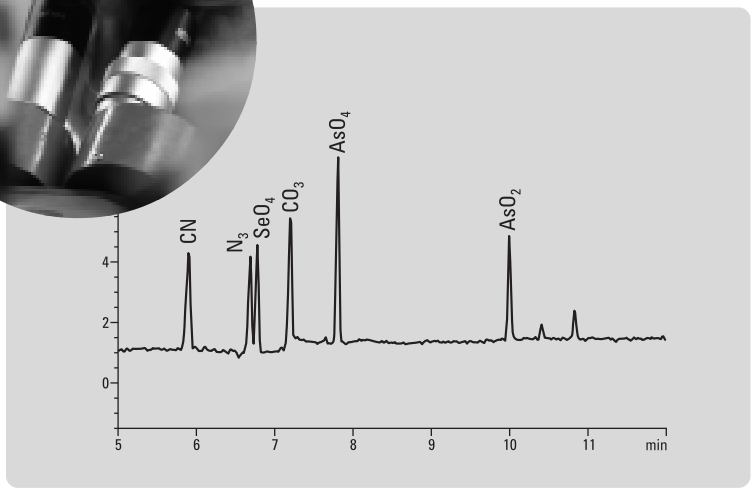
Capillary Electrophoresis

Forensic Anion Solution Kit

PN 5064-8208

Store 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 Forensic Anion Solutions Kit is meant to facilitate the analysis of several toxic and other inorganic anions. Since most analytes of these types 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 Forensic Anion Solution Kit:

Component	Quantity	Part No.
Basic Anion Buffer	5 x 50 ml	5064-8209
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	1 x 10 ml	5062-8524
Bare Fused Silica Capillary (50 µm i.d., l=104 cm, L=112.5 cm)	2/ pk	G1600-64211
CE Water	1 x 500 ml	5062-8578
Product Literature	1	5963-3903E

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

Component	Quantity	Part No.
Buffer vials for CE, glass, 2 ml	100/pk	5182-9697
Sample vials for CE, polypropylene, 100 µl	1000/pk	9301-0978
Vial caps for CE, polyurethane	100/pk	5181-1512
CE Column Cutter*	1	5183-4620

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

Procedures

Sample Preparation

The inorganic anion standard solution supplied contains six anions at 1000 ppm and PO_4^{3-} at 2000 ppm. Prior to use, the standard should be diluted 1:10 with CE water (100 ppm for six anions and 200 ppm for PO_4^{3-} final concentration). For quantitative purposes the diluted standard should be used immediately.
The standard test mixture should be stored at 4 °C.

Buffer Preparation

The Basic Anion Buffer is pre-made and ready to use.

Do not leave the solution uncapped since the Basic Anion Buffer is a highly alkaline solution and will readily absorb gaseous carbonate from the atmosphere. This will cause its pH to drop immediately. Make certain the bottle is capped after use.

Do not store opened bottles which are a third or less full. These should be discarded because gaseous carbonate in the bottle can be adsorbed and will reduce the residual buffer pH.

The buffers should be stored at 20 °C. (Some buffer components may crystallize at lower temperatures.)

Buffer vials

Prepare at least 3 vials (one for capillary flushing and two Home-Vials). When using 2 ml glass vials (PN 5182-0538), fill each vial with 1.4 ml of the buffer.
Since indirect detection buffers have limited buffering capacity the buffer should be replaced every 10 runs when using 2 ml glass vials (PN 5182-0538) or every 5-6 runs when using 1 ml polypropylene vials (PN 5182-0567).

For buffer replacement do not use the replenishment system. The level of dissolved carbonate may increase due to pressurization of the buffer bottle, causing a pH-drop.

Capillary

Only 50 µm i.d. straight capillaries are suitable for this method. Baseline noise is markedly increased if a 75 µm i.d. capillary is used due to the high UV absorptivity of the buffer. Neither bubble cell capillaries nor the High Sensitivity Detection Cell should be used. A 50 µm i.d. capillary (L=112.5cm, l=104 cm) is supplied with the kit.

Capillary conditioning

It has been determined that capillary conditioning with sodium hydroxide degrades separation quality and reproducibility of this method and should therefore be avoided. Prior to first use a new capillary should be flushed only with the run buffer for 15 minutes. Between analyses it is recommended that the capillary is flushed for 2 minutes with buffer from the OutHomeVial then for 2 minutes with buffer from the InHomeVial. This preconditioning helps to avoid baseline fluctuation which is a symptom of buffer exhaustion.

Detection

For ions which have little or no UV absorbance, a detection wavelength of 350 nm with reference at 275 nm is recommended.

Capillary storage

If the capillary is removed from the instrument it should be washed for 15 minutes with CE water or distilled deionized water and then flushed with air (using empty, capped vials). When the capillary is to be reinstalled, it should be flushed with with the run buffer 20 minutes.

Method Summary

The following method can be used to separate the anion standard supplied with the kit as well as other samples. Below are the general analytical conditions followed by details of the method as it should be programmed into the Agilent ChemStation:

Flush new capillary for 15 minutes from a vial filled with the Basic Anion Buffer.

Flush capillary between analyses for 2 minutes with the run buffer in OutHomeVial, then 2 minutes with the run buffer in InHomeVial.

Capillary:	50 µm i.d., l=104 cm, L=112.5 cm (G1600-64211)
Injection:	1. Pressure, 50 mbar for 6 seconds 2. Post-injection of buffer from InHome Vial, 50 mbar for 4 seconds
Power supply polarity:	Negative
Applied voltage:	30 kV
Capillary temperature:	30 °C
Detection wavelength:	Signal 350/20 nm, (i.e. 350 nm with 20 nm bandwidth)
Reference	275/10 nm
Stop Time:	15 minutes

Programming the Method (comment in italic)

HPCE mode:
CE

Home values:

Lift Offset	4	
Cassette Temperature	30.0 °C	
Inlet Home Vial	10	*Vial locations are exemplary only.*
Outlet Home Vial	11	

Conditioning:
Serial processing
No Replenishment used
Do not use replenishment. The level of dissolved carbonate might increase due to pressurization of the buffer bottle, causing a pH-drop.
No Postconditioning used

Preconditioning: use table

Function	Parameter
1 Flush	2.00 min, I: OutHomeVial, O:1
2 Flush	2.00 min, I: InHomeVial, O:1

Conditioning with Out- and InHomeVials between analyses to avoid baseline step.
Set waste vial on position 1. Be careful not to overfill waste vial.

Injecting:
Inject by: use table

Function	Parameter
1 PRESSURE	50.0 mbar, 6.0 sec, I: InjectVial, O:OutHomeVial
	May be increased or decreased depending on results.
2 PRESSURE	50.0 mbar, 4.0 sec, I: InHomeVial, O:OutHomeVial
	The post-sample buffer plug helps to minimize 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	30.0 kV	
Current	150.0 µA	*A 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).* *The current in this method is approximately -40 µA*
Power	System Limit	
Lower alarm limit of current	2 µA	

Time Table:

Store voltage	Yes	
Store current	Yes	*It is recommended to store the current for every analysis. It can be used to identify the source of problems if they occur.*
Store power	No	
Store pressure	No	
Store temperature	Yes	
Stop time	15.00 min	*Adjust as needed when running actual sample*
Post time	Off	
Time Table is empty.		

DAD Signals

Signals

Store	Signal, Bw	Reference, Bw [nm]
A:Yes	350/20	275/10

Spectrum

Store	None

Settings

Stop Time	as HPCE: 15.00 min	
Post Time	Off	
Time table is empty		
Peak width	>0.1 min	
Response time	1.3 sec	*This is recommended to reduce baseline noise.*
Prerun Autobalance	On	
Postrun Autobalance	Off	

Test Electropherogram

Analysis of Inorganic Anion Test Standard

Figure 1 shows a typical electropherogram of the 7-component inorganic anion test mixture (1:10 dilution) using the standard method. If the results are not similar to these please refer to the Troubleshooting section.

The resolution between bromide and chloride is not ideal under these conditions. This can be improved by operating at a temperature of 20 °C.

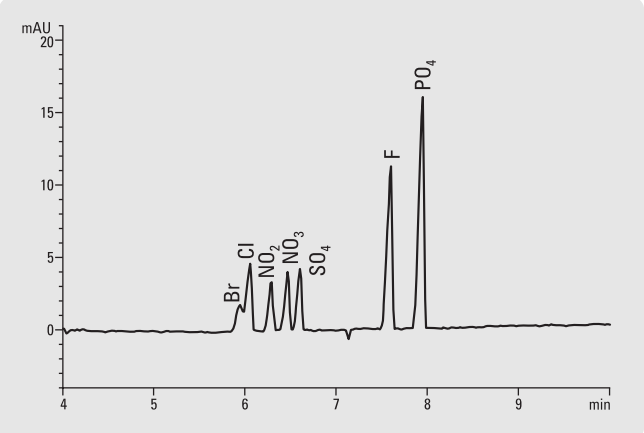


Figure 1: Analysis of 1:10 diluted Inorganic Anion Standard (PO_4^{3-} 200 ppm, others each 100 ppm, delivered with the kit).

Results and Discussion

Inorganic, organic and forensic anion analysis

Figure 2 shows an electropherogram of 50 ppm each of five forensic anions (CN^-, N_3^-, SeO_4^{2-}, AsO_4^{3-}, AsO_2^-) and nine inorganic and organic anions. In order to separate sulfate, azide and selenate, the temperature was set to 30 °C.

Typical % RSD values were in the region of < 0.5 % for migration time and < 4 % for peak areas. The detection limits for all analytes were in the range 3 - 11 ppm with 300 mbar*s pressure injection with a signal-to-noise ratio of 3.

Please be aware that a carbonate peak can appear even if analyzing pure water. This is due to absorption of CO_2 gas from the atmosphere. Therefore quantitative analysis of carbonate is not recommended.

Note that sample concentrations greater than a few 1000 ppm will result in split or badly distorted peak shapes. This is due to the low buffer concentration employed for indirect UV detection. If poor peak shape (and even poor sensitivity) is observed, dilution of the sample is recommended.

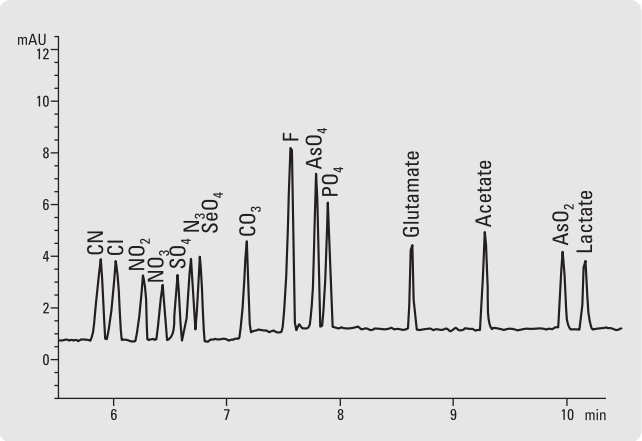


Figure 2: Forensic, inorganic and organic anion test mixture (50 ppm each component). Separation conditions see method section.

Application

Arsenite in curry

The kit is well suited for the screening of forensic anions in food samples. For example, Figure 3 shows the results of the analysis of an arsenite spiked curry sample. In this study 1 % arsenite spiked curry was prepared by adding 1 g of sodium arsenite to 100 g of curry sauce. The curry was diluted 1:100 with deionized water and filtered through 0.22 µm pore membrane prior to analysis by CE.

The electropherogram shows 50 ppm each of standards, curry blank (1:100 dilution) and arsenite spiked curry (1:100 dilution).

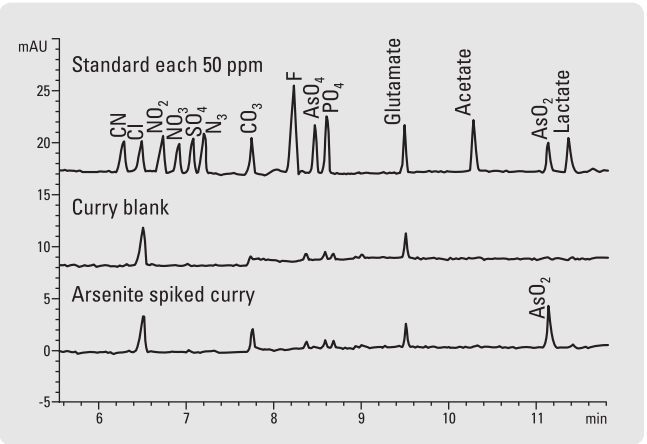


Figure 3: Analysis of arsenite in curry. For separation conditions see method section.

Troubleshooting

Problem	Possible Cause	Solution
Poor resolution or broad/split peaks	Buffer is old	Use new buffer
	Capillary damaged	Replace capillary
	Sample overloaded	Dilute sample
	Capillary too short	Use longer capillary
Peak leading	Buffer absorbed carbonate	Use new buffer
No signal	Sample not injected	Verify no air bubble trapped in bottom of sample vial Verify inlet capillary set correctly
	Capillary damaged	Replace capillary
	Wrong buffer used	Verify buffer
	Wrong setting of power supply polarity	Verify Negative Polarity
	Detection wavelength incorrect	Verify Sig:350/20, Ref.275/10nm
Baseline step	Wrong setting of preconditioning	Use Out- and InHomeVial for preconditioning
Noisy baseline	Wrong setting of response time	Verify Response time 1.3 sec
	Capillary window not adjusted	Examine capillary window
	Capillary window dirty	Examine and Clean with lint-free paper/MeOH
	Lamp is old	Replace lamp
	Buffer pH higher than 12.3	Verify buffer pH
Poor reproducibility	Liquid in outlet tube	Remove outlet tube from pre-puncher
	Buffer overused	Replace buffer
Unstable current	Capillary broken	Replace capillary
	Capillary not filled with buffer	Increase flush time

References

1. Soga, T. and Ross, G. A., Capillary Electrophoretic Determination of Inorganic and Organic Anions using 2,6-pyridinedicarboxylic acid: effect of electrolyte's complexing ability, J. Chromatogr. A 1997, 767, 223-230, Pub. No. 12-5965-8067E.

2. Soga, T. and Heiger, D. N., Simultaneous Determination of Monosaccharides in Glycoproteins by Capillary Electrophoresis, Anal. Biochem. 1998, 261, 73-78, Pub. No. 12-5968-0772E.

Acknowledgement

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