Configuring the Dionex Integrion HPIC System for Fast Determinations of Monosaccharides and Disaccharides Using HPAE-PAD with Eluent Generation

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Key Words

Standard bore, carbohydrates, integrated pulsed amperometric detection, CarboPac PA20 column, RFIC, Reagent-Free IC, electrochemical detection

Goal

Provide application installation instructions to install a fast carbohydrate method using HPAE-PAD on a Thermo Scientific™ Dionex™ Integrion™ HPIC™ system.

Introduction

The latest advancement in ion chromatography (IC) instrumentation, the high-pressure Thermo Scientific Dionex Integrion HPIC system, can operate continuously at 5000 psi for both 4 mm and 2 mm i.d. column formats while using electrolytic eluent generation. The system can take full advantage of the high-efficiency separations offered by smaller particle size separation columns. When combined with the advantages and ease-of-use of a Reagent-Free[™] (RFIC[™]) system, the Dionex Integrion system permits excellent enhanced resolution of closely eluting peaks with excellent reproducibility, thereby yielding greater quantification accuracy and consistently reliable results. The primary focus of this technical note is to provide recommended configuration and installation instructions for the Dionex Integrion HPIC system to achieve fast separations of electro-active analytes at analytical flow rates. Herein, the Dionex Integrion HPIC system is configured for high-performance anionexchange (HPAE) separations with pulsed amperometric detection (PAD) using an electrochemical cell with disposable working electrode for fast determinations of mono- and disaccharides.

The Dionex Integrion HPIC system has new features designed to increase ease-of-use and increase data reliability:

- Separate compartments for the pump, detector (with electrochemical cell), and columns to allow separate temperature control in the other two compartments while installing in the third
- Thermo Scientific[™] Dionex[™] Chromeleon[™]
 Chromatography Data System (CDS) software features automatic device detection to provide easier instrument configuration



- Consumables device tracking capability to allow tracking of electrolytic devices and columns and checks for potentially incompatible format of the installed devices
- Chromeleon CDS software embedded troubleshooting and consumables installation information and videos
- Independent tablet control provides full screen monitoring and allows manual system control

Equipment

- Thermo Scientific Dionex Integrion HPIC system using electrochemical detection includes:
 - Electrochemical Detector (ED)
 - Electrochemical cell, Reference Electrode and Disposable Working Electrode with Gasket
 - Detector Compartment Temperature Control
 - Tablet Control
 - Vacuum Degas Kit, P/N 075522
- Thermo Scientific™ Dionex™ AS-AP Autosampler with temperature control option no trays, (P/N 074926) with AS-AP 1.5/0.3 mL Vial Tray (P/N 074936) and Vial Kit, 1.5 mL Polypropylene with Caps and Septa, 100 each (P/N 079812) or Vial Kit, 0.3 mL Polypropylene with Caps and Septa, 100 each (P/N 055428). Alternatively, AS-AP 10 mL Vial Tray (P/N 074938) and Vial Kit, 10 mL Polystyrene with Caps and Blue Septa, 100 each (P/N 074228)



Software

Thermo Scientific Dionex Chromeleon CM 7.2 SR4 CDS software was used.

The consumables part numbers are shown in Table 1.

 ${\it Table 1. Consumables for the Dionex Integrion HPIC System.}$

| Product Name | Device Description | Part Number |
|---|---|-------------|
| Thermo Scientific™ Dionex™ IC PEEK Viper™ fitting tubing assembly kits | Dionex IC PEEK Viper fitting tubing assembly kit for the Dionex Integrion HPIC system with ED detection: Includes one each of P/Ns: 088805-088807, 088809, 088811 | 088797 |
| Dionex IC PEEK Viper Fitting Tubing Assemblies (Included in Kit; P/N | Guard to separator column: 0.007 × 4.0 in (102 mm) | 088805 |
| | Valve to guard column: 0.007 × 5.5 in (140 mm) | 088806 |
| | EGC Out to CR-TC Eluent In: 0.007 × 6.5 in (165 mm) | 088807 |
| | 0.007×7.0 in (178 mm) – not used in ED | 088808 |
| 088797) | Separator to ED Cell In: 0.007×7.0 in (178 mm), ED | 088809 |
| | 0.007×9.0 in (229 mm) – not used in ED | 088810 |
| | CR-TC Out to Degasser In: 0.007×9.5 in (241 mm) | 088811 |
| Dionex AS-AP Autosampler Vials | Package of 100, polystyrene vials, caps, blue septa, 10 mL | 074228 |
| 4-Port Injection Valve Pod | Install in place of 6-port valve pod. The 4-port pod has an internal sample loop of 4 μ L | 074699 |
| Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator Cartridge* | Eluent generator cartridge when using 4 μm particle columns | 075778 |
| Thermo Scientific™ Dionex™ CR-ATC™ 600 Electrolytic Trap Column* | Continuously regenerated trap column used with Dionex EGC KOH 500 cartridge | 088662 |
| Dionex HP EG Degasser Module* | Degasser module | 075522 |
| Thermo Scientific™ Dionex™ CarboPac™ PA20 Guard | Guard column, 3 mm i.d. | 060144 |
| Dionex CarboPac PA20 Column | Separation column, 3 mm i.d. | 060142 |
| Electrochemical Detector (ED) | Included with Integrion ED. P/N if purchased separately. | 072042 |
| Electrochemical Cell | Includes knob and support block | 072044 |
| Ag/AgCl Reference Electrode | Reference electrode | 061879 |
| Au on PTFE Electrodes | Working electrode, package of six (used for flavored rum liquor; Figure 9) | |
| Carbohydrate Working Electrodes, Au on PEN | Working electrode, package of six (used for Agave nectar sample; Figure 10) | 060139 |
| High Concentration Carbohydrate Analysis Kit | Includes two 62 mil gaskets and matching spacer block for the electrochemical flow cells (used for flavored rum liquor; Figure 9) | |
| 62 mil Gasket | If purchased separately, package of two (used for flavored rum liquor; Figure 9) | |
| 2 mil Gasket | If purchased separately, package of two PTFE gaskets (used for agave nectar sample; Figure 10) | |
| pH Buffer, pH 7 | Reference electrode pH calibration standard | SB107-500** |
| pH Buffer, pH 10 | Reference electrode pH calibration standard | SB115-500** |
| Thermo Scientific™ Dionex™ OnGuard™ II RP Filter Cartridges | L Dovorcod phace cartridge for manual cample proparation (197). | |

 $^{^{\}star}$ High-pressure device recommended for 4 μm particle resin columns.

^{**} Fisher Scientific P/N

| Chromatographic Conditions | | | |
|----------------------------|--|--|--|
| Columns | Dionex CarboPac PA20 guard (3 \times 30 mm) and separation, 3 \times 150 mm | | |
| Eluent | 35 mM KOH; 100 mM KOH wash (see chromatograms for timing) | | |
| Eluent Source | Dionex EGC 500 KOH cartridge with Dionex CR-ATC 600 trap column and high-pressure degasser | | |
| Flow Rate | 0.5 mL/min | | |
| Column Temperature | 30 °C | | |
| Detector Temperature | 30 °C | | |
| Injection Volume | 0.4 μL Internal loop in 4-port injection valve | | |
| Detection | PAD, 4-Potential Carbohydrate Waveform, 2 Hz (Table 2) | | |
| Reference Electrode | Ag/AgCl | | |
| Working Electrode | Au on PTFE, 62 mil gasket | | |

Table 2. 4-Potential carbohydrate waveform (Waveform A*).

| Time (s) | Potential (V) | Integration |
|----------|---------------|-------------|
| 0.00 | +0.10 | - |
| 0.20 | +0.10 | Begin |
| 0.40 | +0.10 | End |
| 0.41 | -2.0 | - |
| 0.42 | -2.0 | - |
| 0.43 | +0.6 | - |
| 0.44 | -0.1 | - |
| 0.50 | -0.1 | - |

^{*}Waveform A is discussed in TN 21.1

Instrument Setup and Installation

The Dionex Integrion HPIC high-pressure-capable system is a Reagent-Free IC (RFIC) system configured for electrochemical detection. The Dionex Integrion HPIC system, Dionex EGC 500 KOH cartridge, HP EG degasser, and Dionex CR-ATC 600 electrolytic trap column are designed for high-pressure conditions up to 5000 psi.

To install this application, connect the Dionex AS-AP autosampler and the Dionex Integrion HPIC system modules as shown in Figure 1.

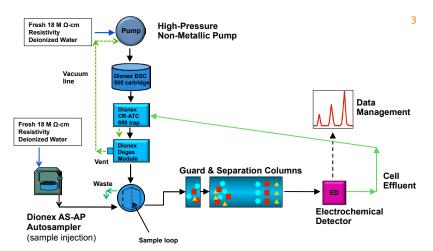


Figure 1. Flow diagram for the Dionex Integrion HPIC system.

Connect the USB cables from the Dionex Integrion HPIC system to the Dionex AS-AP autosampler and to the computer. Open the column compartment door and remove the black ring on the 6-port injection valve. Remove the 6-port valve pod by pulling it straight out. Then, align the notches of the 4-port valve pod to match those of the removed 6-port valve pod and install the 4-port valve pod in the position previously occupied by the 6-port valve pod. Connect the power cables and turn on the instrument and the autosampler.

Configuring the Modules in Chromeleon CDS Software

The configuration for each module is summarized in Table 3.

Table 3. System configuration for Dionex Integrion HPIC system.

| Tab | Action | Result | | |
|------------------------------------|--|---|--|--|
| Integrion HPIC Module | | | | |
| General | Link to USB address | - | | |
| Pump | - | Flow rate and pressure limitations are displayed | | |
| Detectors | - | Automatically detected | | |
| Electrolytic Devices | Automatically detects Dionex eluent generator cartridges Dionex CR-ATC 600 trap columns (Figure 3) | | | |
| Inject Device | - Automatically detected | | | |
| Thermal Controls | - | Automatically detects thermal control options for column, detector, and suppressor | | |
| High-Pressure Valves | - | Automatically detected | | |
| Low-Pressure Valves | - | Automatically detected | | |
| Options | - | Automatically detects Pump Degasser and Seal Wash pump | | |
| Pump Wellness Module | | | | |
| Devices | Click on Devices check box | Activates pressure monitoring feature | | |
| Add Dionex AS-AP Autosampler | | | | |
| Dionex AS-AP Autosampler Module | Add module Link to USB address | Adds and links module to instrument configuration | | |
| Sharing | - | Only if more than one instrument is detected. If this option is present, select Instrument | | |
| Segments / Pump Link | - | Select 10 mL polystyrene vials or 1.5 mL vials for "Red", "Blue", and "Green" | | |
| Options | - | Select Push, select syringe size, select 1.2 mL buffer line, enter the loop size (0.4 µL if using an internal loop) | | |

Electronic Configuration

To configure the system, first start the Chromeleon Instrument Controller program and then select the link, Configure Instruments, which starts the Chromeleon Instrument Configuration Manager. Right-click on the computer name, select Add an Instrument, and enter an appropriate name (for example: Dionex Integrion ED). Select Add a Module, IC: Dionex Integrated Modules, and Integrion HPIC System (Figure 2).

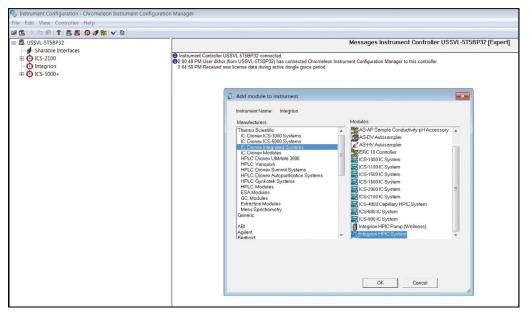


Figure 2. Creating a configuration.

A multi-tabbed program will automatically open (Figure 3). Select *Model Serial Number* to connect. The Chromeleon CDS software automates the system configuration process by automatically detecting the installed devices, including all Dionex Integrion HPIC system devices: the electrolytic devices (Figure 3), detectors, pump degasser, and seal wash (not shown), thereby minimizing data entry during configuration.

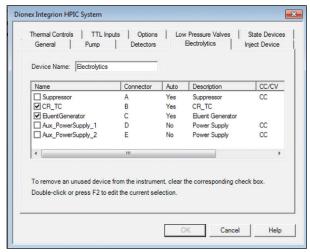


Figure 3. Automatic Detection of electrolytic devices.

To add pressure monitoring capabilities in the configuration, right-click and select *Add a Module, IC: Dionex Integrated Modules, Integrion HPIC Pump Wellness* module and then select the USB address to link the module to the configuration. Select the Devices tab and click on the Pressure Signal(s) box (Figure 4).

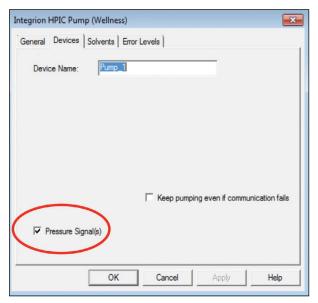


Figure 4. Adding the HPIC Pump Wellness module to the configuration.

Adding Dionex AS-AP Autosampler to the Configuration

Add the Dionex AS-AP autosampler as a module, and select the USB address. In the Segments/Pump Link tab, select the appropriate vial trays for each color zone. In the Options tab, select *Push*, installed syringe size, 1.2 mL for buffer line, and 0.4µL for the sample loop volume. Save the configuration, select check the configuration, and then close the Chromeleon Instrument Configuration program.

Plumbing the High-Pressure Dionex Integrion HPIC System

Tip: To achieve the best chromatography, use the IC PEEK Viper fitting assemblies in the following locations: 1) from the Dionex eluent generator cartridge to the Dionex CR-ATC 600 trap column, 2) from the Dionex CR-ATC 600 trap column to the degas module, 3) from the injection valve to the columns, 4) between the guard and separator columns, 5) from the separator column to the ED cell. Gently tighten IC PEEK Viper fittings to fingertight plus 1/8 clockwise turn with the first use (1/16 clockwise turns after first use).

The Dionex Integrion HPIC system can be run with manually prepared eluents (up to 6000 psi) or RFIC applications at pressures from 2000 to 5000 psi. (Note: Additional pressure can be added for applications that generate < 2000 psi.) The Dionex Integrion system has separate compartments for the pump, detector (with electrochemical cell), and columns to allow separate temperature control in two compartments while installing in the third.

To plumb the high-pressure Dionex Integrion HPIC system, first loosen the waste lines, including the metal-wrapped waste line, in the back of the instrument and direct the free ends to a waste container. Then, connect the pump eluent line to the eluent bottle containing deionized (DI) water previously degassed (vacuum filtration and ultrasonic agitation). To prime the pump, first open the priming knob ¼ turn and press the priming button. Prime the pump until no bubbles are visible and water is flowing at a steady rate out of the pump waste line. Stop the pump and close the priming knob to finger-tight and close the priming waste line. Then, turn the pump back on for use. For more information, review the product manual on the tablet.

Important: Do not remove consumables tracking device tags from the consumable devices. These tags are required for monitoring functionality.

Conditioning Electrolytic Devices

Install the Dionex EGC 500 KOH cartridge and Dionex CR-ATC 600 Continuously Regenerated Anion Trap Column in the Integrion Reservoir Tray compartment. Condition the devices according to instructions in the *Consumables, Install* drop-down menu (Figure 5) (also available in the product manuals and the system installation manual.²⁻⁴). Install a 4 to 6 in. length of black PEEK (0.010 in. i.d.) tubing at the cell outlet when using 4 and 5 mm i.d. columns. (Note: Use a ~ 4 in. length of red PEEK (0.005 in. i.d.) tubing when running 2 and 3 mm i.d. columns.)

0

0

Inventory

Install a CRD on Pump_ECD Install an EGC on Pump_ECD

Install a column on Pump_ECD

<Autogenerated>

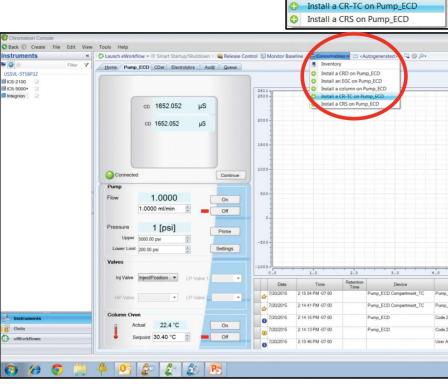


Figure 5. Consumables online installation instructions.

Install Vacuum Line to Degasser Module Vent

Hydroxide eluents require vacuum degassing inline to achieve the optimum analysis conditions for HPAE-PAD. Vacuum degassing is accomplished by connecting the vent line of the degas module to the vacuum pump port on the back of the instrument (Figure 7). In the Dionex Integrion HPIC system, the vacuum pump connector is preinstalled, which eliminates the manual installation used in earlier systems.

To assist in degassing the eluent, install a ¼ in. i.d. air tubing from the degas module vent port to the vacuum connection in the back of the Dionex Integrion HPIC system (Figure 6).



Figure 6. Vacuum connection.

Conditioning Columns

Important: Do not remove consumables tracking device tags on the columns. These tags must remain on the devices to ensure that the Consumables Device Monitoring features are operational.

Condition the columns for 30 min according to the instructions from the *Consumables, Install Column* section (Figure 5). The general practice is to follow the eluent and flow rate conditions listed in the QAR (Quality Assurance Report) while directing the eluent exiting the column to a waste container. Complete the installation according to flow diagram in Figure 1.

Installing and Optimizing the Dionex AS-AP Autosampler

The Dionex AS-AP autosampler needle must be aligned to the injection port. To align the autosampler needle, first select the Sampler tab on the instrument panel, open the align programming by pressing the *Align Tray* button. Follow the commands to align the autosampler needle to the Injection Port and Wash Port (Section B.12 in the Operator's Manual). Then, plumb the wash container containing degassed water to the syringe.

Prime the syringe to flush out any air in the Buffer Wash line and syringe. Initially select a 5000 µL wash volume until a steady flow of water is observed at the Wash Port. Then, calibrate the transfer line volume by following the prompts on the TLV Calibration button. This volume will be recorded automatically. For more information, review Section 5.9 in the Thermo Scientific Dionex IC Series AS-AP Autosampler Operator's Manual.⁷

Electrochemical Cell

Tip: Always wear gloves when handling the electrochemical cell. If this is a new ED cell, disassemble the cell and discard the shipping gasket.

Caution: Do not touch the working electrode with any paper products, as this can contaminate the working electrode.

The ED cell is a three-electrode cell: the cell body as the counter electrode, a reference electrode (pH-Ag/AgCl or PdH), and a working electrode (conventional or disposable). The installation procedures are thoroughly discussed in the ED User's Compendium for Electrochemical Detection.6 The fully assembled cell also includes a yoke block assembly to tighten the cell and a gasket for the working electrode. The installation procedures below describe an electrochemical cell with a disposable working electrode; however, the procedures are similar when using a conventional working electrode. Different gaskets are specified depending on the application. Additionally, a support block is needed when using a disposable working electrode, whereas the conventional working electrode is already installed in a support block.

Note: It is important to use 18 M Ω -cm resistivity DI water for standards, eluent, and autosampler flush solution. Degassing is recommended for DI water intended for eluent used for carbohydrate determinations. (An appropriate degassing method is vacuum filtration.) Using DI water with resistivity less than 18 M Ω -cm can reduce sensitivity, introduce contamination, and affect calibration, thereby resulting in inaccurate quantification. Contamination introduced from samples can affect the chromatography.

Installing the Disposable Electrode

Install the disposable working electrode with the metal face down over the gasket and then install the support block firmly over the working electrode. Install the yoke block by squeezing the tabs and sliding it on the cell body. Align the yoke block parallel to the cell body and rotate the yoke block knob clockwise until you hear three "clicks". The cell with a conventional working electrode is assembled similarly with appropriate gasket. The support block is not needed with the conventional working electrode.

Installing the Electrochemical Cell with a pH-Ag/AgCl Reference Electrode

First, condition the pH-Ag/AgCl reference electrode by removing the storage cap, rinsing the electrode with DI water to remove the potassium chloride solution, and then placing the electrode in a pH 7 buffer solution. Then, calibrate the pH-Ag/AgCl reference electrode (see the Calibrating the Reference Electrode section below). The installation procedures are thoroughly discussed in the ED User's Compendium for Electrochemical Detection.⁶ Now, insert the reference electrode into the cell; make sure that fitting plugs are not installed on the cell inlet and outlet fittings to avoid any hydraulic pressure buildup. Verify that the pH-Ag/AgCl reference electrode O-ring is present and then screw the pH-Ag/AgCl reference electrode into the reference electrode well and tighten it to finger-tight. Orient the cell assembly with the yoke knob on the left and then push the cell onto its mounting location on the ED. If the detector is already installed, connect the reference electrode cable and the cell cable.

Calibrating the Reference Electrode

To calibrate the reference electrode, select pH buffer 7 and the corresponding buffer for the application—pH 10 for basic eluents and pH 4 for acidic eluents. Install the cell into the ED module and connect the yellow cable to the yellow port. Install the reference electrode blue cable into the black port. Immerse the reference electrode in pH 7 buffer to at least mid-level of the electrode. Select the "pH Calibration" button on the ED panel and follow the instructions to calibrate the electrode including using pH 10 buffer. The results appear in the audit trail. Install the reference electrode in the ED flow cell and connect the reference electrode lead to the ED.

Completing the Plumbing

Complete the installation by removing the temporary waste line from the column and installing the 9 in. piece of blue PEEK tubing (P/N 071870) to the column outlet. Allow liquid to flow from the end of the tube and then connect the free end to the cell inlet. Wait 60 s before tightening the connection. Allow liquid to flow through the cell until it flows out the cell outlet. Install the cell in the ED detector module and connect the reference electrode cable (blue to black) and the counter/working electrode cable (yellow to yellow). When using the pH-Ag/AgCl reference electrode, first turn-on the cell using the ED panel and then download the application waveform. Check for leaks by periodically touching an absorbent tissue under the cell. Allow the system to equilibrate until a stable baseline is observed.

Starting the Dionex Integrion HPIC System

To start the system, turn on the pump and immediately turn on both the Dionex EGC 500 KOH eluent generator cartridge and the Dionex CR-ATC trap when liquid is flowing through the device. The system backpressure is dependent on the flow rate and type of column, but the system must be above 2000 psi to support the Dionex EGC cartridges. Typically, columns with 4 µm resin particles have standard operating pressures above 2000 psi and, therefore, do not require backpressure tubing.

However, columns with larger size resin particles will require additional backpressure to maintain a system pressure of > 2000 psi. Set the eluent concentration and the column oven, compartment oven, and cell temperatures as shown in the Chromatographic Conditions section. Allow the system to equilibrate for 30 min. For optimum chromatography, equilibrate until the total background is stable and within ±5 °C of the expected background.

Creating an Instrument Method

To create a new instrument method using the Chromeleon Wizard, select *Create, Instrument Method* and select *Instrument*. Enter the values from the Chromatographic Conditions section and those in Table 4. Save the instrument method.

Table 4. Additional conditions to create a program.

| Page Title | Mode | Action |
|--------------------|---------------------------|--------------------------------------|
| | Injection Mode | Push Full |
| Sampler Options | Loop Overfill | 10 |
| | Injection Wash Property | After Injection |
| | Accept Recommended Values | Click on button |
| | Wait for Temperature | Click box |
| EDet Options | Reference | pH-Ag/AgCl |
| | Select Waveform | Au, 4-Potential Carbohydrate |
| | рН | Lower limit = 10 Upper limit = 14 |
| | Data Collection Rate | 2.00 Hz |
| | Autozero | Yes |

Continuous pH Monitoring

pH is an important parameter in electrochemical detection. Unexpected changes in pH can indicate an unstable reference electrode or improperly manually prepared eluents. To implement continuous monitoring of the pH, open the script section of the method, create an empty row after EDet. Autozero, insert Virtual Channel as the command line, and enter the commands as shown in Figure 7. The pH readings will be visible as one of the channels with each data file. Save the instrument method.

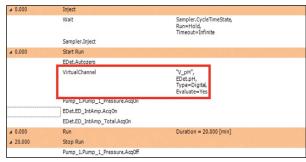


Figure 7. Script commands for continuous pH monitoring.

Consumables Device Monitoring

Important: The sequence will not start if Consumables Device Monitoring detects new devices or incompatibility in the devices.

A new feature of the Dionex Integrion HPIC system is consumables device monitoring tracking. This feature automatically detects the columns and electrolytic devices. Review and approval of the devices is required to start the first sequence on the Dionex Integrion HPIC system and when new consumables are installed. To access this approval, select Consumables and select Inventory (Figure 8). The device monitoring shows the device history, tracking: Part No., Size, Serial Numbers, Manufacture Lot, installed location (On Device), and Best if Use by Date (Figure 9, top highlighted section). Additionally, the device monitoring will provide warnings if there is incompatibility in the devices installed (Figure 8, bottom left). To start the sequence, correct any errors, Approve, and Close page (Figure 8, bottom right). Then select the Instrument Queue tab, and conduct a Ready Check on the sequence and Start.

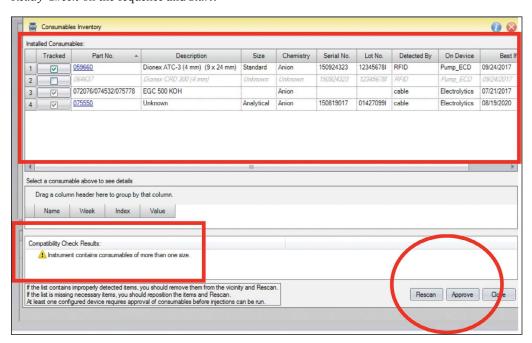


Figure 8. Consumables tracking.

Results and Discussion

Two samples, a flavored rum liquor and an agave nectar, were analyzed for mono- and disaccharides using a Dionex Integrion HPIC system with electrochemical detection. The flavored rum liquor sample was diluted 100× with DI water and the agave nectar sample was diluted 10,000× with DI water. Samples were filtered prior to the injection on the system.

A Dionex CarboPac PA20 strong anion-exchange column was used for this application. The pellicular resin structure of the column consists of 6.5 µm diameter nonporous beads covered with functionalized latex, which allow for excellent mass transfer, resulting in high-resolution chromatography and rapid re-equilibration. Figure 9 shows the sugars separated when a 100x dilute sample of a flavored rum liquor is analyzed on the Dionex CarboPac PA20 column. Glucose, fructose, and sucrose are well resolved and easily quantifiable with concentrations of 302 mg/L, 279 mg/L, and 1080 mg/L, respectively.

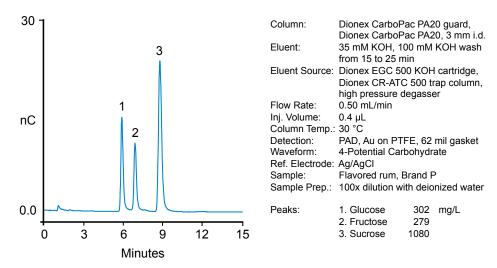


Figure 9. Mono- and disaccharides in a flavored rum liquor sample using a high concentration carbohydrate analysis kit.

Figure 10 shows the sugars separated when a 10,000× diluted sample of agave nectar is run on a Dionex CarboPac PA20 column. Glucose and fructose are all well resolved and easily quantifiable, with glucose at a concentration of 5.1 mg/L and fructose at 24 mg/L.

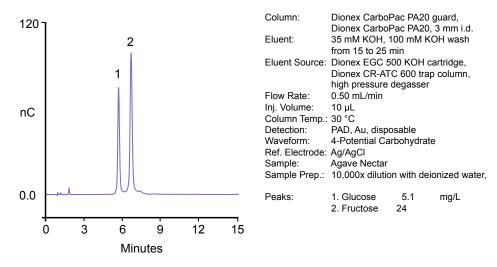


Figure 10. Mono- and disaccharides in agave nectar sample.

Conclusion

This technical note provides installation recommendations for a Dionex Integrion HPIC system configured for electrochemical detection. The study also demonstrates the use of a Dionex CarboPac PA20 column with a Dionex Integrion system with an electrochemical detector for the analysis of rum liquor and agave nectar samples for simple sugars.

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