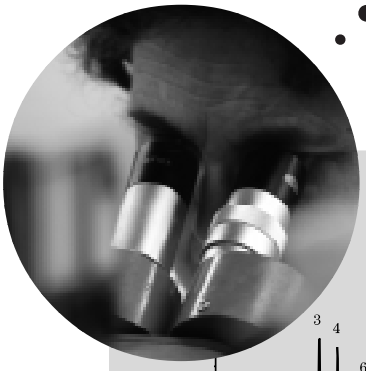
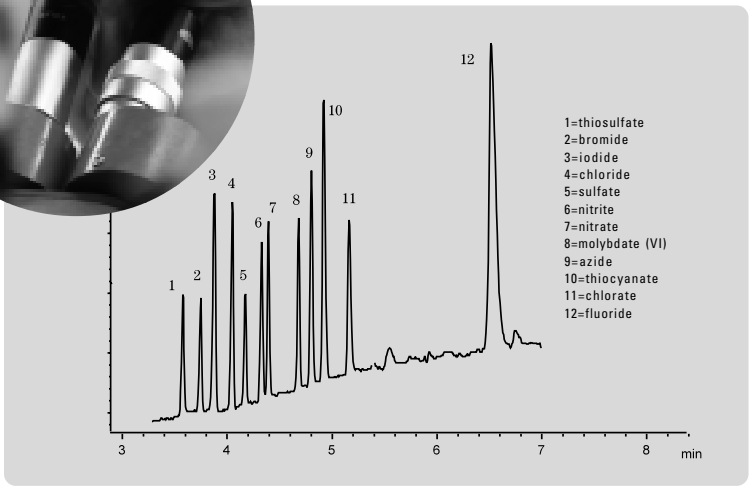
Capillary Electrophoresis

Inorganic Anion Analysis Kit

PN 5063-6511

Store the Inorganic Anion Buffer and the test mixture at 4 °C!





Agilent Technologies
Innovating the HP Way

Upon receipt please verify that all kit contents listed on page 3 are included. If any part is missing, contact your Agilent Technologies sales office.

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Introduction

The Agilent Inorganic Anion Analysis Kit is meant to facilitate the analysis of inorganic anions. Since most analytes of this type do not have significant UV absorption, the method uses indirect UV detection.

The buffer supplied in this kit is pre-made with the pH already adjusted. Therefore, no further preparation is required. Also included are capillaries and a standard test mixture.
To ensure that the kit and instrument are functioning properly, a detailed procedure is given below and some typical electropherograms are shown.

Kit Contents and Other Supplies

The following parts are included in the inorganic anion kit:

Component	Quantity	Part No.
Inorganic Anion Buffer	250 ml	8500-6797
Bare fused silica capillary (50 µm i.d., l=72 cm, L=80.5 cm)	2/pk	G1600-62211
Inorganic Anion Test Mixture Fluoride 1000 ppm, Chloride 1000 ppm, Bromide 1000 ppm, Nitrite 1000 ppm, Nitrate 1000 ppm, Sulfate 1000 ppm, Phosphate 2000 ppm	10 ml	5062-8524
CE Water	500 ml	5062-8578
1.0 N NaOH	250 ml	5062-8576
0.1 N NaOH	250 ml	5062-8575

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) or	100/pk	5181-3375
1 ml (polypropylene)	100/pk	5182-0567
CE sample vials, 100 µl (polypropylene)	1000/pk	9301-0978
CE caps, (polyurethane)	100/pk	5181-1512
Alignment Interface for 50 µm i.d. capillary (color code: green)	1	G1600-60210
optional:		
Bare fused silica capillary, extended light path BF3 (50 µm i.d., l = 56 cm, L = 64.5 cm)	1	G1600-61232
Alignment interface for 50 µm i.d. extended light path capillary, BF3 (color code: red)	1	G1600-60230
CE Column Cutter *	1	5183-4620

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

Procedures

Buffer Preparation

The Inorganic Anion Buffer is premade and ready to use.

The buffer should be stored at 4 °C.

Buffer Vials

Prepare 3 vials (one Flushing Vial and two HomeVials). When using 2 ml glass vials (PN 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 5 runs when using 2 ml glass vials (PN 5182-9697).

Standard Preparation

The supplied Inorganic Anion Test Mixture contains 6 anions at 1000 ppm and phosphate at 2000 ppm. Prior to use, the standard should be diluted 1:100 with CE water (final concentration 10 ppm, 20 ppm phosphate).

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 50 µm i.d. capillary (L=80.5 cm, l=72 cm) is supplied with this kit. For better detection limits in the ppb range, a bubble cell capillary (50 µm i.d., BF3) together with electrokinetic injection may be used. The use of the High Sensitivity Detection Cell is not recommended.

Capillary Conditioning

Prior to first use, a new capillary should be conditioned:
Flush 1 N NaOH for 10 min, flush deionized water for 10 min, flush run buffer for 30 min.

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 newly conditioned.

Method Summary

The following method can be used to separate the Inorganic Anion Test Mixture supplied with the kit, as well as other samples. Below are the general analytical conditions which are followed by a copy of the method as it should be programmed into the Agilent ChemStation:

Capillary :	50 µm i.d., l=72 cm, L=80.5 cm (G1600-62211) The capillary length can be reduced if resolution of analytes exceeds requirements.
Injection:	Pressure, 50 mbar for 4 seconds from sample vial
Applied voltage:	–30 kV
Capillary temperature:	20 °C
Detection wavelength:	Signal 350/80 nm, Reference 245/10 nm

Vial Contents	Carousel Location
Conditioning Vial containing 1 N NaOH	1
Conditioning Vial containing 0.1 N NaOH	2
Wash Vial containing water	3
Flush Vial containing anion buffer	4
Waste vial ¼ full with water or buffer	5
INHOME Vial containing inorganic anion buffer	6
OUTHOME Vial containing inorganic anion buffer	7

Programming the Method (comment in italic)

High Performance Capillary Electrophoresis

Home values:

Lift offset	4	
Cassette Temperature	20.00 °C	*Place buffer vials at position 6 and 7*
Inlet Home Vial	6	
Outlet Home Vial	7	*Vial locations are exemplary only*

Replenishment and Preconditioning:

Serial Processing

Replenishment Entries:

No Replenishment used — *Buffer replenishment can be implemented for repetitive analyses*

Preconditioning Entries:

Function	Parameter	
1 FLUSH	3 .00 min, I:2, O:5	*0.1 NaOH flush between analyses*
2 INLET	I:3	*Raises buffer or water vial to remove residual NaOH*
3 FLUSH	5 .00 min, I:4, O:5	*Buffer flush between analyses.*

Postcondition Entries:

No Postconditioning used

Electric:

Electric	On	
Polarity	Negative	*Negative polarity is used since EOF is reversed*
Voltage	0.00 kV	
Current	100.0 µA	*Current limit is not necessary but may be used to prevent excessive current generation if an error is made (e.g. wrong buffer vial used)*
Power	System Limit	
Low Current Limit	0.00 µA	

Injection:

Inject by PRESSURE 50 mbar, 4.00 sec — *May be increased or decreased depending on result with sample. Alternatively electrokinetic injection can be used for dilute samples (apply "negative" voltage)*

Store Data:

Collect voltage	Yes	
Collect current	Yes	*It is recommended to store current. Current in this method is approxymately –6 µA*
Collect power	No	
Collect pressure	No	
Collect temperature	Yes	

Time entries:

Stop time	20.00 min	*Adjust as needed.*
Post time	Off	

Time Table:

Time [min]	Function	Parameter	
0.30	VOLTAGE	30 kV	*Decrease if capillary length is greatly reduced.*

Diode Array Detector

Settings:

Stop Time	as HPCE: 20.00 min
Post Time	Off
Response Time	0.3 sec
Peakwidth	0.03 min
Prerun Autobalance	ON
Postrun Autobalance	Off

Spectrum:

Store	None	*Spectra may be stored for spectral identification of UV-absorbing anions*

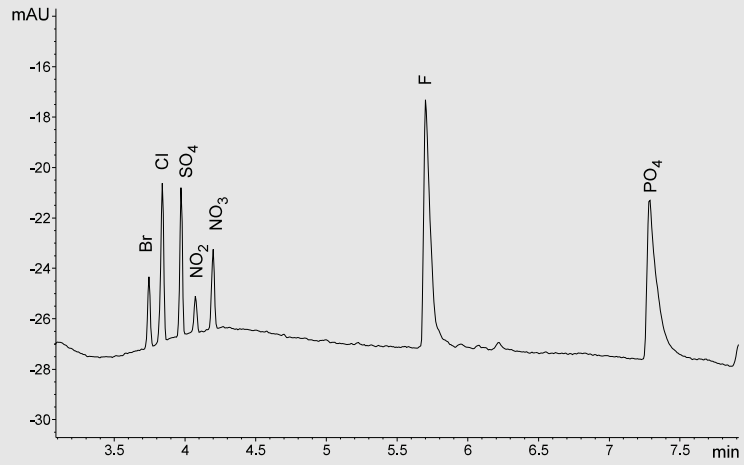
Signals:

Store	Signal,Bw	Reference,Bw [nm]	
A: Yes	350/80	245/10	*See later example of other settings useful for multi-wavelength detection.*

Results and Discussion

Analysis of Anion Standard

An electropherogram of the inorganic anion standard using the standard method with a 56-cm effective length capillary is shown in the following figure. If a 72-cm effective length capillary is used, the test mixture anions have migration times of 6.5 to 12.5 min. If the results are not similar to these, please refer to the Troubleshooting section.



Reproducibility

Migration time reproducibility is highly dependent on use of fresh buffer. Since the indirect detection buffer has limited buffering capacity it should be replaced frequently. For routine use, the replenishment system of the Agilent CE system should be used *(do not use the replenishment reservoir for long-term buffer storage)*.

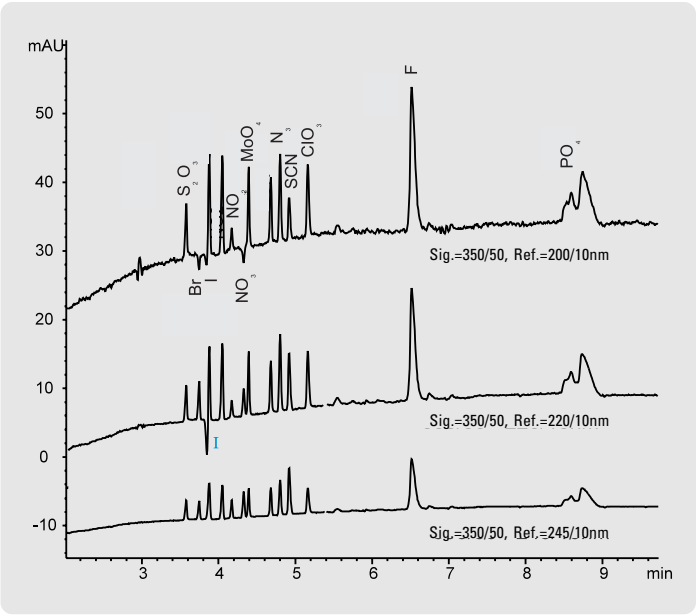
Capillary conditioning also effects migration time stability. As described in the Method section, new capillaries should be conditioned with 1.0 NaOH. Conditioning between analyses may consist of buffer flush only *or* 0.1 N NaOH followed by buffer. Longer conditioning times generally improve migration time stability.

An acidic preconditioning procedure has also been described (1) and may allow shorter overall run times.

Sensitivity

Detection limits may be improved for dilute samples simply by increasing the amount injected. The standard method setting of 50 mbar x 4 sec can be increased 2-3 times or until the peak height does not increase or resolution is lost.

Alternatively, electrokinetic injection can be used. Generally 5-10 kV is applied for 2-30 seconds. With this method a 5-10 fold



improvement may be obtained relative to pressure injection. The dependence of sample loading on the conductivity of the sample matrix limits the utility of this method for quantitative analysis. Internal standards should be used for accurate determinations.

Transient isotachophoresis can also be used to improve sensitivity by allowing injection of larger sample volumes. Most simply, this can be achieved by the addition of 2–10 mM octanesulfonate to the sample (2).

Injection of concentrated samples (e.g. 250-500 ppm) may result in severe peak distortion. Prepare diluted samples to determine optimum operating concentrations.

Contaminants should be minimized when analyzing very dilute samples. Always use fresh buffer and ultrapure reagents. It may be necessary to soak vials and caps in CE water prior to use.

Multi-wavelength detection

Diode-array detection provides the advantage of peak identification for UV-absorbing anions. Detection at various wavelengths is illustrated in the following example.

At 245 nm none of the species absorbs. Iodide shows absorbtion at 220 nm which results in a negative peak in the indirect UV detection system. Finally at 200 nm iodide, bromide, and nitrate absorb and give negative peaks. This type of detection can be used for peak identification as well as for providing reference peaks in the electropherogram.

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 or cut inlet end
	Capillary broken at window	Replace capillary
Poor resolution or broad peaks	Capillary not equilibrated	Flush and repeat analysis
	Sample overloaded	Reduce sample concentration or amount injected
No signal	Wrong setting of power supply polarity	Verify injection and that migration is toward anode
	Detection wavelength incorrect	Verify Sig: 350/80, Ref: 245/10nm detection
	Sample not injected	Verify no air bubble trapped in bottom of sample vial
Noisy baseline	Dissolved air in buffer	degas buffer
	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 lintfree paper/MeOH
	Lamp is old	Replace if more than 650-750 hours
Poor reproducibility	Capillary not equilibrated	Increase flush time with buffer
	Buffer overused	Replace buffer

References

1. Ehmann, T. et al., J. Chromatogr. A, 816 (**1998**) 261–275

2. Jandik, P. and Bonn, G., Capillary Electrophoresis of Small Molecules and Ions, VCH Publishers, Inc., New York, **1993**.

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