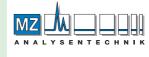
## MIMTARY BECHNICAL REPORT TROTA

## An Innovative Size Exclusion Column Compatible with GFC/GPC

World's first silica-diol SEC column designed to work with LC and LC-MS compatible volatile salts

# Intrada SEC



AUTHORIZED DISTRIBUTOR

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Compatible with hydrophilic polymers like proteins (GFC) Compatible with hydrophobic polymers like polystyrene (GPC) Uses volatile solvents as standard eluents

Extended lifetime under pH 1-8

Spherical porous silica / 3um particle / 30nm pore / Diol phase / pH 1-8 / MW up to 1MDa

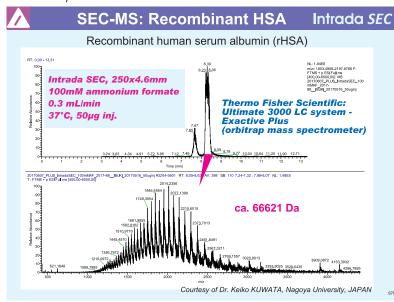
#### **Does not require NaCl for protein separation** Monomer 66 kDa **BSA (Bovine Serum Albumin)** Dimer 132 kDa monomer **ELSD UV 280nm** monomer Intrada SEC, 250x4.6mm **Protein separation** 100mM ammonium formate using Volatile eluent volatile eluent 0.3mL/min (5MPa) 37 °C 10 µL (1mg/mL) dimer dimer 5 10 0 5 10 min n min

Intrada SEC is a wide pore (300A), fully porous silica material with a bonded diol substitution stationary phase. This innovative technology overcomes some traditional problems with diol-silica columns, such as the need for use of high concentrations of non-volatile salts in the eluent and poor lifetime due to undesired residual silanols effect. The next generation Intrada SEC column allows both aqueous durability like a polymer-based SEC column and the mechanical strength of a silica-based SEC column. This column can be used with volatile salts, such as 100mM ammonium formate, as the eluent for aqueous polymer analytes, making it compatible with ELSD or MS detection.

Another benefit of the technology behind the Intrada SEC column is that it can be used for both aqueous GFC (proteins, polysaccharides and nucleic acids etc.) and non-aqueous GPC (polystylene, PVAc etc.), making it a real unified "SEC" column.

#### 🗖 Ability to use volatile salt eluents may change SEC history

Traditionally, it has been required to use 100mM phosphate buffer + 300mM NaCl as an eluent for diolsilica SEC columns due to an undesired ionic effect, caused by exposed residual surface silanols. But use of high concentrations of inorganic salt is too harsh for use with most LC systems and also their non-volatile nature makes them incompatible for use with an MS detector. The innovative Intrada SEC column with a novel diol substitution technology allows for the use of 100mM ammonium formate instead, which is a volatile eluent that is compatible with MS and ELSD detection. This truly revolutionary design may change the history of SEC.



Intrada SEC may be the best SEC column for use on MS detectors, due to its use of volatile eluents.

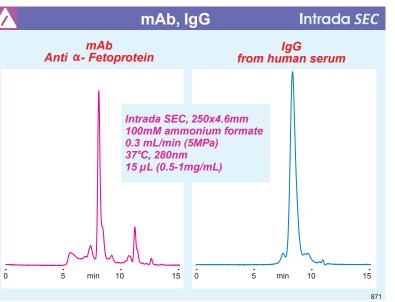
The figure to the left shows recombinant human serum albumin (rHSA) monomer and dimer separation on LC-MS. The molecular weight of the monomer was directly analyzed as 66621Da, which is more accurate than the traditional caliburation curve method, which is based on the hypothesis that all of the proteins are globular shape.

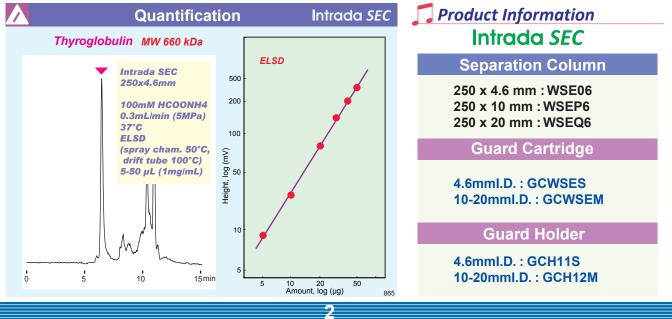
We feel this shows that SEC-MS using Intrada SEC column will likely be an improved method for MW determination in the future.

Determination of mAb purity is critical in antibody drug science. To further support this work, Intrada SEC columns allow the separation of not only monomers but also oligomers and even fragments, as the figure to the right shows.

For purification needs for products such as biopolymers, antibodies or enzymes, full preparative column dimensions are available (10 - 20 mm ID). Further adding to the convenience of using Intrada SEC for purification work, this unique material is designed to work conveniently with HPLC instruments, due to its ability to utilize volatile buffer eluents.

Polymer quantification is also possible with Intrada SEC columns, as is shown below, where accurate quantification of a 660 kDa large protein is performed. Improved separation performance and accuracy can also be achieved by using our u-shaped multicolumn connections, which enable up to four columns to be connected in series.



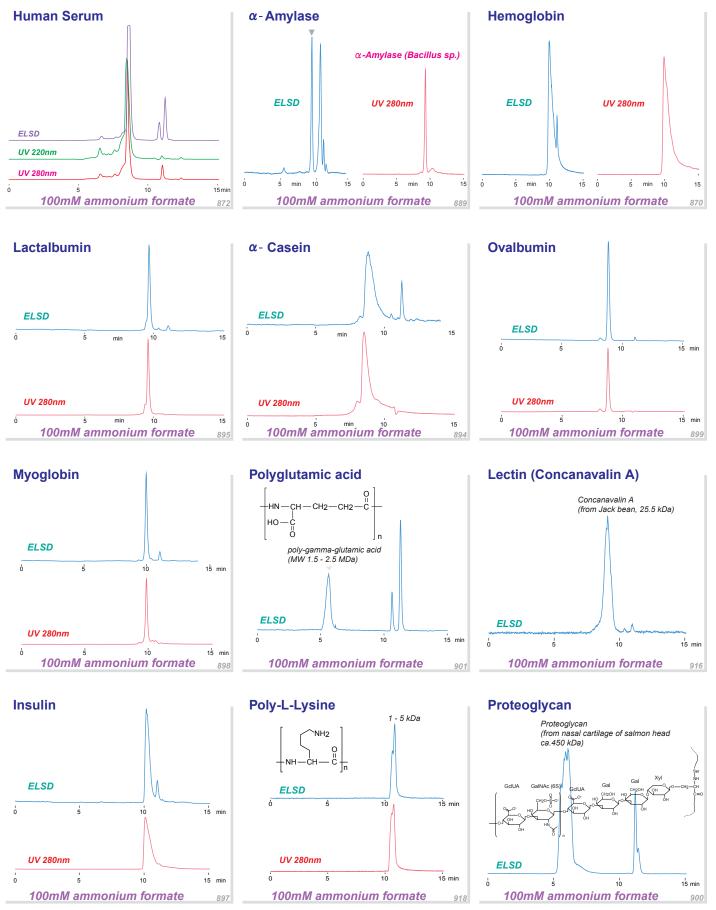


Intrada SEC

### Versatile SEC column for peptides, proteins, bio- and synthetic polymers

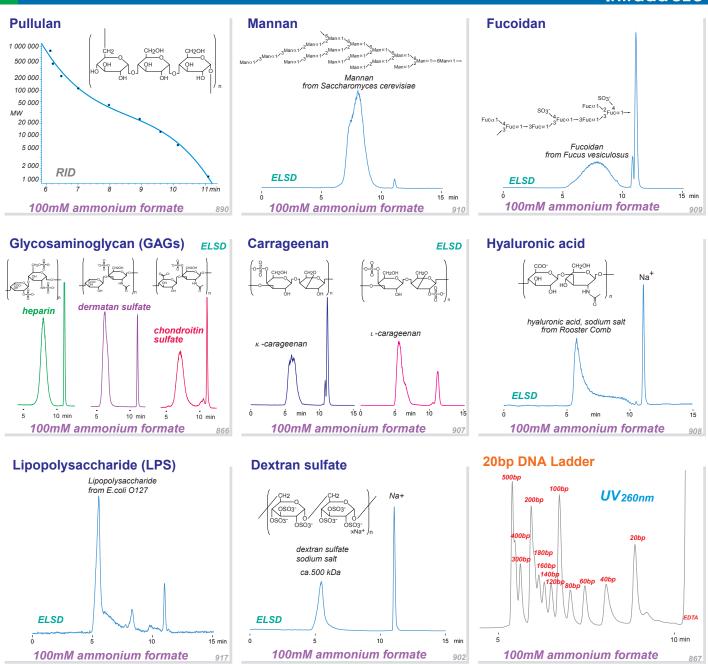
Peptides, Proteins / Bio-Polymers

Nearly all bio-polymers are compatible with the use of volatile 100mM ammonium formate as the eluent solution, as shown in the examples below.



Intrada SEC, 250x4.6mm, 0.3mL/min, UV or ELSD

#### Intrada SEC



#### Synthetic polymers

Synthetic polymers have a wide range of polarities and solubilities, so it is necessary to optimize both the sample solvent as well as the eluent, independently. Sample solvent should have a high solubility for the polymer, and the eluent should have a higher polarity than the sample, to ensure adequate elution.

