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Diamond Analytics Application Note: DA1014-A

FLARE C18 Mixed-Mode Column: Separation of Apo-Transferrin and Bovine Serum Albumin (BSA) by LC-MS

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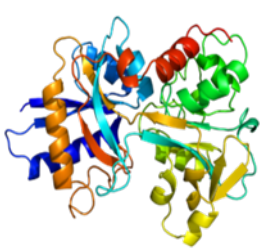
Introduction

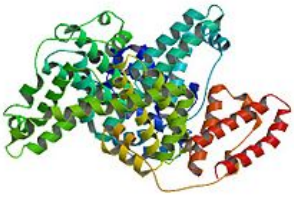
Proteins are polymeric species composed of amino acid residues that fold into complex three-dimensional structures. The ca. 20 amino acids nature uses in proteins have a variety of side chains that influence protein structure, e.g., smaller or larger, acidic or basic, nonpolar or polar, etc.[1] Many proteins have been extensively analyzed to understand their roles in organisms. In this application note, bovine serum albumin (BSA) and apo-transferrin were separated by LC-MS using the FLARE mixed-mode column from Diamond Analytics.

Analytes:



1. Apo-transferrin

http://en.wikipedia.org/wiki/Transferrin



2. BSA

http://en.wikipedia.org/wiki/Bovine_serum_albumin

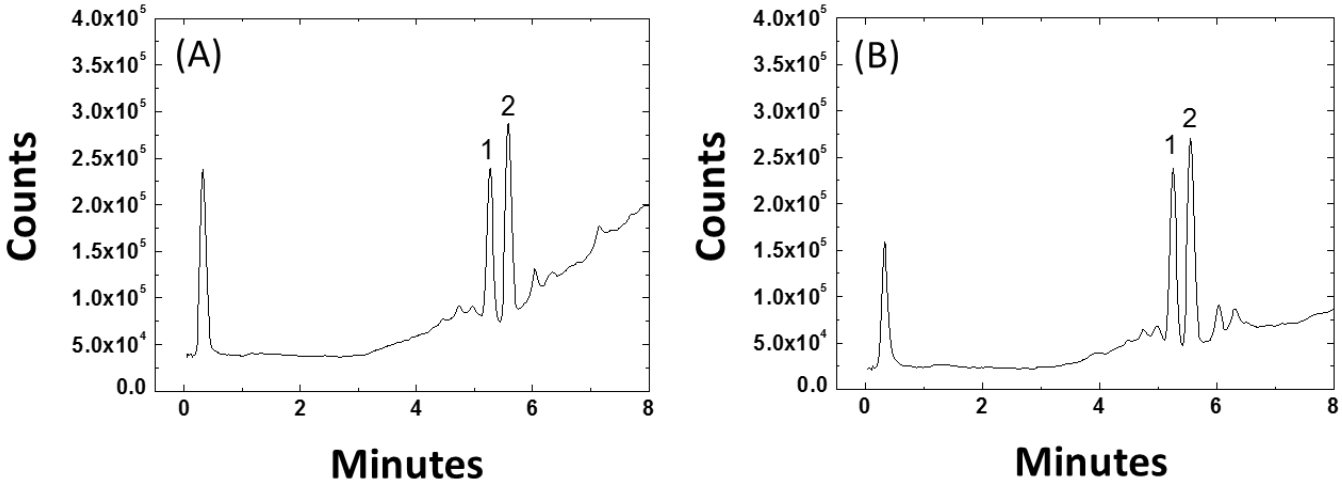


Figure 1. Separations of apo-transferrin (1) and BSA (2). (A) 0.1% TFA in buffers A and B, and (B) 0.1% TFA in buffer A and 0.0875% TFA in buffer B.

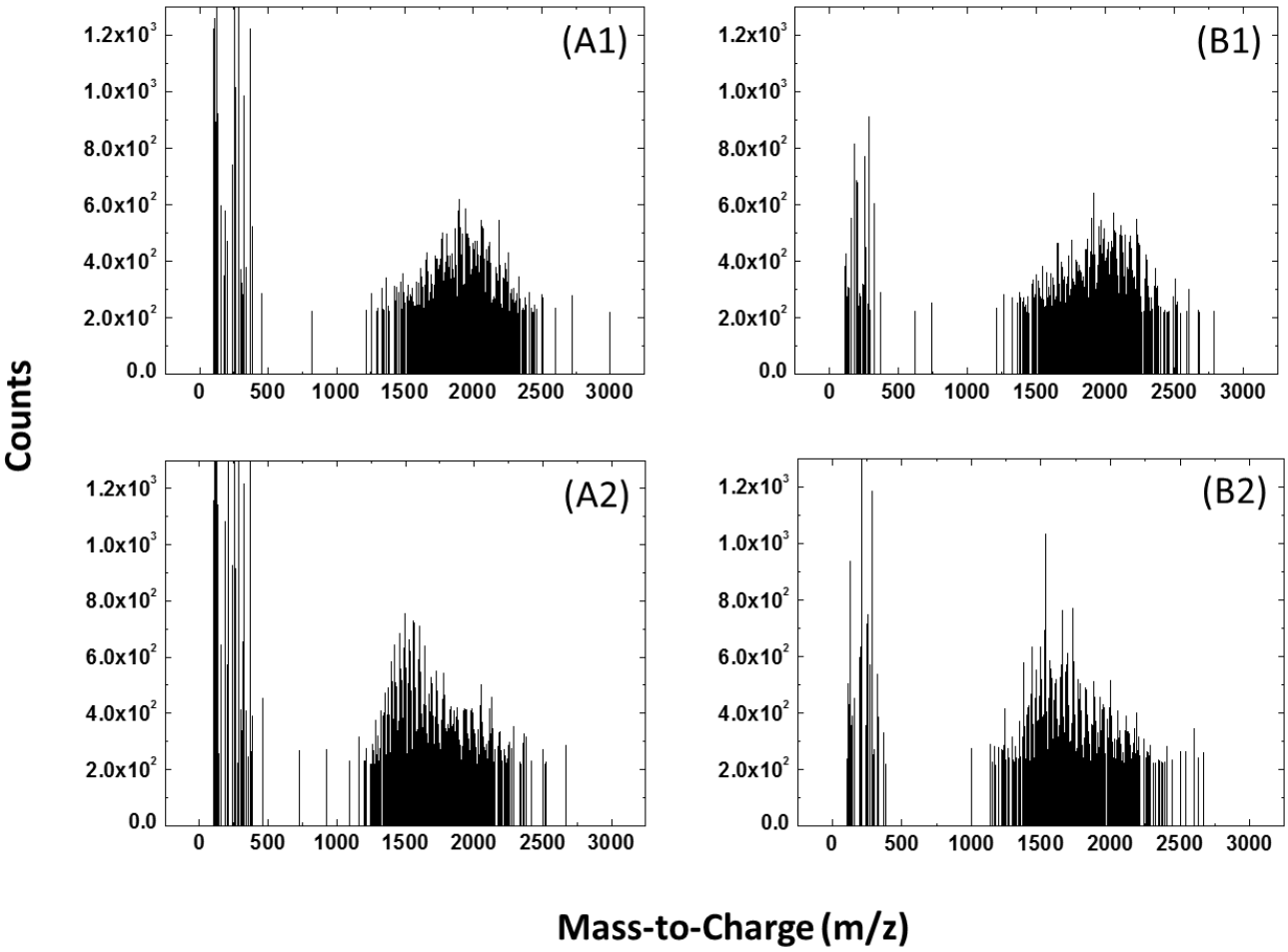


Figure 2. Mass spectra of apo-transferrin (A1 and B1) and BSA (A2 and B2) from the corresponding peaks in the chromatograms in Figure 1 (A) and (B).

The FLARE C18 mixed-mode (MM), core-shell column from Diamond Analytics is made of carbon, nanodiamond, and polymer, and is suitable for operation under high temperature and elevated pH conditions.[2-4] Because the amine based polymer in its stationary phase is positively charged at low pH and neutral at high pH, the column has mixed-mode properties, which allow it to retain acidic analytes at low pH and basic analytes at high pH.[5]

Conclusions

BSA and apo-transferrin have been separated by LC-MS using the Diamond Analytics FLARE C18 mixed-mode column by gradient elution and with commonly used reagents: water, ACN, and TFA. The concentration of the TFA additive affects the baseline.

Experimental

HPLC system: Agilent 1260 Infinity coupled with an Agilent 6230 TOF LC-MS in positive ion mode

Analytes: Apo-transferrin and BSA (ca. 0.5 mg/mL in ACN)

LC Conditions:

Column dimensions: 2.1 x 50 mm (id: TM0001 08/14/14 TB)

Injection volume: 10.0 µL

Flow rate: 0.4 mL/min

Temperature: 30 °C

A: 1000 mL H_2O + 1 mL TFA

B: 1000 mL ACN + 1 mL or 0.875 mL TFA

Gradient:

0 min	100% A	0% B
8 min	0% A	100% B
8.1 min	100% A	0% B
20 min	End	

MS conditions:

Gas temperature: 300 °C

Drying gas: 5 L/min

Nebuilizer: 35 psig

Capillary voltage: 3500 V

Fragmentor: 150 V

Skimmer: 65 V

OCT 1Rf Vpp: 750 V

References

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[3] Saini G., Jensen D. S., Wiest L. A., Vail M. A., Dadson A., Lee M. L., et al. Core-shell Diamond as a Support for Solid-phase Extraction and High-performance Liquid Chromatography. Analytical Chemistry. 2010;82(11):4448-56.

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