Errata

Product Manual for Dionex IonPac™ ICE-AS6 Columns
034961-07

For new orders of the following parts discussed in this manual, please use the updated part numbers listed below.

Part	Old Part Number in this manual	Updated Part Number to use for new orders
PROD,COL,IP,ICE-AS6	*046023*	*079798*

PRODUCT MANUAL

IonPac® ICE-AS6

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PRODUCT MANUAL

for the

IONPAC® ICE-AS6 ANALYTICAL COLUMN

(9 x 250 mm, P/N 046023)

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SECTION 1 - IONPAC ICE-AS6 ION EXCLUSION CHROMATOGRAPHY

The IonPac ICE-AS6 is an ion exclusion column designed for efficient separation of low molecular weight aliphatic organic acids including hydroxy- substituted organic acids, aliphatic alcohols and glycols.

The IonPac ICE-AS6 is uses an ion exclusion mechanism, Donnan exclusion (see Figure 1, "The Ion Exclusion Separation"), which allows retention and separation of weakly ionized acids based on differences in pKa's. Strong inorganic acid anions are not retained by the stationary phase and elute in the void volume of the column. In addition to Donnan exclusion, the ICE-AS6 has hydrophobic functional groups within the resin structure which promote adsorption and hydrogen bonding. These two additional retention mechanisms allow resolution of organic acids which are poorly resolved by ion exclusion alone, such as tartrate (pK_1 = 2.82) from citrate (pK_1 = 2.87) and glycolate (pK_1 = 3.63) from lactate (pK_1 = 3.66) from hydroxyisobutyric acid (pK_1 = 3.72).

In the ion exclusion mechanism shown in Figure 1, "The Ion Exclusion Separation," below, the sulfonated surface is a hydrated, negatively charged boundary called a Donnan membrane which is permeable only to neutral molecules. Totally dissociated inorganic acids, such as dilute hydrochloric acid, can not penetrate the membrane due to electrostatic repulsion and are totally excluded from the stationary phase. This volume is called the exclusion volume, Ve. In contrast, neutral molecules can diffuse into the resin pores. The total permeation volume, Vp, for water is observed as a small negative peak.

The undissociated form of a weak carboxylic acid exhibits a greater retention than water due to adsorption to the resin. In general, the higher the pKa of the acid, the greater the retention. The longer the aliphatic carboxylic acid, the longer its retention time. On the ICE-AS6, the adsorption mechanism, in addition to Donnan exclusion, is very significant. The addition of a solvent such as acetonitrile can be used to reduce retention times of highly retained hydrophobic carboxylic acids. Stronger carboxylic acids such as di- and tricarboxylic acids (i.e. oxalic or citric) have low retention and elute between the exclusion and total permeation volumes.

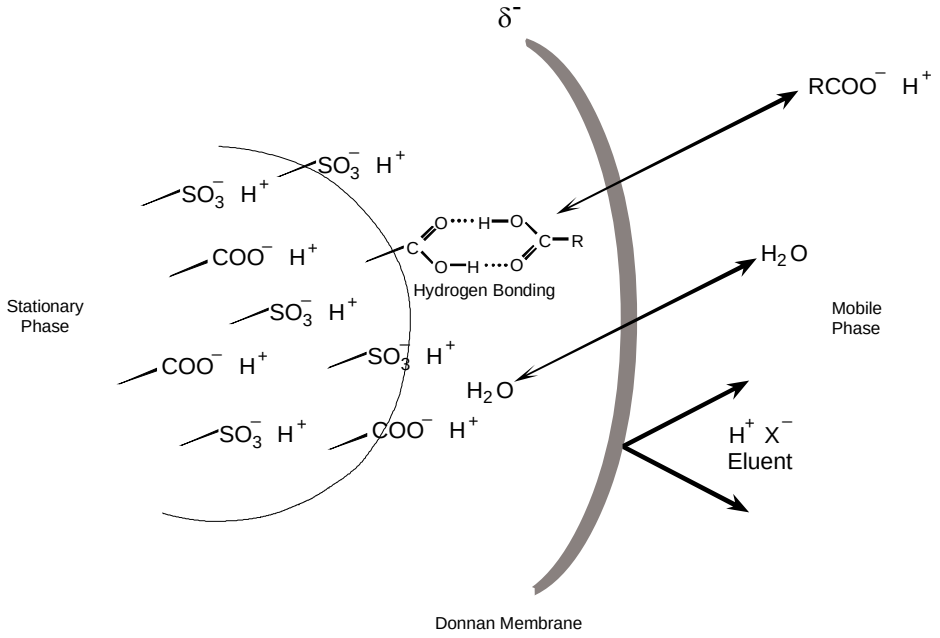


Figure 1
The Ion Exclusion Separation

ICE-AS6 columns are stable between pH 0 - 7 (do not run eluents with cations other than hydronium ion through the ICE-AS6) and are compatible with eluents containing 0 - 15% HPLC solvents such as methanol, acetonitrile or 5% isopropanol. The ICE-AS6 is composed of a 8 µm cross-linked styrene/acrylate/divinylbenzene resin which is functionalized to produce sulfonate and carboxylate groups. The ion exchange capacity of the 9 x 250 mm analytical column is 27 meq/column. The IonPac ICE-AS6 also has intermediate hydrophobicity when compared to the ICE-AS1. The ICE-AS6 also has intermediate solvent compatibility (15%) when compared to the ICE-AS1 (50%).

The ICE-AS6 can be operated at flow rates up to 1.25 mL/min. When setting up the analytical system, observe the special precautions listed in Section 3, “Installation.” PEEK (polyetheretherketone) is used to make column hardware. PEEK has excellent chemical resistance to most organic solvents and inorganic solutions. Concentrated sulfuric acid and concentrated nitric acid will attack PEEK. Tetrahydrofuran at concentrations of greater than 15% is not compatible with PEEK systems. The ICE-AS6 Analytical Columns have minimum efficiencies for sulfate of 12,000 plates/column (for acetic acid), under standard operating conditions. The ICE-AS6 operates at a back pressure between 400-600 psi (2.76-4.14 MPa) at 1.0 mL/min with the test eluent. However, the column is capable of operating at back pressures up to 900 psi (6.20 MPa).

This manual assumes that you are familiar with the installation and operation of the DIONEX Ion Chromatograph (IC) and the AMMS-ICE MicroMembrane™ Suppressor. If you do not understand the operation of the system, take the time to familiarize yourself with the various system components before beginning an analysis.

The IonPac ICE-AS6 Analytical Column has 10-32 threaded PEEK end fittings for use with ferrule/bolt liquid line fittings. If your system is otherwise configured, refer to, “DIONEX Liquid Line Fittings.”

Always remember that assistance is available for any problem that may be encountered during the shipment or operation of DIONEX instrumentation and columns through any of the DIONEX Worldwide Offices listed in “DIONEX Worldwide Offices.”

SECTION 2 - IONPAC ICE-AS6 EXCLUSION CHROMATOGRAPHY SYSTEM

Condition	Operation Summary
Eluent Flow Rate:	Typically 1.0 mL/min
Anion MicroMembrane:	Anion-ICE MicroMembrane Suppressor II, AMMS-ICE II (P/N 037107)
	Do not run the AMMS-ICE II Suppressor over 40°C. Place outside of oven if application requires higher temperatures
Injection Loop:	10 - 50 µL
System Void Volume:	Minimize dead volumes. Switching valves, couplers can be used.
	The GM-3 Gradient Mixer should be used for gradient analysis on systems other than DX-300 HPLC Pump.
Detectors:	AD20 or AD25 Absorbance Detector (10 µL, 6-mm)
	CD20 or CD25 Conductivity Detector (9 µL, 10 mm or 7.5 µL, 6-mm) with a DS3 Temperature Controller (P/N 44130)
	DIONEX Back pressure Regulator 75 psi (0.52 MPa) rating (P/N 039760) or Tubing (see Table 1) Ensure 50 - 75 psi (0.34-0.52 MPa) back pressure.
	Either the TS-1 or the TS-2 can be used.

SECTION 3 - INSTALLATION

3.1 System Requirements

The IonPac ICE-AS6 Analytical Column is designed to be run on any DIONEX Ion Chromatograph equipped with suppressed conductivity detection.

3.2 System Void Volume

For best performance, all of the tubing installed between the injection valve and detector should be 0.005" (P/N 044221) ID PEEK tubing or 0.010" ID PEEK tubing (P/N 042260). Minimize the lengths of all connecting tubing and remove all unnecessary switching valves and couplers. If you need assistance in properly configuring your system contact the DIONEX Worldwide Office nearest you (see “DIONEX Worldwide Offices”).

3.3 The Injection Loop

Table 1
Smallest Injectable Volumes (µL)

Valve Type	Using 0.012" ID Tefzel Tubing	Using 0.007" ID Tefzel Tubing	Using 0.010" ID PEEK Tubing	Using 0.005" ID PEEK Tubing
DIONEX BF2 Valve (8 µL Internal Volume) (10 cm Loop)	15.2	10.5	13.1	9.2
DIONEX MicroInject Valve (10.5 µL Internal Volume) (14 cm Loop)	20.5	14.0	17.6	12.2
Rheodyne Microinjection Valve Model 9126 (0.8 µL Internal Volume) (10 cm Loop)	8.0	3.3	5.9	2.0

For most applications on a 4-mm analytical system, a 10 - 50 µL injection loop will be sufficient. DIONEX recommends that a 50 µL injection loop be used.

3.4 Eluent Storage

IonPac ICE-AS6 columns are designed to be used with acid eluent systems and isocratic analysis. Storage under a helium atmosphere ensures contamination free operation and proper pump performance (nitrogen can be used if eluents do not contain solvents). Acidic eluents that contain acetonitrile should be made fresh daily. Acetonitrile slowly hydrolyzes in acidic solutions.

3.5 Anion MicroMembrane Suppressor-ICE II Requirements

An Anion-ICE MicroMembrane Suppressor II (AMMS-ICE II, P/N 037107) should be used instead of an ASRS-ULTRA or AMMS III for ion exclusion applications that require suppressed conductivity detection. It is compatible with all solvents and concentrations with which the systems and columns are compatible. Use DIONEX Cation Regenerant Solution, Tetrabutylammonium hydroxide (TBAOH, P/N 039602). Dilute as required for the example applications.

This manual assumes that you are familiar with the installation and operation of the Ion Chromatograph and the AMMS-ICE II Suppressor. If you do not understand the operation of your system, take the time to familiarize yourself with the operator's manuals for the products before beginning an analysis. For detailed information on the operation of the AMMS-ICE II Suppressor, see Document No. 03266.

SECTION 4 - OPERATION

4.1 General Operating Conditions

The selectivity of the IonPac ICE-AS6 Analytical Column has been designed to separate a broad range of low molecular weight organic acid anions in less than 20 minutes. The ICE-AS6 column consists of a cross-linked (8%), microporous, intermediate hydrophobic resin that has been sulfonated. The nature of the cross-linked polymeric structure of the packing material makes ICE-AS6 columns compatible with pH 0 - 7 eluents (see Section 4.1.1, "IonPac ICE-AS6 Operation Precautions) and 0 - 15% organic solvent eluents. The ICE-AS6 can be used with any suppressible ionic eluent that does not exceed the capacity of the AMMS-ICE II.

4.1.1 IonPac ICE-AS6 Operation Precautions

CAUTION

15% maximum Solvent Concentration

When using solvents over 5%, use a gradient over 30 minutes to achieve the maximum solvent concentration (a step change will cause fronting). When the maximum concentration of solvent is achieved, rinse the column for at least 30 minutes.

DO NOT use hydroxide eluents

Filter and Degas Eluents

Filter Samples

Maximum Recommended Operating Pressure is 900 psi (6.20 Mpa)

4.1.2 Solvents

The ICE-AS6 column is more sensitive to solvents than the ICE-AS1 column. It is recommended that only acetonitrile or methanol be used in either eluents or cleanup solutions. See "Column Care" for recommended cleanup approaches. The maximum amount recommended is 15% of either acetonitrile or methanol, and only as a last resort. Try starting with 5% at first and then increment by 5% for each experiment (up to but not higher than 15%). Make sure that for amounts higher than 5%, you use a 30-minute gradient to reach the desired concentration (see Section 4.1.1, "IonPac ICE-AS6 Operation Precautions" above). Use a gradient to ramp back down as well, otherwise the column may be damaged. After returning to 100% aqueous it will take about an hour to achieve a stable baseline. The ICE-AS6 column is moderately hydrophobic and therefore solvents take longer to wash out before achieving equilibration.

Solvents and water should be premixed in concentrations to allow proper mixing by the gradient pump and to minimize outgassing. Ensure that all of the inorganic chemicals are soluble in the highest solvent concentration to be used during the analysis.

When using a solvent in an ionic eluent, column generated back pressures will depend on the solvent used, concentration of the solvent, the ionic strength of the eluent and the flow rate used. The column back pressure will vary as the composition of water-methanol and water-acetonitrile mixture varies. The practical back pressure limit for the IonPac ICE-AS6 columns is 900 psi (6.20 MPa).

Table 2
HPLC Solvents for Use with IonPac ICE-AS6 Columns

Solvent	Maximum Operating Concentration*
Acetonitrile	15%
Methanol	15%
Isopropanol	5%

* See Section 4.1.1, "IonPac ICE-AS6 Operation Precautions."

4.2 Chemical Purity Requirements

Obtaining reliable, consistent and accurate results requires eluents that are free of ionic and spectrophotometric impurities. Chemicals, solvents and deionized water used to prepare eluents must be of the highest purity available. Maintaining low trace impurities and low particle levels in eluents also help to protect your ion exchange columns and system components. DIONEX cannot guarantee proper column performance when the quality of the chemicals, solvents and water used to prepare eluents has been compromised.

4.2.1 Inorganic Chemicals

Reagent Grade inorganic chemicals should always be used to prepare ionic eluents. Whenever possible, inorganic chemicals that meet or surpass the latest American Chemical Society standard for purity should be used. These inorganic chemicals will detail the purity by having an actual lot analysis on each label. The analyses performed in Section 5, “Example Applications,” use heptafluorobutyric acid obtained from Fluka Chemie AG.

4.2.2 Solvents

Since solvents used with the IonPac ICE-AS6 columns are added to ionic eluents to modify the ion exclusion process, the solvents used must be free of ionic impurities. However, since most manufacturers of solvents do not test for ionic impurities, it is important that the highest grade of solvents available be used. Currently, several manufacturers are making “ultrahigh” purity solvents that are compatible for HPLC and spectrophotometric applications. These “ultrahigh” purity solvents will usually ensure that your chromatography is not affected by ionic impurities in the solvent. Currently at DIONEX, we have obtained consistent results using High Purity Solvents manufactured by Burdick and Jackson and Optima Solvents by Fisher Scientific.

4.2.3 Deionized Water

The deionized water used to prepare eluents should be **Type I Reagent Grade Water** with a specific resistance of 18.2 megohm-cm. The deionized water should be free of ionized impurities, organics, microorganisms and particulate matter larger than 0.2 µm. Bottled HPLC-Grade Water (with the exception of Burdick & Jackson) should not be used since most bottled water contains an unacceptable level of ionic impurities.

4.3 Eluent Preparation

4.3.1 Acid Eluent Preparation

The acidic eluents used with the IonPac ICE-AS6 columns are stable and require no special storage. Always prepare eluents with Type I Reagent Grade Water (see Section 4.2.3, “Deionized Water”) the has been properly degassed (see Section 4.3.2, “Degassing Deionized Water”).

4.4 Eluents Containing Solvents

When mixing solvents with water remember to mix solvent with water on a volume to volume basis. If a procedure requires an eluent of 10% acetonitrile, prepare the eluent by adding 100 mL of acetonitrile to an eluent reservoir. Then add 900 mL of deionized water to the acetonitrile in the reservoir. Using this procedure to mix solvents with water will ensure that a consistent true volume/volume eluent is obtained. Premixing water with solvent will minimize the possibility of outgassing.

NOTE

As a rule degass the aqueous component of the eluent and then add the solvent component. Avoid excessive purging or degassing of eluents containing solvents if possible, since it is possible that a volatile solvent can be “boiled” off from the solution.

4.5 Anion Suppression Regenerant Preparation

The regenerant used with the AMMS-ICE II Suppressor when used with the IonPac ICE-AS6 to perform the analyses in Section 5, “Example Applications,” is 5 mM tetrabutylammonium hydroxide (TBAOH). Use DIONEX Cation Regenerant Solution (P/N 039602). Dilute 50 mL of the 0.1 M Cation Regenerant Solution to 1 L with degassed Type I Reagent Grade Water. Prepare several liters of the regenerant.

For a guide to properly adjusting the regenerant flow rate, see Document No. 032661, the Product Manual for the AMMS-ICE II.

4.6 Organic Acid Standard Solution Preparation

For ion exclusion chromatography, the acid forms of organic acids are typically use to prepare standards rather than the sodium or potassium salts.

SECTION 5 - EXAMPLE APPLICATIONS

The IonPac ICE-AS6 may be used isocratically for chromatographing a large number of carboxylic acids.
If your sample or standard contains organic acids, adding chromate (about 10 mg/L) will help stabilize them from bacterial degradation at room temperature.

NOTE

The chromatograms in this section were obtained using columns that reproduced the Production test Chromatogram (see Section 5.3, "Production Test Chromatogram") on optimized Ion Chromatographs (see Section 3, "Installation"). Different systems will differ slightly in performance due to slight differences in column sets, system void volumes, liquid sweep-out times of different components and laboratory temperatures.

Before attempting any of the following example applications, take the time to ensure that your system is properly configured. Ensure that all of the eluents have been made from high purity reagents and deionized water. All water used in the preparation of eluents should be degassed, deionized water. For chemical purity requirements see Section 4.2, "Chemical Purity Requirements."

Run synthetic standards to calibrate and confirm the operation of your system. This column has a very high loading capacity and can handle a large number of dirty samples. If after running complex samples, the ICE-AS6 shows signs of fouling, refer to the column cleanup protocols in, "Column Care."

5.1 Preparation of Eluents

The standard eluent for the example applications presented in this section is 0.4 mM heptafluorobutyric acid. It was prepared from heptafluorobutyric acid (same as perfluorobutyric acid) obtained from FLUKA Chemie AG (P/N 77249). It is >99% (GC) purity with a molecular weight of 214.04 and a density of 1.652. It is supplied in 10.0 mL bottles (16.52 g).

Heptafluorobutyric Acid Stock Solution (0.0772 M):	Dilute the entire contents of one 10.0 mL bottle to 1 L.
Heptafluorobutyric Acid Eluent (0.4 mM):	Dilute 5.2 g of the stock solution (0.0772 M) to 1 L.

If you prefer to work from a 0.100 M stock solution, dilute 21.40 g of the >99% purity heptafluorobutyric acid to 1 L. The eluent can then be made by diluting 4.0 g of the 0.100 M stock solution to 1 L to obtain the 0.4 mM heptafluorobutyric acid eluent. Heptafluorobutyric acid was chosen for minimum background conductivity. However, other 0.4 mM monoprotic acids can be used (see Section 5.5, "Comparison of Different acids used on the IonPac ICE-AS6").

5.2 pKa Values of Selected Organic Acids

The following tables list the pKs of selected organic acids in alphabetical order and in ascending order of pK. Organic acids elute in approximately the order of ascending pK but additional hydrogen bonding and adsorption variables modify the elution order slightly so that the elution order of the acids is not strictly in order of ascending pK. The tables plus the example applications are designed to give the chromatographer a simple method for estimated the ability of the ICE-AS6 to separate various combinations of organic acids.

Table 3
Organic Acids with pKa Values In Order of Increasing pK_1

Compound	pK_1	pK_2	pK_3
Terephthalic			
Squaric (3,4-dihydroxy-3-cyclobutene-1,2-dione	0.40	3.10	
Trichloroacetic	0.66		
Mellitic (benzenehexacarboxylic)	0.70	2.21	3.52
Dichloroacetic	0.87		
Oxalic (ethanedioic)	1.04	3.82	
Nitroacetic	1.46		
Maleic (cis-butenedioic)	1.75	5.83	
Ketoglutaric (2-oxopentanedioic)	1.85	4.44	
Orotic (uracil-6-carboxylic)	1.96	9.34	
Citraconic (cis-methylbutenedioic)	2.20	5.60	
Pyruvic (2-oxopropanoic)	2.26		
Trimellitic (benzene-1,2,4-tricarboxylic)	2.40	3.71	5.01
Fluoroacetic	2.59		
Mesaconic (trans-methylbutene)	2.61		
Cyanoacetic	2.63		
Malonic (propanedioic)	2.65	5.28	
Chloroacetic	2.68		
Gentistic (5-hydroxysalicylic)	2.70		
Dithiotartaric (2,3-dimercaptobutanedioic)	2.71	3.48	8.89
Bromoacetic	2.72		
Phthalic (benzene-1,2-dicarboxylic)	2.75	4.93	
Diglycolic (oxydiacetic)	2.79	3.93	
Salicylic (2-hydroxybenzoic)	2.81	13.40	
Tartaric (d-2,3-dihydroxybutanedioic)	2.82	3.95	
Fumaric (trans-butenedioic)	2.85	4.10	
Citric (2-hydroxypropane-1,2,3-tricarboxylic)	2.87	4.35	5.69
Iodoacetic	2.98		
Isocitrate (dl-1-hydroxypropane-1,2,3-tricarboxylic)	3.02	4.28	5.75
Mucic	3.08	3.63	
Mandelic (l-phenylhydroxyacetic)	3.19		
Galacturonic	3.23	11.42	
Malic (l-hydroxybutanedioic)	3.24	4.71	
Thiomalic (dl-mercaptobutanedioic)	3.30	4.60	10.38
Quinic (1,3,4,5-tetrahydroxycyclohexanecarboxylic)	3.36		
Thioglycolic (mercaptoacetic)	3.42	10.11	
Thiolactic (dl-2-mercaptopropanoic)	3.48	10.08	
Hippuric (n-benzoylglycine)	3.50		
Glyceric (dl-2,3-dihydroxypropanoic)	3.52		
Formic (methanoic)	3.55		
Glycolic (hydroxyacetic)	3.63		
Lactic (d-2-hydroxypropanoic)	3.66		
Itaconic (methylenebutanedioic)	3.68	5.14	
2-Hydroxyisobutyric	3.72		
Benzoic	4.00		
Succinic (butanedioic)	4.00	5.24	
Ascorbic	4.03	11.34	
4-Hydroxybenzoic	4.10	9.96	
Vinylacetic (but-3-enoic)	4.12		
Glutaric (pentanedioic)	4.13	5.03	
Acrylic (propenoic)	4.26		
Adipic (hexanedioic)	4.26	5.03	
Pimelic (heptanedioic)	4.31	5.08	
3-Mercaptopropanoic	4.34	10.84	
Azelaic (nonanedioic)	4.39	5.12	
Anisic (4-methoxybenzoic)	4.48		
Acetic (ethanoic)	4.56		
Isovaleric (3-methylbutanoic)	4.58		
Butanoic	4.63		
Isobutyric (2-methylpropionic)	4.63		
Valeric (pentanoic)	4.64		
Propanoic	4.67		
Crotonic (trans-but-2-enoic)	4.69		
Pivalic (2,2-dimethylpropanoic)	4.83		
Caproic (hexanoic)	4.85		
Octanoic	4.89		
Uric (2,6,8-trihydroxypurine)	5.61		
Guanidine	13.54		

Table 4
Organic Acids with pKa Values in Alphabetic Order

Compound	pK_1	pK_2	pK_3
Acetic (ethanoic)	4.56		
Acrylic (propenoic)	4.26		
Adipic (hexanedioic)	4.26	5.03	
Anisic (4-methoxybenzoic)	4.48		
Ascorbic	4.03	11.34	
Azelaic (nonanedioic)	4.39	5.12	
Benzoic	4.00		
Bromoacetic	2.72		
Butanoic	4.63		
Caproic (hexanoic)	4.85		
Chloroacetic	2.68		
Citraconic (cis-methylbutenedioic)	2.20	5.60	
Citric (2-hydroxypropane-1,2,3-tricarboxylic)	2.87	4.35	5.69
Crotonic (trans-but-2-enoic)	4.69		
Cyanoacetic	2.63		
Dichloroacetic	0.87		
Diglycolic (oxydiacetic)	2.79	3.93	
Dithiotartaric (2,3-dimercaptobutanedioic)	2.71	3.48	8.89
Fluoroacetic	2.59		
Formic (methanoic)	3.55		
Fumaric (trans-butenedioic)	2.85	4.10	
Galacturonic	3.23	11.42	
Gentistic (5-hydroxysalicylic)	2.70		
Glutaric (pentanedioic)	4.13	5.03	
Glyceric (dl-2,3-dihydroxypropanoic)	3.52		
Glycolic (hydroxyacetic)	3.63		
Guanidine	13.54		
2-Hydroxyisobutyric	3.72		
4-Hydroxybenzoic	4.10	9.96	
Hippuric (n-benzoylglycine)	3.50		
Iodoacetic	2.98		
Isobutyric (2-methylpropionic)	4.63		
Isocitrate (dl-1-hydroxypropane-1,2,3-tricarboxylic)	3.02	4.28	5.75
Isovaleric (3-methylbutanoic)	4.58		
Itaconic (methylenebutanedioic)	3.68	5.14	
Ketoglutaric (2-oxopentanedioic)	1.85	4.44	
Lactic (d-2-hydroxypropanoic)	3.66		
Maleic (cis-butenedioic)	1.75	5.83	
Malic (l-hydroxybutanedioic)	3.24	4.71	
Malonic (propanedioic)	2.65	5.28	
Mandelic (l-phenylhydroxyacetic)	3.19		
Mellitic (benzenehexacarboxylic)	0.70	2.21	3.52
3-Mercaptopropanoic	4.34	10.84	
Mesaconic (trans-methylbutene)	2.61		
Mucic	3.08	3.63	
Nitroacetic	1.46		
Octanoic	4.89		
Orotic (uracil-6-carboxylic)	1.96	9.34	
Oxalic (ethanedioic)	1.04	3.82	
Phthalic (benzene-1,2-dicarboxylic)	2.75	4.93	
Pimelic (heptanedioic)	4.31	5.08	
Pivalic (2,2-dimethylpropanoic)	4.83		
Propanoic	4.67		
Pyruvic (2-oxopropanoic)	2.26		
Quinic (1,3,4,5-tetrahydroxycyclohexanecarboxylic)	3.36		
Salicylic (2-hydroxybenzoic)	2.81	13.40	
Squaric (3,4-dihydroxy-3-cyclobutene-1,2-dione	0.40	3.10	
Succinic (butanedioic)	4.00	5.24	
Tartaric (d-2,3-dihydroxybutanedioic)	2.82	3.95	
Terephthalic			
Thioglycolic (mercaptoacetic)	3.42	10.11	
Thiolactic (dl-2-mercaptopropanoic)	3.48	10.08	
Thiomalic (dl-mercaptobutanedioic)	3.30	4.60	10.38
Tricarballylic	3.48	4.50	5.82
Trichloroacetic	0.66		
Trimellitic (benzene-1,2,4-tricarboxylic)	2.40	3.71	5.01
Uric (2,6,8-trihydroxypurine)	5.61		
Valeric (pentanoic)	4.64		
Vinylacetic (but-3-enoic)	4.12		

5.3 ICE-AS6 Elution Plots

5.3.1 ICE-AS6 Retention Time vs. Eluent Strength

The following plot illustrates the effect of eluent strength on retention time for a large group of low molecular weight organic acids. Predicted separations can be obtained from a plot of column selectivity versus eluent strength. The actual resolution of separation though is also dependent on the efficiency of the column.

Below 0.2 mM Heptafluorobutyric acid (PFBA), the retention times of all of the analytes fall off very quickly. On close examination, there is a minimum eluent concentration required to maintain peak symmetry which in this case is also approximately 0.2 mM heptafluorobutyric acid.

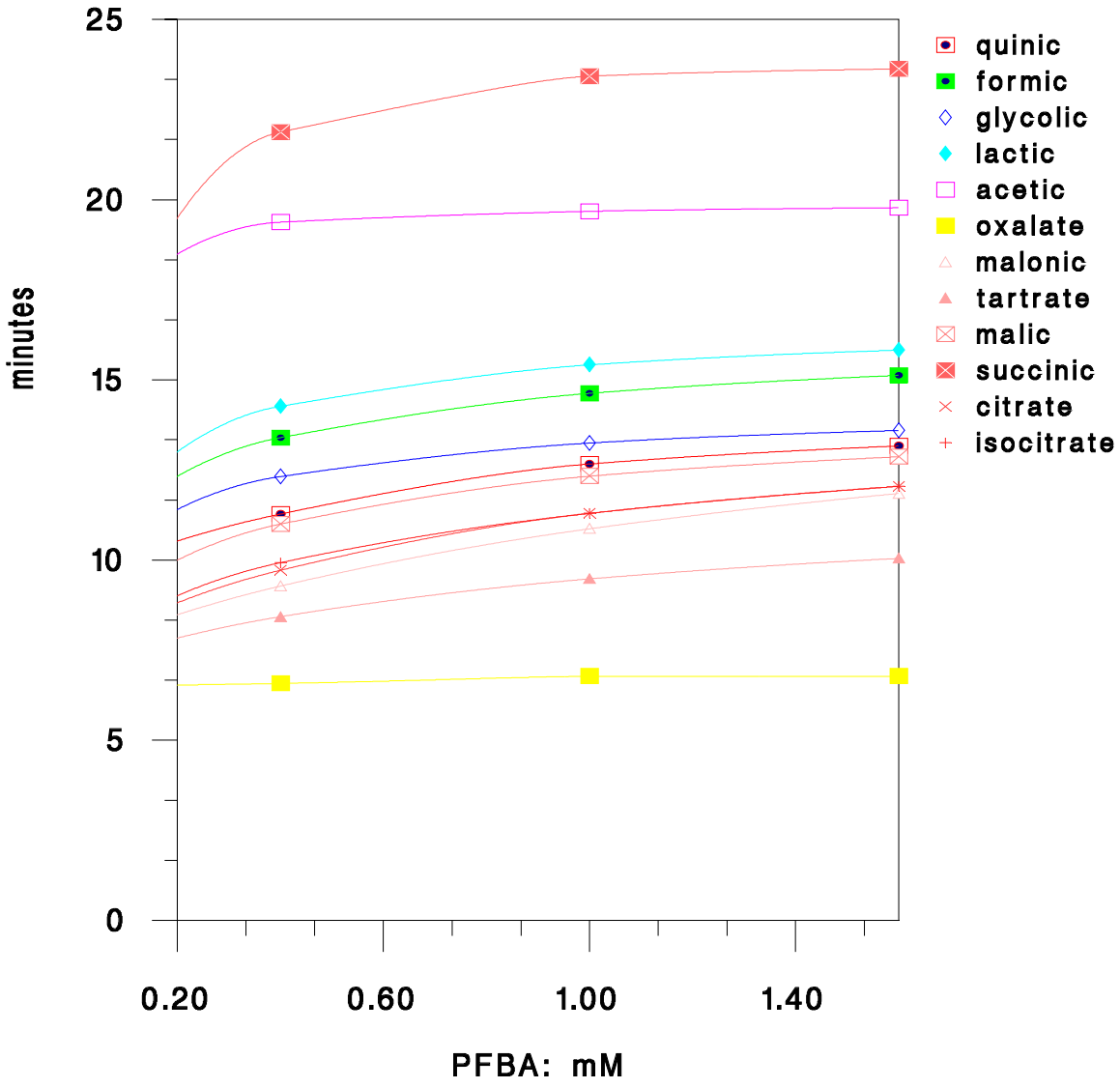


Figure 2
ICE-AS6 Retention Time vs. Eluent Strength

5.3.2 ICE-AS6 Retention Time vs. Temperature

Temperature can effect the retention time and the selectivity of organic acids. Dionex tests all ion-exclusion columns at 19°C to ensure reproducible retention times. As can be seen from the plot below, retention times decrease slightly with increasing temperature. For example, glutaric and propionic acids switch elution order at approximately 35°C and succinic and acetic at 30°C. Fumaric acid is also strongly effected showing a very rapid decrease in run time with increasing temperature. Laboratories can have widely varying temperatures throughout the day (some as high as ±5°C), it is advisable to use temperature control to maintain reproducible retention times. This can be done with any of the Dionex temperature control devices such as the LC30, LC25, or the AS50 Thermal Compartment. If a temperature control device is not available, it is recommended to keep the system away from any heating/cooling vents located in the laboratory to minimize wide temperature swings. This will keep wide temperature swings to a minimum within the column area. See section 5.6.3 for comparison runs.

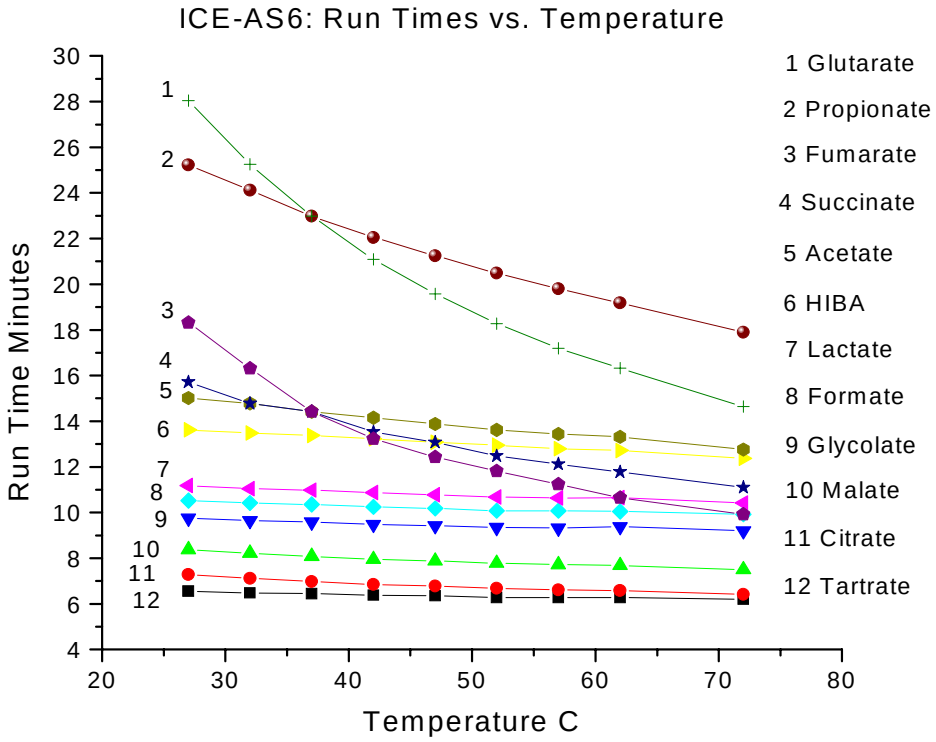


Figure 3
ICE-AS6 Retention Time vs. Temperature

5.4 Production Test Chromatogram

Isocratic elution of organic anions has been optimized on the IonPac ICE-AS6 Analytical Column. To guarantee that all IonPac ICE-AS6 Analytical Columns meet high quality and reproducible performance specification standards, all columns undergo the following production control test.

The large dip in the baseline which occurs at approximately 5 minutes is the total exclusion volume of the column. This is the volume of liquid in the column which is external to the resin beads. The total permeation or void volume of the column, the combined resin bead internal and external liquid volume, creates a baseline disturbance at approximately 13 minutes. This baseline disturbance, however, can only be seen at sensitivities higher than 1 µS full scale.

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor II, AMMS-ICE II
Regenerant:	5 mN Tetrabutylammonium hydroxide
Expected Background Conductivity:	23 - 25 µS
Expected System Operating Back pressure:	< 500 psi (3.45 MPa)
Temperature:	19°C
Storage Solution:	Eluent, 0.4 mM Heptafluorobutyric acid

	Acid	pKa	mg/L
1.	Oxalic	1.04, 3.82	5.0
2.	Tartaric	2.82, 3.95	10.0
3.	Citric	2.87, 4.35, 5.69	15.0
4.	Malic	3.24, 4.71	20.0
5.	Glycolic	3.63	10.0
6.	Formic	3.55	10.0
7.	Lactic	3.66	10.0
8.	HIBA	3.72	30.0
9.	Acetic	4.56	25.0
10.	Succinic	4.00, 5.24	25.0

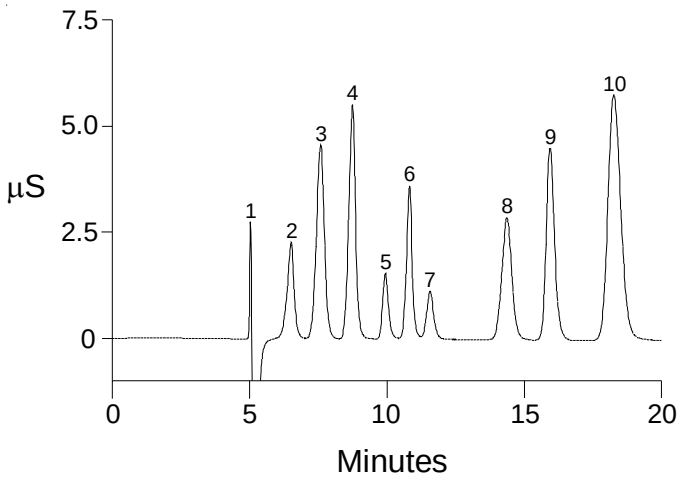


Figure 4
IonPac ICE-AS6 Production Test Chromatogram

5.5 Full IonPac ICE-AS6 Organic Acid Chromatogram

Fumarate, propionate and glutarate will also elute using the Production Test Chromatogram conditions if the run is allowed to proceed to 40 minutes.

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor II, AMMS-ICE II
Regenerant:	5 mN Tetrabutylammonium hydroxide
Expected Background Conductivity:	23 - 25 µS
Expected System Operating Back pressure:	< 500 psi (3.45 MPa)
Temperature:	19°C
Storage Solution:	Eluent, 0.4 mM Heptafluorobutyric acid

	Acid	pKa	mg/L
1.	Oxalic	1.04, 3.82	5.0
2.	Tartaric	2.82, 3.95	10.0
3.	Citric	2.87, 4.35, 5.69	15.0
4.	Malic	3.24, 4.71	20.0
5.	Glycolic	3.63	10.0
6.	Formic	3.55	10.0
7.	Lactic	3.66	10.0
8.	HIBA	3.72	30.0
9.	Acetic	4.56	25.0
10.	Succinic	4.00, 5.24	25.0
11.	Fumaric	2.85, 4.10	35.0
12.	Propionic	4.67	50.0
13.	Glutaric	4.13,5.03	40.0

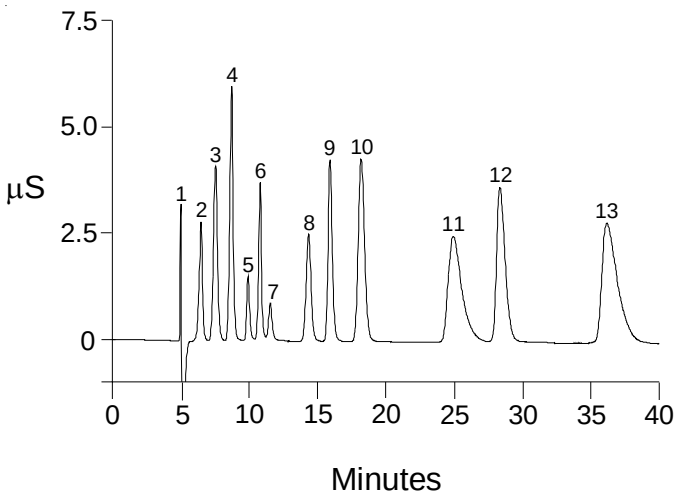


Figure 5
Full IonPac ICE-AS6 Organic Acid Chromatogram

5.6 Comparison of Different Acids Used on the IonPac ICE-AS6

The following examples demonstrate that at constant concentration, several different acids are observed to yield chromatograms of identical selectivity, sensitivity and runtime.

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	See Chromatogram (0.4 mM each)
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor II, AMMS-ICE II
Suppressor Regenerant:	5 mN Tetrabutylammonium hydroxide
Expected Background Conductivity:	23 - 25 µS
Expected System Operating Back Pressure:	< 500 psi (3.45 MPa)
Temperature:	19°C

	Acid	pKa	mg/L
1.	Oxalic	1.04, 3.82	5.0
2.	Tartaric	2.82, 3.95	10.0
3.	Citric	2.87, 4.35, 5.69	15.0
4.	Malic	3.24, 4.71	20.0
5.	Glycolic	3.63	10.0
6.	Formic	3.55	10.0
7.	Lactic	3.66	10.0
8.	HIBA	3.72	30.0
9.	Acetic	4.56	25.0
10.	Succinic	4.00, 5.24	25.0

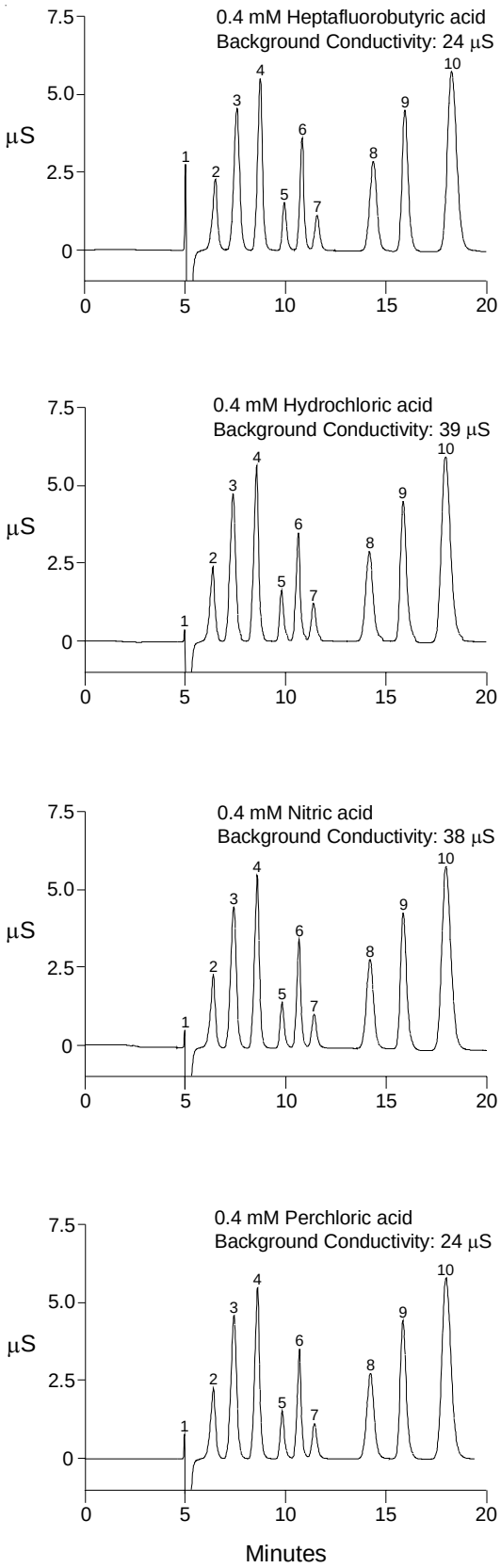


Figure 6
Comparison of Different Acids used with the IonPac ICE-AS6

5.7 Retention Times Versus Eluent acid Concentration

This example compares predicted separations obtained from a plot of column selectivity versus eluent strength to the actual chromatographic separations, see Section 5.3.1, "ICE-AS6 Retention Time vs. Eluent Strength." For the first estimate of the best separation based on retention times only, the selectivity chart is used. The actual resolution of separation though is also dependent on the efficiency of the column.

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	See Chromatogram
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor II, AMMS-ICE II
Regenerant:	5 mN Tetrabutylammonium hydroxide
Expected Background Conductivity:	See Chromatogram
Expected System Operating Back Pressure:	<440 psi (3.03 MPa)
Temperature:	19°C

	Acid	pKa	mg/L
1.	Oxalic	1.04, 3.82	5.0
2.	Tartaric	2.82, 3.95	10.0
3.	Citric	2.87, 4.35, 5.69	15.0
4.	Malic	3.24, 4.71	20.0
5.	Glycolic	3.63	10.0
6.	Formic	3.55	10.0
7.	Lactic	3.66	10.0
8.	HIBA	3.72	30.0
9.	Acetic	4.56	25.0
10.	Succinic	4.00, 5.24	40.0

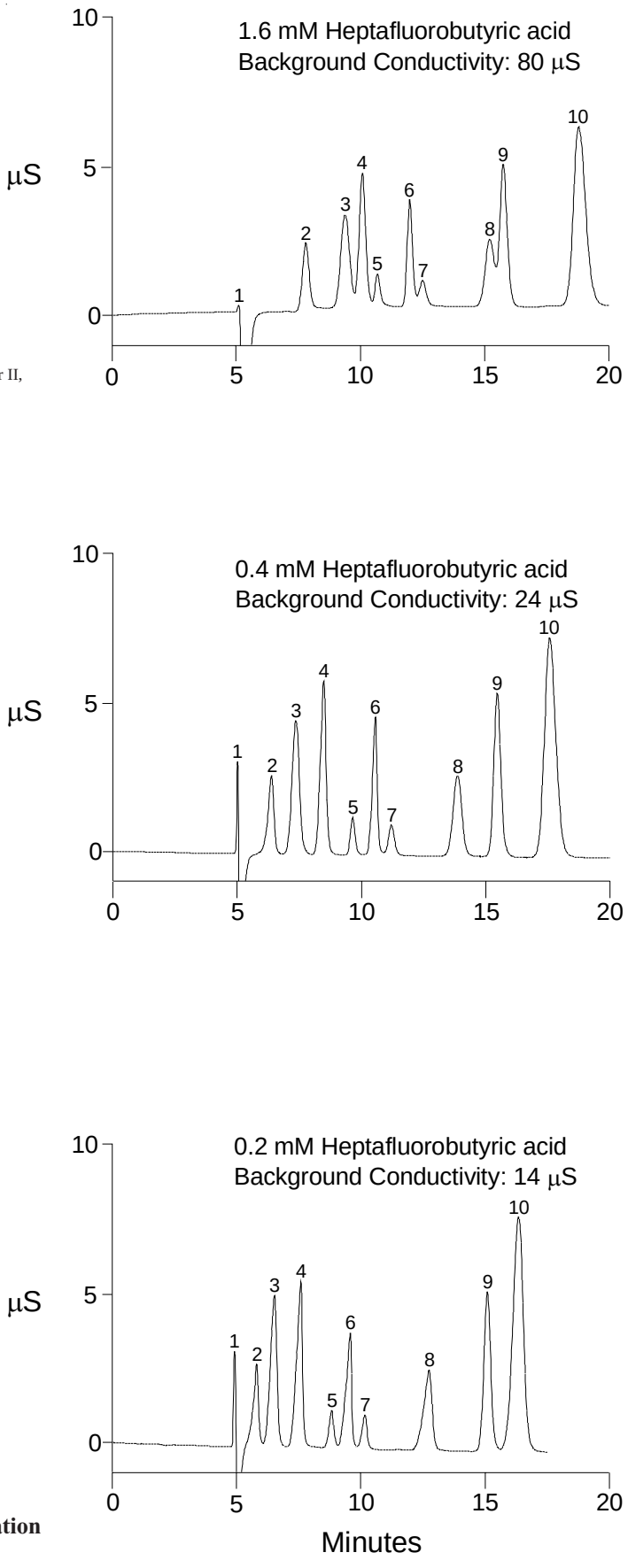


Figure 7
IonPac ICE-AS6 Retention Time versus Acid Concentration

5.8 Analysis of a Coffee Sample

The following analysis of coffee demonstrates one of the most complex samples. Because of the complexity of the sample, concentrated coffee samples were used for the fouling studies.

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor II, AMMS-ICE II
Regenerant:	5 mN Tetrabutylammonium hydroxide
Expected Background Conductivity:	23 - 25 µS
Expected System Operating Back pressure:	< 500 psi (3.45 MPa)
Temperature:	19°C

	Acid	pKa
1.	Inorganics	
2.	Inorganics	
3.	Maleic	1.75, 5.83
4.	Malonic	2.65, 5.28
5.	Citric	2.87, 4.35, 5.69
6.	Malic	3.24, 4.71
7.	Quinic	3.36
8.	Glycolic	3.63
9.	Formic	3.55
10.	Lactic	3.66
11.	Unknown	
12.	Acetic	4.56
13.	Unknown	
14.	Shikimic	
15.	Fumaric	3.55
16.	Propionic	4.83

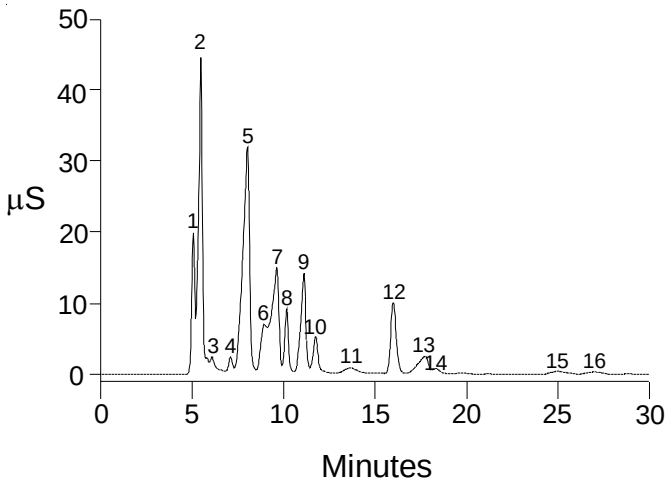


Figure 8
IonPac ICE-AS6 Analysis of Coffee

5.9 Fouling Resistance of IonPac ICE-AS6 Columns

The following fouling runs used a 10X concentrated instant coffee solution. After 100 injections of the column with the concentrate, the standard 10 organic acids were analyzed to demonstrate the degree of column degradation. Note that after the 100 injections of the 10X coffee concentrate the pressure had increased from 420 to 510 psi (2.89-3.51 MPa).

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor II, AMMS-ICE II
Regenerant:	5 mN Tetrabutylammonium hydroxide
Expected Background Conductivity:	23 - 25 µS
Expected System Operating Back pressure:	See Chromatogram
Temperature:	19°C

	Acid	pKa	mg/L
1.	Oxalic	1.04, 3.82	5.0
2.	Tartaric	2.82, 3.95	10.0
3.	Citric	2.87, 4.35, 5.69	15.0
4.	Malic	3.24, 4.71	20.0
5.	Glycolic	3.63	10.0
6.	Formic	3.55	10.0
7.	Lactic	3.66	10.0
8.	HIBA	3.72	30.0
9.	Acetic	4.56	25.0
10.	Succinic	4.00, 5.24	40.0

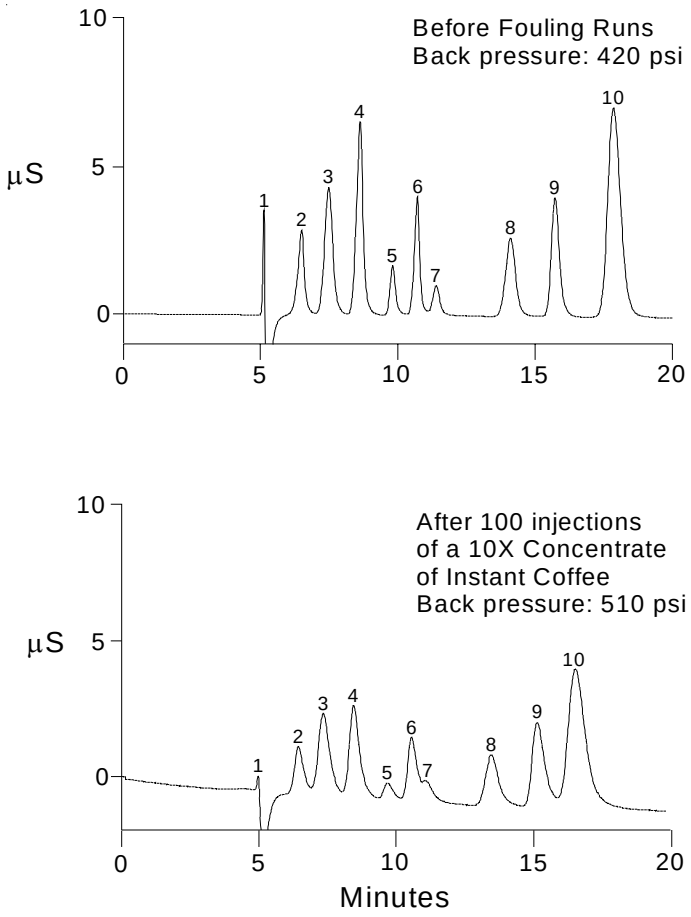


Figure 9
Fouling Resistance of IonPac ICE-AS6 Columns

5.10 Analysis of Milk

The following example demonstrates the analysis of milk on the IonPac ICE-AS6.

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor II, AMMS-ICE II
Regenerant:	5 mN Tetrabutylammonium hydroxide
Expected Background Conductivity:	23 - 25 µS
Expected System Operating Back pressure:	< 500 psi (3.45 MPa)
Temperature:	19°C

	Acid	pKa
1.	Inorganics	
2.	Inorganics	
3.	Citric	2.87, 4.35, 5.69
4.	Formic	3.55

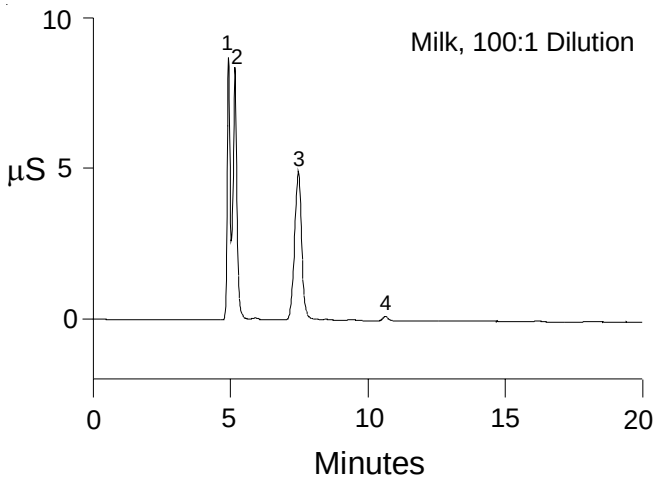
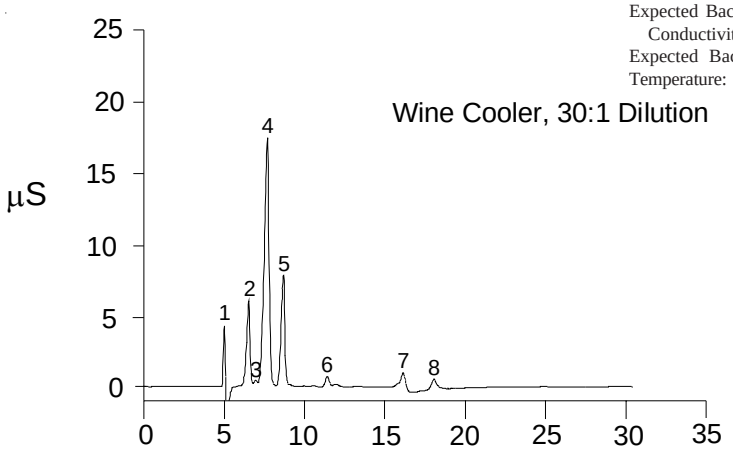


Figure 10
IonPac ICE-AS6 Analysis of Milk

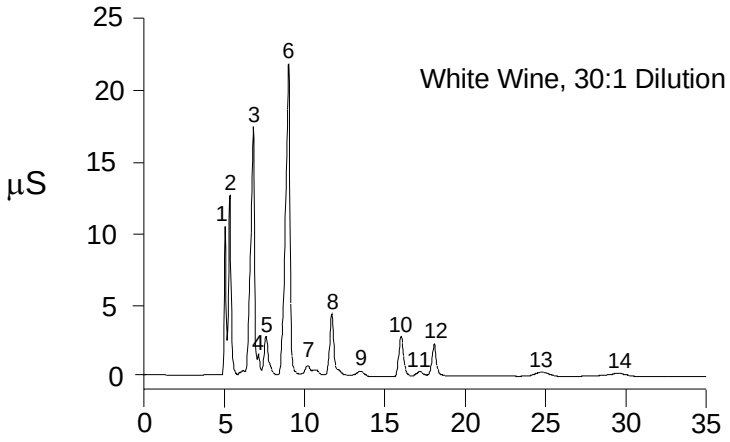
5.11 Analysis of Wine Samples

The following samples demonstrate the analysis of different wine samples on the IonPac ICE-AS6.

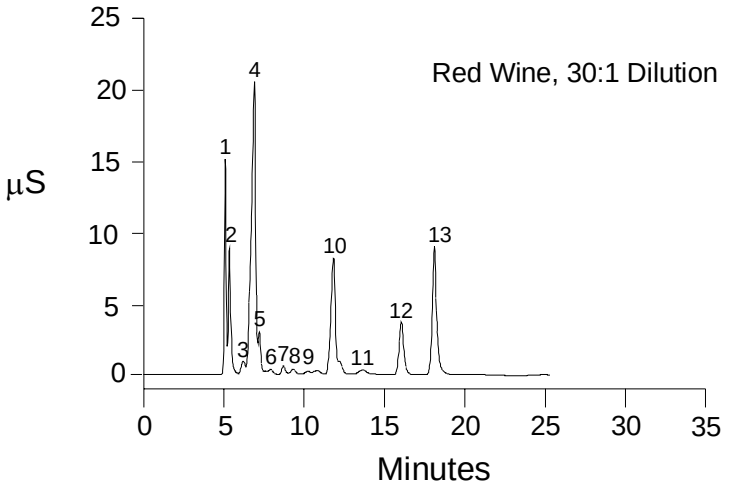
Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor II, AMMS-ICE II
Regenerant:	5 mN Tetrabutylammonium hydroxide
Expected Background Conductivity:	23 - 25 µS
Expected Backpressure:	< 500 psi (3.45 MPa)
Temperature:	19°C



	Acid	pKa
1.	Inorganics	
2.	Tartaric	2.82, 3.95
3.	Malonic	3.19
4.	Citric	2.87, 4.35, 5.69
5.	Malic	3.24, 4.71
6.	Lactic	3.66
7.	Carbonic	
8.	Succinic	4.00, 5.24



	Acid	pKa
1.	Inorganics	
2.	Inorganics	
3.	Tartaric	2.82, 3.95
4.	Galacturonic	3.23,11.42
5.	Citric	2.87, 4.35, 5.69
6.	Malic	3.24, 4.71
7.	Glycolic	3.63
8.	Lactic	3.66
9.	Unknown	
10.	Acetic	4.56
11.	Unknown	
12.	Succinic	4.00, 5.24
13.	Fumaric	3.55
14.	Propionic	4.83



	Acid	pKa
1.	Inorganics	
2.	Inorganics	
3.	Unknown	
4.	Tartaric	2.82, 3.95
5.	Galacturonic	3.23,11.42
6.	Citric	2.87, 4.35, 5.69
7.	Malic	3.24, 4.71
8.	Unknown	
9.	Glycolic	3.63
10.	Lactic	3.66
11.	Unknown	
12.	Acetic	4.56
13.	Succinic	4.00, 5.24

Figure 11
IonPac ICE-AS6 Analysis of Wine Samples

5.12 Analysis of Jack Daniels Lynchburgh Lemonade

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor II, AMMS-ICE II
Regenerant:	5 mN Tetrabutylammonium hydroxide
Expected Background Conductivity:	23 - 25 µS
Expected System Operating Back pressure:	< 500 psi (3.45 MPa)
Temperature:	19°C

	Acid	pKa
1.	Inorganics	
2.	Citric	2.87, 4.35, 5.69
3.	Carbonic	

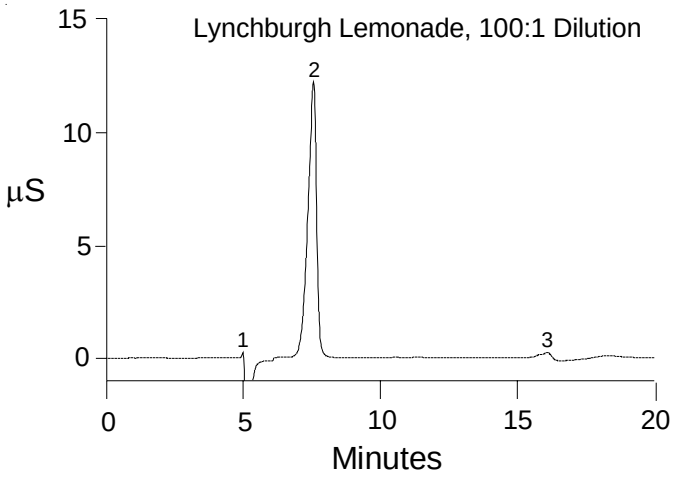


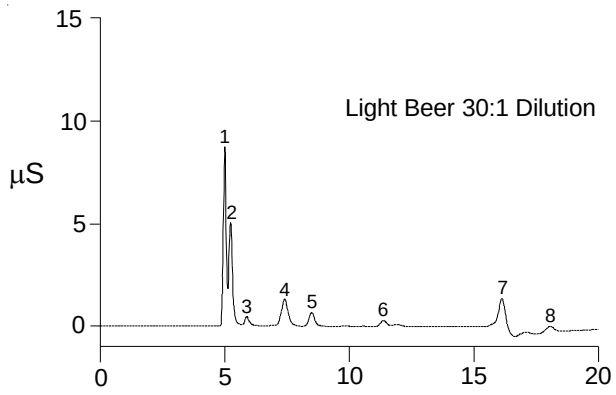
Figure 12
IonPac ICE-AS6 Analysis of Jack Daniels Lynchburgh Lemonade

5.13 Analysis of Beer Samples

The following examples demonstrate the analysis of beer samples on the IonPac ICE-AS6.

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor II, AMMS-ICE II
Regenerant:	5 mN Tetrabutylammonium hydroxide
Expected Background Conductivity:	23 - 25 µS
Expected System Operating Back pressure:	< 500 psi (3.45 MPa)
Temperature:	19°C

	Acid	pKa
1.	Inorganics	
2.	Inorganics	
3.	Maleic	1.75, 5.83
4.	Citric	2.87, 4.35, 5.69
5.	Malic	3.24, 4.71
6.	Lactic	3.66
7.	Carbonic	
8.	Succinic	4.00,5.24



	Acid	pKa
1.	Inorganics	
2.	Inorganics	
3.	Maleic	1.75, 5.83
4.	Citric	2.87, 4.35, 5.69
5.	Malic	3.24, 4.71
6.	Formic	3.55
7.	Lactic	3.66
8.	Carbonic	
9.	Succinic	4.00,5.24

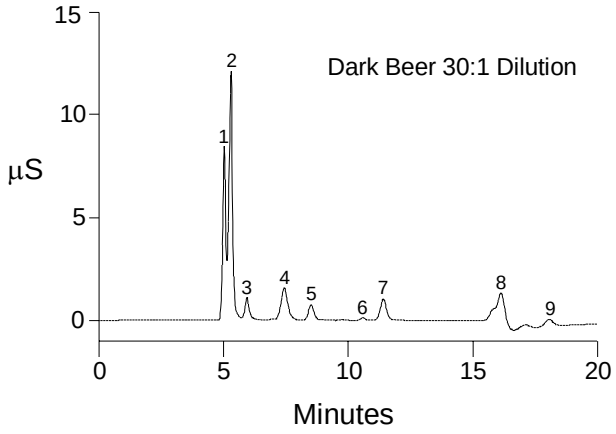


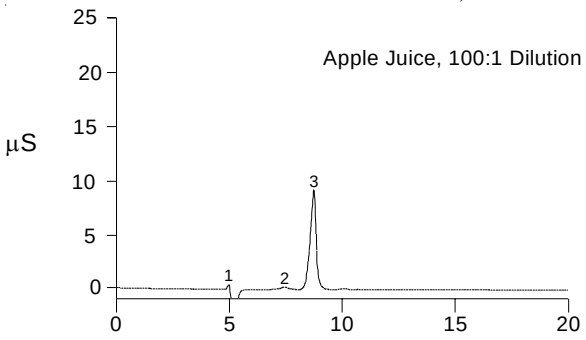
Figure 13
IonPac ICE-AS6 Analysis of Beer Samples

5.14 Analysis of Fruit Juice Samples

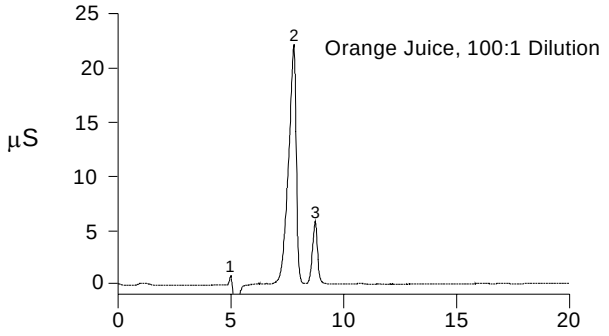
The following examples demonstrate the analysis of various fruit juices on the IonPac ICE-AS6.

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor II, AMMS-ICE II
Regenerant:	5 mN Tetrabutylammonium hydroxide
Expected Background Conductivity:	23 - 25 µS
Expected System Operating Backpressure:	< 500 psi (3.45 MPa)
Temperature:	19°C

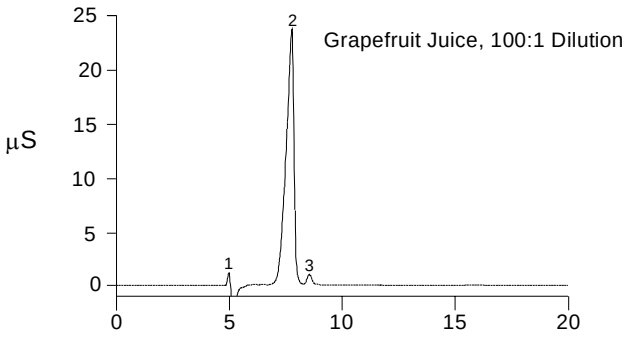
	Acid	pKa
1.	Oxalic	1.04, 3.82
2.	Citric	2.87, 4.35, 5.69
3.	Malic	3.24, 4.71



	Acid	pKa
1.	Oxalic	1.04,3.82
2.	Citric	2.87, 4.35, 5.69
3.	Malic	3.24, 4.71



	Acid	pKa
1.	Inorganics	
2.	Citric	2.87, 4.35, 5.69
3.	Malic	3.24, 4.71



	Acid	pKa
1.	Citric	2.87, 4.35, 5.69
2.	Malic	3.24, 4.71

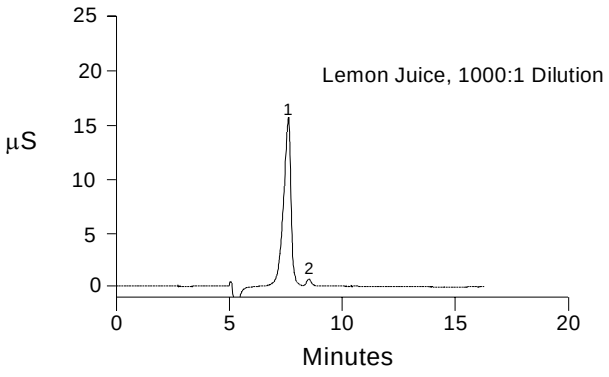


Figure 14
IonPac ICE-AS6 Analysis of Fruit Juices

	Acid	pKa
1.	Inorganics	
2.	Tartaric	2.82, 3.95
3.	Galacturonic	3.23, 11.42
4.	Citric	2.87, 4.35, 5.69
5.	Malic	3.24, 4.71
6.	Unknown	

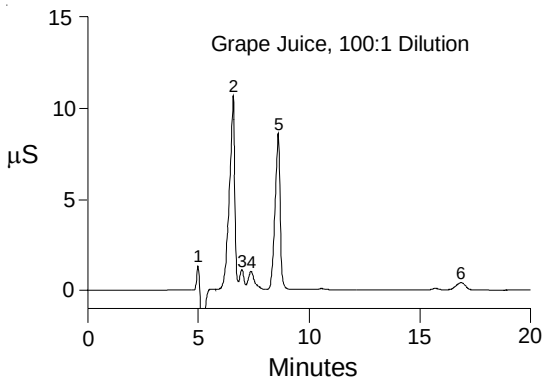


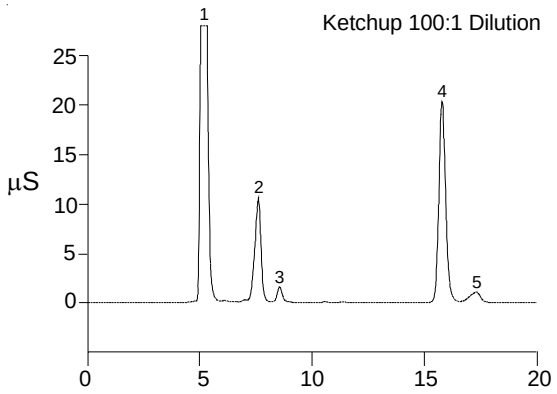
Figure 14 (continued)
IonPac ICE-AS6 Analysis of Fruit Juices

5.15 Analysis of Tomato Product Samples

The following examples demonstrate the analysis of tomato products on the IonPac ICE-AS6.

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor II, AMMS-ICE II
Regenerant:	5 mN Tetrabutylammonium hydroxide
Expected Background Conductivity:	23 - 25 µS
Expected System Operating Backpressure:	< 500 psi (3.45 MPa)
Temperature:	19°C

	Acid	pKa
1.	Inorganics	
2.	Citric	2.87, 4.35, 5.69
3.	Malic	3.24, 4.71
4.	Acetate	4.56
5.	Unknown	



	Acid	pKa
1.	Inorganics	
2.	Malonic	2.65,5.28
3.	Citric	2.87, 4.35, 5.69
4.	Malic	3.24, 4.71
5.	Formic	3.55
6.	Unknown	

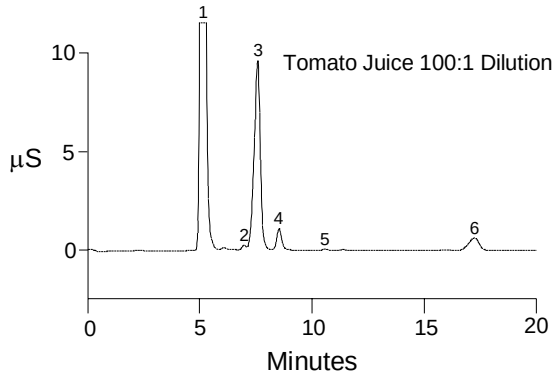


Figure 15
IonPac ICE-AS6 Analysis of Tomato Products

5.16 Analysis of Mustard

The following example demonstrates the analysis of mustard on the IonPac ICE-AS6.

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor II, AMMS-ICE II
Regenerant:	5 mN Tetrabutylammonium hydroxide
Expected Background Conductivity:	23 - 25 µS
Expected System Operating Back pressure:	< 500 psi (3.45 MPa)
Temperature:	19°C

	Acid	pKa
1.	Inorganics	
2.	Citric	2.87, 4.35, 5.69
3.	Malic	3.24, 4.71
4.	Acetic	4.56

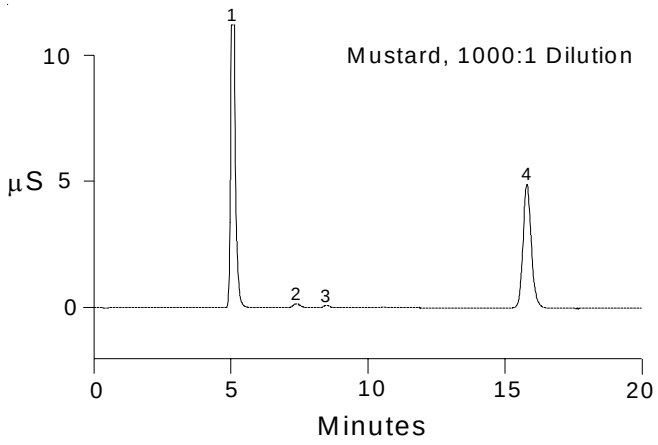


Figure 16
IonPac ICE-AS6 Analysis of Mustard

5.17 Analysis of Boric Acid and Carbonic Acid

The following examples demonstrate the analysis of a sample containing boric acid and carbonic acid on the IonPac ICE-AS6.

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor II, AMMS-ICE II
Regenerant:	5 mN Tetrabutylammonium hydroxide
Expected Background Conductivity:	23 - 25 µS
Expected System Operating Backpressure:	< 500 psi (3.45 MPa)
Temperature:	19°C

	Acid	pKa	mg/L
1.	Galacturonic	3.23, 11.42	30.0
2.	Threonic		15.0
3.	Quinic	3.63	20.0
4.	Boric		5.0
5.	Chloroacetic	2.68	10.0
6.	Tricarballylic	3.48, 4.50, 5.82	5.0
7.	Carbonic		50.0
8.	Shikimik		50.0
9.	Itaconic	3.68, 5.14	15.0
10.	Acrylic	4.26	30.0

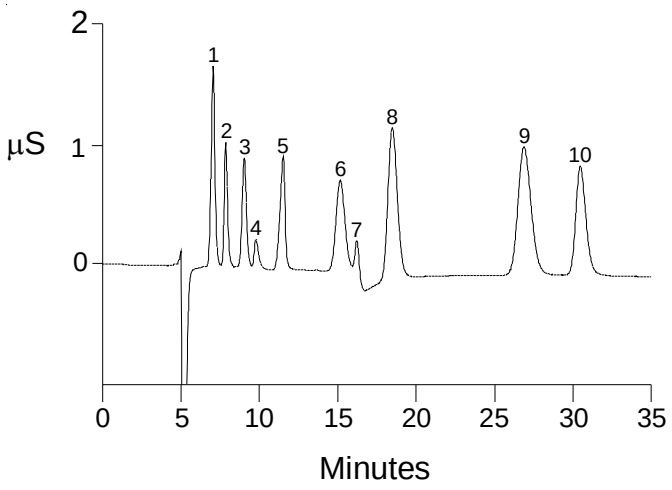


Figure 17
IonPac ICE-AS6 Analysis of Boric Acid and Carbonic Acid

5.18 Analysis of 0.25% Sulfuric Acid

The following example demonstrates the analysis of organic acids in 0.25% sulfuric acid on the IonPac ICE-AS6.

Sample Loop Volume:	50 µL, 0.25% Sulfuric Acid
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	1.0 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor II, AMMS-ICE II
Regenerant:	5 mN Tetrabutylammonium hydroxide
Expected Background Conductivity:	73 - 75 µS
Expected System Operating Backpressure:	< 500 psi (3.45 MPa)
Temperature:	19°C

	Analyte	mg/L
1.	Citrate	0.5
2.	Malic	1.0
3.	Glycolic	1.0
4.	Lactic	1.0
5.	Acetic	1.0
6.	Succinic	1.0

where 1 mg/L = 1 ppm

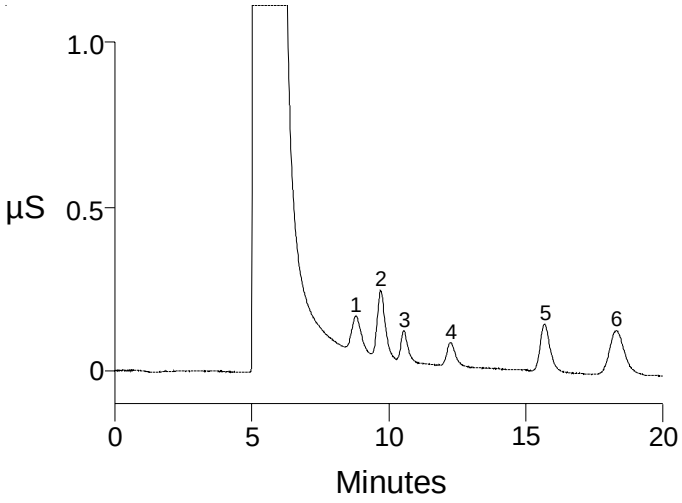


Figure 18
IonPac ICE-AS6 Analysis of 0.25% Sulfuric Acid

5.19 Analysis of 0.50% Sodium Chloride

The following example demonstrates the analysis of organic acids in 0.50% sodium chloride on the IonPac ICE-AS6.

Sample Loop Volume:	50 µL, 0.5% Sodium Chloride
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	1.0 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor II, AMMS-ICE II
Regenerant:	5 mN Tetrabutylammonium hydroxide
Expected Background Conductivity:	73 - 75 µS
Expected System Operating Backpressure:	< 500 psi (3.45 MPa)
Temperature:	19°C

	Analyte	mg/L
1.	Tartrate	1.0
2.	Citrate	0.5
3.	Malic	1.0
4.	Glycolic	1.0
5.	Lactic	1.0
6.	Acetic	1.0
7.	Succinic	1.0

where 1 mg/L = 1 ppm

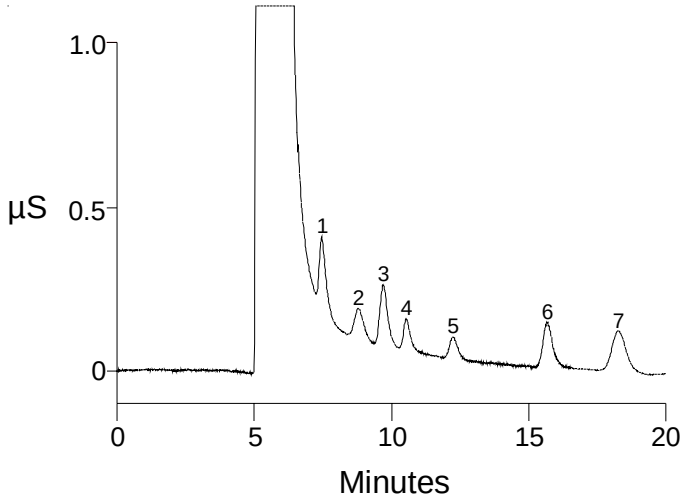


Figure 19
IonPac ICE-AS6 Analysis of 0.25% Sodium Chloride

5.20 Comparison of UV versus Suppressed Conductivity Detection

The following example demonstrates the comparison of UV detection (at 210 nm) and suppressed conductivity detection of organic acids on the IonPac ICE-AS6.

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor II, AMMS-ICE II
Regenerant:	5 mN Tetrabutylammonium hydroxide
Expected Background Conductivity:	23 - 25 µS
Expected System Operating Backpressure:	< 500 psi (3.45 MPa)
Temperature:	19°C

	Acid	pKa	mg/L
1.	Oxalic	1.04, 3.82	5.0
2.	Tartaric	2.82, 3.95	10.0
3.	Citric	2.87, 4.35, 5.69	15.0
4.	Malic	3.24, 4.71	20.0
5.	Glycolic	3.63	10.0
6.	Formic	3.55	10.0
7.	Lactic	3.66	10.0
8.	HIBA	3.72	30.0
9.	Acetic	4.56	25.0
10.	Succinic	4.00, 5.24	40.0
11.	Fumaric	2.85, 4.10	35.0
12.	Propionic	4.67	50.0
13.	Glutaric	4.13, 5.03	40.0

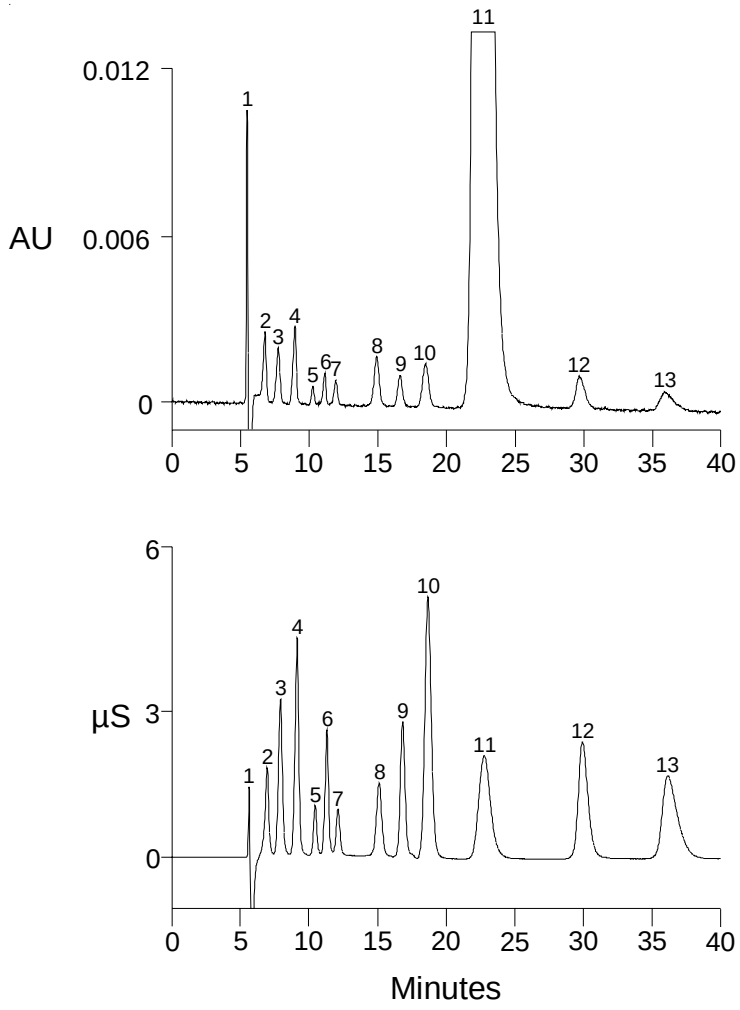


Figure 20
Comparison of UV detection and Suppressed Conductivity Detection

5.21 Detection of Alcohols Using Pulse Amperometric Detection

The top chromatogram illustrates pulsed amperometric detection of alcohols in which the eluent and the sample are carefully degassed. A woven reaction coil can be used to reduce oxygen levels with similar results.

The middle chromatogram illustrates the appearance of the total permeation volume baseline dip when the eluent and sample are not thoroughly degassed.

The bottom chromatogram illustrates the repositioning of the total permeation volume baseline dip with the addition of an IonPac NG1 in front of the IonPac ICE-AS6. The added permeation volume of the IonPac NG1 provides improved resolution of methanol and propylene glycol.

Sample Loop Volume:	100 µL (sample made up in eluent and degassed)
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	75 mM $HClO_4$ (made fresh daily and sonicated under vacuum for 15 minutes to degas)
Eluent Flow Rate:	1.5 mL/min
Detection:	PED with 3 mm Platinum Electrode
Waveform	Integration
Temperature:	19°C

Time (sec)	Poten (V)	Begin (sec)	End (sec)
0.00	0.30	0.01	0.21
0.30	0.30		
0.31	1.40		
0.51	1.40		
0.52	-1.40		
0.92	-1.40		

	Analyte	mg/L
1.	Sorbitol	1000
2.	Erythritol	1000
3.	Glycerol	1000
4.	Ethylene Glycol	1000
5.	Methanol	1000
6.	Propylene Glycol	1000
7.	Ethanol	1000
8.	2-Propanol	1000
9.	1-Propanol	1000

where 1 mg/L = 1 ppm

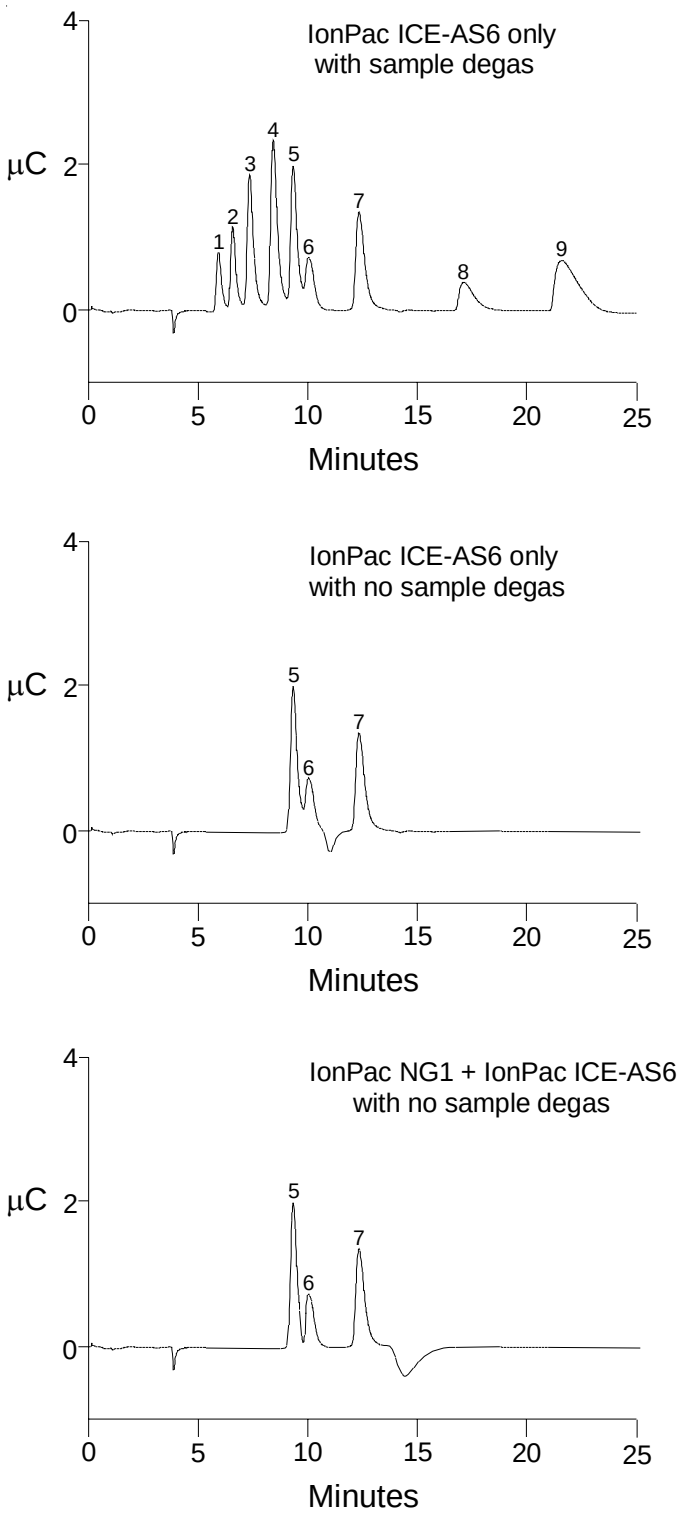


Figure 21
Detection of Alcohols Using Pulsed Amperometric Detection

5.22 Analysis of Selected Inorganic Ions

The following examples demonstrate the analysis of selected inorganic ions on the IonPac ICE-AS6.

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor II, AMMS-ICE II
Regenerant:	5 mN Tetrabutylammonium hydroxide
Expected Background Conductivity:	23 - 25 µS
Expected System Operating Backpressure:	< 500 psi (3.45 MPa)
Temperature:	19°C

Analyte
1. Nitrate/Chloride/Sulfate
2. Phosphate
3. Fluoride (HF_2^-)
   (Total concentration: ~ 300 mg/L)

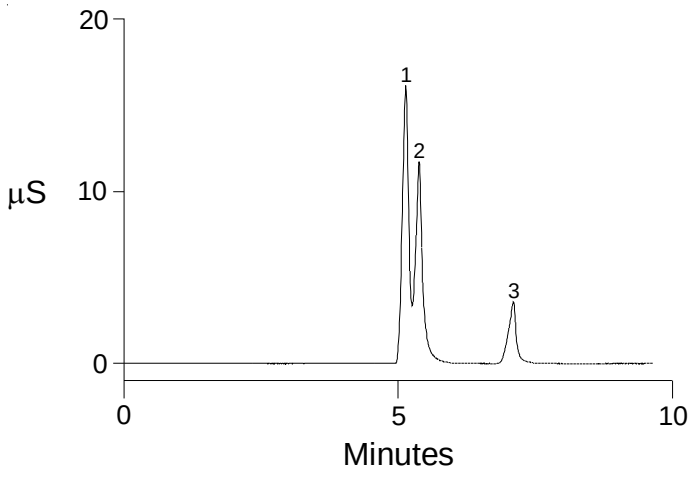


Figure 22
IonPac ICE-AS6 Analysis of Selected Inorganic Anions

SECTION 6 - TROUBLESHOOTING GUIDE

The purpose of the Troubleshooting Guide is to help you solve operating problems that may arise while using the IonPac ICE-AS6 column. For more information on problems that originate with the Ion Chromatograph (IC) or the AMMS-ICE MicroMembrane Suppressor (AMMS-ICE), refer to the Troubleshooting Guide in the appropriate operator's manual. If you cannot solve the problem on your own, contact the DIONEX Worldwide Office nearest you (see "DIONEX Worldwide Offices").

Table 5
ICE-AS6 Troubleshooting Summary

Observation	Cause	Action	Reference Section
High Back Pressure	Unknown Component	Isolate Blockage	6.1.1
	Plugged Column Bed Supports	Replace Bed Supports	6.1.2
	Plugged System Hardware	Unplug, Replace	Component Manual
High Background Conductivity	**AMMS-ICE Not Suppressing**	Check Regenerant Flow Rate Check Eluent Flow Rate	6.2.4.A, Component Manual 6.2.4.B, Component Manual
	Contamination Bad Eluents	Remake Eluents	6.2, 6.3.2.B, 6.3.3.A
	Contaminated Column	Clean Column	6.2.3, 6.3.2.C, 6.3.2.D, 6.4.A, 6.4.B, Column Care
	Contaminated AMMS-ICE	Clean Suppressor	6.2.4.C, Component Manual
	Hardware Operation Proportioning Valve	Service Valve	Component Manual
Poor Peak Resolution Poor Efficiency	Large System Void Volumes Sluggish Injection Valve	Replumb System Service Valve	6.3.1.B, Component Manual 6.3.3.C, 6.4.C, Component Manual
	Column Headspace	Replace Column	6.3.1.A
	Column Overloading	Reduce Sample Size	6.3.3.B
Fronting Peaks	Column Overloading	Reduce Sample Size	6.3.3.B
Tailing Peaks	Contaminated AMMS-ICE	Clean Suppressor	6.2.4.C, Component Manual
Short Retention Times	Flow Rate Too Fast Bad Eluents	Recalibrate Pump Remake Eluents	6.3.2.A 6.3.2.B
	Column Contamination	Clean Column	6.2.2, 6.3.2.C, 6.3.2.D, 6.4.A, 6.4.B, Column Care
Spurious Peaks	Column Contamination	Pretreat Samples	6.3.2.C, 6.4.A
	Sluggish Injection Valve	Service Valve	6.3.3.C, 6.4.C, Component Manual

6.1 High Back Pressure

6.1.1 Finding the Source of High System Pressure

Total system pressure when using the IonPac ICE-AS6 Analytical Column at 1.0 mL/min should be less than 700 psi (4.80 MPa) when using the eluent used to generate the test chromatogram. Refer to Section 4.1.2, "Solvents," to see how solvent concentration can affect the column operating pressure. If the system pressure is higher than 800 psi (5.52 MPa), it is advisable to find out what is causing the high system pressure.

The system should be used with a High-Pressure In-Line Filter (P/N 044105) for eluents. The filter should be positioned between the pump pressure transducer and the injection valve.

A. Make sure that the pump is set to the correct eluent flow rate. Higher than recommended eluent flow rates will cause higher pressure. Measure the pump flow rate if necessary with a stop watch and graduated cylinder.

B. Find out what part of the system is causing the high pressure. It could be a piece of tubing that has plugged or whose walls are collapsed, an injection valve with a plugged port, a column with particulates plugging the bed support, a plugged High-Pressure In-Line Filter, the AMMS-ICE II, or the detector cell.

To find out which part of the chromatographic system is causing the problem, disconnect the pump eluent line from the injection valve and turn the pump on. Watch the pressure; it should not exceed 50 psi (0.34 MPa). Continue adding the system's components (injection valve, column(s), AMMS-ICE II and the detector) one by one, while watching the system pressure. The pressure should increase up to a maximum of 550 psi (3.79 MPa) at a flow rate of 2.0 mL/min when the column(s) are connected. The AMMS-ICE II may add up to 130 psi (0.90 MPa). No other components should add more than 100 psi (0.69 MPa) of pressure. Refer to the appropriate manual for cleanup or replacement of the problem component.

6.1.2 Replacing Column Bed Support Assemblies

If the column inlet bed support is determined to be the cause of the high back pressure, it should be replaced. To change the inlet bed support assembly, refer to the following instructions, using one of the two spare inlet bed support assemblies included in the Ship Kit.

A. Disconnect the column from the system.

B. Carefully unscrew the inlet (top) column fitting. Use two open end wrenches, If the resin appears soupy, carefully remove excess moisture by touching a paper towel to the edge of the resin bed. Allow water (not resin) to soak into the paper towel.

C. Remove the bed support assembly. Turn the end fitting over and tap it against a benchtop or other hard, flat surface to remove the bed support and seal assembly. If the bed support must be pried out of the end fitting, use a sharp pointed object such as a pair of tweezers, but be careful that you **DO NOT SCRATCH THE WALLS OF THE END FITTING.** Discard the old bed support assembly.

D. Place a new bed support assembly into the end fitting. Make sure that the end of the column tube is clean and free of any particulate matter so that it will properly seal against the bed support assembly. Use the end of the column to carefully start the bed support assembly into the end fitting.

IonPac ICE-AS6 Columns	**(P/N)**
Analytical Column	046023
Bed Support Assembly	048238
Zitex® Bed Support	048297
End Fitting	048298

CAUTION

CAUTION

If the column tube end is not clean when inserted into the end fitting, particulate matter may obstruct a proper seal between the end of the column tube and the bed support assembly. If this is the case, additional tightening may not seal the column but instead damage the column tube or the end fitting. Carefully wipe the sealing surfaces clean before assembling

E. **Screw the end fitting back onto the column.** Tighten it fingertight, then an additional 1/4 turn (25 in x lb). Tighten further only if leaks are observed.

F. Reconnect the column to the system and resume operation.

NOTE

DO NOT attempt to remove the outlet column fitting as the resin will extrude out of the column and ruin it.

6.2 High Background or Noise

In a properly working system, the background conductivity levels expected for several eluent systems are shown below:

ELUENT	EXPECTED BACKGROUND CONDUCTIVITY
0.2 mM heptafluorobutyric acid	13 - 15 µS
0.4 mM heptafluorobutyric acid	23 - 25 µS
1.6 mM heptafluorobutyric acid	78 - 82 µS

6.2.1 Preparation of Eluents

A. Make sure that the eluents and the regenerant are made correctly.

B. Make sure that the eluents are made from chemicals with the recommended purity.

C. Make sure that the deionized water used to prepare the reagents has a specific resistance of 18.2 megohm-cm.

6.2.2 A Contaminated Guard or Analytical Column

Remove the IonPac ICE-AS6 Analytical Column from the system. If the background conductivity decreases, then the column is the cause of the high background conductivity, clean the column as instructed in “Column Care.”

6.2.3 Contaminated Hardware

To eliminate the hardware as the source of the high background conductivity, bypass the AMMS-ICE and pump deionized water with a specific resistance of 18.2 megohm-cm through the system. The background conductivity should be less than 2 µS. If it is not, check the detector/conductivity cell calibration by injecting deionized water directly into it. See the appropriate manual for details.

6.2.4 A Contaminated Anion-ICE MicroMembrane Suppressor II, AMMS-ICE II

If the above items have been checked and the problem persists, the AMMS-ICE II is probably causing the problem.

A. Check the regenerant flow rate at the REGEN OUT port of the AMMS-ICE II. For the example isocratic applications, this flow rate should be 3 - 5 mL/min.

B. Check the eluent flow rate. For most applications, the eluent flow rate should be 0.8 mL/min. Refer to the Anion-ICE MicroMembrane Suppressor II Product Manual (Document No. 032661) to ensure that the eluent is within

C. The AMMS-ICE II may be contaminated. Prepare fresh regenerant solution. If the background conductivity is high after preparing fresh regenerant, you probably need to clean or replace your AMMS-ICE II. Refer to the "Anion-ICE MicroMembrane Suppressor II Product Manual" (Document No. 032661) for assistance.

6.3 Poor Peak Resolution

Poor peak resolution can be due any or all of the following factors.

6.3.1 Loss of Column Efficiency

A. Check to see if headspace in the analytical column. This may be due to improper use of the column such as submitting it to high pressures. Remove the column's top end fitting (see Section 6.1.2, "Replacing Column Bed Support Assemblies"). If the resin does not fill the column body all the way to the top, it means that the resin bed has collapsed, creating a headspace. 1 - 2 mm of headspace is the maximum allowable before the column demonstrates significant losses of efficiency. If more than 2 mm of headspace is observed, the column must be replaced.

B. Extra-column system effects can result in sample band dispersion, decreasing peak efficiencies. Make sure you are using PEEK tubing with an ID of no greater than 0.010" to make all eluent liquid line connections between the injection valve and the detector cell inlet on 4-mm systems. Check for leaks.

6.3.2 Poor Resolution Due to Shortened Retention Times

Even with adequate system and column efficiency, resolution of peaks will be compromised if analytes elute too fast.

A. Check the eluent flow rate. See if it is different than the flow rate specified by the analytical protocol. Measure the eluent flow rate after the column using a stopwatch and graduated cylinder. Wait at least 5 minutes before making the measurement to allow time for the pump pressure feedback to engage.

B. Check to see if the eluent compositions and concentrations are correct. For isocratic analysis, an eluent that is too strong will cause the peaks to elute later. Prepare fresh eluent. If you are using a gradient pump to proportion the final eluent from concentrated eluents in two or three different eluent reservoirs, the composition of the final eluent may not be accurate enough for the application. Use one reservoir containing the correct eluent composition to see if this is the problem. This may be a problem when one of the proportioned eluents is less than 5%.

C. Column contamination can lead to a loss of column efficiency. Cationic contamination nonionic contamination or metal ions might be concentrating on the column. Refer to "Column Care," for recommended column cleanup procedures.

Possible sources of column contamination are impurities in chemicals, in the deionized water or from the sample matrix being used. Be especially careful to make sure that the recommended chemicals are used. The deionized water should have a specific resistance of at least 18.2 megohm-cm.

D. If run times are reduced to the point that resolution is lost, clean the column (see "Column Care"). After cleaning the column, reinstall it in the system and let it equilibrate with eluent for about 30 minutes. The column is equilibrated when consecutive injections of the standard give reproducible retention times. The original column capacity should be restored by this treatment, since the contaminants should be eluted from the column. If you need assistance in solving resolution problems, contact the DIONEX Worldwide Office nearest you (see "DIONEX Worldwide Offices").

6.3.3 Loss of Front End Resolution

If poor resolutions and efficiencies are observed for the very early eluting peaks near the system void volume compared to the later eluting peaks, check the following:

A. Improper eluent concentration may be the problem. Remake the eluent as required for your application. Ensure that the water and chemicals used are of the required purity.

B. Column overloading may be the problem. Reduce the amount of sample ions being injected onto the analytical column by either diluting the sample or injecting a smaller volume onto the column.

C. Sluggish operation of the injection valve may be the problem. Check the air pressure and make sure there are no gas leaks or partially plugged port faces. Refer to the valve manual for instructions.

D. Improperly swept out volumes anywhere in the system prior to the guard and analytical columns may be the problem. Swap components, one at a time, in the system prior to the analytical column and test for front-end resolution after every system change.

6.4 Spurious Peaks

A. Column fouling may be the problem. If the samples contain an appreciable level of aromatic weak acids or fatty acids (larger than 6 carbons) and the column is used with a weak eluent system, these anions may contaminate the analytical column. The retention times for the analytes will then decrease and spurious, inefficient (broad) peaks can show up at unexpected times. Clean the column as indicated in "Column Care."

B. If you need assistance in determining the best way to clean strongly retained solutes in your specific sample matrix from the IonPac ICE-AS6 columns, contact the DIONEX Worldwide Office nearest you (see "DIONEX Worldwide Offices").

C. Baseline disturbances may be caused when an injection valve is actuated. This baseline upset can show up as a peak of varying size and shape. It will happen when the injection valve needs to be cleaned or retorqued (see valve manual). Check to see that there are no restrictions in the tubing connected to the valve. Also check the valve port faces for blockage and replace them if necessary. Refer to the Valve Manual for troubleshooting and service procedures. Small baseline disturbances at the beginning or at the end of the chromatogram can be overlooked as long as they do not interfere with the quantification of the peaks of interest.

If cleaning and retorquing the valve does not help, replace the valve. Use a DIONEX High Pressure Injection Valve (P/N 037142) or a DIONEX High Pressure Inert Valve (P/N 037143) as required.

For DX-300 systems equipped with a Rheodyne Microinjection Valve, Model 9126 (DIONEX P/N 044697), consult the accompanying manual for service instructions.

D. When doing trace analysis with solvents in the eluent, a solvent peak will appear around the total exclusion volume of the column. This occurs at 12-15 minutes after injection when operating at a flow rate of 1.0 mL/min. This is a suppressor phenomenon that can be avoided by making the solvent concentration of the sample the same as the solvent concentration of the eluent.

6.5 Split Peaks

Split Peaks are Low Sample Concentrations. A high sample pH can cause peak splitting. This problem can occur for early eluting peaks such as citrate at concentrations below 1 mg/L (1 ppm). If you observe split peaks, adjust the pH of the sample to less than pH 3-4 using hydrochloric acid, sulfuric acid or other acid or by passing the sample through an OnGuard-H (P/N 039596).

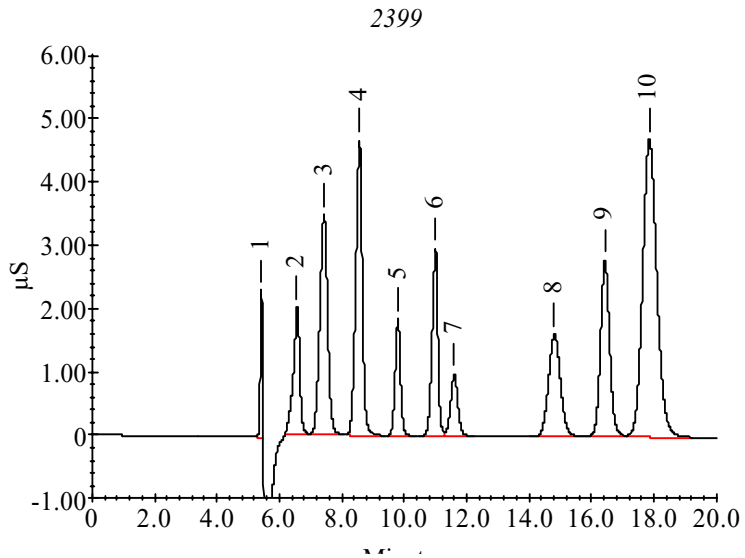
APPENDIX A - QUALITY ASSURANCE REPORT

Quality Assurance Report - IonPac ICE-AS6 Analytical Column - 9 x 250 mm

IonPac® ICE-AS6
Analytical (9 x 250 mm)
Product No. 046023

Serial No. : 2399 **Pressure (PSI) : 560** **Date : 8/15/00 2:57:21 PM**

File Name : C:\PEAKNET\DATA\EXAMPLES\46023 ICE-AS6_A008.DXD



Eluent: 0.4 mM Heptafluorobutyric acid

Flow Rate: 1.0 mL/min

Detection: Suppressed Conductivity at 10 µSFS

Injection Volume: 50 µL

Temperature: 19° C

Storage Solution: Eluent

Peak Information : Found Components

Peak No.	Retention Time	Name	(mg/L)	Efficiency	Asymmetry (10%)	Resolution
1	5.42	Oxalic	5.0	21667	1.1	4.57
2	6.57	Tartaric	10.0	5423	0.6	2.11
3	7.43	Citric	15.0	4195	0.9	2.73
4	8.57	Malic	20.0	8158	0.8	3.57
5	9.80	Glycolic	10.0	15830	0.9	3.69
6	11.00	Formic	10.0	16759	0.8	1.59
7	11.60	Lactic	10.0	12252	1.0	5.74
8	14.81	HIBA	30.0	7112	1.1	2.63
9	16.43	Acetic	25.0	15233	1.1	2.09
10	17.85	Succinic	40.0	7453	1.2	n/a

APPENDIX B - COLUMN CARE

Recommended Operation Pressures
Operating a column above its recommended pressure limit can cause irreversible loss of column performance. The maximum recommended operating pressure for IonPac ICE-AS6 columns is 900 psi (6.20 MPa).

Column Start-Up
The column is shipped in eluent (0.4 mM acid) storage solution.
Pump fresh eluent through the column with the column disconnected from the suppressor for about 20 minutes to clean out any polymer leach from the resin. Simply run effluent into a beaker. You will notice an initial brown color, which should turn clear within a few minutes. Repeat this process if the column is unused for longer than a week. This will prevent damaging the suppressor"

Prepare the eluent shown on the test chromatogram, install the column in the chromatography module and test the column performance under the conditions described in the test chromatogram. Continue making injections of the test standard until consecutive injections of the standard give reproducible retention times. Equilibration is complete when consecutive injections of the standard give reproducible retention times.

CAUTION

It is important to maintain a constant temperature for reproducibility. The IonPac ICE-AS6 is tested at 19 ± 1° C. If the column is exposed to temperature variations of 2° C or more, selectivity changes may be observed.

Column Storage
For both short-term storage and long-term storage, eluent (0.4 mM acid) should be used as the storage solution. Flush the column for a minimum of 10 minutes with the storage solution. Cap both ends securely, using the plugs supplied with the column. When putting the column back into service after storing it, pump fresh eluent through the column with the column disconnected from the suppressor for about 20 minutes to clean out any polymer leach from the resin. Polymer leach from the column can severely contaminate the suppressor.

Column Conditioning
For sample matrices that contain organic solvent content, it is recommended to condition the column with the following procedure:

A. Disconnet the column and direct the column effluent to a waste container.
B. Rinse the column for 90 minutes with 0.5 mN sulfuric acid and 10% acetonitrile.
C. Rinse the column for 30 minutes with eluent.
D. Reconnect the column to the suppressor.

Column Cleanup
The following column cleanup protocols have been divided into three general isocratic protocols to remove acid-soluble, base-soluble or organic contaminants. They can be combined into one gradient protocol if desired but the following precautions should be observed.
Always ensure that the cleanup protocol used does not switch between eluents which may create high pressure eluent interface zones in the column. High pressure zones can disrupt the uniformity of the packing of the column bed and irreversibly damage the performance of the column. High pressure zones in the column can be created by pumping successive eluents through the column that are not miscible. The precipitation of the salts in solvents during column rinses can result in very high pressure zones. High viscosity mixing zones can be created between two eluents having solvents with a very high energy of mixing.
When in doubt, always include short column rinse steps to reduce the solvent content of the eluent to 5% levels and the ionic strength of the eluent to 5 mM levels to avoid creating high pressure zones in the column that may disrupt the uniformity of the column packing.

Choosing the Appropriate Cleanup Solution

A. **Iron contamination of the ICE-AS6 results in a decease in peak heights.** However, successive injections of citrate samples will remove the iron resulting in increasing peak heights.

Citric acid solutions in the concentration range of 2 to 5 mM will remove a variety of metals. If after citric acid treatment, the chromatography still suggests metal contamination, treatment with chelating acids such as oxalic acid is recommended.

B. **Organic solvents can be used if the contamination is nonionic and hydrophobic.** The degree of nonpolar character of the solvent should be increased as the degree of hydrophobicity of the contamination within the range of acceptable solvents.

CAUTION

Start with 5% solvent initially, using the recommended gradient in Section 4.1.1, "IonPac ICE-AS6 Operation Precautions". If this amount is not sufficient, try 5% increases in solvent for each wash. Do not use higher than 15% solvent, otherwise the column performance will be degraded irreversibly.

C. **Acid solutions such as 5 to 10 mM HCl can be used with compatible organic solvents to remove contamination that is ionic and hydrophobic.** The acid suppresses ionization and ion exchange interactions of the contamination with the resin. The organic solvent then removes the subsequent nonionic and hydrophobic contamination. See Section B above.

NOTE

A frequently used cleanup solution is 5 mM heptafluorobutyric acid in 10% acetonitrile. This solution must be made immediately before use because the acetonitrile will decompose in the acid solution during long term storage. Regardless of the cleanup solution chosen, use the following cleanup procedure in "Column Cleanup Procedure", to clean the ICE-AS6.

Column Cleanup Procedure

A. **Prepare a 500 mL solution of cleanup solution.** Use the guidelines in "Choosing the Appropriate Cleanup Solution".

B. **Disconnect the AMMS-ICE from the IonPac ICE-AS6 Analytical Column. Connect the ICE-AS6 directly to the pump.** Double check that the eluent flows in the direction designated on the column label. Direct the effluent from the outlet line of the ICE-AS6 to a separate waste container.

C. **Set the pump flow rate to 0.50 mL/min.**

D. **Pump the cleanup solution through the column for 60 minutes.**

E. **If your cleanup solution contains a solvent between 10 and 15%, use a gradual gradient which reaches a maximum solvent concentration after 30 minutes. Wash the column for 30 minutes and then ramp back down to the eluent over 15 to 30 minutes.**

G. **Reconnect the AMMS-ICE to the ICE-AS6 Analytical Column and connect the ICE-AS6 to the injection valve.**

H. **Equilibrate the ICE-AS6 with eluent before resuming normal operation.**