Agilent
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Capillary Electrophoresis

Organic Acids Analysis Kit

PN 5063-6510

Upon receipt, please verify that all kit contents listed on page 4 are included. If any part is missing, contact your Agilent Technologies sales office.

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Introduction

This Organic Acids Analysis Kit is meant to facilitate the analysis of common organic acids and some inorganic anions. Since most compounds of these types do not absorb significantly in the UV, detection is performed indirectly.

The buffer supplied in this kit is pre-made, and does not require any special preparation. Also included are capillaries and an organic acids test mixture. To insure that the kit and instrument are functioning properly, a detailed procedure and test electropherogram are given.

Kit contents and other supplies

The following parts are included in the Organic Acids Kit:

Component	Quantity	Part no.
Organic Acids Buffer	250 mL	8500-6785
Organic Acids Test Mixture	20 mL	8500-6900
Capillary (75 µm id, l = 72 cm, L = 80.5 cm)	2 pk	G1600-62311
CE Water	500 mL	5062-8578
1.0 N NaOH	250 mL	5062-8576

The following Agilent parts should be ordered separately when used with the Agilent CE system:

Component	Quantity	Part no.
CE buffer vials, 2 mL glass*	100/pk	5181-3375
CE sample vials, polypropylene, 250 µL	1,000/pk	9301-0978
CE caps, polyurethane Alignment Interface for 75 µm id capillary (color code: blue)	100/pk	5181-1512
CE Column Cutter**		5183-4620

* It is recommended to use glass vials rather than polypropylene vials.

** Can be used if the capillary length needs to be reduced.

Procedures

Buffer preparation

- The Organic Acids Buffer is pre-made and ready to use.
- The buffer should be stored at room temperature.

Buffer vials

Prepare three vials (one Flushing Vial and two HomeVials). When using 2-mL glass vials (p/n 5182-9697), fill each vial with 1.2 mL of the buffer. Also prepare a waste vial (filled with 300 µL CE water).

Since buffers used for indirect UV detection have limited buffering capacity, the buffer should be replaced every five runs when using 2-mL glass vials (p/n 5182-9697).

Standard preparation

The supplied Organic Acids Test Mixture contains three organic acids at 1,000 ppm. Before use, the standard should be diluted 1:40 with CE water (final concentration 25 ppm).

If intended for quantitative analysis, the diluted standard should be freshly prepared and used immediately. **The standard test mixture stock solution should be stored at 4 °C.**

Capillary

A 75 µm id capillary (l = 72 cm, L = 80.5 cm) is supplied with this kit. This method should not be used with the High Sensivity Cell.

Capillary conditioning

Avoid capillary conditioning with sodium hydroxide since this degrades the performance of this application.

Before first use, a new capillary should be flushed for 15 minutes at 1 bar with the run buffer only. Between analyses, it is recommended that the capillary is flushed for 4 minutes with buffer from the Flushing Vial.

Capillary storage

Before the capillary is removed from the instrument for long-term storage, it should be flushed with water (for 10 minutes) followed by a flush with air (for 10 minutes, using empty, capped vials). When the capillary is to be reinstalled, it must be flushed with the run buffer for 15 minutes.

Method summary

The following method can be used to separate the Organic Acids Test Mixture supplied with the kit, as well as other samples. Below are the general analytical conditions followed by step-by-step instructions of the method as it should be programmed into the Agilent ChemStation:

Parameter	Value
Capillary	75 µm i.d., l = 72 cm, L = 80.5 cm (G1600-62311)
Injection	1. Pressure, 50 mbar for 2 seconds from sample vial 2. Post-injection of buffer from InHome Vial, 50 mbar for 4 seconds
Applied voltage	−25 kV
Capillary temperature	20 °C
Detection wavelength	Signal 350/20 nm, Reference 200/10 nm
Preconditioning	Buffer flush for 4 minutes at 1 bar before each run

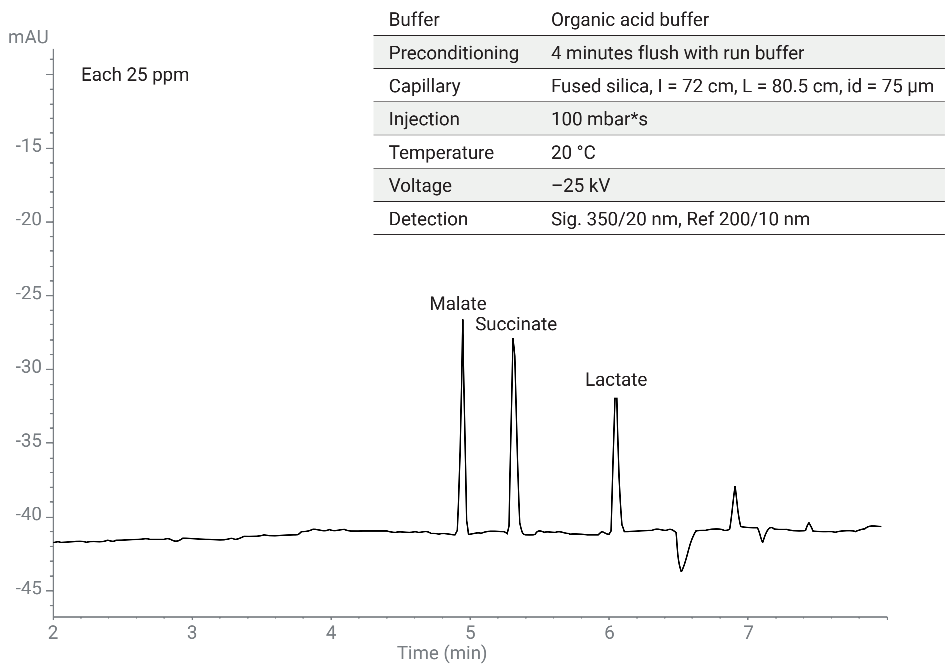
Programming the method

HPCE mode		
CE		
Home Values		
Lift offset (G1600A only)	4	
Cassette temperature	20.0 °C	
Inlet Home Vial	3	Place the buffer vials at position 3 and 4. Vial locations are exemplary only
Outlet Home Vial	4	
Conditioning		
Serial processing		
No replenishment used		
No postconditioning used		
Preconditioning	Use table	
Function	**Parameter**	
1 Flush	4 .00 min, I:1, O:2	Place flushing vial at position 1 and waste vial at 2. Be careful not to overfill waste vial.
Injecting		
Inject by	Use table	
Function	**Parameter**	
1 Pressure	50 mbar, 2.0 seconds I: InjectVial O: OutHomeVial	May be increased or decreased depending on result with sample. Alternatively electrokinetic injection can be used for dilute samples **Note:** be sure to apply negative voltage
2 Pressure	50 mbar, 4.0 seconds I: InHomeVial O: OutHomeVial	The post injection minimizes sample loss upon application of voltage. A voltage ramp is used for the same purpose.
Electric		
Switch electric	On	
Polarity	Negative	Negative polarity is used since EOF is reversed.
Voltage	25.00 kV	
Power	System limit	
Current	100 µA	Current limit is not necessary but may be used to prevent excessive current generation if an error is made (for example, wrong buffer vial used).
Lower alarm limit of current	2 µA	
Time table		
Store voltage	Yes	
Store current	Yes	It is recommended to store current. The current in this method is approximately 11 µm.
Store power	No	
Store pressure	No	
Store temperature	Yes	
Stop time	10.00 minutes	Adjust as needed when running actual sample.
Post time	Off	
Time table is empty		

DAD			
Signals			
Store	Signal, Bw	Reference, Bw (nm)	Adjust (increase) reference wavelength for detection of ions absorbing at 200 nm.
A: Yes	350/20	200/10	
Spectrum			
Store	none		Spectra may be stored for spectral identification of UV- absorbing anions.
Settings			
Stop time	as CE: 10.00 minutes		
Post time	Off		
Time table is empty.			
Peak width	>0.1 minutes		
Response time	1.3 seconds		
Prerun autobalance	On		
Postrun autobalance	Off		

Test electropherogram

Figure 1 shows an electropherogram of the 3-component Organic Acids Test Mixture using the standard method. If the results are not similar to these, please refer to the Troubleshooting section.



Buffer	Organic acid buffer
Preconditioning	4 minutes flush with run buffer
Capillary	Fused silica, l = 72 cm, L = 80.5 cm, id = 75 µm
Injection	100 mbar*s
Temperature	20 °C
Voltage	−25 kV
Detection	Sig. 350/20 nm, Ref 200/10 nm

Figure 1. Analysis of 25 ppm Test Mixture (supplied).

Results and discussion

Reproducibility, linearity, and sensitivity

The reproducibility, linearity, and sensitivity of the method were determined using a 13-component test mixture (not supplied) containing nine organic acids and four inorganic anions. Figure 2 shows the electropherogram and details of this sample.

Buffer	Organic acid buffer
Preconditioning	4 minutes flush with run buffer
Capillary	Fused silica, l = 72 cm, L = 80.5 cm, id = 75 µm
Injection	100 mbar*s
Temperature	20 °C
Voltage	−25 kV
Detection	Sig. 350/20 nm, Ref 200/10 nm

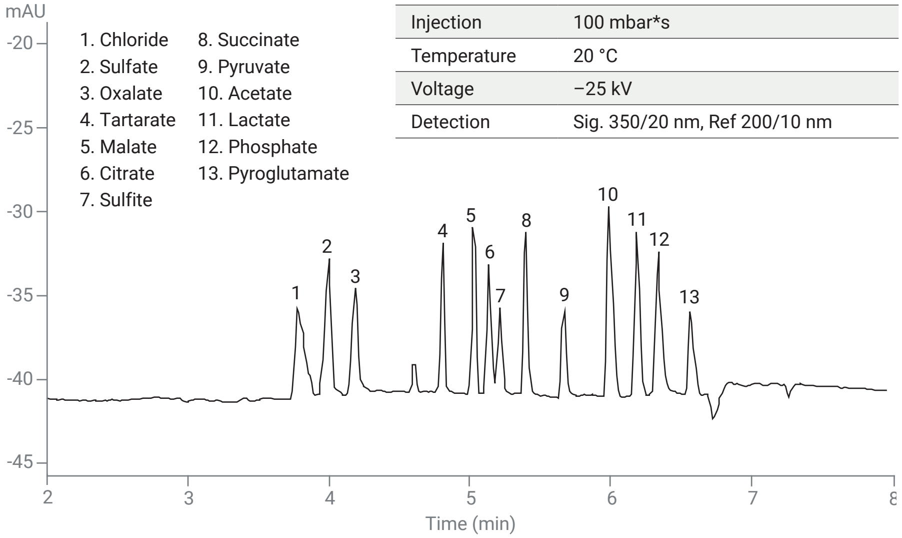


Figure 2. Organic and inorganic anion test mixture (25 ppm each component).

Table 1 shows the reproducibility obtained for migration time and peak area as reflected by the %RSD (n = 6). The calibration curves for all inorganic and organic anions were linear over the range 5–50 mg/L, with correlation coefficients better than 0.9992. The detection limits for all analytes were in the range 0.9–2.5 mg/L with 100 mbar·s pressure injection at a signal-to-noise ratio of 3.

Table 1. Reproducibility and detection limits.

Compound	%RSD (n = 6) Migration time	%RSD (n = 6) Peak area	Detection limit (mg/L)
Chloride	0.03	1.8	1.9
Sulfate	0.03	1.9	1.4
Oxalate	0.04	0.4	1.8
Tartarate	0.03	2.2	1.4
Malate	0.03	1.5	1.2
Citrate	0.03	1.0	2.2
Sulfite	0.04	1.5	2.4
Succinate	0.03	1.5	1.2
Pyrurate	0.04	2.0	2.4
Acetate	0.03	1.4	0.9
Lactate	0.05	1.8	1.2
Phosphate	0.05	1.5	1.8
Pyroglutamate	0.06	2.1	2.5

Note that sample concentrations greater than a few mg/mL will result in split or badly distorted peak shapes. This is a result of the low buffer concentration used for indirect-UV detection. If poor peak shape (and even sensitivity) is observed, dilution of the sample is recommended.

Migration time reproducibility is highly dependent on capillary conditioning and the use of fresh buffer. Since indirect detection buffers have limited buffering capacity, the buffer should be replaced every 2–3 runs when using 1-mL polypropylene vials (p/n 5182-0567), or every 5–7 runs when using 2-mL glass vials (p/n 5181-3375). For routine use, the replenishment system of the Agilent CE system could be used. Be certain to thoroughly clean the replenishment system before use.

Detection of UV-absorbing ions

Ions that absorb in the low UV require modification of the diode-array reference wavelength. For example, nitrate ions could not be observed using a detection wavelength of 350 nm with reference at 200 nm because nitrate ions have their own absorption at 200 nm. Using a signal wavelength of 350 nm with reference at 275 nm enabled detection of nitrate ions between the sulfate and oxalate peaks, albeit with decreased sensitivity.

Peak shape of inorganic anions

The Organic Acids Buffer can also be used for simultaneous analysis of organic acids and some inorganic anions. However, the peak shape of chloride and other small anions is not ideal since the mobilities are not well matched to the mobility of the Organic Acids Buffer. This can be improved using the buffer contained in the Agilent Inorganic Anion Analysis Kit (p/n 5063-6511).

Further optimization of separation

The buffer supplied has been optimized to allow the separation and analysis of the widest number of analytes. Tables 2 and 3 show values of analyte mobility using the standard conditions. Values of mobility offer important insight into feasibility of separations. Analytes with very similar mobility values may be difficult to separate.

Since many organic acids have multiple pK_a's, adjusting the pH can dramatically alter mobility and, therefore, migration time. This is illustrated for a number of ions in Figure 3 and Table 4.

Table 2. Inorganic anion mobility.

Inorganic anion	Mobility (10^{-4} cm^2/Vs)	Inorganic anion	Mobility (10^{-4} cm^2/Vs)
$S_2O_2^{2-}$	-7.26	BrO_3^-	-5.16
Cl^-	-7.20	F^-	-5.08
Br^-	-7.05	ClO_2^-	-4.76
SCN^-	-6.74	S_2^-	-4.41
NO_2^-	-6.69	SO_3^{2-}	-4.38
SO_4^{2-}	-6.64	PO_2^{3-}	-4.01
$NO3^-$	-6.50	IO_3^-	-3.73
I^-	-6.40	PO_3^{3-}	-3.58
ClO_2^-	-5.71	PO_4^{3-}	-3.06
BF_4^-	-5.41		

Table 3. Organic anion mobility.

Organic anion	Mobility (10^{-4} cm^2/Vs)	Organic anion	Mobility (10^{-4} cm^2/Vs)
Oxalate	-6.23	Propionate	-2.09
Fumarate	-5.16	Pyroglutamate	-2.87
Tartarate	-5.10	Levulinate	-2.76
Formate	-5.03	*n*-Butyrate	-2.68
α-Ketoglutarate	-4.70	*n*-Pentanoate	-2.36
Malonate	-4.68	Ascorbate	-2.35
Malate	-4.63	D-Mannuronate	-2.33
Citrate	-4.49	D-Glucuronate	-2.28
Succinate	-4.06	D-Galacturonate	-2.24
Malcinate	-4.03	*n*-Hexanoate	-2.09
Glutarate	-3.94	NANA	-1.76
Pyruvate	-3.02	NGNA	-1.75
Glycolate	-3.73	*n*-Heptanoate	-1.59
Acetate	-3-38	*n*-Octanoate	-0.58
Lactate	-3.23		

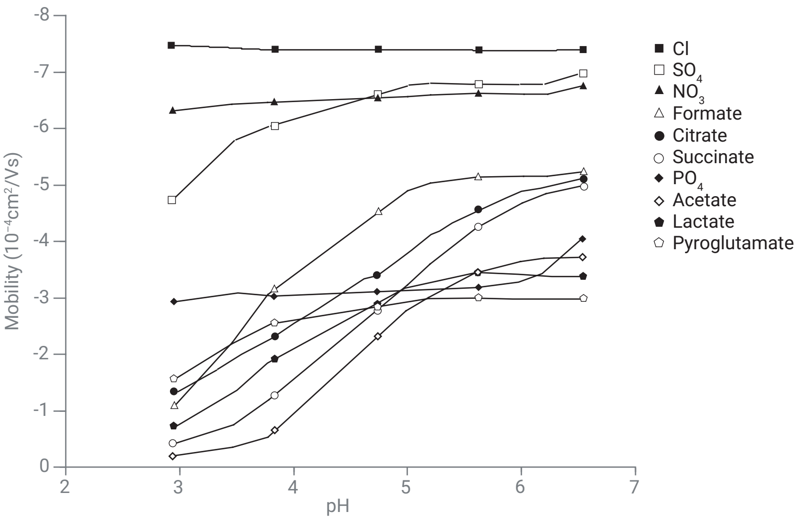


Figure 3. Mobility as a function of buffer pH.

Table 4. pK_a of various anions.

Compound	pK_{a1}	pK_{a2}	pK_{a3}
Sulfate	-3	1.92	
Nitrate	-1.34		
Oxalate	1.27	4.27	
Formate	3.75		
Malate	3.4	5.05	
Citrate	3.13	4.76	6.4
Succinate	4.21	5.64	
Pyruvate	2.49		
Acetate	4.76		
Lactate	3.86		
Phosphate	2.12	7.2	12.36

Applications

Beer and wine analysis

Beer analysis

The kit is well suited for the analysis of inorganic and organic anions in beer and wine samples. Figure 4 shows an electropherogram of a beer analysis. Sample preparation was very simple and comprised only of degassing the beer by sonication and 1:5 dilution with deionized water before injection. A well defined electropherogram was obtained without interference from matrix compounds. The concentration of ions in the sample ranged 9–511 mg/L, and the method showed good agreement with data obtained from conventional techniques (ion chromatography for inorganic anions and liquid chromatography for organic acids), with a correlation coefficient of 0.993. Acceptable reproducibility was obtained, with %RSD values (n = 5) for migration times better than 0.3 % and for peak areas better than 3.4 %, except for the low concentration oxalate and formate, which were difficult to integrate. Refer to reference 1 for more details.

Buffer	Organic acid buffer
Preconditioning	4 minutes flush with run buffer
Capillary	Fused silica, l = 72 cm, L = 80.5 cm, id = 75 µm
Injection	100 mbar*s
Temperature	20 °C
Voltage	–25 kV
Detection	Sig. 350/20 nm, Ref 200/10 nm

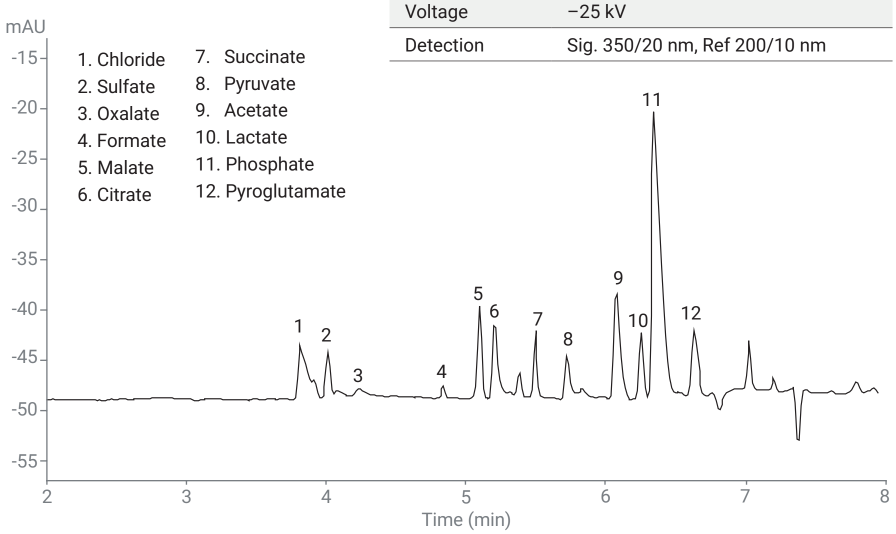


Figure 4. Beer analysis.

Wine analysis

The organic acid content of table wines is an important factor in determining the quality of the finished product. The result of white wine analysis is shown in Figure 5. Peaks were identified by their migration times, and the concentrations of tartarate, malate, citrate, succinate, pyruvate, acetate, and lactate were calculated as 1.6, 4.5, 0.42, 0.74, 0.11, 0.05, and 0.84 g/L, respectively.

Buffer	Organic acid buffer
Preconditioning	4 minutes flush with run buffer
Capillary	Fused silica, l = 72 cm, L = 80.5 cm, id = 75 µm
Injection	100 mbar*s
Temperature	20 °C
Voltage	–25 kV
Detection	Sig. 350/20 nm, Ref 200/10 nm

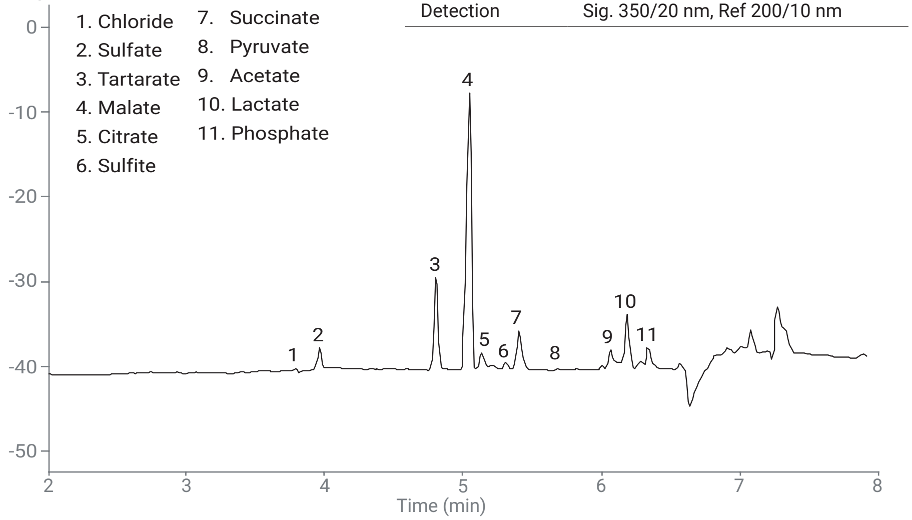


Figure 5. Wine analysis.

Short alkyl-chain carboxylic acids

Figure 6 shows an electropherogram of 50 mg/L each of short-chain (C1–C8) carboxylic acid standards. These compounds are often of significant environmental interest. Eight carboxylic acids were rapidly well separated.

Buffer	Organic acid buffer
Preconditioning	4 minutes flush with run buffer
Capillary	Fused silica, l = 72 cm, L = 80.5 cm, id = 75 µm
Injection	100 mbar*s
Temperature	20 °C
Voltage	–25 kV
Detection	Sig. 350/20 nm, Ref 200/10 nm

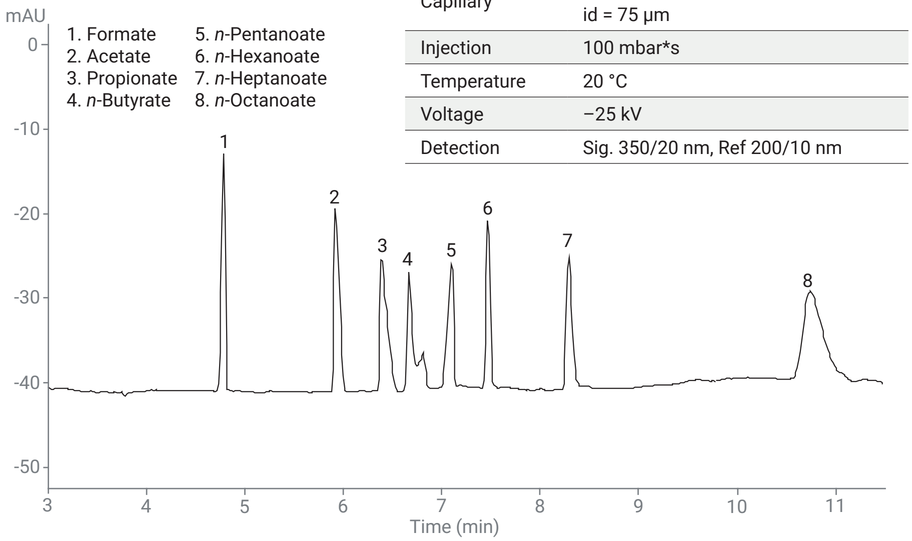


Figure 6. Short alkyl-chain carboxylic acids.

Halides, oxyhalides, and phoshoric compounds

Inorganic anions including halide, oxyhalide, and phosphoric compounds could also be separated. Figure 7 shows the separation of a standard solution containing 20 mg/L of each anion.

Buffer	Organic acid buffer
Preconditioning	4 minutes flush with run buffer
Capillary	Fused silica, l = 72 cm, L = 80.5 cm, id = 75 µm
Injection	100 mbar*s
Temperature	20 °C
Voltage	–25 kV
Detection	Sig. 350/20 nm, Ref 200/10 nm

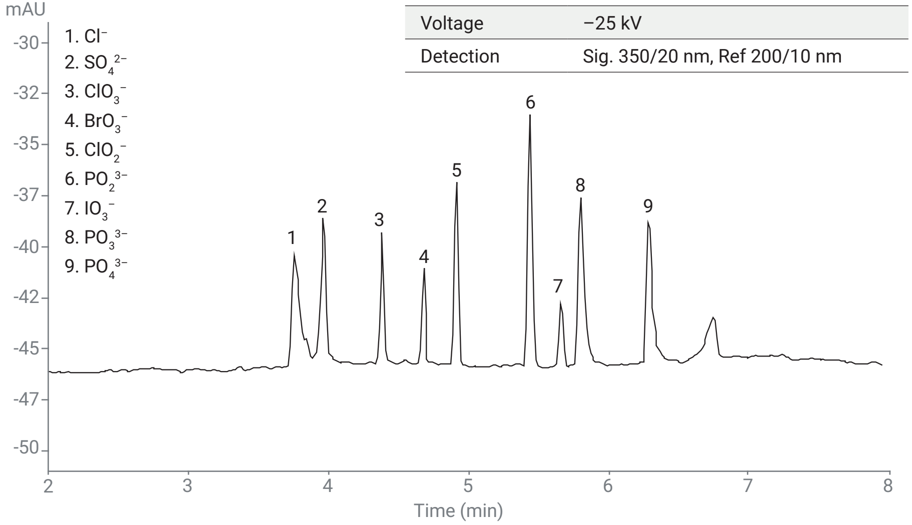


Figure 7. Halides, oxyhalides, and phosphoric compounds.

Troubleshooting

Problem	Possible cause	Solution
Unstable current	Capillary not filled with buffer	Increase flush time
	Air bubbles in buffer	Ultrasonicate buffer
	Capillary clogged	Remove, flush with syringe Cut inlet end
	Capillary broken at window	Replace capillary
Poor resolution or broad/split peaks	Capillary not equilibrated	Flush and repeat analysis
	Sample overloaded	Reduce sample concentration or amount injected
No signal	Wrong setting of power supply	Verify injection and polarity migration is toward anode
	Detection wavelength incorrect	Verify Sig: 350/20, Ref: 200/10 nm detection
	Sample not injected	Verify no air bubble trapped in bottom of sample vial
Noisy baseline	Buffer contains particulates	Filter through 0.45 µm filter
	Alignment interface occluded	Examine under microscope and clean with MeOH
	Capillary window dirty	Examine and clean with lint-free paper/MeOH
	Lamp is old	Replace if more than 650-750 hours
Poor resolution	Damaged capillary	Replace capillary
	Sample Overloading	Dilute sample
	Capillary too short	Replace capillary
Poor reproducibility	Capillary not equilibrated	Increase flush time with buffer
	Buffer overused	Replace buffer

Reference

1. Soga, T., Analysis of Inorganic and Organic Anions by Capillary Zone Electrophoresis, *Agilent Technologies Application Note*, publication number 12-5965-5744E, **1996**.

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