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Using the AS Automated Sampler in the Simultaneous, Sequential, and Concentrate Modes

INTRODUCTION

The AS Autosampler is enhanced with new features to speed throughput and simplify sample pretreatment. This technical note describes these features and how to select the AS operating mode that best suits the application. Each operating mode is demonstrated with an example application that includes step-by-step instructions for setting up the AS, summarizes expected performance, and provides troubleshooting tips.

The AS can perform injections using a sample loop or a concentrator column. Whenever a direct injection of 1–1000 µL will provide sufficient sensitivity for the analytes in a matrix, a sample loop should be used. The AS offers three modes for direct injection using a sample loop: Normal, Simultaneous, and Sequential.

• To make a direct injection onto a single-channel system (a system with only one injection valve and separation column), choose Normal mode. In the Normal mode, you can make a full-loop or a partial-loop injection. Details are provided in section A.1.

To make direct injections onto a dual-channel system (a system with two injection valves and two separation columns), the AS offers two approaches to increase sample throughput:

• In the Simultaneous mode, the AS uses a tee junction to split the sample equally from a single vial between two sample loops installed on two separate valves. These two equal portions of the sample are then injected simultaneously onto two separate systems or channels. Only full-loop injections are allowed in Simultaneous mode, but the two sample loops can be of different sizes. Simultaneous mode is used whenever two different analyses must be performed on a single sample. The throughput for both systems in the Simultaneous mode is determined by the application with the longest run time. Details of the Simultaneous mode are provided in section A.2.

• In the Sequential mode, the AS uses a diverter valve to direct the sample to one of the two systems. The samples are injected sequentially and injections are independent. Only full-loop injections are allowed in Sequential mode, but the two sample loops can be of different sizes. The Sequential mode is used whenever two independent applications must be performed on the same sample, or on different samples. Throughput for each system in the Sequential mode is independent of the other. Details of the Sequential mode are provided in section A.3.

When a sample volume greater than 1000 μL is required to achieve sufficient sensitivity for the analytes, or when matrix elimination is needed, a concentrator column should be used. The AS offers two modes for injection using a concentrator column.

- To use a concentrator on a single system, choose the AS Concentrate mode. In the Concentrate mode, 100 to 8000 μL can be concentrated depending on the sample syringe size. There are two options to choose from: (1) concentrate and inject; or (2) concentrate, flush the concentrator column with a solution, and inject. Details are provided in section B.1.

- To use two separate concentrators on two separate systems, select the AS Sequential Concentrate mode. In Sequential Concentrate mode, the AS uses a diverter valve to direct the sample to one of the two systems. The samples are concentrated sequentially and injections are independent. The throughput for each system in the Sequential Concentrate mode is independent of the other. Details of the Sequential Concentrate mode are provided in section B.2.

BEFORE GETTING STARTED
Software and Firmware Requirements
Sequential, Sequential Concentrate, and Concentrate with Reagent Flush modes are available to users of Chromeleon® 6.70 or higher. If using an AS50 (USB), ensure that firmware 2.0.0 has been installed on the autosampler in order to access all modes.

Program Wizard
All programs for the applications discussed below were generated using the Chromeleon program wizard. To ensure accurate syntax for the various features, use the program wizard to create new programs.

Autosampler USB Connection
Section B.3.8 of the AS autosampler manual (Doc#065051) details connection of the AS autosampler to the server via a USB connection. In an ICS-3000 system, the AS will typically be connected via an internal hub located on the DC detector compartment.

Syringe and Needle Assembly Installation Notes
When a syringe or needle assembly is replaced, ensure that the two are compatible. The larger syringes, 5 mL and 10 mL, require the 8.5-mL tubing assembly, while all smaller syringes require the 1.2-mL assembly. After installation, it is crucial to ensure that the syringe and tubing are free of air. The presence of air in the tubing can cause back flow through the injection port and increase carryover and other problems. Careful observation to see if there is any liquid on top of the inject port or on top of vials will help determine if there is a problem. Always prime the assembly and flush the lines according to section B.3.13 of the AS Autosampler manual (Doc#065051).

Inject Port Tubing Notes
Do not attempt to cut or extend the pink injection port tubing. Make sure that it is free from any places where it may become crimped, such as door hinges, because this may affect the delivery of samples.

Evaluation Criteria:
Good indicators of autosampler performance are peak area precision, sample carryover, and a linear relationship between amount actually injected and peak area. The applications discussed below include some or all of these measurements to provide a benchmark for expected performance. For example, a well performing AS doing full-loop injections should deliver peak area precision of <0.4% relative standard deviation (RSD) for seven repeated injections. Poor peak area precision is usually symptomatic of inadequate flushing, incomplete sample loop filling, or dilution of sample with flush water.

A. DIRECT INJECTION METHODS
1. Normal Mode
Choose Normal sample mode from the AS or AS50 (USB) System Parameters screen and enter the Loop Volume of the installed sample loop in the Plumbing Configuration screen. The autosampler provides three options to make precise injections from a variety of vial sizes. If the amount of sample is limited, two of these options allow the AS to inject a volume less than the sample loop volume: (1) partial-loop injection, and (2) partial loop, limited sample injection. Details on Normal mode injections can be found in the AS Autosampler Manual (Doc#065051), sections 3.12.4, 3.12.5, and 3.12.6.

To perform a full-loop injection, enter an Injection Volume into the Chromeleon Sequence that is equal to or greater than the Loop Volume entered on the autosampler Plumbing Configuration Screen. To flush the sample loop, the autosampler withdraws at least four times the volume of the sample loop from the vial. The volume injected is equal to the sample loop volume. If you prefer a larger flush volume, enter into the Sequence a volume greater than four times the Loop Volume. Full-loop injections deliver the best results in terms of reproducibility, carryover, and linearity.

To perform a partial-loop injection, enter an Injection Volume into the Chromeleon Sequence that is less than the Loop Volume entered on the autosampler Plumbing Configuration screen and enter a Cut Volume on the System Parameters screen that is greater than 0 μL. The autosampler withdraws the injection volume plus 2x the cut volume specified (settable from 1–30 μL). The amount injected is equal to the Injection Volume specified in the Sequence. For best results, specify an injection volume less than half the loop volume. (The cut volume is positioned before and after the sample. To deliver the best possible reproducibility, the cut volume is not injected, but is "cut" from the sample to be injected by the injection valve.

Partial-loop, limited sample is recommended only when necessary to withdraw the minimum amount of sample from the vial. To perform a partial-loop injection, enter an Injection Volume into the Chromeleon Sequence that is less than the Loop Volume entered on the autosampler Plumbing Configuration screen and enter a Cut Volume of 0 μL on the System Parameters screen. The autosampler withdraws and injects exactly the Injection Volume specified in the Sequence. For best results, specify an Injection Volume less than half the Loop Volume.

2. Simultaneous Mode

Use the Simultaneous mode to perform two different analyses on every sample. Simultaneous mode saves time both during the run and in sample preparation. Simultaneous mode allows full-loop injections only.

In Simultaneous mode, the AS uses a tee junction mounted to the interior wall of the AS to divide each sample between two injection valves. This tee is available as part of the Simultaneous Upgrade Kit (Dionex P/N 063742). Simultaneous mode requires a 5-mL (P/N 053915) or 10-mL (P/N 055068) sample syringe, Chromeleon 6.60 or higher and AS firmware 1.0.0 or higher, (AS50 (USB) firmware 2.0.0 or higher). Simultaneous injections are also possible using AS50 (USB) firmware 1.0.0 and Chromeleon 6.60, although the user interface is somewhat different from the AS Autosampler. See the AS50 (USB) manual.

As a demonstration application, we used an AS autosampler and a dual ICS-3000 system for Simultaneous determination of cations and anions. System preparation, operating conditions, and expected performance are summarized.

PREPARING THE SYSTEM

Install the AS as detailed in section 3.12.7 of the AS Autosampler manual (Doc#065051). From the AS front panel, enter the system parameters screen (press Menu, 5, 5) and change Sample mode to Simultaneous. Install a 5- or 10-mL syringe on the sample syringe pump and enter the corresponding syringe size into the AS on the Plumbing Configuration screen. Enter a flush and syringe speed of four. Syringe speed is set in the Chromeleon PGM. Route the pink inject-port tubing into the fitting at the back of the tee junction. Trim two pieces of 0.02-in. diameter orange PEEK tubing to the minimum length required to reach the injection valves. The length of the two tubes should be the same and, thus, must be equal to the line length needed to reach the valve of the system that is farthest away from the tee. Connect each arm of the tee to the sample port of each injection valve. Install a sample loop on each injection valve in the ICS-3000 DC and enter the largest of the two-sample loop volumes used on the Plumbing Configuration screen. Trim two pieces of 0.03-in. diameter green PEEK tubing to fit from each of the injection valves back to the autosampler drip tray. Each line should be the same length. Connect each one to the waste port on an injection valve. Before routing the waste lines to the AS drip tray, balance the backpressure through both arms of the tee junction by using the following procedure.

BALANCING BACKPRESSURES

Good injection accuracy and precision require that both sample loops be thoroughly flushed with sample during the LOAD operation. To ensure good AS performance, use a sample volume at least as large as recommended in the AS Autosampler Manual (Doc#065051, section 3.12.7) (550 μL + 5x sum of both sample loops). Balance the backpressure through both arms of the tee junction by using the following procedure to compare the amount of liquid flushed through each sample loop, and then add or subtract tubing as needed to balance the flows.

Fill the Flush reservoir with degassed deionized water, prime the sample syringe and needle assembly tubing to expel air bubbles, and program the AS to FLUSH 2 mL with both inject valves in the LOAD position. Collect the sample waste from each green waste line in separate 10-mL vials labeled "System 1" and "System 2". Weigh the amount of water in each vial. Repeat twice to check for consistency. The amount of water collected from the two arms of the tee should be 1.0 ± 0.1 g. If the difference between the

vials is greater than 0.2 g, then add a length of .010-in. i.d. tubing to increase the backpressure to the arm of the tee with the higher volume delivered, either between the tee and injection valve, or to the injection valve sample waste line.

After balancing the backpressure through the two injection valves, route the 0.03-in. green tubing waste lines back to the autosampler drip tray. Insert the waste line from the first injection valve into the small round opening at the right front corner of the drip tray. Insert the waste line from the second injection valve into the larger drain line opening. Position both waste line ends level with, or slightly below, the needle port top in order to minimize siphoning of the sample liquid.

Complete the setup of the anion application according to Dionex Application Note 135, Part B, and the cation application according to Dionex Application Note 141. Allow both systems to equilibrate.

Open the Chromeleon Server Configuration program and create a single timebase that includes the AS and other instrument modules. In Simultaneous mode, modules are not shared across multiple timebases. It is important to correctly link one EGC Cartridge, one injection valve, and one CD detector to each pump.

Create a new program by using the program wizard in Chromeleon. Pay particular attention to which system (1 or 2) each screen in the wizard pertain so that suppressor settings, flow rate, and gradients are set correctly. The program wizard detects to what mode the AS is set and so will default to the correct settings in the majority of cases. Use a FLUSH volume of 4000 μL.

Create a new Sequence and enter at least as much injection volume as recommended in the AS manual (550 μL + 5x sum of both sample loops) or 2000 μL as the injection volume (minimum recommended 800 μL). If there is sufficient sample, we recommend using the largest practical volume.

EQUIPMENT

ICS-3000 Ion Chromatography system consisting of:
    DP Dual Pump
    DC Detector Compartment with two CD
        conductivity cells
    AS Autosampler
    EG Eluent Generator
    Chromeleon 6.70 software

REAGENTS AND STANDARDS

Deionized (DI) water — 18 MΩ-cm or better
Magnesium chloride (1.48 g), potassium sulfate (2.08 g), sodium fluoride (3.40 g) salts (Fisher Scientific) in one liter DI water. Sample consists of 1/1000 dilution of this stock standard.

CHROMATOGRAPHY CONDITIONS

System 1:

Columns:	IonPac® CG16, 5 mm
	IonPac CS16, 5 mm
Column Temperature:	40 °C
Eluent:	30 mM MSA
Flow Rate:	1.0 mL/min
Loop Volume:	25 μL
Detection:	Suppressed conductivity CSRS® ULTRA II, 4 mm, AutoSuppression® recycle mode, 88 mA current
CD Cell Temperature:	45 °C
Expected Background:	<0.5 μS
Run Time:	25 min
Flush Volume:	4000 μL

System 2:

Columns:	IonPac AG14, 4mm
	IonPac AS14, 4mm
Column Temperature:	30 °C
Eluent:	3.5 mM CO_3^{2-}/ 1.0 mM HCO_3^{-} - EGC
Flow Rate:	1.2 mL/min
Loop Volume:	25 μL
Detection:	Suppressed conductivity AAES®, 4 mm, AutoSuppression recycle mode, 70 mA current
CD Cell Temperature:	35 °C
Expected Background:	17 μS
Run Time:	25 min
Flush Volume:	4000 μL

TYPICAL PERFORMANCE

AS performance was evaluated by measuring peak area precision and response. Peak area precision is expected to be better than 0.4% RSD for full-loop injections of 30 replicates. Poor peak area precision is usually symptomatic of inadequate flushing, incomplete sample loop filling, or dilution of sample with flush water. Table 1 shows that for n = 30 runs of the three anions tested, an area relative standard deviation (RSD) of between 0.17% and 0.32% was achieved. For n = 30 runs of the three cations tested, an area RSD between 0.19% and 0.28% was achieved. Peak area responses were comparable to normal, full-loop injections.

TABLE 1. PEAK AREA PRECISION OBTAINED FOR SIMULTANEOUS INJECTIONS OF ANIONS AND CATIONS		
Analyte	**Amount Injected (mg/L)**	**Precision RSD (%) (n = 30)**
Fluoride	1.5	0.17
Chloride	1.8	0.23
Sulfate	0.7	0.32
Sodium	1.8	0.19
Potassium	0.6	0.28
Magnesium	0.6	0.24

Sample carryover occurs when residual analytes from an injected sample appear in the chromatogram of the next sample run. Carryover is caused by inadequate flushing of the sampling needle assembly, sampling needle (interior and exterior), injection port, injection port tubing, tee junction, or sample loop. Similarly, because all of these components except the injection valve are shared by a dual system using Simultaneous mode, inadequate flushing of the shared components can cause sample crossover from one channel to the other channel of a dual system. Both sample carryover and sample crossover are measured by analyzing a concentrated standard followed by a deionized water sample. The amount of analyte (or peak area) appearing in the deionized water sample should be less than 0.01% of the analyte seen in the concentrated standard. If carryover or crossover exceeds 0.01%, increase the flush volume until carryover and crossover are reduced to a level acceptable for the application.

3. Sequential Mode

Sequential mode offers greater flexibility than Simultaneous mode. Use the Sequential mode to perform two analyses independently on different samples. For example, set up the same application on two channels of a dual system and use Sequential mode to double throughput. Or, set up different applications on each channel and use Sequential mode to efficiently process separate samples, with independent sample preparation steps and run times. The Sequential mode even allows one AS to service two independent systems. As with the Simultaneous mode, Sequential mode allows full-loop injections only.

In Sequential mode, the AS uses a diverter valve mounted inside the AS to divert samples, one at a time, to a specified injection valve. After the sequential injection, the runs continue in parallel at the same time. Using the AS in Sequential mode requires a diverter valve kit (Dionex P/N 063294) mounted inside the AS, Chromeleon 6.70 or higher, and AS firmware 1.0.0 or higher (AS50 (USB) firmware 2.0.0 or higher). Any size sample syringe can be used with Sequential mode, except for the 2.5-mL syringe size. Install the AS as detailed in section 3.12.9 of the AS Autosampler manual (Doc#065051).

As a demonstration application, we used an AS autosampler and a dual ICS-3000 system for sequential determination of carbohydrates and amino acids. System preparation, operating conditions, and expected performance are summarized.

PREPARING THE SYSTEM

Install the AS as detailed in section 3.12.9 of the AS Autosampler manual (Doc#065051). From the AS front panel, enter the system parameters screen (press Menu, 5, 5) and change sample mode to Sequential. Enter the correct sample syringe size (the application cited here uses a 1-mL syringe, P/N 055066) and flush speed of 3. Typical flush speeds for sequential injection should be 2 or 3. Use speed 2 for a loop size of 2.5 μL. These 2.5-μL loops should be made from a 4-in. length of .007-in. i.d. PEEK tubing (violet color) P/N 063405. Tubing of .005-in. i.d. in a 2.5-μL loop will provide too much backpressure for the AS sample delivery system. Route the pink inject-port tubing into the "s" position on the diverter valve. Install a 25-μL sample loop on one injection valve in the ICS-3000 DC and a 10-μL loop on the second valve. Enter the greater of the two sample loop volumes (25 μL) on the plumbing configuration screen. Trim two pieces of 0.03-in. diameter green PEEK tubing to fit from each of the injection valves back to the autosampler

drip tray. Each line should be the same length. Connect each one to the waste port on an injection valve. Route them to the AS drip tray and insert the waste line from the first injection valve into the small round opening at the right front corner of the drip tray and insert the waste line from the second injection valve into the larger drain line opening. Trim two pieces of 0.013-in. diameter blue PEEK tubing to the minimum length to fit to the injection valves. The length of the two tubes should be the same and must be equal to the line length needed to reach the valve of the system that is farthest away from the tee. Connect each line to positions 1 and 2 on the diverter valve. Calibrate the total inject-port tubing volume according to section 5.9 of the AS Autosampler Manual (Doc#065051), and then connect to the injection valves. Double check the plumbing by setting the diverter valve to position 1; FLUSH with 1 mL of water and make sure that water exits from the correct injection valve.

Important! Pay particular care to the calibration of the inject-port tubing volume. Incorrectly measuring or entering this value will cause poor precision and accuracy.

Complete the setup of the carbohydrate application according to Dionex Technical Note 40 and the amino acids application according to Dionex Application Note 163.

In server configuration, create two timebases and add the AS to one of them. On the AS Properties screen, choose the "sharing" tab of the AS, and select the two timebases that are to share the AS. Other instruments can be configured in the same way, except for the DP. To share the DP, navigate to the "devices" tab and assign each pump to the correct timebase.

Create a new program for each system, by using the program wizard in Chromeleon. The program wizard learns from the Server Configuration that the AS is in Sequential mode and introduces three new sets of commands to synchronize sharing between the two timebases. Modify the default commands, if necessary, as explained.

SEQUENTIAL COMMANDS
1) Sampler.AcquireExclusiveAccess
By default, this command is placed at the start of each program so that the selected timebase will acquire exclusive access to the AS. The AS will ignore commands from the other timebase and will not be available to begin another run until exclusive access is relinquished. By default, the command Sampler.ReleaseExclusiveAccess is placed 5 min

after injection. Change the time for release of exclusive access as appropriate, but do not change to less than 2 min. Remember that making it longer than 5 min will further delay the start of the next run on the opposite channel.

2) Compartment TC.AcquireExclusiveAccess
This command is used for ICS-3000 Thermal Compartments only. In a dual zone DC there is an additional command, Column TC.AcquireExclusiveAccess. By default, the program wizard checks the Detector Compartment properties in the Server Configuration to see which timebase is assigned thermal control of each TC and automatically places this command at the start of the program for that timebase—no command is placed in the program of the other timebase. The command to release exclusive access is placed at the end of the program.

3) Sampler DiverterValve.Position 1
The default is Position 1; change to Position 2 as appropriate.

Create a new Sequence for each system and insert the desired parameters as with normal mode injections.

EQUIPMENT
ICS-3000 Ion Chromatography system consisting of:
    DP Dual Pump
    DC Detector compartment with two ED electro chemical cells
    AS Autosampler
    EG Eluent generator
    Equipment control and data processing performed with Chromeleon 6.70 software

REAGENTS AND STANDARDS
Deionized (DI) water — 18 MΩ-cm or better
Sodium hydroxide 50% (w/w) aqueous solution (Fisher Scientific)
Threonine (Sigma T-8375)
MonoStandard, mix of six (Dionex P/N 043162)

CHROMATOGRAPHY CONDITIONS
System 1:

Columns:	CarboPac™ PA20 and guard
Temperature:	30 ˚C
Eluent:	10 mM KOH (from EG)
Flow Rate:	0.5 mL/min
Loop Volume:	10 μL

Detection: Integrated pulsed amperometric detection (IPAD) using standard quadruple potential waveform (Dionex Technical Note 40)

Background: 23 nC

Run Time: 30 min

Flush Volume: 250 μL

System 2:

Columns: AminoPac PA10 and Guard, 2 mm

Temperature: 30 °C

Eluent: 50 mM NaOH

Flow Rate: 0.25 mL/min

Loop Volume: 25 μL

Detection: Pulsed amperometric detection (PAD) using waveform from Technical Note 163

Background: 50 nC

Run Time: 10 min

Flush Volume: 250 μL

ELUENT PREPARATION

Pipette 2.62 mL 50% (w/w) NaOH into 500 mL degassed deionized water. Bring to 1 L by the careful addition of degassed DI water.

Plumb, calibrate, and set the autosampler according to section 3.12.9 of the AS Autosampler Manual (Doc#065051).

TABLE 2. PEAK AREA PRECISION OBTAINED FOR SEQUENTIAL INJECTIONS OF MONOSACCHARIDES AND THREONINE		
Analyte	Amount Injected (pmol)	Precision RSD (%) (n = 30)
Fucose	100	0.47
Galactosamine	100	1.27
Glucosamine	100	0.91
Galactose	100	0.32
Glucose	100	0.52
Mannose	100	0.79
Threonine	125	0.54

TYPICAL PERFORMANCE

AS performance was evaluated by measuring peak area precision and sample carryover. Table 2 shows that for n=30 runs of the six carbohydrates tested, an area relative standard deviation (RSD) of between 0.47% and 1.3% was achieved. For n=30 runs of threonine, an area RSD of 0.54 was achieved. The precision for both applications is in line with the precision described in the application notes.

Sample carryover can occur if the sampling needle assembly, sampling needle (interior and exterior), injection port, injection port tubing, diverter valve, or sample loop are not adequately flushed between injections. Because all of these components except the injection valve are shared by a dual system using Sequential mode, inadequate flushing of the shared components can cause sample crossover from

TABLE 3. SAMPLE CARRYOVER AND CROSSOVER OBTAINED FOR SEQUENTIAL INJECTIONS OF MONOSACCHARIDES AND THREONINE				
Analyte	Carryover		Crossover	
	Amount Injected (pmol)	Carryover (%)	Amount Injected (pmol)	Crossover (%)
Fucose	10,000	0.013	25,000	0.001
Galactosamine	10,000	0.021	25,000	0.001
Glucosamine	10,000	0.016	25,000	0.001
Galactose	10,000	0.013	25,000	0.002
Glucose	10,000	0.016	25,000	0.002
Mannose	10,000	0.011	25,000	0.002
Threonine	10,000	0.004	5,000	0.001

one system to the next. Sample carryover and sample crossover were measured by analyzing a concentrated standard followed by a deionized water sample of equal volume. The results are summarized in Table 3. The amount of analyte (or peak area) appearing in the deionized water sample should be less than 0.01% of the analyte seen in the concentrated standard. If carryover or crossover exceeds 0.01%, increase the flush volume until carryover and crossover are reduced to a level acceptable for the application. In this example, the carryover ranged from about 0.01–0.02% when the flush volume between sample injections was 250 µL, and was considered acceptable for this application.

B. CONCENTRATE METHODS

1. Normal Concentrate Mode

When low detection limits are required, sometimes injections of up to 1000 µL do not provide enough sensitivity. Furthermore, interference from other compounds in the matrix can obscure the peak of interest. Concentrate mode is a new feature that enables the AS to concentrate directly onto a TAC-ULP1 column. The AS can inject the concentrated sample or perform a reagent flush to eliminate the matrix before injecting. The Concentrate feature simplifies and improves sample concentration by eliminating the extra pump, sample loop, and valve required by previous approaches.

Using the AS in Concentrate mode requires Chromeleon 6.70 or higher, and AS firmware 1.0.0 or higher (AS50 (USB) firmware 2.0.0 or higher). To use the AS in Concentrate mode requires a 1-, 5-, or 10-mL sample syringe. All syringe speeds are allowable with the 1-mL sample syringe; only use speeds 1 or 2 with a 5- or 10-ml sample syringe. Syringe speed is set in the Chromeleon PGM. Install the AS as detailed in section 3.12.8 of the AS Autosampler manual (Doc#065051).

USING CONCENTRATOR COLUMNS

The maximum pressure limit for the sample syringe is 100 psi. Pressures above this may cause leaking around the needle port or may reduce the lifetime of the sample syringe. The pressure should remain well below 100 psi if a syringe speed of 1 or 2 is used with a 5- or 10-mL sample syringe and the TAC-ULP1 concentrator column. Dionex does not recommend using a syringe speed greater than 2 in Concentrator mode or concentrators other than the TAC-ULP1. When using a concentrator other than that specified here, be sure to check that the backpres-

sure produced on the syringe does not exceed 100 psi. Refer to section 3.14 of the AS Autosampler manual (Doc#065051) for further details.

To minimize contamination, thoroughly rinse vials, caps, and septa with DI water, wear gloves when handling sample vials, and ensure that the system is well flushed prior to use. Use a plastic flush reservoir, soak it in DI water overnight before first use, and fill it daily with degassed deionized water. Install the TAC-ULP1 so that sample elution is in the direction opposite to sample loading, as diagrammed in the AS autosampler manual. For more information on using concentrator columns, see Technical Note 8.

As a demonstration application, trace anion concentration was performed using a TAC-ULP1 concentrator on an ICS-3000 system. System preparation, operating conditions, and expected performance are summarized below.

PREPARING THE SYSTEM

From the AS front panel, enter the system parameters screen (press Menu, 5, 5) and change sample mode to Concentrate. Install a 1-, 5-, or 10-mL sample syringe and enter the corresponding syringe size into the AS on the Plumbing Configurations screen. Enter a flush speed of 4 in the AS system parameters screen.

Install the consumables for each system, and allow the systems to equilibrate for about one hour under the run conditions specified above.

Create a new program through Chromeleon program wizard. A Concentrate command will be included with the inject valve set to the Load position. Create a sequence and specify an injection volume of 6900 µL.

EQUIPMENT

ICS-3000 Ion Chromatography system consisting of:
- DP Dual Pump
- DC Detector compartment with two CD conductivity cells
- AS Autosampler
- EG Eluent generator
- Equipment control and data processing performed with Chromeleon 6.70 software

REAGENTS AND STANDARDS

Deionized (DI) water — 18 MΩ-cm or better

Dionex Seven Anion Standard-II 5000-fold with DI water (P/N 057590)

CHROMATOGRAPHY CONDITIONS

Columns:	IonPac AG18, 4 mm
	IonPac AS18, 4 mm
Column Temperature:	30 ˚C
Eluent:	23 mM KOH - EGC
Flow Rate:	1.0 mL/min
Volume Concentrated:	6900 uL
Detection:	Suppressed conductivity ASRS ULTRA II, 4 mm AutoSuppression Recycle mode, 57 mA current
CD Cell Temperature:	35 °C
Expected Background:	<1.0 μS
Run Time:	30 min
Flush Volume:	9999 μL

TYPICAL PERFORMANCE

Performance of the AS for this example application is summarized in Table 4. For most of the anions tested, the peak area precision was better than 1% (RSD). Precision was slightly worse for formate because of low-level contamination. Sample carryover after injection of a concentrated chloride standard was consistently under 0.1%.

TABLE 4. PEAK AREA PRECISION OBTAINED IN NORMAL CONCENTRATE MODE		
Analyte	**Amount Injected (µg/L)**	**Precision RSD (%) (n = 7)**
Fluoride	2.5	0.61
Acetate	12.5	0.70
Formate	5	2.17
Chlorite	25	0.29
Chloride	15	0.23
Nitrite	25	0.24
Bromide	50	0.38
Sulfate	50	0.31
Nitrate	50	0.32

2. SEQUENTIAL CONCENTRATE MODE

Sequential Concentrate mode allows all the trace-level benefits of working with a concentrator column and combines it with the time saving gained by using Sequential mode. In Sequential Concentrate mode, the AS uses a diverter valve mounted inside the AS to concentrate samples, one at a time, on a TAC-ULP1 concentrator column mounted in the sample loop position of a specified injection valve. The AS can further treat the TAC-ULP1 through Reagent Prime, Reagent Flush, and other commands, or simply inject the concentrated sample. After the sequential injection, the runs continue at the same time in parallel.

Using the AS in Sequential mode requires a diverter valve kit (Dionex P/N 063294) mounted inside the AS, Chromeleon 6.70 or higher, and AS firmware 1.0.0 or higher (AS50 (USB) firmware 2.0.0 or higher). To use the AS in Concentrate mode requires a 1-, 5-, or 10-mL sample syringe. All syringe speeds are allowable with the 1-mL sample syringe; only use speeds 1 or 2 with a 5- or 10-mL sample syringe. Syringe speed is set in the Chromeleon PGM. Install the AS as detailed in section 3.12.8 and 3.12.9 of the AS Autosampler Manual (Doc#065051).

USING CONCENTRATOR COLUMNS

The maximum pressure limit for the sample syringe is 100 psi. Pressures above this may cause leaking around the needle port or may reduce the lifetime of the sample syringe. The pressure should remain well below 100 psi if a syringe speed of 1 or 2 is used with a 5- or 10-mL sample syringe and the TAC-ULP1 concentrator column. Dionex does not recommend using a syringe speed greater than 2 in Concentrator mode or concentrators other than the TAC-ULP1. When using a concentrator other than that specified here, be sure to check that the backpressure produced on the syringe does not exceed 100 psi. Refer to section 3.14 of the AS Autosampler Manual (Doc#065051) for further details.

To minimize contamination thoroughly rinse vials, caps, and septa with DI water, wear gloves when handling sample vials, and ensure that the system is well flushed prior to use. Use a plastic flush reservoir, soak it in DI water overnight before first use, and fill it daily with degassed deionized water.

Install the TAC-ULP1 so that sample elution is in the direction opposite to sample loading, as diagrammed in the AS autosampler manual. For more information on using concentrator columns, see Technical Note 8.

As a demonstration application, we performed trace anion concentration using a TAC-ULP1 concentrator on a dual ICS-3000 system, using hydroxide and carbonate eluents. System preparation, operating conditions, and expected performance are summarized below.

PREPARING THE SYSTEM

Install the AS as detailed in section 3.12.9 of the AS Autosampler manual (Doc#065051). From the AS front panel, enter the system parameters screen (press Menu, 5, 5) and change sample mode to Sequential + Concentrate and flush speed to 2 or 3. Enter the Plumbing Configuration screen and change sample syringe size to match the installed syringe (the application cited here uses a 1-mL syringe, P/N 055066).

Route the pink inject-port tubing into the "s" position on the diverter valve. Install a TAC-ULP1 concentrator column on each injection valve in the ICS-3000 DC. Trim two pieces of 0.03-in. i.d. green PEEK tubing to fit from each of the injection valves back to the autosampler drip tray. Each line should be the same length. Connect each one to the waste port on an injection valve. Route them to the AS drip tray and insert the waste line from the first injection valve into the small round opening at the right front corner of the drip tray, and insert the waste line from the second injection valve into the larger drain line opening.

Trim two pieces of 0.013-in. i.d. blue PEEK tubing to the minimum length to fit to the injection valves. The length of the two tubes should be the same and must be equal to the line length needed to reach the valve of the system that is farthest away from the tee. Connect each line to positions 1 and 2 on the diverter valve. Calibrate the total inject-port tubing volume according to section 5.9 of the AS Autosampler manual (Doc#065051), and then connect to the injection valves. Double check the plumbing by setting the diverter valve to position 1, FLUSH with 1 mL of water, and make sure that water exits from the correct injection valve.

Install the consumables for each system and complete the application setup according to the conditions specified below.

In Server Configuration, create two timebases and add the AS to one of them. On the AS Properties screen select the "sharing" tab of the AS, and select the two timebases that are to share the AS. Other instruments can be configured in the same way, except for the DP. To share the DP, navigate to the "devices" tab and assign each pump to the correct timebase.

Create a new program for each system by using the program wizard in Chromeleon. The program wizard learns from the Server Configuration that the AS is in Sequential mode and introduces three new sets of commands to synchronize sharing between the two timebases. Modify the default commands, if necessary, as explained.

SEQUENTIAL COMMANDS

4) Sampler.AcquireExclusiveAccess

By default, this command is placed at the start of each program so that the selected timebase will acquire exclusive access to the AS. The AS will ignore commands from the other timebase and will not be available to begin another run until exclusive access is relinquished. By default, the command Sampler.ReleaseExclusiveAccess is placed 5 min after injection. Change the time for release of exclusive access as appropriate, but do not change to <2 min, and remember that making it longer than 5 min will further delay the start of the next run on the opposite channel.

5) Compartment_TC.AcquireExclusiveAccess

This command is used for ICS-3000 Thermal Compartments only. In a dual zone DC there is there is an additional command, Column TC.AcquireExclusiveAccess. By default, the program wizard checks the Detector Compartment properties in the Server Configuration to see which timebase is assigned Thermal Control of each TC and automatically places this command at the start of the program for that timebase. No command is placed in the program of the other timebase. The command to release exclusive access is placed at the end of the program.

6) Sampler DiverterValve.Position 1

The default is Position 1. Change to Position 2 as appropriate.

Create a new Sequence for each system and insert the desired parameters as with normal mode injections.

Important! Pay particular attention to the calibration of the inject-port tubing volume. Incorrectly measuring or entering this value will lead to greater variability of results and a decrease in recovery.

EQUIPMENT

ICS-3000 Ion Chromatography system consisting of:
- DP Dual Pump
- DC Detector compartment with two CD Conductivity Cells
- AS Autosampler
- EG Eluent generator
- Equipment control and data processing performed with Chromeleon 6.70 software

REAGENTS AND STANDARDS

Deionized (DI) water — 18 ⬜⬜-cm or better

CHROMATOGRAPHY CONDITIONS

System 1:

Columns:	IonPac AG19, 4 mm
	IonPac AS19, 4 mm
Column Temperature:	30 °C
Eluent:	10–45 mM KOH (EG)
Flow Rate:	1.0 mL/min
Loop Volume:	1000 uL
Detection:	Suppressed conductivity ASRS ULTRA II, 4 mm AutoSuppression Recycle mode, 117 mA current
CD Cell Temperature:	30 °C
Expected Background:	<0.5 μS
Run Time:	30 min + 7 min equilibration
Flush Volume:	5000 μL

System 2:

Columns:	IonPac AG14, 4 mm
	IonPac AS14, 4 mm
Column Temperature:	30 °C
Eluent:	9 mM CO_3^{2-} (EG)
Flow Rate:	0.25 mL/min
Injection Volume:	1000 μL
Detection:	Suppressed conductivity AAES, 4 mm, AutoSuppression Recyle mode, 100 mA current
CD Cell Temperature:	40 °C
Expected Background:	~23 μS
Run Time:	25 min
Flush Volume:	5000 μL

TYPICAL PERFORMANCE

Performance of the AS Autosampler for the AS19 application is summarized in Tables 5 and 6. For n=30 runs of the seven anions tested, we achieved peak area relative standard deviations (RSD) between 0.39% and 1.34%. Sample carryover after injecting a concentrated standard was under 0.02% for chloride, phosphate, and sulfate. Other anions were not detectable in the carryover. Sample crossover from the AS14 system to the AS19 system was less than 0.01% for all anions.

TABLE 5. PEAK AREA PRECISION OBTAINED FOR SEQUENTIAL CONCENTRATION OF ANIONS ON AS19 AND AS9-HC (AS19 RESULTS)		
Analyte	Amount Injected (µg/L)	Precision RSD (%) (n = 30)
Fluoride	17	1.34
Chloride	80	0.5
Nitrite	80	0.63
Bromide	80	0.43
Nitrate	80	0.39
Phosphate	170	1.07
Sulfate	80	0.80

TABLE 6. SAMPLE CARRYOVER AND CROSSOVER OBTAINED FOR SEQUENTIAL CONCENTRATION OF ANIONS ON AS19 AND AS9-HC				
Analyte	Carryover		Crossover	
	Amount Injected (mg/L)	Carryover (%)	Amount Injected (mg/L)	Crossover (%)
Chloride	3.3	0.002	100	0.007
Phosphate	6.6	0.016	100	0.015
Sulfate	3.3	0.006	100	0.005

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