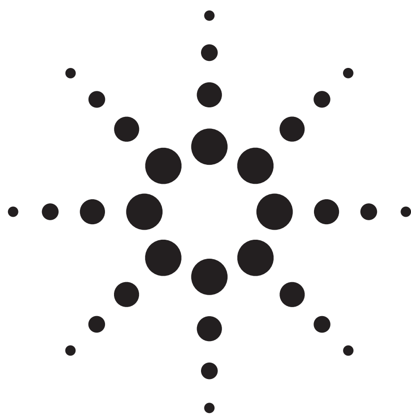
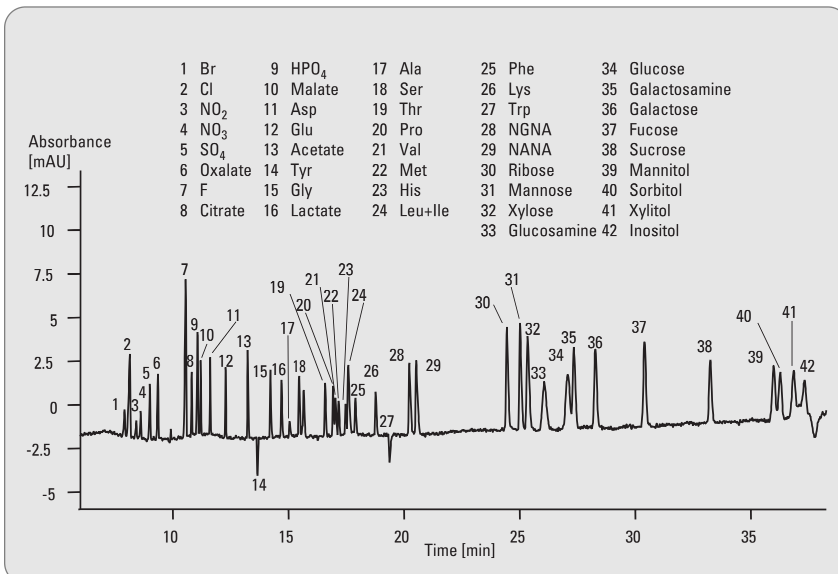


Simultaneous analysis of inorganic anions, organic acids, amino acids and carbohydrates using the Agilent Basic Anion Buffer

Application Note

Food

Tomoyoshi Soga

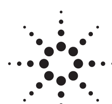


Introduction

This application note describes extended applications of the Agilent Basic Anion Buffer. It facilitates the analysis of many anions including inorganic anions, organic acids, amino acids and carbohydrates. The method described here is useful for the screening analysis of anions in food and beverage samples.

To separate these anions simultaneously, a highly alkaline pH condition is used to confer a negative charge not only on inorganic and organic anions but also on amino acids and carbohydrates, and promote their migration towards the anode. Electroosmotic flow (EOF) is reversed in the direction of the anode by adding quaternary ammonium salt to the electrolyte. This is necessary to have both anion migration and EOF in the same direction (towards the anode) and ensure anion migration past the detector.

In this method, indirect UV detection is employed to visualize anions which have little or no chromophore. The Agilent Basic Anion Buffer is pre-made with the pH already adjusted, therefore no further preparation is required. Detailed method parameters and some typical electropherograms are shown.



Agilent Technologies

Innovating the HP Way

Necessary supplies

The following parts are necessary for the simultaneous analysis of anions:

Component	Quantity	Part No.
Agilent Basic Anion Buffer*	50 ml	5064-8209
Product literaure	1	5968-7715E

* For this buffer, only 50-µm id straight capillaries are usable.

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

Component	Quantity	Part No.
Fused silica capillary (id = 50 µm, l=104 cm L=112.5 cm)	1 pk	G1600-64211
CE buffer vials 2 ml (glass*)	100/pk	5182-9697
CE sample vials, 100 µl (polypropylene)	1000/pk	9301-0978
CE caps (polyurethane)	100/pk	5181-1512
CE water	500 ml	5062-8578

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

Procedures

Buffer preparation

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

Do not leave the solution uncapped since the 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 pH of the residual buffer.

The buffer should be stored at room temperature (not less than 20 °C, since some buffer components may crystallize at lower temperatures).

Buffer and waste vials

Prepare 3 vials (one flushing vial and two home vials). When using 2-ml glass vials (PN 5182-9697), fill each vial with 1.4 ml of the buffer. Also prepare a waste vial (filled with 300-ml CE water or deionized water).

Since buffers used for indirect UV detection have limited buffering capacity, the buffer should be replaced every 8 runs when using 2-ml glass vials (PN 5182-9697).

For this application, do not use the replenishment system for buffer replacement. The level of dissolved carbonate may increase due to pressurization of the buffer bottle, causing a pH drop. Also, crystallization of the buffer might block the tubes.

Standard Preparation

Individual stock solutions of inorganic and organic anions should be prepared from their sodium salts or free acids. Since carbohydrates are unstable, they should be prepared shortly before use.

For amino acids, individual stock solution of Tyr, Cys-Cys, Asp, Trp, Leu, Ile and Phe should be prepared at a concentration of 10 g/l in 0.1 M NaOH. Other amino acids should be prepared in 0.01 M HCl. The working mixture standard should be prepared by diluting stock solutions with deionized water.

If a commercially available amino acid standard mixture in 0.1 M HCl is used, Arg may not be detected since the pKa value of Arg is 10.76. Arg is positively charged in 0.1 M HCl and migrates

toward the opposite direction of the detector. In this case, the standard solution should be diluted with 0.01 M NaOH.

The concentration of the standard mixture should be in the range of 50 to 1000 mg/l to obtain good peak shape and sensitivity.

Sample Preparation

For actual samples, dilution with deionized water is necessary in order to reduce the conductivity of the samples, for example, 1:50 dilution for soy sauce.

If the sample contains proteins and the migration times increase from run to run, removal of the proteins is recommended by using centrifugal filtering through a 30-kDa cutoff filter.

Capillary

Only 50-µm id straight capillaries are suitable for this method. Baseline noise is markedly increased if a 75-µm id 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 id capillary (L=112.5 cm, l=104 cm) is recommended.

Capillary conditioning

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

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 be flushed for 4 minutes with buffer from the flushing vial.

Capillary storage

If the capillary is removed from the instrument it should be washed for 10 minutes with deionized water and then flushed with air for 10 minutes. When the capillary is to be reinstalled, it is necessary to flush with the run buffer for at least 15 minutes.

Method summary

The following method can be used to separate most inorganic anions, organic acids, amino acids and carbohydrates simultaneously. Below are the general analytical conditions. The method as it should be entered into the Agilent ChemStation is shown on the following page.

Capillary	Fused silica id = 50 µm, l=104 cm, L=112.5 cm (G1600-64211)
Injection	1. Pressure: 50 mbar for 6 seconds from sample vial 2. Post-injection of buffer from InHome vial, 50 mbar for 4 s
Applied voltage:	- 30 kV
Capillary temperature	15 °C
Detection wavelength	Signal 350/20 nm, reference 230/10 nm
Preconditioning	Buffer flush for 4 min at 1 bar prior to each run

Programming the method

HPCE mode: CE

Home values:

Lift Offset	4	
Cassette Temperature	15.00 °C	
Inlet Home Vial	10	*Place the buffer vials at position 10 and 11.*
Outlet Home Vial	11	*Vial locations are exemplary only.*

Replenishment and Preconditioning:

Serial processing

Replenishment Entries:

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.*

Preconditioning Entries:

Function Parameter	
1 Flush 4.00 min, I: 9, O:1	*Place flushing vial at 9 and waste vial at 1. Remember to monitor the waste vial volume for overflow.*

Postcondition Entries:

No Postconditioning used

Injection Table Entries:

Function Parameter	
1 PRESSURE 50.0 mbar, 6.0 sec, I: InjectVial, O:OutHomeVial	*May be increased or decreased depending on sample concentration.*
2 PRESSURE 50.0 mbar, 4.0 sec, I: InHomeVial, O:OutHomeVial	*The post injection plug helps to minimize sample loss upon application of voltage. A voltage ramp is used for the same purpose.*

Electric:

Electric	On	
Polarity	Negative	*Negative polarity is used since EOF is reversed.*
Voltage	30.0 kV	
Current	150.0 mA	*A current limit is not necessary but may be used to prevent excessive current generation in case the wrong vial is used.*
Power		System Limit
Low Current Limit		2 μA

Store Data:

Collect voltage Yes	
Collect currentYes	*It is recommended to store the current for every analysis. Current in this method shows from -30 to -40 μA.*
Collect power	No
Collect pressure	No
Collect temperature	Yes

Time entries:

Stop time	40.00 min *Adjust as needed when running actual sample.*
Post time	Off
Time Table is empty.	

<u>Diode array detector</u>

Settings:

Stop Time	as HPCE: 40.00 min
Post Time	Off
Response Time 1.3 sec	*This is recommended to reduce baseline noise.*
Peak width >	0.1 min
Prerun Autobalance	On
Postrun Autobalance	Off

Spectrum:

Store	None

Signals:

Store	Reference
Signal, Bw	Reference, Bw [nm]
A: Yes 350/20	230/10

Results and discussion

Simultaneous analysis of inorganic anions, organic acids, amino acids and carbohydrates

Figure 1 shows a typical electropherogram of a 43-component anion sample including seven inorganic anions, five organic acids, 16 amino acids and 15 carbohydrates using the standard method. If the results are not similar to these please refer to the section *Troubleshooting* in this note.

In this method most inorganic anions and carbohydrates can be separated. However, migration times of several organic acids such as tartarate, succinate, malate and α-ketoglutarate are close. This separation can be improved by using the Organic Acids Analysis Kit from Agilent Technologies (PN 5063-6510).

With respect to amino acids, Leu and Ile cannot be resolved. Although Arg is not observed in figure 1, Arg can be detected at approximately 35 minutes if the sample is dissolved in an alkaline solution. Since Tyr and Trp have UV absorbance at 230 nm, they are recorded as negative peaks. Ser migrates just before a system peak.

Electrophoretic mobilities at 20 °C

The effective mobilities of 82 compounds including nine inorganic anions, 23 organic acids, 18 amino acids and 32 carbohydrates were determined by this method at 20 °C and are listed in table 1. If the mobilities of the compounds of interest are close, they are difficult to separate. In this case investigating a change of temperature or pH is recommended.

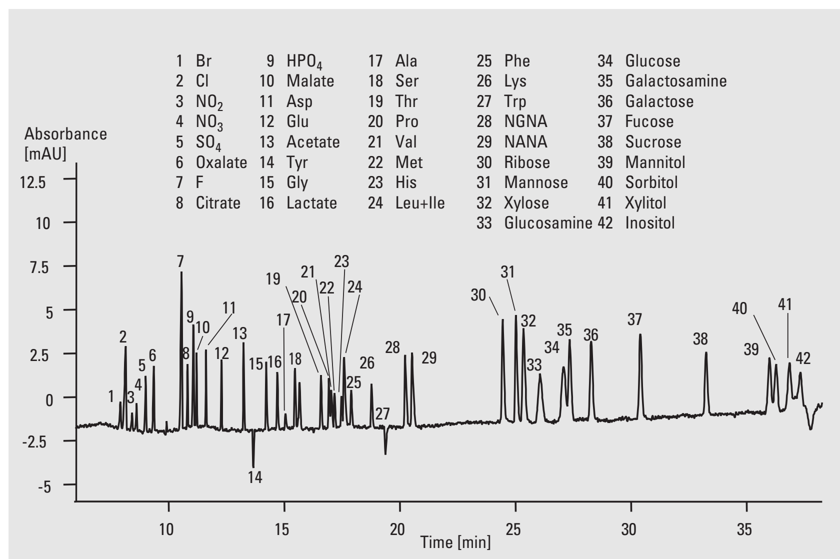


Figure 1
Analysis of 43-component anion standard mixture

Buffer	Agilent Basic Anion Buffer (PN 5064-8209)
Sample	Cl 110 mg/l, carbohydrates 200 mg/l each, others 50 mg/l each
Capillary	fused silica, l=104 cm, L=112.5 cm, id= 50 µm
Injection	300·mbar·s
Temperature	15 °C
Voltage	-30 kV
Detection	signal 350/20 nm, reference 230/10 nm

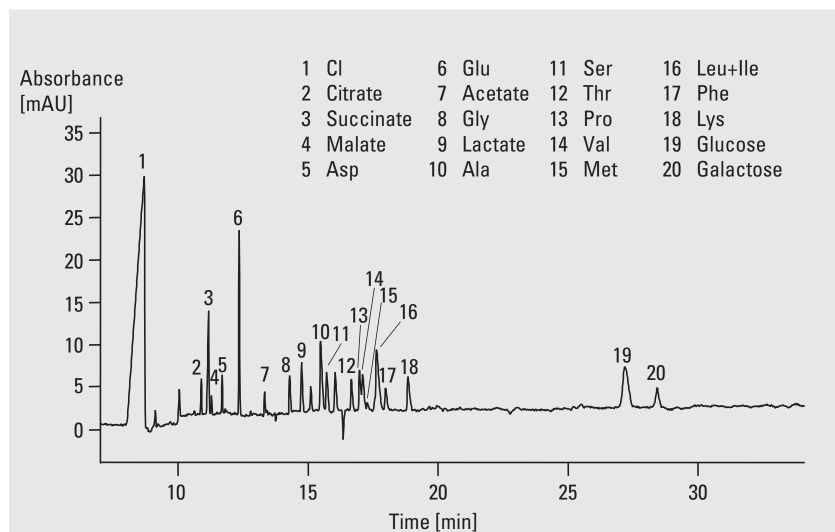
Compound	Mobility (X10-4 cm2/Vs)	Compound	Mobility (X10-4 cm2/Vs)	Compound	Mobility (X10-4 cm2/Vs)	Compound	Mobility (X10-4 cm2/Vs)
Bromide	-7.181	Acetate	-3.589	n-Hexanoate	-2.385	Glucosamine	-0.846
Chloride	-6.983	Pyruvate	-3.540	Galacturonic	-2.337	Mannosamine	-0.832
Nitrite	-6.648	CysCys	-3.514	His	-2.310	Lactose	-0.774
Nitrate	-6.442	Glycolate	-3.495	Leu	-2.300	Arabinose	-0.764
Sulfate	-6.140	Tyr	-3.493	Ile	-2.300	Glucose	-0.761
Oxalate	-5.784	Gly	-3.260	Phe	-2.220	Maltose	-0.731
Ascorbate	-5.409	n-Propionate	-3.111	n-Heptanoate	-2.149	Galactosamine	-0.725
Malonate	-5.093	Lactate	-3.041	Gluconate	-2.060	Lactulose	-0.676
Fluoride	-4.990	Borate	-2.963	Lys	-2.026	Galactose	-0.659
Formate	-4.911	Ala	-2.858	Trp	-1.910	Fucose	-0.485
Citrate	-4.775	Ser	-2.795	NGNA	-1.719	Sucrose	-0.291
Pyrophosphate	-4.760	n-Butyrate	-2.781	n-Octanoate	-1.707	Raffinose	-0.284
Phosphate	-4.677	Levulinate	-2.729	NANA	-1.675	Mannitol	-0.134
Tartarate	-4.584	Mannuronic	-2.674	ManNAc	-1.221	Trehalose	-0.121
Succinate	-4.565	Pyroglutamate	-2.631	Ribose	-1.037	Sorbitol	-0.118
Malate	-4.520	n-Pentanoate	-2.578	Fructose	-0.983	Galactitol	-0.104
a-Ketoglutarate	-4.513	Thr	-2.542	GlcNAc	-0.975	Xylitol	-0.086
Asp	-4.418	Glucuronic	-2.497	Mannose	-0.966	Erythritol	-0.076
Glutarate	-4.196	Pro	-2.450	Xylose	-0.935	Inositol	-0.064
Glu	-4.084	Val	-2.444	GalNAc	-0.919		
Adipate	-3.934	Met	-2.389	Rhamnose	-0.904		

Table 1
Electrophoretic mobilities of anions at 20 °C

Applications

Soy sauce analysis

Figure 2 shows the analysis of a soy sauce. The sample was diluted 1:50 with CE water. Centrifugal filtering through a 30 kDa cutoff filter was applied to remove proteins and peptides. A well-defined electropherogram was obtained without interference from other matrix compounds. Satisfactory reproducibilities were obtained for all compounds with RSD values (n=5) for migration times better than 0.3 % and for peak areas between 0.6 and 5.4 %.



Buffer	Agilent Basic Anion Buffer (PN 5064-8209)
Sample	soy sauce, 1:50 diluted with water, ultrafiltration with 30-kDa cutoff filter
Preconditioning	4 min with run buffer
Capillary	fused silica, l=104 cm, L=112.5 cm, id= 50 µm
Injection	300 mbar·s
Temperature	15 °C
Voltage	-30 kV
Detection	signal 350/20 nm, reference 230/10 nm

Figure 2
Analysis of soy sauce

Pineapple analysis

This method was applied to the analysis of organic acids and carbohydrates in pineapple. In the agriculture industry, technical experts are trying to develop a new crossbreed of fruits. Since the content of organic acids and carbohydrates determines the taste of the pineapple juice, their analysis can help to characterize the product.

If citrate and malate concentrations are high, the taste tends to be sour. If the carbohydrate concentration is high, the taste is sweet. Determination of these compounds is traditionally performed using two HPLC methods. However, the method described here enables the simultaneous analysis of both organic acids and carbohydrates in a much shorter time (less than 18 min) and in a single run. In order to reduce the analysis time, a shorter length capillary was used for this sample. Squeezed pineapple juice was diluted 50-fold with CE water prior to injection. Figure 3 shows a result of the analysis.

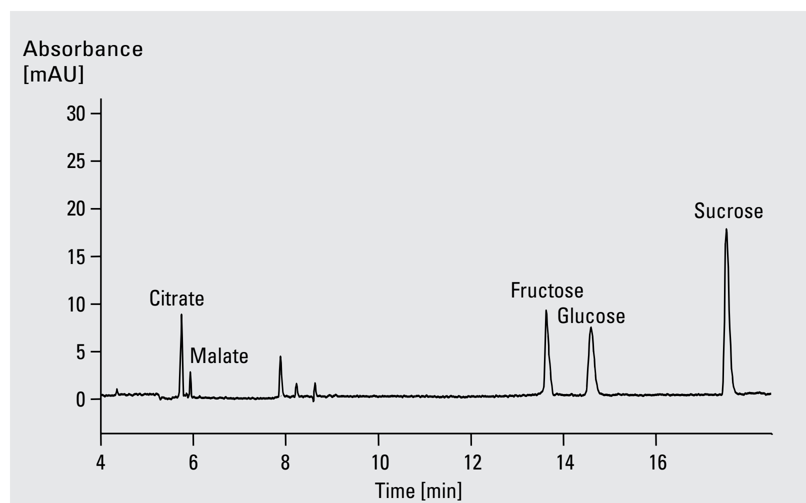


Figure 3
Analysis of pineapple

Buffer	Agilent Basic Anion Buffer (PN 5064-8209)
Sample	pineapple, 1:50 diluted with water,
Capillary	fused silica, l=72 cm, L=80.5 cm, id= 50 µm
Injection	300 mbar·s
Temperature	20 °C
Voltage	-25 kV (reversed polarity)
Detection	signal 350/20 nm, reference 275/10 nm

Troubleshooting

Problem	Possible Cause	Solution
Poor resolution or broad/split peaks	Buffer is old Capillary damaged Sample overloaded Capillary too short	Use new buffer Replace capillary Dilute sample Replace capillary
Peak leading	Buffer absorbed carbonate	Use new buffer
No signal	Sample not injected Capillary damaged Wrong buffer used Wrong setting of power supply polarity Detection wavelength incorrect	Verify no air bubble trapped in bottom of sample vial Verify inlet capillary set correctly Replace capillary Verify buffer Check that polarity is negative Verify signal: 350/20, reference: 230/10 nm
Noisy baseline	Wrong setting of response time Capillary window not adjusted Capillary window dirty Lamp is old Buffer pH higher than 12.3	Verify response time 1.3 sec DAD Examine capillary window Examine and clean with lint-free paper/MeOH Replace lamp Verify buffer pH
Poor reproducibility	Buffer overused	Replace buffer
Unstable current	Capillary broken Capillary not filled with buffer	Replace capillary 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, Agilent publication number 5965-8067E.

2.
Soga, T. and Heiger, D. N. “Simultaneous Determination of Monosaccharides in Glycoproteins by Capillary Electrophoresis”, *Anal. Biochem.*, **1998,** 261, 73-78, Agilent publication number 5968-0772E.

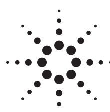
3.
Soga, T. and Ross, G. A. “Simultaneous Determination of Inorganic Anions, Organic Acids, Amino Acids and Carbohydrates by Capillary Electrophoresis” *J. Chromatogr.* , **1999,** 837, 231-239, Agilent publication number 5968-4470E.

Tomoyoshi Soga is application chemist at Yokogawa Analytical Systems, Japan. Agilent Technologies recognizes his efforts in the development of this work.

For more information on our products and services, visit our website at:
http://www.agilent.com/chem

Copyright © 1999 Agilent Technologies
All Rights Reserved. Reproduction, adaptation or translation without prior written permission is prohibited, except as allowed under the copyright laws.

Published in Germany 01/2000
Publication Number 5968-7715E



Agilent Technologies
Innovating the HP Way