OIV-MA-AS313-19 Determination of the principal organic acids of wines and sulphates by capillary electrophoresis

Type II method (for organic acids)

Type III method (for sulphate)

1. Introduction

Tartaric, malic and lactic acids and sulphates are separated and assayed by capillary electrophoresis after simple dilution and addition of an internal standard.

2. Title

Determination of the principal organic acids of wines and sulphates by capillary electrophoresis

3. Scope

Capillary electrophoresis can be used to assay the tartaric and malic acid in musts, as well as the tartaric, malic and lactic acids and sulphates in wines that have been diluted, degassed and filtered beforehand if need be.

4. Definitions

4.1. Capillary electrophoresis

Capillary electrophoresis: all the techniques that use a capillary tube of very small diameter with an appropriate buffer solution to effectively separate small and large electrically charged molecules in the presence a high-voltage electric current.

4.2. Buffer for electrophoresis

Solution containing one or more solvents and aqueous solutions with suitable electrophoretic mobilities to buffer the pH of the solution.

4.3. Electrophoretic mobility

Aptitude of an ion to move quickly under the effect of an electric field.

4.4. Electroosmotic flow

Flow of solvent in the buffer solution along the internal wall of the capillary tube due to displacement of the solvated ions under the effects of the field and the electric charges of the silica.

5. Principle

Separations of the aqueous solutions of a mixture by capillary electrophoresis are obtained by differential migrations in a buffered electrolyte referred to as a buffer.

The electrophoresis takes place in a silica tube with an inside diameter ranging between 25 and 75 µm. The aqueous solutions to be separated are simultaneously driven by 2 forces that can act in the same direction or in the opposite direction. These two forces are caused by the electric field and the electroosmotic flow.

The electric field is represented by the voltage in volts applied between the electrodes brought to within one centimetre of the capillary tube, and is expressed in $V.cm^{-1}$. Mobility is a characteristic of ions. The smaller the molecules, the greater their electrophoretic mobility.

If the internal wall of the capillary tube is not coated, the negative electric charges of the silica fix part of the cations of the buffer. The solvation and displacement towards the cathode of part of the cations of the buffer create the electroosmotic flow. The pH of the buffer and additives can be chosen in order to control the direction and the intensity of the electroosmotic flow.

The addition of a chromophoric ion in the buffer can be used to obtain negative peaks that quantitatively represent the solutions to be separated which do not absorb at the used wavelength.

6. Reagents and products

6.1. Chemically pure grade products for analysis at least at 99%

6.1.1. Sodium sulphate or Potassium sulphate

6.1.2. L-tartaric acid

6.1.3. D,L- malic acid

6.1.4. Monohydrated citric acid

6.1.5. Succinic acid

6.1.6. D,L Lactic acid

6.1.7. Sodium dihydrogenophosphate

6.1.8. Sodium gluconate

6.1.9. Sodium chlorate

6.1.10. Dipicolinic acid

6.1.11. Cethyltrimethyl ammonium bromure

6.1.12. Acetonitrile for HPLC

6.1.13. Deionized ultra filtered pure water

6.1.14. Sodium hydroxide

6.2. Solutions

6.2.1. Calibration stock solution

Solution in pure water (6.1.13) of different acids and sulphates to be measured (6.1.1 to 6.1.6) at exact known concentrations ranging between 800 and 1200 mg l^{-1}
Solution to be kept at +5° C for a maximum of 1 month

6.2.2. Internal standard solution

Solution of sodium chlorate (6.1.9) at approximately 2 g l^{-1} in pure water (6.1.13)
Solution to be kept at +5° C for a maximum of 1 month

6.2.3. Calibration solution to be injected

In a graduated 50-ml class "A" flask using class "A" pipettes, deposit:

- 2 ml of calibration solution (6.2.1)
- 1 ml of internal standard solution (6.2.2)
- Adjust solution to 50 ml with pure water (6.1.13)

Homogenize by agitation
Solution to be prepared each day

6.2.4. Sodium hydroxide solutions

6.2.4.1. Sodium hydroxide solution M

In a 100-ml flask place 4g of sodium hydroxide (6.1.14)
Adjust with pure water (6.1.13)
Shake until completely dissolved.

6.2.4.2. sodium hydroxide solution 0.1M

In a 100 ml flask place 10 ml of sodium hydroxide M (6.2.4.1)
Adjust with pure water (6.1.13)
Homogenise.

6.2.5. Electrophoretic buffer solution

In a graduated 200-ml class "A" flask, place:

- 0.668 g of dipicolinic acid (6.1.10)
- 0.364 g of cethyltrimethyl-ammonium bromide. (6.1.11)
- 20 ml of acetonitrile (6.1.12)
- Approximately 160 ml of pure water (6.1.13)
- Shake until complete dissolution (if need be, place in ultrasound bath to eliminate any aggregated material)

- Bring M sodium hydroxide solution M (6.2.4.1) to pH 5.64 and then 0.1M sodium hydroxide (6.2.4.2)
- Make up to 200 ml with pure water (6.1.13)
- Homogenize by agitation
- Solution to be prepared each month.
- Store at laboratory temperature.

This buffer can be replaced by equivalent commercial product.

7. Apparatus

The capillary electrophoresis apparatus required for these determinations basically comprises:

- A sample changer
- Two bottles (phials) containing the buffer
- A non-coated silica capillary tube, internal diameter 50 µm, length 60 cm, between the inlet of the capillary tube and the detection cell. Depending on the apparatus, an additional 7 to 15 cm are required so that the outlet of the capillary tube is immersed in the centre of another bottle
- A high voltage DC power supply capable of outputting voltages of -30 to + 30 kV. The electrodes immersed in the two bottles where the outlets of the capillary tube emerge are connected to the terminals of the generator
- A pressurization system capable of circulating the buffer in the capillary tube and enabling the injection of the test specimen
- A UV detector
- A data acquisition system

8. Preparation of samples for tests

8.1. Degassing and filtration

The samples rich in carbon dioxide are degassed for 2 min with ultra-sound. Turbid samples are filtered on a membrane with an average pore diameter of 0.45 µm.

8.2. Dilution and addition of internal standard

Place 2 ml of sample in a graduated flask of 50 ml. Add 1 ml of internal standard

solution (6.2.2). Adjust to 50 ml with pure water (6.1.13)
Homogenize.

9. Procedure

9.1. Conditioning of a new capillary tube (for example)

- Circulate pure water (6.1.13) in the opposite direction (from the outlet of the capillary tube towards the inlet flask) for 5 min at a pressure of approximately 40 psi (2.76 bar or 276 kPa)
- Circulate 0.1M sodium hydroxide (6.2.4.2) in the opposite direction for 5 min at the same pressure
- Circulate pure water (6.1.13) in the opposite direction (from the outlet of the capillary tube towards the inlet flask) for 5 min at the same pressure
- Repeat the cycle of circulating pure water, 0.1M sodium hydroxide , pure water
- Circulate electrophoretic buffer (6.2.5) in the opposite direction for 10 min

9.2. Reconditioning a capillary tube in the course of use (optional)

- When the quality of the separations becomes insufficient, new conditioning of the capillary tube is essential. If the results obtained are still not satisfactory, change capillary tube and condition it.

9.3. Checking the quality of the capillary tube (optional)

- Analyse 5 times the calibration solution under the recommended analysis conditions.

9.4. Separation and detection conditions (for example)

- Light the detector lamp 1 hour before the start of the analyses
- Rinse the capillary tube by circulating the buffer for 3 min in the opposite direction at a pressure of 40 psi
- Pressure inject the samples (prepared at 8.1) at 0.5 psi for 6 to 15 seconds
- The polarity is regulated such that the anode is on the detector side
- Apply a voltage from 0 to 16 kV in 1 min then 16 kV for approximately 18 min (the duration of separation can slightly vary depending on the quality of the capillary tube)

- Maintain the temperature at + 25 C°
- Detection in the ultraviolet is at 254 Nm
- Rinse the capillary tube by circulating the electrophoretic buffer (6.2.5) for 2 min in the opposite direction at a pressure of 40 psi
- Change the electrophoretic buffer (6.2.5) contained in the inlet and outlet flasks at least every 6 injections

5. Order that the analyses are to be carried out (for example)

Change the electrophoretic buffer (6.2.5) for every new series of analyses

The sequence of analysis in order contains:Analysis of reference material (external concentration sample known for different acids to be measured)

Analysis of samples prepared in 8.2,chromatograms should look like those presented in appendix A

At the end of analysis, rinse with pure water (6.1.13) 10 mm in opposite direction (outlet of capillary tube toward the inlet)

Switch off detector lamp

10. Calculation of results

The calculations are based on the surface areas of the peaks obtained after integration.

The surface areas of the peaks of the aqueous solutions of the calibration solution (6.2.3) are corrected by taking into account the variations in the surface areas of the peaks of the internal standard. The response factor for each acid is calculated.

The surface areas of the peaks of the internal standard and the peaks of the aqueous solutions are read off for each sample. The surface areas of the aqueous solutions to be assayed are recalculated by taking into account variations in the surface areas of the peaks of the internal standard a second time in order to obtain "corrected" surface areas.

The corrected surface areas are then multiplied by the value of the corresponding response factor.

It is possible to use an automatic data management system, so that they can be controlled in accordance with the principles described above as well as with the best practices (calculation of response factor and / or establishment of a calibration curve).

Calculation formula

The abbreviations used to calculate the concentration in an acid are given in the following table:

Surfaces are expressed by the whole numbers of integration units.

The concentrations are given in g/L (only indicate to two decimal places).

ABBREVIATIONS		
	REFERENCE SOLUTION	SAMPLE
SURFACE AREAS OF TITRATED PEAKS	S_{AR}	S_{AE}
INTERNAL STANDARD PEAKS	S_{EIR}	S_{EIE}
CONCENTRATION	C_{AR}	C_E

The calculation formula is:

$$C_E = \frac{C_{AR} \times S_{AE} \times S_{EIR}}{S_{AR} \times S_{EIE}}$$

Whenever possible, a duplicate analysis is used to highlight a possible error in the recognition of the peaks or inaccuracy of integration. The sample changer makes it possible to carry out the analyses in automatic mode day and night.

11. Precision

11.1. Organization of the tests

Interlaboratory trials and correspondent results are described in appendix B1 and B2

11.2. Measurement of precision

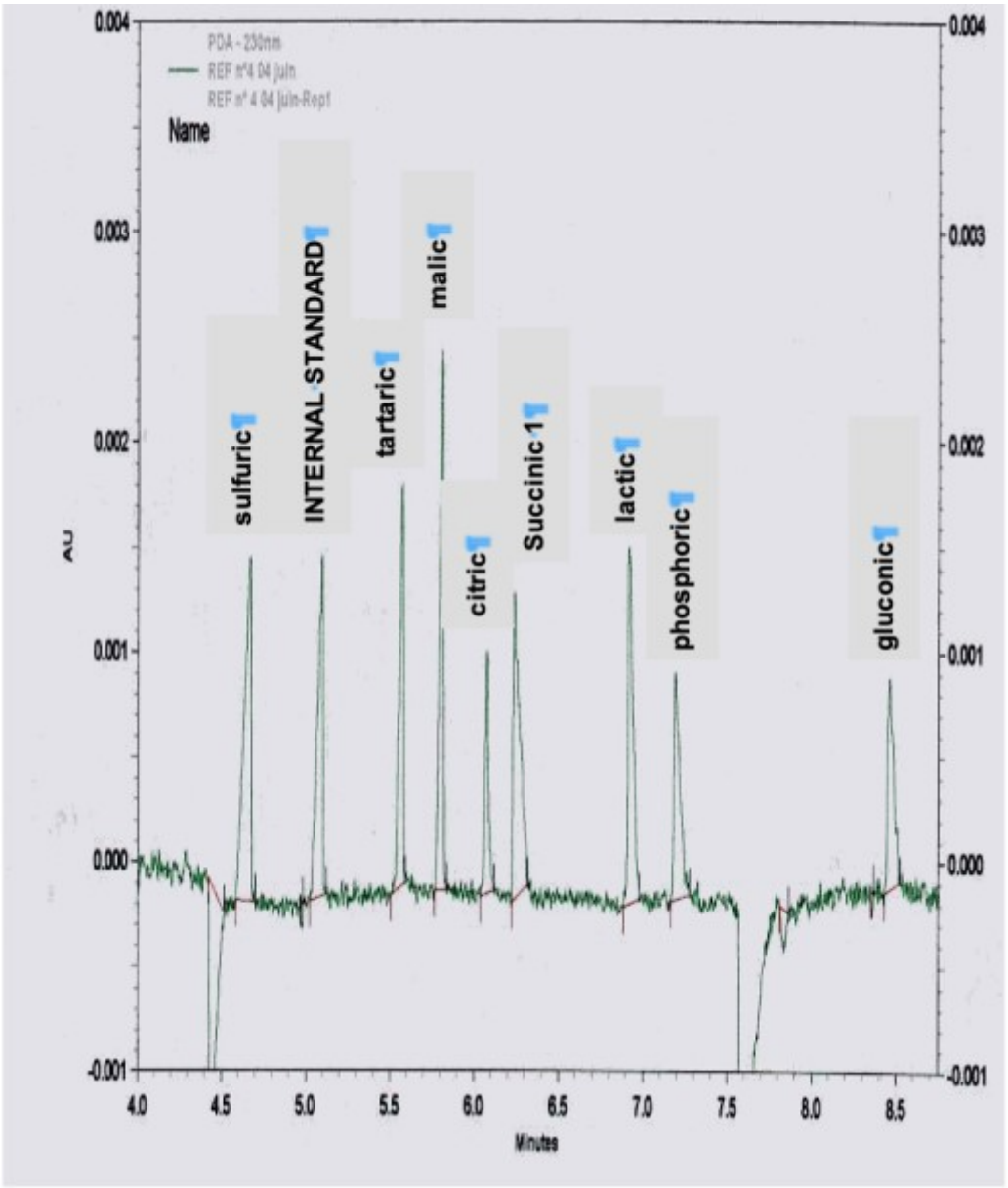
Assessement of precision by interlaboratory trials

Number of laboratories involved: 5

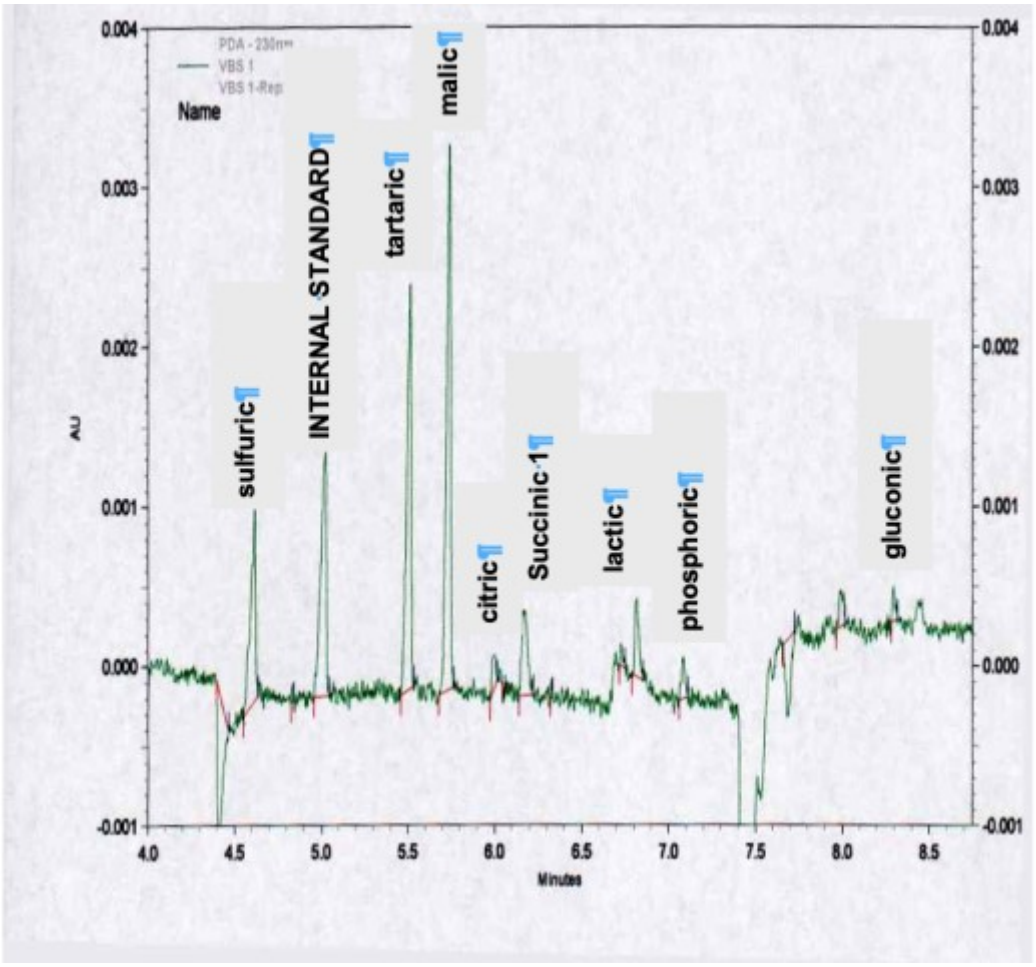
Results expressed in mg / L			
	TARTRIC ACID	**MALIC ACID**	**LACTIC ACID**
Average values of concentrations			
	1395	1884	1013
Average values of standard deviations in repeatability	38	54	42
Average values of standard deviations in reproducibility	87	113	42

12. Appencides

Appendix A: Electrophoregram of a standard solution of ACI



Electropherogram of a wine



Appendix B1

Statistic data obtained from the results of the interlaboratory trials (2006)

According to ISO 5725-2:1994, the following parameters have been defined during an interlaboratory trial. This trial has been conducted by the laboratory « Direction Générale de la Consommation et de la Répression des Fraudes de Bordeaux (France). »

Year of interlaboratory trial: 2006

Number of laboratories: 5

Number of samples: 8 double-blind (2 dry white wines, 2 sweet white wines, 2 rosé wines and 2 red wines)

INTERLABORATORY TESTS

Determination of TARTRIC ACID by capillary electrophoresis

Identification of the sample	dry white wines		liquorous white wines		rosé wines		red wines	
	A+D	B+C	E+F	G+H	I+J	K+L	M+N	O+P
Number of laboratories taking part	5	5	5	5	5	5	5	5
Number of results accepted	5	5	4	5	5	5	4	4 5
Average value in mg/l	1943	2563	1440	255	553	1885	1373	1148
Accepted value in mg/l	1943	2563	1387	2217	1877	1593	1370	1830
Standard deviation of repeatability (Sr)	27	25	106	23	40	31	25	24
Repeatability coefficient of variation	1.4	1.0	7.7	1.0	2.2	1.9	1.8	1.3
Limit of repeatability (r)	77	70	298	65	113	88	70	66
Standard deviation of reproducibility (SR)	96	128	174	80	57	55	52	53
Reproducibility coefficient of variation in %	4.9	5	12.6	3.6	3	3.5	3.8	2.9
Reproducibility limit (R)	268	359	488	223	160	154	145	148

INTERLABORATORY TESTS

Determination of MALIC ACID by capillary electrophoresis

Identification of the sample	dry white wines		liquorous white wines		rosé wines		red wines	
	A+D	B+C	E+F	G+H	I+J	K+L	M+N	O+P
Number of laboratories taking part	5	5	5	5	5	5	5	5
Number of results accepted	5	5	5	5	5	5	4	4 5
Average value in mg/l	2571	1602	1680	2539	3524	2109	173	869
Accepted value in mg/l	2571	1602	1680	2539	3524	2109	177	869
Standard deviation of repeatability (Sr)	54	19	113	35	61	109	7	32
Repeatability coefficient of variation	2.1	1.2	6.7	1.4	1.7	5.2	4.1	3.7
Repeatability limit (r)	151	54	315	99	170	305	20	89
Standard deviation of reproducibility (SR)	90	51	171	97	279	142	21	53
Reproducibility coefficient of variation in %	13.6	9.8	41	39.6	14.7	9	14.1	7.6
Limit of reproducibility (R)	252	142	479	273	782	397	59	148

INTERLABORATORY TESTS

Determination of LACTIC ACID by capillary electrophoresis
results in mg/l

Sample identification	dry white wines		liquorous white wines		rosé wines		red wines	
	A+D	B+C	E+F	G+H	I+J	K+L	M+N	O+P
Number of laboratories taking part	5	5	5	5	5	5	5	5
Number of results accepted	4	5	5	5	5	5	4	4 5
Average value in mg/l	659	1324	258	255	553	1885	2066	1148
Accepted value in mg/l	650	1324	258	255	553	1885	2036	1148
Standard deviation of repeatability (Sr)	20	42	20	39	27	99	75	16
Repeatability coefficient of variation	3.1	3.2	7.8	15.1	4.8	5.3	3.7	16,0
Repeatability limit (r)	57	117	56	108	75	278	211	46
Standard deviation of reproducibility (SR)	20	42	20	39	27	99	75	16
Reproducibility coefficient of variation in %	13,6	9,8	41	39,6	14,7	9	14,1	7,6
Reproducibility limit (R)	247	363	296	283	227	475	802	243

Appendix B2

Statistic data obtained from the results of the interlaboratory trials (sulphates 2010)

According to ISO 5725-2:1994, 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: 2010-2011

Number of laboratories: 7 (one laboratorysent two sets of results obtained by means of two different instruments)

Number of samples: 6 double-blind

Indicators	White wine (A/G)	Rosé (B/F)	Rosé (C/O)	Red wine (D/M)	Liquor wine (E/N)	Liquor wine (I/K)	White wine (H/Q)	Red wine (J/P)	Liquor wine (L)
Number of groups	7	7	6	7	8	7	7	7	8
Number of repetitions	2	2	2	2	2	2	2	2	2
Minimum (g/L K_2SO_4)	0,71	0,34	0,40	0,62	1,79	1,06	1,38	1,96	2,17
Maximum (g/L K_2SO_4)	0,88	0,54	0,52	0,75	2,40	1,35	1,70	2,30	2,85
Repeatability variation s_r^2	0,0012	0,0011	0,0001	0,0016	0,0063	0,0013	0,0036	0,0015	0,0053
Intergroup variation s_L^2	0,00148	0,00230	0,00163	0,00055	0,01952	0,01082	0,00668	0,01744	0,03552
Reproducibility variation s_R^2	0,0027	0,0034	0,0018	0,0022	0,0258	0,0122	0,0103	0,0189	0,0408
Mean (g/L K_2SO_4)	**0,78**	**0,43**	**0,44**	**0,69**	**2,01**	**1,19**	**1,49**	**2,15**	**2,41**
Standard deviation of Repeatability (g/L K_2SO_4)	**0,04**	**0,03**	**0,01**	**0,04**	**0,08**	**0,04**	**0,06**	**0,04**	**0,07**
Limit r (g/L K_2SO_4)	0,100	0,093	0,031	0,115	0,224	0,103	0,170	0,109	0,206
Repeatability CV	5%	8%	3%	6%	4%	3%	4%	2%	3%
Standard deviation of Reproducibility (g/L K_2SO_4)	**0,05**	**0,06**	**0,04**	**0,05**	**0,16**	**0,11**	**0,10**	**0,14**	**0,20**
Limit R (g/L K_2SO_4)	0,148	0,165	0,118	0,132	0,454	0,312	0,287	0,389	0,572
Reproducibility CV	7%	14%	10%	7%	8%	9%	7%	6%	8%
HORRAT	1,1	2,1	1,5	1,1	1,6	1,7	1,3	1,3	1,7

13. Bibliography

- ARELLANO M., COUDERC F. and PUIG .L (1997): Simultaneous separation of organic and inorganic acids by capillary zone electrophoresis. Application to wines and fruit juices. Am. J. Enol. Vitic., 48, 408-412.
- KANDL T. and KUPINA S. (1999): An improved capillary electrophoresis procedure for the determination of organics acids in grape juices and wine. Am. J. Enol. Vitic., 50, 155-161.
- KLAMPF C.F. (1999): Analysis of organic acids and inorganic anions in different types of beer using capillary zone electrophoresis. J. Agric. Food Chem., 47,

987-990.