RESOLUTION OIV-OENO 687-2023

VALIDATION OF SORBIC ACID ASSAY METHOD IN WINES USING LIQUID CHROMATOGRAPHY (OENO 06/2006)

WARNING: this resolution modifies the following resolution:
- *OENO 6/2006*

THE GENERAL ASSEMBLY,

IN VIEW of article 2, paragraph 2 iv of the Agreement of 3rd April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING the proposal of the "Methods of Analysis" Sub-commission,

CONSIDERING the resolution OENO 06/2006 "Determination of sorbic, benzoic and salicylic acid content in wine by the use of high-performance liquid chromatography", adopted in 2006

CONSIDERING that this validation concerns only the sorbic acid,

DECIDES to add the annex A: Validation of Sorbic Acid Assay Method in Wines Using Liquid Chromatography, and to change the sorbic acid method from type IV to type II.

DECIDES to modify the resolution OENO 06/2006 "Determination of sorbic, benzoic and salicylic acid content in wine by the use of high-performance liquid chromatography" and consequently method OIV-MAAS313-20 with the following modifications, where the additions can be seen in bold letters and the deleted parts are strikethrough:

2. Scope

All wines and grape musts, especially those likely to contain only traces of sorbic, benzoic or salicylic acid. (demonstration from 1 mg/l).

3. Principle

The antiseptics are determined using HPLC by direct injection of the sample into a column functioning by isocratic reversed-phase partition chromatography with ultraviolet detection at a wavelength of 235 nm [1].

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4.11. 50% vol. hydro-alcohol solution or other suitable concentration: put 500 ml of absolute alcohol (4.10) into a 1-litre flask and dilute to volume with distilled water (4.1)

5.7. Detector capable of functioning at an ultraviolet rating of 235 nm (1) and fitted with a circulating tank for HPLC (for example, 8 □l for 1 cm of optical thickness)

A 5- □m stationary phase HPLC column of the silica-type with immobilisation by octadecyl groups (C18), length 20 cm, inside diameter 4 mm (1)

6.2. Degas the elution solvent (4.6) for 5 minutes using the ultrasonic bath (5.2) or any other suitable way.

6.3. Filter the solvent using the device in (5.43)

8. Calculation

Having located the peaks of the acids to be determined in the sample, compare the peak area with those of the acids of a surrogate solution (4.13) with a known concentration C.

For example, let s be the peak area of the acid to be determined, and S is the peak area of the solution (4.13) with concentration C

$$X_{in\ the\ sample} = C \times \frac{s}{S}\ in.\ mg/l$$

It is also possible to use a calibration curve and determine the concentration by interpolation.

9. Characteristics of the method

9.1. For Sorbic acid

Interlaboratory trial and correspondent results are described in annex A.

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- r = 0.0148 x + 0.5498
- R = 0.0936 x + 1.5542
- x: sorbic acid concentration (mg/l)

9.2. For Benzoic and Salicylic acids

	~~Sorbic acid~~	Benzoic acid	Salicylic acid
Linearity range	~~0 to 200 mg/l~~	0 to 200 mg/l	0 to 200 mg/l
Accuracy (rate of recuperation)	~~> 90 %~~	> 90 %	> 90 %
Répétabilité : r*	~~2%~~	3%	8%
Reproducibility: R*	~~8%~~	9%	12%
Detection limit	~~3 mg/l~~	3 mg/l	3 mg/l
Quantification limit	~~5 mg/l~~	6 mg/l	7 mg/l
Uncertainty	~~11%~~	12%	13%

ANNEX A - Statistic data obtained from the results of the interlaboratory trials

The following parameters have been defined during an interlaboratory trial. This trial has been conducted by the laboratory " Instituto dos Vinhos do Douro e do Porto (Portugal). "

Year of interlaboratory trial: 2020

1. Laboratories: 23 laboratories from 14 different countries

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HBLA und Bundesamt für Wein- und Obstbau Klosterneuburg	Austria
Department of Agriculture	Cyprus
Czech Agriculture and Food Inspection Authority	Czech Republic
Comité Interprofessionnel du Vin de Champagne	France
Laboratoires Dubernet	France
Service Commun des Laboratoires-Laboratoire de Montpellier	France
Service Commun des Laboratoires-Laboratoire de Bordeaux	France
Landesuntersuchungsamt, Institut für Lebensmittelchemie	Germany
Lebensmittel- u. Veterinärinstitut Braunschweig/Hannover	Germany
National Food Chain Safety Office	Hungary
Unione Italiana Vini Servizi	Italy
Vassanelli Lab s.r.l.	Italy
AsureQuality Ltd Laboratory Services	New Zealand
Arcus Norway AS	Norway
ASAE-Autoridade de Segurança Alimentar e Económica	Portugal
Instituto dos Vinhos do Douro e do Porto	Portugal
RUDN University	Russian Federation
National Laboratory of Health, Environment and Food (Novo Gorica)	Slovenia
National Institute of Chemistry	Slovenia
National Laboratory of Health, Environment and Food (Novo Mesto)	Slovenia
Laboratorio Arbitral Agroalimentario.	Spain
Estación Enológica de Haro- La Rioja	Spain
Cukurova University Food Engineering Dept.	Turkey

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2. Number of samples: 6 samples in duplicate

- 2 white wines: A/G, C/K
- 1 white wine - late harvest: I/O
- 1 red wine: D/M
- 1 rosé wine: B/F
- 1 red fortified wine (alcoholic strength - 19 % vol.): E/N

3. Analytical conditions

The analytical conditions are summarized in Table 1.

4. Individual results

The individual results for Sorbic Acid (mg/L) are summarized in Table 2.

5. Data Analysis

- Statistical analysis was performed according to the recommendations of the OIV to obtain precision data
- The values below the quantification limit were treated as normal values.
- Determination of outliers was assessed by Cochran and Grubbs test
- The results are summarized in tables 3 and 4.
- Repetibility and reproducibility limit according to sorbic acid concentration are represented in figure 1.
- The Z-score for each laboratory was evaluated against the global values for the different samples.The results are in figure 2.

TABLE 1 – ANALYTICAL CONDITIONS

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Lab. Code	Calibration Curve	Column	Detection	Mobile phase	Flow rate (mL/min)	Column temperature (°C)	Injection volume (µL)
1	yes	Altima C18LL 5 µm 250 x 4.6 mm	▫ = 235 nm	Water pH=2.0 / MeOH/ THF (650/280/70)	1	ambient	20
2	yes	C18 250 x 4.6 Luna Phenomenex	▫ = 235 nm	0.389 Ammonium acetate in 1L water + 250 mL ACN (pH=4 adjusted w/ CH_3COOH)	1.25	35	20
3	yes	Poreshell 120 EC-C18 .150 x 4.4 mm; 2.7 µm	▫ = 235 nm	A: H2O 0.7 % THF (pH=2) /B: MeOH (80 % A e 20 % B)	1.5	40	5
4	yes	RP-18 (250-4)	▫ = 254 nm	Ammonium acetate buffer (pH 4.4): MeOH = 70:30	1	40	20
6	yes	PerfectSil Target ODS-3 HD. 5 µm. 250 x 4.6 mm	▫ = 235 nm	ACN:Buffer solution (35:65); Buffer solution = Ammonium acetate (1.8 g/l) + Acetic acid. pH=4.3	1	40	2
7	yes	PoroShell C18 4.6 x 75 mm; 2.7 µm	▫ = 235 nm	Isocratic: 80 % H_2O acid. with sulfuric acid; 20 % ACN	1.5	25	20
8	yes	Rezex-ROA-Organic acid H+ (8 %) -300 x 7.8 mm	▫ = 254 nm	Elution solvent Sulfuric acid = 0.025M	1	90	50
9	yes	HICROM Ultrasphere 5 µm ODS column. 250 x 4.6 mm	▫ = 235 nm	650 mL H_2O pH 2 with 280 mL MeOH + 7 mL THF	0.6	25	20

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10	yes	Teknokroma Trace Excel 120 ODSB 5 µm. 20 x 0.4 cm	▫ = 235 nm	650 mL H_2O pH 2 (HCl 0.1M) + 280 mL MeOH + 7 mL THF	1	24	20
11	yes	C18 5 µm. 200x4 mm	▫ = 235 nm				
12	yes	RP18 Chromolith 100 x 4.6 mm; 5 µm	▫ = 235 nm	770 Water pH=2.3 (0.8 mL H_3PO_4): isopropanol 200 mL. ACN 30 mL	1	25	10
13	yes	Phenomenex Gemini 5 µm. C18; 150 x 2 mm	▫ = 240 nm	0.925 g ammonium acetate + 1.2 L H_2O + 0.8 L MeOH (pH 5.5 acetic acid)	0.4	40	10
15	yes	C18 5 µm. 200 x4 mm	▫ = 235 nm	90 % v/v Water 15mL Acetic acid+ 15 g ammonium acetate 10 % v/v MeOH	1	40	10
16	yes	Prodigy 5 µm ODS-3 100. 150 x 4.6 mm w/ C18 guard column	▫ = 261 nm	80 % v/v H_2O pH=4 (pure acetic acid) + 0.005M ammonium acetate 20 % v/v ACN	1.2	40	50
17		Kinetex; RP-C18 150x4.6 mm; 5 µm	▫ = 230 nm	11/89. MeOH: Buffer (Buffer = 3.0 g KH2PO4 +3.0 g K2HPO4+ 100 mL water)	0.6	40	6
18	yes	C18 Luna. Phenomenex	▫ = 224 nm	95 % phosphate buffer: 5 % ACN	1.2	ambient	20
19	yes	Phenomenex Aqua C18 125A 250 x 4.6 mm; 5 µm	▫ = 235 nm	0.005M ammonium acetate (pH 4): ACN = 8:2	1	25	2
20	yes	Zorbax Eclipse XDB-C18 4.6 x 150 mm. 5 µm	▫ = 235 nm	650 mL H_2O pH 2 (HCl 0.1M) + 280 mL MeOH + 7 mL THF	1	ambient	5

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21	yes	RP-18 Nucloedur 100-C18. 3 µm. 50 mm	◻ = 262 nm	A:B = 80:20; A: ammonium acetate. 0.002 mol/l. pH= 4.1 (adjusted by conc. Acetic acid); B: MeOH/ACN 2:1	1	60	6
22	yes	HYPERSIL ODS 250X4.6 mm. 5 µm	◻ = 235 nm		1.8	40	20
23	yes	RP-C18 . 150 x 4.6 mm; 5 µm	◻ = 235 nm		1	25	20
24	yes	RP-C18 Purospher .125 x 4 mm; 5 µm	◻ = 235 nm	MeOH / 0.02M sodium acetate buffer pH 4.4 = (30/70 v/v)	0.8	40	5
26	yes	Kinetex XB-C18 100 x 4.6 mm; 2.6 µm	◻ = 254 nm	Water 0.1 % TFA. ACN 0.1 % TFA	1.5	30	10

TABLE 2 - INDIVIDUAL RESULTS FOR SORBIC ACID (mg/L)

	White wine (A)		Rosé (B)		White Wine (C)		Red wine (D)		Fortified wine (E)		Rosé (F)		White wine (G)		White wine (I)		White wine (K)		Red wine (M)		Fortified wine (N)		White wine(O)	
Laboratory code	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
1	36.3	35.5	226.0	221.0	88.0	86.0	5.2	5.3	1.7	1.8	226,0	224.0	36.1	35.9	125.0	124.0	90.0	87.0	4.7	4.6	1.8	2.0	126.0	126.0
2	33.1	33.2	210.7	208.3	80.9	81.4	4.5	4.4	1.7	2.4	210.4	209.5	33.0	32.5	116.0	114.9	81.2	80.6	4.3	5.7	2.0	2.4	116.3	116.1
3	34.1	34.5	216.8	215.5	84.7	84.5	5.3	5.2	2.5	2.3	216.3	217.2	34.5	34.3	119.8	120.1	84.5	84.3	5.7	5.7	2.4	2.4	120.5	120.2
4	34.7	35.0	223.1	224.7	89.3	88.6	5.1	5.1	2.1	2.2	220.0	221.6	34.5	34.8	123.1	123.2	86.7	86.4	5.2	5.2	2.1	2.1	123.1	123.5
6	34.3	34.6	214.2	215.2	83.9	84.0	5.5	5.6	1.9	2.0	215.0	215.6	34.6	34.7	120.6	120.7	83.6	83.9	6.2	6.5	2.0	2.1	120.1	121.1
7	34,0	35.0	217.0	218.0	84.0	85.0	5.0	6.0	3.0	3.0	218.0	218.0	35.0	35.0	122.0	123.0	86.0	84.0	5.0	5.0	2.0	2.0	122.0	122.0
8	33.9	33.4	216.8	218.5	86.2	83.8	4.5	4.5	2.0	2.0	218.8	215.5	35.2	33.9	122.0	118.9	84.2	84.3	4.3	4.1	2.0	2.0	124.5	123.3
9	32.5	32.5	197.4	198.2	81.0	80.7	3.4	3.4	1.4	1.4	197.0	195.8	32.1	32.2	112.5	112.2	79.3	79.4	3.3	3.3	1.3	1.2	113.5	113.7
10	34.1	34.1	216.0	217.0	83.7	83.6	5.7	5.6	2.2	2.1	216.0	216.0	34.2	34.0	118.0	119.0	83.4	83.4	5.6	5.8	2.1	2.1	120.0	119.0
11	36.8	37.0	227.2	229.9	90.2	90.4	4.6	4.8	2.1	1.9	224.2	225.6	37.1	36.8	127.4	127.0	88.4	89.0	4.0	4.3	2.1	1.9	128.0	128.5

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12	31.0	31.0	207.0	202.0	78.0	78.0	4.0	3.0	2.0	2.0	209.0	209.0	32.0	32.0	111.0	111.0	79.0	78.0	4.0	5.0	2.0	2.0	109.0	109.0
13	35.0	35.0	219.0	220.0	86.0	87.0	6.0	6.0	3.0	3.0	220.0	220.0	35.0	36.0	124.0	124.0	85.0	87.0	7.0	7.0	3.0	3.0	123.0	123.0
15	32.0	32.0	200.0	199.0	79.0	78.0	4.0	4.0	1.7	1.6	200.0	199.0	32.0	32.0	111.0	111.0	79.0	78.0	4.0	4.0	1.7	1.6	111.0	111.0
16	31.3	29.1	213.1	214.2	84.7	84.2	4.3	4.3	1.9	1.9	217.3	216,0	30.3	31.0	120.1	119.6	83.5	84.2	4.2	4.6	2.0	2.0	119.3	120.1
17	35.6	35.4	217.8	221.4	87.0	86.0	5.2	5.4	2.3	2.4	215.5	219.5	35.6	35.3	122.2	123.4	85.9	86.5	5.4	5.5	2.7	2.5	122.3	123.9
18	35.0	37.0	235.0	246.0	88.0	85,0	6.1	5.2	7.5	7.7	220.0	209.0	36.0	37.0	124.0	125.0	94.0	88.0	6.5	6.6	7.8	7.4	127.0	128.0
19	31.0	30.0	183.0	187.0	71.0	72.0	8.0	8.0	-2.0	0.0	177.0	179.0	30.0	27.0	105.0	106.0	67.0	71.0	4.0	5.0	-1.0	-1.0	116.0	115.0
20	36.2	34.8	217.4	217.0	84.9	84.4	7.1	8.9	1.9	2.7	219.0	217.1	35.1	34.4	121.2	119.0	85.8	84.3	5.6	5.9	2.4	2.3	121.9	119.2
21	32.2	32.2	204.1	204.2	79.3	79.1	4.3	4.4	1.9	2.0	204.9	204.4	32.6	32.3	111.9	112.4	78.6	78.7	4.3	4.4	1.9	2.0	115.0	114.2
22	34.0	34.0	216.0	216.0	83.0	83.0	6.0	6.0	3.0	3.0	216.0	217.0	34.0	34.0	121.0	121.0	83.0	83.0	6.0	6.0	3.0	3.0	121.0	121.0
23	35.3	35.0	219.3	217.6	87.3	86.1	4.1	4.5	1.6	1.7	219.3	217.9	35.4	35.9	124.3	123.0	87.0	86.0	3.9	4.1	1.6	1.8	124.6	123.5
24	34.2	34.0	214.0	216.0	81.8	82.1	4.1	4.1	2.7	2.8	215.0	215.0	33.7	33.8	117.0	118.0	83.7	83.4	4.1	4.1	2.8	2.7	119.0	119.0
26	35.1	35.2	214.4	214.5	86.2	86.2	5.0	5.1	2.2	2.2	214.9	214.2	35.0	35.1	122.3	122.5	86.0	86.3	5.1	5.1	2.2	2.2	122.2	122.5

TABLE 3- RESULTS FOR SORBIC ACID (mg/L)

	White wine		Rosé		White wine		Red wine		Fortified wine		White wine	
Laboratory code	A	G	B	F	C	K	D	M	E	N	I	O
1	35.90	36.00	223.50	225.00	87.00	88.50	5.25	4.65	1.75	1.90	124.50	126.00
2	33.15	32.25	209.50	209.95	81.15	80.80	4.45	5.00	2.05	2.20	115.45	116.20
3	34.30	34.40	216.15	216.75	84.60	84.40	5.25	5.70	2.40[c)]	2.40[c)]	119.95	120.35
4	34.85	34.65	223.90	221.00	88.95	86.55	5.10	5.20	2.15	2.10	123.15	123.30
6	34.45	34.65	214.70	215.30	83.95	83.75	5.58[c)]	6.34	1.95[c)]	2.08[c)]	120.65	120.60
7	34.50	35.00	217.50	218.00	84.50	85.00	5.50	5.00	*3.00*[b)]	*2.00*[b)]	122.50	122.00
8	33.65	34.55	217.65	217.15	85.00	84.25	4.50	4.20	2.00	2.00	120.45	123.90
9	32.49	32.16	197.83	196.38	80.81	79.40	3.42	3.32	1.41	1.25	112.38	113.56
10*	34.10	34.10	216.50	216.00	83.65	83.40	5.66	5.68	2.15[c)]	2.13[c)]	118.50	119.50
11*	36.90	36.95	228.55	224.90	90.30	88.70	4.70	4.15	2.00[c)]	2.00[c)]	127.20	128.25
12	31.00	32.00	204.50	209.00	78.00	78.50	3.50	4.50	2.00	2.00	111.00	109.00
13	35.00	35.50	219.50	220.00	86.50	86.00	6.00	7.00	3.00	3.00	124.00	123.00

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15*	32.00	32.00	199.50	199.50	78.50	78.50	4.00	4.00	1.65	1.65	111.00	111.00
16	30.20	30.65	213.65	216.65	84.45	83.85	4.28	4.38	1.94	1.96	119.85	119.70
17	35.53	35.44	219.61	217.53	86.51	86.19	5.30	5.47	2.38	2.61	122.82	123.12
18	36.00	36.50	240.50[b)]	214.50[b)]	86.50[b)]	91.00[b)]	5.65	6.55	*7.60*[a)]	*7.60*[a)]	124.50	127.50
19	30.50[b)]	28.50[b)]	185.00[a)]	98.00[a)]	71.50[a)]	69.00[a)]	8.00[b)]	4.50[c)b)]	-1.00[c)a)]	-1.00[c)a)]	105.50[b)]	115.50[b)]
20	35.50	34.75	217.20	218.05	84.65	85.05	8.00[b)]	5.75[b)]	2.30[c)]	2.35[c)]	120.10	120.55
21	32.20	32.45	204.15	204.65	79.20	78.65	4.36	4.36	1.94	1.94	112.15	114.60
22	34.00	34.00	216.00	216.50	83.00	83.00	6.00	6.00	3.00	3.00	121.00	121.00
23	35.15	35.65	218.45	218.60	86.70	86.50	4.30	4.00	1.65[c)]	1.70[c)]	123.65	124.05
24	34.10	33.75	215.00	215.00	81.95	83.55	4.10 [c)]	4.10[c)]	2.75[c)]	2.75 [c)]	117.50	119.00
26	35.13	35.06	214.45	214.58	86.21	86.15	5.07	5.07	2.17	2.17	122.37	122.35

a) Values rejected by Grubbs test on mean outliers

b) Values rejected by Cochran test variance outliers

c) Value identified by the laboratory below Quantification Limit

TABLE 4 - PRECISION DATA

Indicators	White wine (A/G)	Rosé (B/F)	White wine (C/K)	Red wine (D/M)	Fortified wine (E/N)	White wine (I/O)
Number of laboratories	22	21	21	21	20	22
Number of repetitions	2	2	2	2	2	2
Minimum (mg/L)	30.20	196.38	78.00	3.32	1.25	109.00
Maximum (mg/L)	36.95	228.55	90.30	7.00	3.00	128.25
Repeatability variance s_r^2	0.0942	1.5249	0.4191	0.1201	0.0037	0.9397
Intergroup variance s_L^2	2.6370	55.1140	9.7049	0.6418	0.1793	22.4914
Reproducibility variance s_R^2	2.7312	56.6389	10.1240	0.7619	0.1830	23.4311
Average (mg/L)	**34.16**	**214.72**	**83.96**	**4.92**	**2.15**	**120.07**

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Repeatability standard deviation (mg/L)	0.31	1.23	0.65	0.35	0.06	0.97
Repeatability limit (mg/L)	0.869	3.495	1.832	0.981	0.172	2.743
Repeatability RSD %	0.9	0.6	0.8	7.0	2.8	0.8
Reproducibility standard deviation (mg/L)	1.65	7.53	3.18	0.87	0.43	4.84
Reproducibility limit (mg/L)	4.677	21.298	9.005	2.470	1.211	13.699
Reproducibility RSD %	4.8	3.5	3.8	17.7	19.9	4.0
HORRAT	0.5	**0.5**	**0.5**	**1.4**	**1.4**	0.5

FIGURE 1 – CORRELATION BETWEEN SORBIC ACID CONCENTRATION AND PRECISION

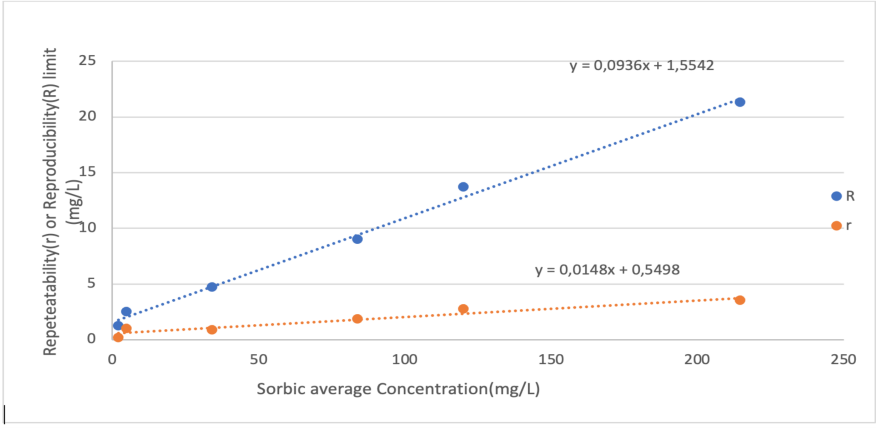


FIGURE 2 - Z-SCORES REPRESENTATION WITHOUT OUTLIERS

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***LAI -lower action limit; LAS - upper action limit; LSI - lower warning limit; LSS - upper warning**

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[1] For sorbic acid analysis the column and the wavelength are given as an example.

Certified in conformity Jerez, 9th June 2023
The Director General of the OIV
Secretary of the General Assembly
Pau ROCA

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