I. INTRODUCTION

Mass spectrometry is a powerful tool for intact protein analysis. However, the quality of analyses can be compromised by salts in the sample that interfere with ionization and/or form adducts with the investigated proteins. It is therefore important to remove or significantly minimize the introduction of extraneous salt ions into the mass analyzer. The use of on-line, desalting devices can efficiently perform this function with minimal sample handling.

Waters MassPREP™ On-line Desalting Cartridges (2.1 x 10 mm, P/N 186002785) contain a polymer-based, reversed-phase packing material that can be used to effectively desalt protein samples prior to mass spectrometry. (Note: The purchase and use of Waters Sentry™ 2.1 x 10 mm Guard Column Holder Kit (P/N WAT097958) is required for this application as shown in Figure 1.) The phenyl phase material contained in these on-line devices successfully “traps” proteins allowing the salts to be washed to waste prior to elution of the desalted protein into the mass spectrometer. With an optimized LC/MS method, cycle times as low as 3 minutes are achievable. These on-line devices, when used with an appropriate HPLC System injector and injector wash solution, can be used for the sequential analyses of different samples (See Figures 2 and 3).

This Care and Use manual provides suggested methods for the successfully use of this product. For the latest list of MassPREP, BioSuite™ or NanoEase™ column offerings, go to www.waters.com/lifesciences.

II. INSTALLING THE MASSPREP ON-LINE DESALTING CARTRIDGE INTO THE SENTRY 2.1 X 10 MM GUARD COLUMN HOLDER

1. Unscrew the two halves of the Sentry 2.1 x 10 mm Guard Column Holder.

2. Remove the old MassPREP On-line Desalting Cartridge, if present.
3. Install the new MassPREP On-line Desalting Cartridge, noting the flow arrow direction on the device. This should match the flow arrow direction on the analytical column.

4. Reassemble the Sentry 2.1 x 10 mm Guard Column Holder. Hand-tighten only.

5. Connect the 2.1 x 10 mm Guard Column Holder containing the MassPREP On-line Desalting Cartridge onto HPLC or LC/MS System using appropriate connectors (e.g., PEEK™ tubing and connectors). Prior to use, the MassPREP On-line Desalting Cartridge reversed-phase packing material must be “wet” with organic solvent prior to equilibration with 95% Eluent A/5% Eluent B. This can be accomplished by running a “blank injection” (i.e., buffer injection followed by running the separations gradient) or by washing the column with a minimum of five column volumes of 50% Eluent B followed by a ten column volume equilibration at 95% Eluent A/5% Eluent B prior to sample injection. While equilibrating, check the holder and all connections for leaks.

III. RECOMMENDED LC/MS SYSTEM CONFIGURATION TO MINIMIZE MS SOURCE CONTAMINATION

The presence of non-volatile salts (e.g., NaCl) can suppress protein ionization and foul the ionization source of the mass spectrometer. It is therefore important to remove or significantly minimize the introduction of these compounds into the mass analyzer. Figure 2 details an LC/MS configuration that can effectively be used to load sample onto the column and elute salt to waste prior to valve switching and protein elution into the mass analyzer.

**Position 1: Sample Loading and Salt Elution to Waste**

**Position 2: Sample Elution to Mass Spec**

*Figure 1: Sentry Guard Holder (P/N WAT097958) and MassPREP On-line Desalting Cartridge (P/N 186002785).*

*Figure 2: LC/MS Valve Switching Configuration for use with MassPREP On-line Desalting Cartridge.*
IV. SUGGESTED METHOD FOR MASS ANALYSIS USING MASSPREP ON-LINE DESALTING CARTRIDGE

(Considerations to Minimize Protein Carryover Between Injections):

HPLC injector design, composition of injector wash solution, and number of injector wash cycles between injections are some of the factors that can effectively minimize protein carryover between sample analyses. Waters MassPREP On-line Desalting Cartridges, when used with an appropriately performing HPLC System and injector wash protocol, can be used for the sequential analyses of different samples due to the lack of protein carryover from prior injections. Figure 3 shows the mass analyses results obtained from buffer blank and intact protein injections [bovine serum albumin (BSA) and IgG monoclonal antibody (mAb)] processed through the MassPREP On-line Desalting Cartridge using the Waters instrumentation and separation method detailed below:

a. Instrumentation

HPLC System: Alliance® 2796 Separations Module

Needle Wash
Solution Composition: H$_2$O (35%) / Isopropanol (5%) Acetonitrile (60%)

Number of Needle Washes: Single

Needle Wash Time: 15 sec needle exterior/3 sec inject port

Mass Spectrometer: Q-tof

Ionization mode: ES +

Capillary voltage: 3300

Cone voltages: 30 for BSA mass analysis 35 for mAb mass analysis

Source temp: 150 °C

Desolvation temp: 350 °C

Gas flow: 500 L/Hr

Cone gas: 50 L/Hr

b. Separation method:

Eluent A: H$_2$O with 0.1% Formic Acid

Eluent B: Acetonitrile with 0.1% Formic Acid

Flow: 0.4 mL/minute

Initial Column Conditioning:

Prior to use, the MassPREP On-line Desalting Cartridge reversed phase packing material must be “wet” with organic solvent prior to equilibration with 95% Eluent A/5% Eluent B. This can be accomplished by running a “blank injection” (i.e., buffer injection followed by running the separations gradient) or by washing the cartridge with a minimum of five column volumes of 50% Eluent B followed by a ten cartridge volume equilibration at 95% Eluent A/5% Eluent B prior to sample injection.

Sample Loading/Salt Elution:

Load sample onto cartridge while delivering 95% Eluent A/5% Eluent B. The protein sample will bind to the cartridge while the contaminating salt passes through unretained to waste.

Sample Elution to Mass Spec:

At approximately 0.5 min, begin increase Eluent B concentration from 5 to 80% in 1.5 min. Hold at 80% B for an additional 1.0 min to allow protein to completely elute from cartridge into MS system.

Column re-equilibration:

At approximately 3.0 min, re-equilibration cartridge with ten cartridge volumes of 95% Eluent A/5% Eluent B prior to next sample injection.
Figure 3: Results from final 4 injections on MassPREP On-line Desalting Cartridge from a 100 injection overnight sequence noting absence of protein carryover between injections. Test series consisted of repetitive injections of PBS, IgG monoclonal antibody (mAb) and BSA.
V. GUIDELINES FOR USE AND TROUBLESHOOTING

1. The amount of intact protein to load onto a MassPREP On-line Desalting Cartridge to obtain acceptable mass analysis results depends upon a number of variables. These include the type of mass analyzer used, the ionization efficiency of analyzed protein and MS source, the potential ionization suppression effect of ion-pairing reagents used in the separation eluent (e.g., TFA), etc. In general, between 1 and 5 µg of injected protein such as BSA or an IgG monoclonal antibody should yield good mass detector signal and excellent protein recovery from the MassPREP On-line Desalting Cartridge. Proportionately less material should be injected when analyzing smaller molecular weight proteins to prevent overloading the On-line Desalting Cartridge and or mass detector.

2. The maximum recommended operating pressure for MassPREP On-Line Desalting Cartridge, when configured WITHOUT pre-column tubing, is approximately 150 psi. Use of pressure generating tubing immediately before the MassPREP On-Line Desalting Cartridge is required to generate sufficient UPLC System back pressure for proper functioning. Thus, the total reported UPLC System back pressure generated when BOTH the pre-column tubing and MassPREP On-Line Desalting Cartridge are configured for an application is approximately 5700 psi at a flow of 0.5 mL/min with a 5% ACN/0.1% FA eluent.

3. Results obtained with Waters MassPREP On-line Desalting Cartridges can vary depending upon the physical characteristics of the analyzed proteins as well as on the performance characteristics of the HPLC (or LC/MS System), injector, and injector wash protocol used.

VI. MISCELLANEOUS

Recommended Storage:
For overnight storage, continuously flush the cartridge with the mobile phase at slow flow (e.g., 0.1 – 0.2 mL/min). If the cartridge is not to be used for several days, store the cartridge in 50% acetonitrile (CH$_3$CN) without any eluent additives such as formic acid or TFA. Do not store the MassPREP On-line Desalting Cartridges in buffered eluents. Completely seal the stored column to avoid evaporation and drying of the bed.