %	HorRat (r)
Acesulfame	83 % – 120 %	0.9 % – 5.0 %	0.05 – 0.20
Aspartame	68 % – 110 %	1.8 % – 21.6 %	0.12 – 0.87
Cyclamate	84 % – 116 %	0.6 % – 3.9 %	0.04 – 0.16
Saccharin	80 % – 126 %	1.1 % – 3.5 %	0.04 – 0.18
Stevioside	60 % – 112 %	0.8 % – 11.8 %	0.04 – 0.60
Sucralose	70 % – 119 %	1.7 % – 15.4 %	0.10 – 0.63

11.6. Intermediate Precision

Intermediate precision was evaluated by analyzing samples spiked with 50 µg/L, 250 µg/L and 1000 µg/L in different moments spanning throughout several days. The

results are presented in the following tables. Count represents the number of points considered for the determination of the mean values and respective standard deviation (Std. Dev.). The recovery percentage, the relative standard deviation (RSD%) and the Horwitz ratio (HorRat) are also displayed for each case.

Table 12 – Intermediate precision values for potassium acesulfame at 3 spiking levels

Acesulfame	White wine (W)			Red (R) and Rosé wines (Ro)					
Sample	W1	W2	W3	Ro1	Ro2	Ro3	R1	R2	R3
Count	12	12	11	12	12	12	12	12	12
Mean µg/L	46	223	928	54	252	1089	53	252	1113
Std. Dev.	6.1	31.4	76.6	6.1	23.6	66.3	2.4	9.1	41.3
Recovery %	91 %	89 %	93 %	108 %	101 %	109 %	106 %	101 %	111 %
RSD% IP	13.2 %	14.1 %	8.2 %	11.3 %	9.4 %	6.1 %	4.5 %	3.6 %	3.7 %
HorRat	0.53	0.72	0.52	0.46	0.48	0.38	0.18	0.18	0.23

Table 13 - Intermediate precision values for aspartame at 3 spiking levels

Aspartame	White wine (W)			Rosé wine (Ro)			Red (R)		
Sample	W1	W2	W3	Ro1	Ro2	Ro3	R1	R2	R3
Count	11	10	10	11	12	12	11	12	12
Mean µg/L	57	281	1190	41	202	841	41	222	998
Std. Dev.	5.5	21.8	91.9	4.1	12.9	73.9	7.5	15.4	43.5
Recovery %	114 %	113 %	119 %	82 %	81 %	84 %	83 %	89 %	100 %
RSD % IP	9.6 %	7.7 %	7.7 %	10.0 %	6.4 %	8.8 %	18.1 %	6.9 %	4.4 %
HorRat	0.38	0.40	0.49	0.40	0.32	0.55	0.73	0.36	0.27

Table 14 - Intermediate precision values for sodium cyclamate at 3 spiking levels

Cyclamate	White wine (W)			Rosé wine (Ro)			Red (R)		
Sample	W1	W2	W3	Ro1	Ro2	Ro3	R1	R2	R3
Count	10	10	10	11	12	12	12	12	12
Mean µg/L	48	237	1011	40	210	918	49	226	999
Std. Dev.	5.5	27.3	134.3	2.5	20.1	70.7	1.3	7.4	26.6
Recovery %	97%	95%	101%	80%	84%	92%	98%	91%	100%
RSD% IP	11.3%	11.5%	13.3%	6.3%	9.6%	7.7%	2.7%	3.3%	2.7%
HorRat	0.45	0.59	0.84	0.25	0.49	0.48	0.11	0.17	0.17

Table 15 - Intermediate precision values for saccharin at 3 spiking levels

Saccharin	White wine (W)			Red (R) and Rosé wine (Ro)					
Sample	W1	W2	W3	Ro1	Ro2	Ro3	R1	R2	R3
Count	11	10	10	12	12	12	12	12	12
Mean µg/L	51	241	1010	56	270	1166	44	195	857
Std. Dev.	2.6	8.4	36.8	2.8	10.7	47.6	3.0	8.3	31.7
Recovery %	103%	96%	101%	112%	108%	117%	88%	78%	86%
RSD % IP	5.0%	3.5%	3.6%	5.1%	4.0%	4.1%	6.9%	4.3%	3.7%
HorRat	0.20	0.18	0.23	0.20	0.20	0.26	0.28	0.22	0.23

Table 16 - Intermediate precision values for stevioside at 3 spiking levels

Stevioside	White wine (W)			Rosé wine (Ro)			Red (R)		
Sample	W1	W2	W3	Ro1	Ro2	Ro3	R1	R2	R3

Count	11	10	10	12	12	12	12	12	12
Mean µg/L	35	232	977	31	210	921	41	208	905
Std. Dev.	6.5	45.8	184.1	8.1	45.4	184.4	3.1	22.2	84.5
Recovery %	70%	93%	98%	61%	84%	92%	81%	83%	91%
RSD% IP	18.5%	19.7%	18.8%	26.4%	21.6%	20.0 %	7.6%	10.7%	9.3%
HorRat	0.74	1.01	1.19	1.06	1.10	1.26	0.31	0.55	0.59

Table 17 - Intermediate precision values for sucralose at 3 spiking levels

Sucralose	White wine (W)			Red (R) and Rosé wine (Ro)					
Sample	W1	W2	W3	Ro1	Ro2	Ro3	R1	R2	R3
Count	10	11	11	10	11	12	12	12	12
Mean µg/L	51	197	776	51	295	1196	42	228	1069
Std. Dev.	10.4	41.6	137.5	11.9	48.7	184.5	5.3	18.0	51.3
Recovery %	101%	79%	78%	101%	118%	120%	85%	91%	107%
RSD % IP	20.5%	21.1%	17.7%	23.5%	16.5%	15.4%	12.5%	7.9%	4.8%
HorRat	0.82	1.08	1.12	0.94	0.84	0.97	0.50	0.40	0.30

Table 18 - Intermediate precision summary table

Compound	Recovery	RSD%	HorRat
Acesulfame	89 % – 111 %	3.6 % – 14.1 %	0.18 – 0.72
Aspartame	81 % – 119 %	4.4 % – 18.1 %	0.27 – 0.73

Cyclamate	80 % – 101 %	2.7 % – 13.3 %	0.11 – 0.84
Saccharin	78 % – 117 %	3.5 % – 6.9 %	0.18 – 0.28
Stevioside	61 % – 98 %	7.6 % – 26.4 %	0.31 – 1.26
Sucralose	78 % – 120 %	4.8 % – 23.5 %	0.30 – 1.12

12. Bibliography

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Appendix

A1. Quantitation performance for a wine sample spiked with 50 µg/L of each sweetener

Sweetener	S/N
Acesulfame K	789.4
Aspartame	586.5
Cyclamate Na	282.5
Saccharin	24.3
Sucralose	80.5
Stevioside	224.1

A2 . *Sweeteners identification data* - additional transitions given as guidance

Sweetener	Additional transition in ESI negative.
Ace sulfame K	162 > 82
Aspartame	293 > 200
Cyclamate Na	178 > 96
Saccharin	182 > 106
Sucralose	397 > 361
Stevioside	641 > 479 641 > 317