# PRODUCT MANUAL

## for

# InGuard™ Cartridges

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# Product Manual

# for

# InGuard™ Cartridges

InGuard Ag (P/N 074038)
InGuard H (P/N 074037)
InGuard Na (P/N 074036)
InGuard HRP (P/N 074034)
InGuard Na/HRP (P/N 074035)



Document No. 065345
Revision 01
November 2009

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# SECTION 1 – INTRODUCTION

InGuard™ products are designed for in-line sample pretreatment. An autosampler or pump is used to pass the sample through the cartridge to remove matrix components. The InGuard chemistries are suitable for matrix elimination of a wide variety of sample types. Matrix elimination is a method of sample preparation that removes certain matrix species from a sample, typically by specifically binding matrix species to a solid phase. Interfering compounds can be removed before the analyte determination. The number of samples that can be treated with an InGuard product depends on the matrix composition, concentration of compounds to be removed and the sample injection volume. The cartridges have very low backpressure but the chemistries usually function optimally at flow rates between 0.5 and 2 mL/min. Since these products treat samples before chromatographic separation, they are suitable for both standard bore and microbore IC systems.

The InGuard line of sample pretreatment cartridges includes five chemistries in 9 x 24 mm PEEK cartridges. The cartridges have 10-32 end fittings and can be used with standard fittings in an ion chromatography (IC) system. The InGuard cartridges contain some OnGuard phases, e.g., Ag, H, Na and some newly developed phases, e.g., HRP and Na/HRP. The cartridges can be used singly or in series for multiple chemistries in an IC system. In this automated process, only the sample injection volume is treated for analysis. The cartridge size has been optimized for volume and capacity so that, depending on the application, one cartridge will treat from about 40 to more than 200 samples in an automated method. The limited sample volume of generally from 5 to 100 µL which is treated and then trapped in a concentrator, is far less sample than that necessary in comparable offline, e.g. OnGuard, methods.

# SECTION 2 – CARTRIDGES AND USAGE

## 2.1. Cartridge Overview

This manual describes recommended automated sample pretreatment procedures and examples of use for each of the cartridge types. A few minutes of pre-washing can be accomplished in the IC system on startup so manual cleaning is not necessary. Several valve configurations are provided. Selection of a valve configuration depends on the available hardware and on the matrix elimination chemistry used in the application.

Table 1 provides an overview of the InGuard cartridge products and Table 2 provides guidance on expected capacity.

**Table 1**
**Recommended Cartridge Applications**

| Cartridge | Functional Group(s) | Mode of Use | Example Applications |
|---|---|---|---|
| Ag | Ag+-form sulfonate | Ion Exchange | Removal of halides |
| Na | Na+-form sulfonate | Ion Exchange | Removal of cations |
| H | H+-form sulfonate | Ion Exchange | Removal of cations<br>pH adjustment |
| HRP | Hydrophilic divinylbenzene | Adsorption, π- π bonding | Removal of hydrophobic species, azo and cyano-containing species |
| Na/HRP | Dual Functionality | Ion exchange (Na) and Adsorption | Remove $Ca^{2+}$ (Na) and lipids (HRP) from dairy |

**Table 2**
**InGuard Capacity**

| Cartridge Type<br>9 x 24 mm PEEK | meq/cartridge |
|---|---|
| Ag | 5-5.5 |
| H | 5-5.5 |
| Na | 5-5.5 |
| HRP | 2 g resin |
| Na/HRP | 50%Na/50% HRP |

### 2.1.1. Example calculation of resin capacity

A 1% sodium chloride sample contains 10 g NaCl/L sample. Of this 10 g NaCl, 35.45/58.45 is chloride, or 6.1 g. To convert to mEq, 6.1 g chloride/ 35.45 g/mole = 0.17 mol or 170 mmol or 170 mEq/1000 mL or 0.17 mEq/mL. A 100 µL injection of 1% NaCl contains 0.017 mEq chloride. A cartridge containing 5 mEq of Ag+ can treat a maximum of 5/0.017 = 290 samples of 1% NaCl. Other matrix components such as bromide and phosphate will also use some of this capacity. Some of the silver chloride precipitate will re-dissolve, as discussed in the application. The figure below shows the amount of chloride remaining after treatment, from a sample containing 250 ppm chloride, compared to no sample treatment.

The cartridge inlet is marked with the product label and must be connected towards the loading device as it contains a flow distributor. The outlet contains a standard bed support to contain the resin in the cartridge.

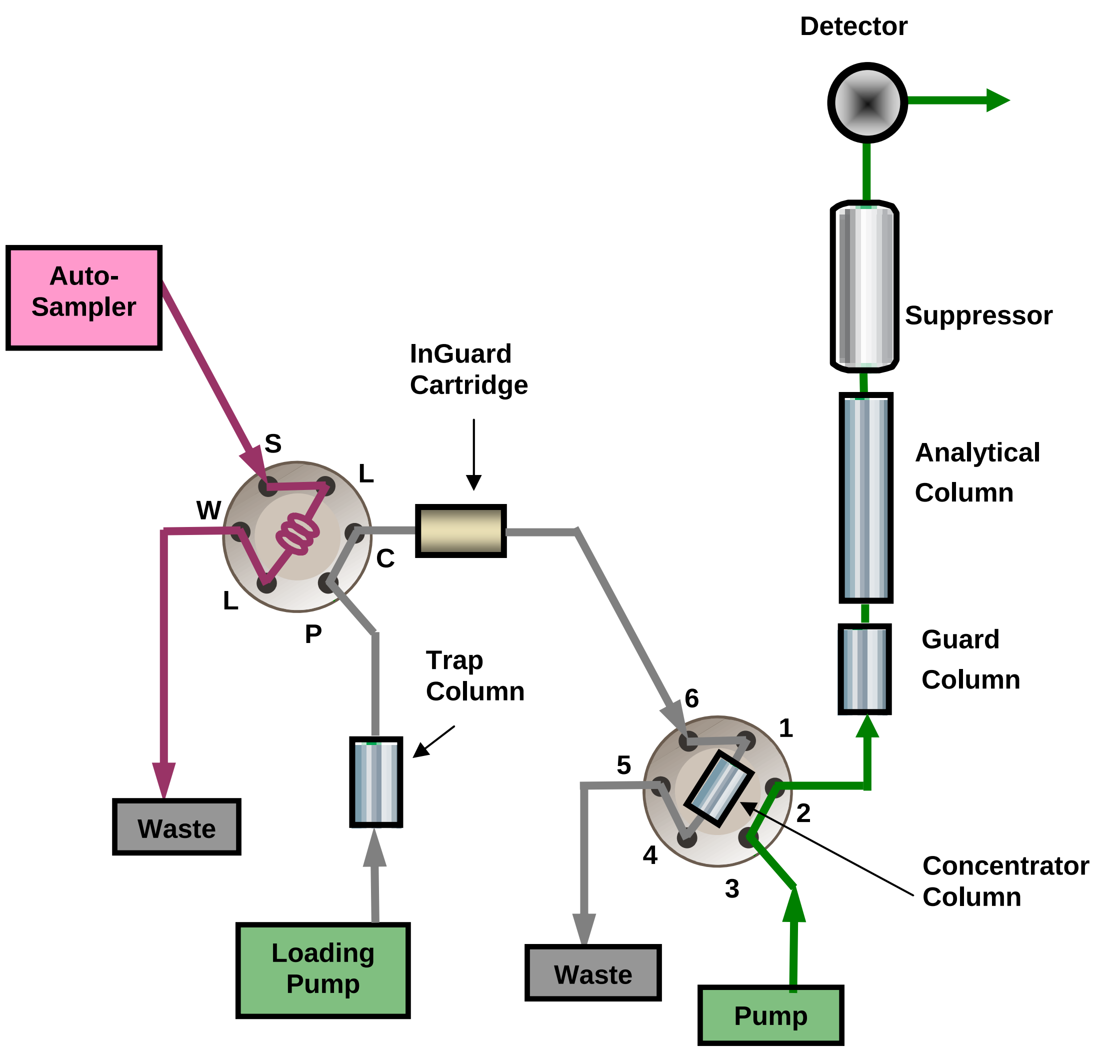
**CAUTION** ***Flow must always be in the indicated direction; otherwise, damage to the chromatographic system may occur.***

## 2.2. System Configurations

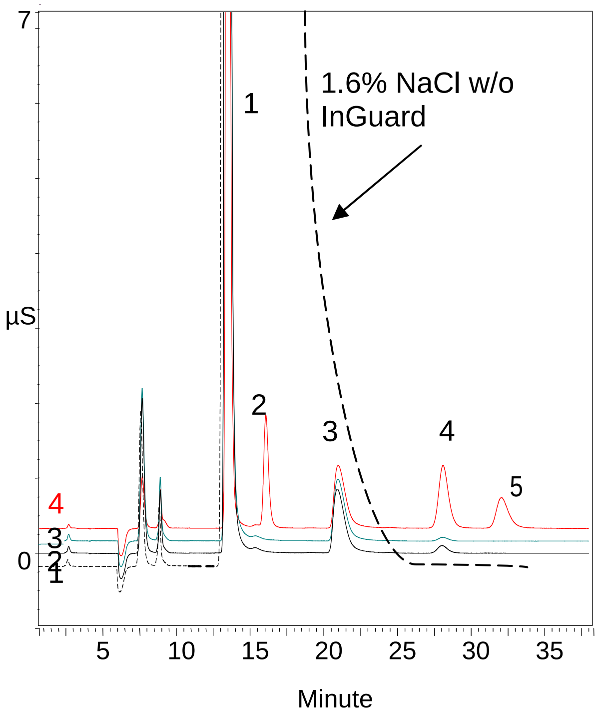
Optimum performance is achieved when the InGuard cartridge(s) is used to treat the contents of an injection loop and the treated sample is then loaded onto a concentrator column. This configuration is shown in Figure 1 and can be accomplished on any IC with two 6-port, a 6-port and a 10-port, or two 10-port valves. By using a defined loop volume and a concentrator column, the need to time sample preparation in order to obtain an undiluted injection volume is avoided. In addition, smaller sample volumes can be treated thus extending the life of the InGuard cartridge.

**Figure 1**
**Preferred Configuration 1:**
**Two 6-port Valves and Two Pumps**



**Column:** IonPac AG/AS15, 4-mm
**Eluent:** 23 mM KOH
**Flow rate:** 1 mL/min
**Sample Prep:** InGuard Ag and Na
**Injection vol:** 100 uL
**Detection:** Suppressed conductivity, ASRS 300




**Sample**
1.6% NaCl blank without InGuard
1.6% NaCL blank with InGuard Ag and Na
Water blank with InGuard
Std of 2ppm nitrite, sulfate, nitrate in 1.6% NaCl using InGuard

| Peaks: | mg/L |
|---|---|
| Chloride | |
| Nitrite | 2 |
| Carbonate | |
| Nitrate | 2 |
| Sulfate | 2 |

The key aspects of the system shown in Figure 1 include:

1. Two independently controlled valves such as 2 injection valves or 2 high pressure auxiliary valves from an ICS-3000 DC module; or an injection valve and auxiliary valve in an ICS-1100/1600/2100 (ICS-1100 series) instrument.
2. Two pumps such as from an ICS-3000 DP; or ICS-3000 SP or an ICS-1100 series instrument plus an AXP pump. The second pump is a used to load the loop contents through the InGuard column and onto the concentrator. The backpressure of the InGuard and concentrator columns together should be under 120 psi.
3. An ATC-3 column (for anion analysis) or an Electrolytic Water Purifier (EWP, P/N 071553) is used to clean the water used to load the concentrator. If an EWP is used the loading pump is not required.
4. Sample volume is controlled by the autosampler/injection loop with a pressure limit of about 100 psi.
5. Eluent (DI water) reservoirs can be pressurized with 5 psi of clean Helium or Nitrogen in order to minimize blanks.
6. Note that flow across the concentrator column to the analytical column should be in the reverse direction to the flow across the concentrator when it is being loaded. This minimizes peak broadening in the analysis.

Figure 2 shows a plumbing configuration where the cell effluent is recycled for sample prep. The Electrolytic Water Purifier (EWP) is used to clean the cell effluent water for trace work.

**Figure 2**
**Preferred Configuration 2:**
**Two 6-port Valves, one pump and Electrolytic Water Purifier**

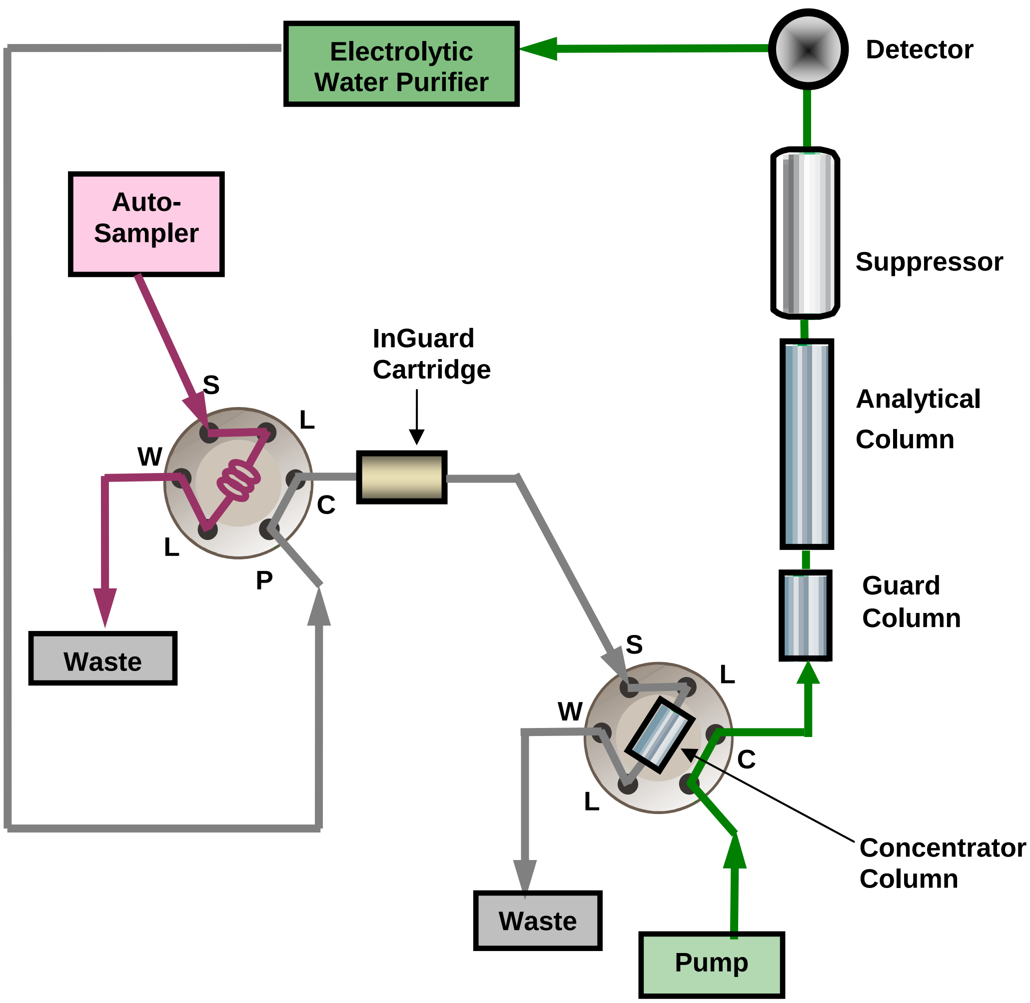


Figure 3 shows a plumbing configuration where a 10-port valve replaces the second of the 6-port valves in Figure 1. In this arrangement water does not flow over the InGuard cartridges between runs so the re-dissolution of silver halide precipitates (formed during the removal of halides in the matrix) is much reduced. This configuration usually produces a higher sulfate blank than the constant flow configurations.

**Figure 3**
**Preferred Configuration 3:**
**One 6-port and One 10-port Valve and Two Pumps with Stopped Flow**

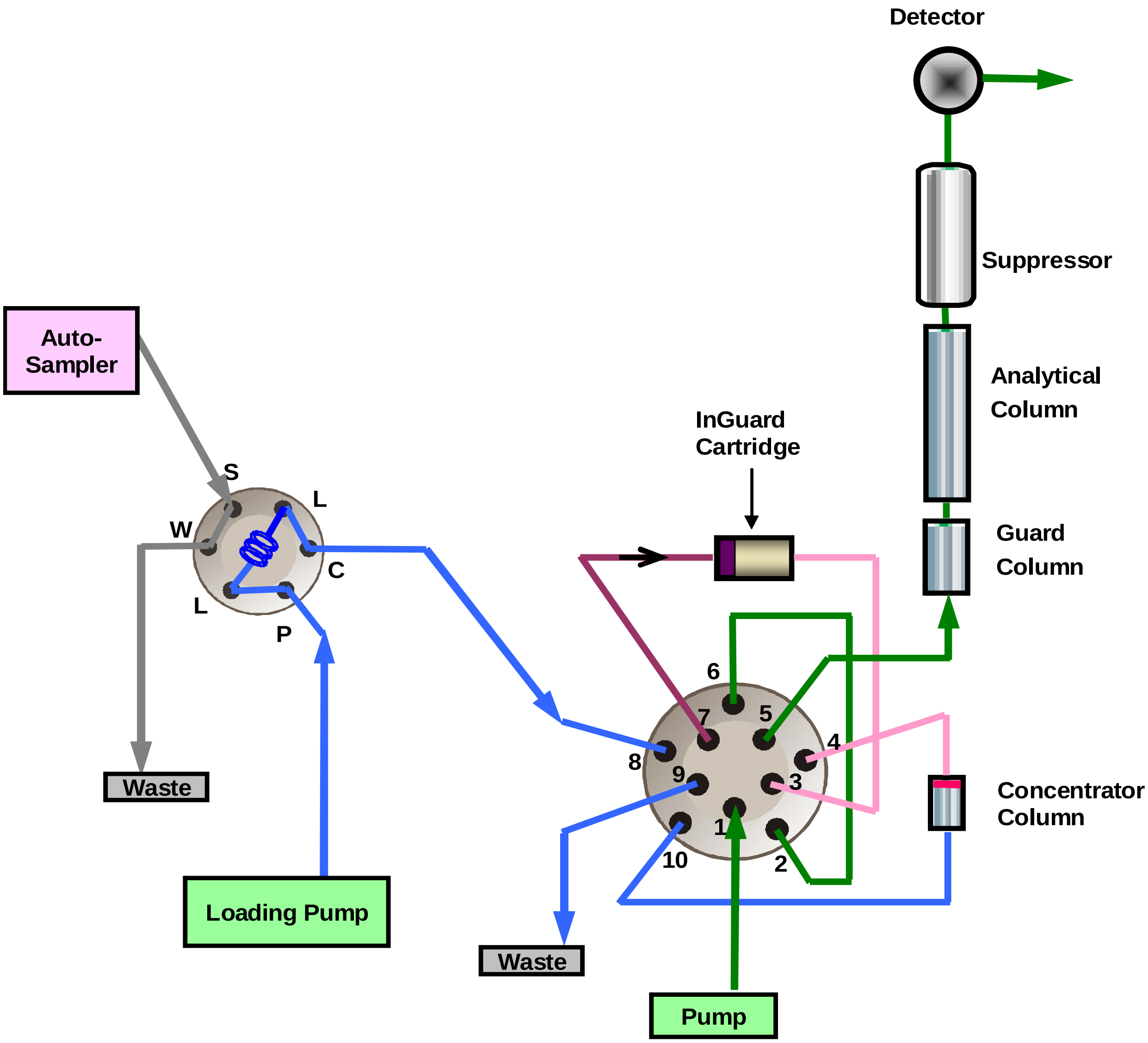
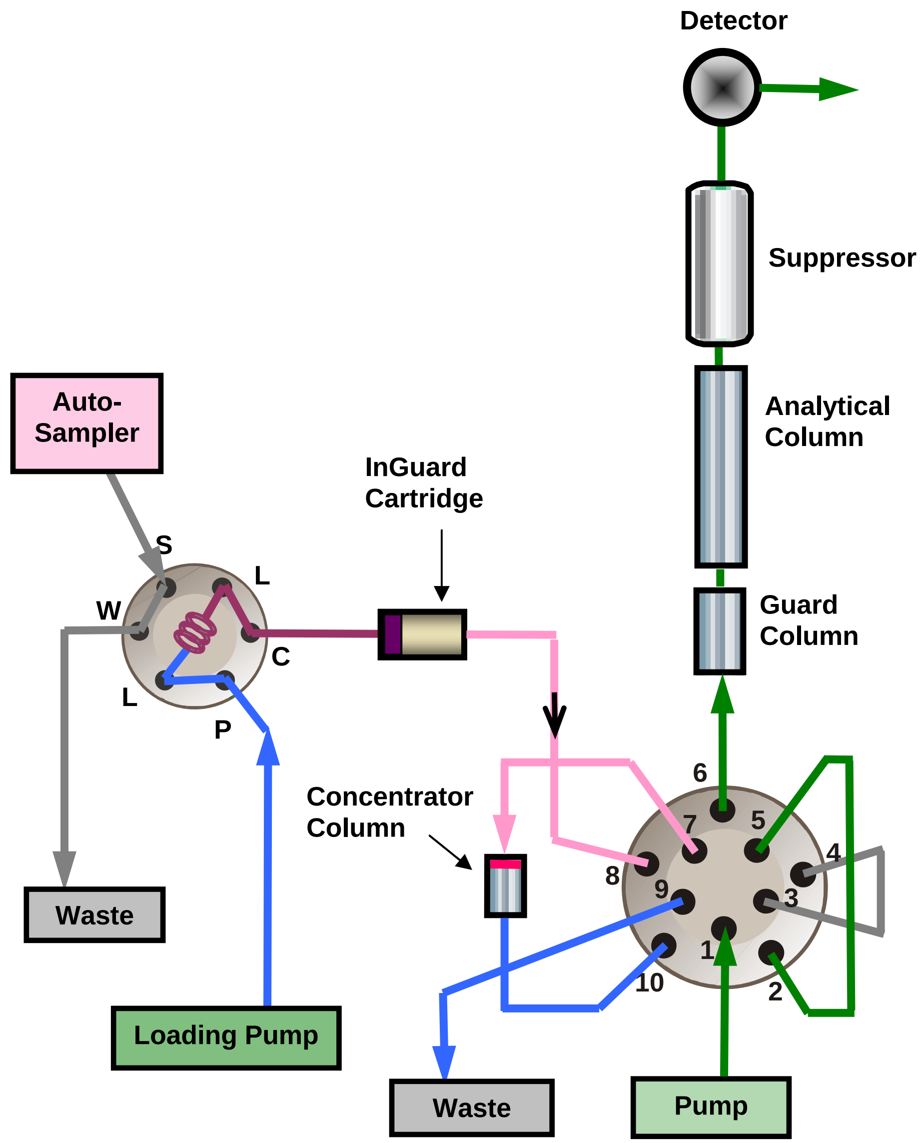


**Figure 4**
**Preferred Configuration 4:**
**One One 6-port and One 10-port Valve and Two Pumps with Constant Wash**

This configuration is used to minimize sulfate blank although the chloride peak will be larger than when a configuration with stopped flow is used.



## 2.3. Use of Concentrator Columns

The use of concentrator columns in this system allows the collection of all treated sample ions for analysis using standard injection volumes. InGuard cartridges have sufficiently low backpressure to be used on an AS autosampler directly in line with the analytical columns, but more sample must be treated in order to obtain an undiluted sample segment for analysis.

## 2.4. Standard InGuard Use Conditions

The InGuard cartridge format is a 9 x 24 mm I.D PEEK column body with 10-32 end fittings for easy installation in an IC system. The cartridges contain approximately 2.2 g of resin. The backpressure generated at 2 mL/min flow is less than 50 psi which is compatible with the backpressure limits of syringe-type autosamplers including the AS and AS-DV. Depending on the exact chemistry and types of samples treated, some cartridges can be regenerated.

The cartridge inlet is marked with the product label and must be connected towards the loading device as it contains a flow distributor. The outlet contains a standard bed support to contain the resin in the cartridge.

***Backwards flow will result in loss of resin, plugging of the chromatograph and possible damage to it.***

**NOTE**

The volume necessary for complete sample loading through the InGuard cartridge(s) and onto the concentrator column can be determined by an experiment as shown in Table 3. Enough volume needs to be pumped through the InGuard cartridge to displace the entire sample, while at the same time displacing the minimum possible amount of the trapped matrix.

This is particularly noticeable when using an Ag cartridge to remove halides. Silver halides will redissolve in excess loading water so only enough transfer water should be used to fully transfer the sample. For halide removal with tandem Ag and Na cartridges in series with a 100 µL sample loop, a loading volume of 2-4 mL is sufficient.

**Table 3**
**Optimizing Sample Prep Time using Two InGuard Cartridges**

| InGuard Ag and Na cartridges with 100 µL sample loop | Loading volume (mL) | Peak Area (µS*min) 50 ppb Nitrate in 16,000 ppm Chloride | Peak Area (µS*min) Chloride residual from 16,000 ppm Chloride |
|---|---|---|---|
| | 1 (0.5 mL/min x 2 min) | 0 | 3 |
| | 1.5 | 0.022 | 7 |
| | 2.0 | 0.028 | 19 |
| | 4.0 | 0.025 | 36 |

**Table 4**
**General use parameters for InGuard cartridges.**

| Type | Pump 2 Loading Solvent | AS* and Pump 2 Maximum Flow rate (mL/min) | Void volume (µL) | Expected Backpressure (psi) |
|---|---|---|---|---|
| Ag | DI water | 2 | 850 | 50 |
| H | DI water | 2 | 850 | 50 |
| Na | DI water | 2 | 850 | 50 |
| HRP | 100% DI water to 100% solvent | 2 | 850 | 50 |
| Na/HRP | DI Water | 2 | 850 | 50 |

* AS Syringe Speed setting of 2, 3 or 4 is recommended.

## 2.5. InGuard Ag, Na and H

InGuard Ag cartridges contain 40 µm diameter Ag+ form sulfonated styrene-divinylbenzene (S-DVB) resin beads, the same material used in OnGuard II Ag cartridges. Matrix elimination using this cartridge is based on the very low solubility of silver salts, halides and some other anions. Since the salts are not infinitely insoluble, the precipitate can re-dissolve to some extent. The chemical process for this matrix elimination requires the displacement of Ag+ from the resin by sample cations and the subsequent precipitation in the ion exchange bed. The small amount of silver ion that redissolves should be trapped using a Na+ or H+ InGuard cartridge placed after the InGuard Ag cartridge. This will avoid fouling of downstream cation exchangers including the suppressor. The use of the Na or H cartridge after the Ag cartridge should be standard practice.

InGuard Ag cartridges have a fixed capacity and cartridge lifetime which will depend on sample matrix, injection volume, etc. Due to the consumption of silver as discussed above, the halide matrix peak will gradually increase over the lifetime of the cartridge. It is not recommended to re-use partially expended Ag cartridges as the size of the chloride peak from re-dissolution of AgCl, will be higher.

InGuard Na and H cartridges contain 40 µm Na+ form or H+ form S-DVB resin beads, the same as that used in OnGuard II cartridges. They remove cations including metals by cation exchange governed by the standard selectivity of sulfonated resins for cations. A partial selectivity series for these resins is $H^+ < Na^+ < K^+ < Ca^{2+}$ < transition metals (as cations); multiply charged cations are more highly retained than monovalent ions. Relative concentration also affects selectivity; ions present in higher concentration can elute ions present in relatively lower concentration.

### 2.5.1. Determination of Nitrite in Brine

The most common application for the Ag cartridge is the removal of chloride from high chloride samples for the determination of ppm concentrations of other anions. As noted above, the Ag cartridge must be used in tandem with an InGuard Na or H cartridge. The Na cartridge will not acidify the sample as does InGuard H. It should be chosen for situations where the sample is pH sensitive, for example, in the determination of nitrite. Nitrite is readily oxidized to nitrate at low pH.

The system can be configured as shown in Figure 1, Figure 2 or Figure 3.

Figure 1 shows a system configured with two 6-port valves, two pumps and features a continuous wash of the InGuard cartridges throughout the run. The Figure 2 configuration is very similar except that the system uses only one pump and the detector cell outlet fluid is used in a recycle arrangement with an Electrolytic Water Purifier. The Figure 3 system uses one 6-port valve and one 10-port valve with two pumps. In this arrangement the flow of the pump supplying DI water for the sample preparation is diverted during the run so that no water flows over the InGuard cartridge stack during a run. This helps minimize dissolution of the silver halide precipitates.

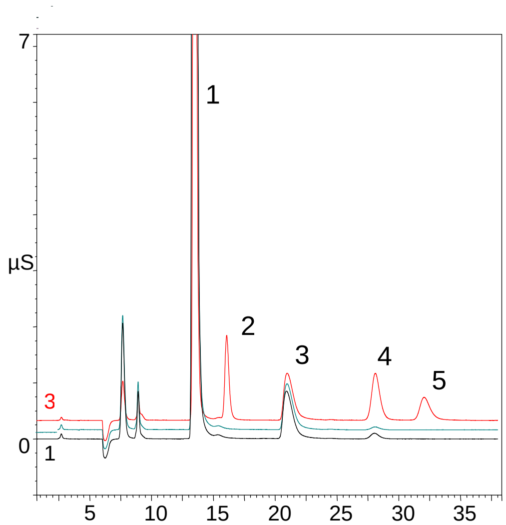
The cartridges can be used with standard bore or microbore columns since they are used for sample pretreatment during loading of a concentrator column. The sulfate blank will be approximately 50 ppb, depending on flow rate and the age of the cartridge.

Figure 5 is an overlay of a water blank, a 1.6% NaCl blank and a standard containing 2 ppm nitrite, sulfate and nitrate in 1.6% NaCl.

**Figure 5**
**Analysis of Nitrite, Nitrate and Sulfate in Brine**

| | |
|---|---|
| **Column:** | IonPac® AG15 guard column and AS15 analytical column (4 x 250 mm) |
| **Suppressor:** | SRS 300, 4-mm, external water mode (15 psi) |
| **Concentrator:** | TAC-LP1 concentrator column |
| **Eluent:** | 23 mM KOH |
| **Flow rate:** | 1 mL/min |
| **Column compartment temp:** | 30 °C |
| **Injection volume:** | 100 µL |
| **Sample rep:** | InGuard Ag followed by InGuard Na |
| **Sample loading pump:** | DI water, 1 mL/min |
| **Concentrator loading time:** | 4 min. |




**Sample**

- 1.6% NaCl blank
- Water blank
- Std of 2 ppm nitrite, sulfate, nitrate in 1.6% NaCl

| **Peak** | **Analyte** | **mg/L** |
|---|---|---|
| 1 | Chloride | - |
| 2 | Nitrite | 2 |
| 3 | Carbonate | - |
| 4 | Nitrate | 2 |
| 5 | Sulfate | 2 |

## 2.6. InGuard HRP and Na/HRP

InGuard HRP cartridges contain a 35 µm hydrophilic reversed phase resin based on divinylbenzene. The material has a surface area of about 300 $m^2/g$. Since this material is water-wettable, 100% aqueous samples can be applied without disruption of the column bed. This material can be used to remove organic matrix material over a wide range of hydrophobicity.

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InGuard Na/HRP contains a blend of $Na^+$-form sulfonated resin and HRP resin to provide the dual functionality of removing both cations including metals and organic contaminants from a sample. This cartridge is designed to provide general purpose cleanup of samples for anion analysis.

### 2.6.1. Removal of Organics from Whole Milk

Figure 6 shows the determination of nitrite in whole milk after the removal of organic materials such as lipids and proteins using InGuard HRP. The goal of sample preparation in this application is to achieve a stable retention time for the analytes. This is achieved by removal of organic material that fouls the analytical column. InGuard Na/HRP can also be used for this application to remove both $Ca^{2+}$ and organic components of the milk. Table 5 provides results from 180 injections of whole milk on one HRP cartridge using the method described below. The combination of the AG15 and AS20 is effective for resolving the nitrite and nitrate both from each other and from matrix.

  

**Figure 6**
**Analysis of Nitrite and Nitrate in Whole Milk**

**Column:** IonPac® AG15 guard column and IonPac® AS20 (4x50mm)
**Eluent source:** Eluent generator
**Gradient steps:**

| Time (min) | conc. (mM) |
|---|---|
| -20.0 | 50 |
| -5.1 | 50 |
| -5.0 | 14 |
| 0.0 | 14 |
| 17.1 | 14 |
| 17.1 | 50 |
| 20.0 | 50 |

**Flow rate:** 1.0 mL/min
**Sample volume:** 10 µL
**Column oven:** 30 °C
**Detection:** Suppressed conductivity; ASRS® 300 4-mm, external water mode.
**Sample:** Treated whole milk spiked with 40 ppb nitrite.
**Peaks:**
1. Nitrite
2. Nitrate

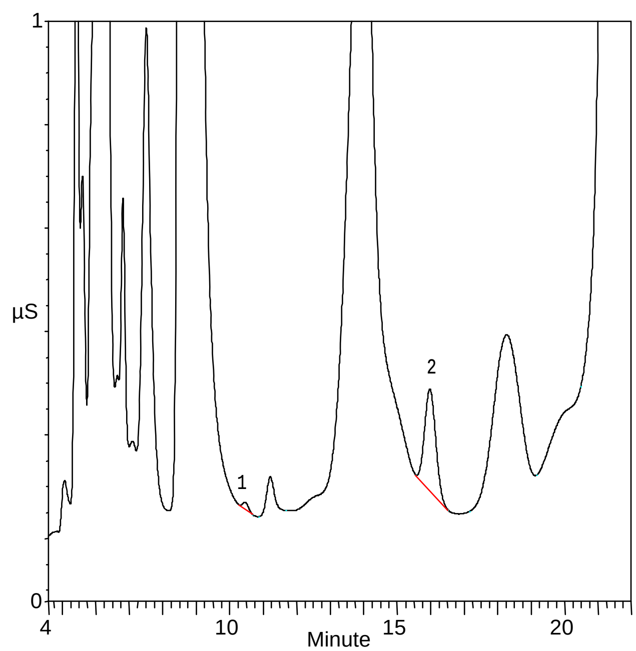


**Table 5**
**Summarized Results from 180 Injections of Whole Milk**

| Injection no. | % Retention time reduction (nitrate) from Injection 1 |
|---|---|
| 1-60 | <0.4% |
| 61-180 | <1.25% |

Samples were prepared by dilution with an equal volume of 100% acetonitrile. Samples were allowed to stand for 30 minutes. The supernatant was sampled and this fraction analyzed by IC (10µL injection volume). The fouling rate was measured by calculating the retention time reduction of nitrate detected by the IC. Based on this data, the capacity limit using this method was determined to be approximately 60 samples/cartridge.

# Appendix A. Example Chromeleon Program, Dual Injection Valves in ICS-3000 DC Column Compartment

The following steps in a PGM will load a loop, perform the sample preparation, load the concentrator column and start the analysis, using the valving shown in Figure 1, where both valves are installed in an ICS-3000 DC column compartment. Valve nomenclature follows the default conventions of the Chromeleon Server Configuration.

```
;this valving pumps water through the sample loop and the InGuard column; eluent is
pumped through the analytical column and concentrator column

            InjectValve_1.State =                 InjectPosition
            InjectValve_2.State =                 InjectPosition

 0.000

;this valving connects the AS to the sample loop, water goes to InGuard; eluent is
pumped through analytical column and concentrator column

            InjectValve_1.State =                 LoadPosition
            InjectValve_2.State =                 InjectPosition
            Load
            Wait                                  CycleTimeState
            Inject
            Wait                                  InjectState
            Sampler.ReleaseExclusiveAccess

;this valving directs loading water through the sample loop, the InGuard and the
concentrator column to waste; eluent flows directly to analytical column

0.1         InjectValve_1.State =                 InjectPosition
            InjectValve_2.State =                 LoadPosition

; this valving places concentrator column in line with eluent and the analytical
column; the time point below (4.0)can be adjusted to optimize sample loading to
concentrator (see section 2.4)

4.0         InjectValve_1.State =                 LoadPosition
            InjectValve_2.State =                 InjectPosition
            CD_1.AcqOn
            Autozero
```

The system configuration is set up so that the Left High Pressure Valve is controlled by the AS autosampler and the Right High Pressure Valve is controlled by the ICS-3000 DC module (setup in Server Configuration). This is a logically convenient arrangement if the AS is physically located to the left of the ICS-3000 DC.

Other valve configurations are possible, of course, including having one valve located in the ICS-3000 DC column compartment and the other installed in an ICS-3000 DC Automation Manager. In this case, the second valve's default name from Server Configuration would be AM_HP1, states A and B. The flow path for AM_HP1 State B should be verified to be through the concentrator column, in the reverse direction to the loading flow.

  

# Appendix B. Example Chromeleon Program, ICS-1100/1600/2100 with Auxiliary Valve.

The following steps in a PGM will load a loop, perform the sample preparation, load the concentrator column and start the analysis, using the valving shown in Figure 1 where both valves are installed in an ICS-1100, ICS-1600 or ICS-2100.

```
;this valving pumps water through the sample loop and the InGuard column; eluent is
pumped through the analytical column and concentrator column

            Pump_InjectValve.State =                InjectPosition
            Valve_2.State =                         B

 0.000

;this valving connects the AS to the sample loop, water goes to InGuard; eluent is
pumped through analytical column and concentrator column

            Pump_InjectValve.State =                LoadPosition
            Valve_2.State =                         B
            Load
            Wait                                    CycleTimeState
            Inject
            Wait                                    InjectState
            Sampler.ReleaseExclusiveAccess

;this valving directs loading water through the sample loop, the InGuard and the
concentrator column to waste; eluent flows directly to analytical column

0.1         Pump_InjectValve.State =                InjectPosition
            Valve_2.State =                         A

; this valving places concentrator column in line with eluent and the analytical
column; the time point below can be adjusted to optimize sample loading to
concentrator (see section 2.4)

4.0         Pump_InjectValve.State =                LoadPosition
            Valve_2.State =                         B
            CD_1.AcqOn
            Autozero
```

The system configuration is set up so that the left high ressure valve is controlled by the AS autosampler as the default injection valve and the right high pressure valve (auxiliary valve) is controlled by the ICS module. This is a logically convenient arrangement if the AS is physically located to the left of the IC. The flow path for Valve_2 State B should be verified to be through the concentrator column, in the reverse direction to the loading flow.

  