

# Waters

## Desalting of Proteins Using MassPREP™ On-line Desalting Cartridges Prior to Mass Spectrometry

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**L**C-MS is a powerful tool used for the characterization of proteins. Reversed-phase HPLC is the most common mode of chromatography coupled with mass spectrometry. This separation technique can also be an effective method for the desalting of proteins. Pharmaceutical biotherapeutics and other proteins are often prepared or stored in salt-containing buffer solutions such as PBS (phosphate buffered saline). These buffer solutions contain salts that interfere with ESI-MS analysis by suppressing ionization and by forming adducts. Therefore, separation of proteins from non-volatile salts is an important step prior to introduction into the mass spectrometer. Here we show a rapid, on-line desalting procedure with cycle times less than five min. The MassPREP™ On-Line Desalting Cartridge is a  $2.1 \times 10$  mm device packed with polymer sorbent and was evaluated using acidic, (bovine serum albumin), basic, (cytochrome c), and large globular (monoclonal antibody) protein samples. Parameters such as column carryover, loading, and lifetime

were investigated. The results of this study show no sample carry-over and excellent lifetime for repeated sample analysis under the conditions tested.

### Instrumentation

Divertor valve: Waters Selection Valve

HPLC System: Alliance® 2796 Separations Module

Needle Wash Solution: H<sub>2</sub>O (35%) / Isopropanol (5%) / Acetonitrile (60%)

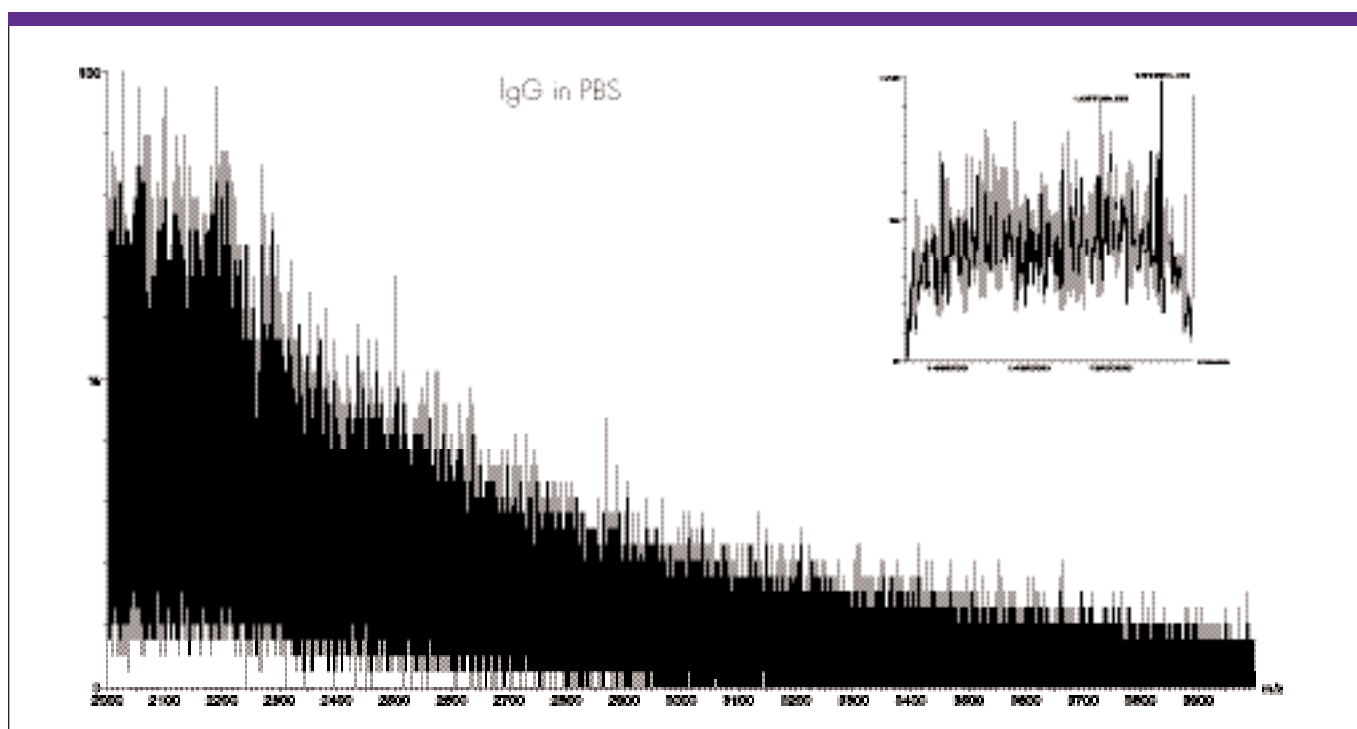
Mass Spectrometer: Waters® Micromass® Q-tof micro

Ionization mode: ES +

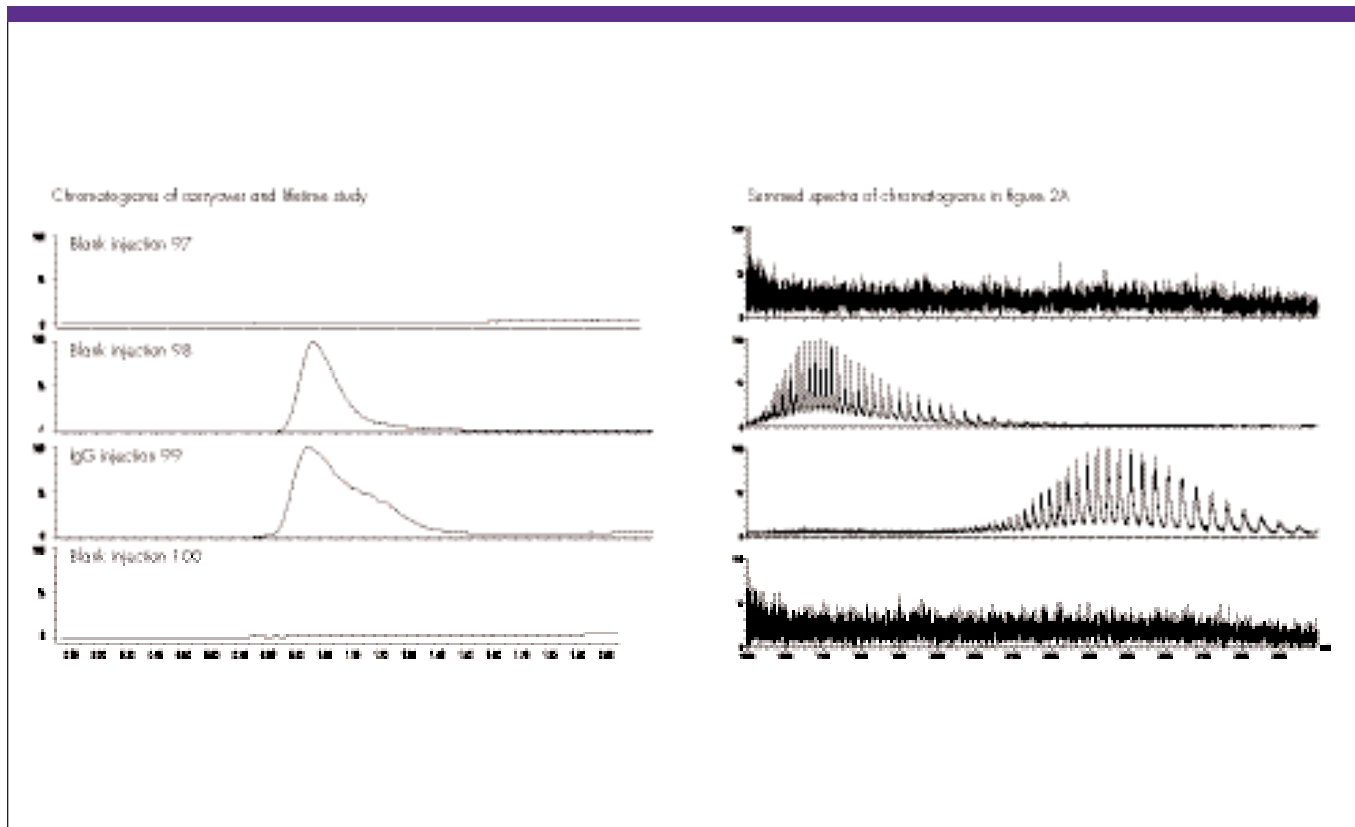
Divertor valve, HPLC system, and mass spectrometer were all controlled by Waters® MicroMass® Masslynx™ software

### Sample preparation:

Bovine serum albumin (BSA), cytochrome c and monoclonal IgG, were obtained from Sigma Aldrich Inc. St. Louis, Missouri. All pro-



**Figure 1A and 1B:** Desalting of monoclonal IgG. The sample in PBS gives no recognizable protein mass spectrum. After desalting, the sample gives a typical protein spectrum that can be deconvoluted using the Waters® Micromass® MaxENT™ 1 algorithm to reveal isoforms of IgG.



**Figures 2A and 2B:** A series of injections consisting of a blank, 1  $\mu\text{g}$  BSA, 1  $\mu\text{g}$  monoclonal IgG, and blank was repeated 25 times for a total of 100 injections. The performance of the cartridge is unaltered over this series. There is no evidence of carryover or background interference in the blank injection representing the 100th injection.

teins were dissolved in phosphate buffered saline (PBS) at a concentration of 1  $\mu\text{g}/\mu\text{L}$ .

#### Separation method:

Eluent A:  $\text{H}_2\text{O}$  with 0.1% Formic Acid

Eluent B: Acetonitrile with 0.1% Formic Acid

Flow: 0.4 mL/min

#### Conclusion:

Waters MassPREP™ On-Line Desalting Cartridges are an effective tool for the successful desalting of acidic, basic, and large globular proteins (data not shown). The desalted proteins yielded abundant ESI-MS signal suitable for deconvolution and protein characteriza-

tion. Studies determining loading capacity and carryover showed that up to 10  $\mu\text{g}$  of monoclonal IgG was successfully loaded with no breakthrough (data not shown) and no detected carryover in a subsequent analysis. The same results were obtained for BSA and cytochrome c for injected masses of up to 5  $\mu\text{g}$ . Further, no loss of performance was observed after 100 consecutive injections were performed. The overall MassPREP™ desalting strategy provides an easy, fast (< 5 min), and reproducible approach for the effective desalting of proteins stored in physiological salt buffers prior to high-resolution structural analysis.

Table 1.

Time	Eluent A	Eluent B	Valve Position
0.0	95	5	Waste
0.5	95	5	Waste
2.0	20	80	Mass Spectrometer
3.0	95	5	Waste

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