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Evaluation of Reversed Phase Columns for Protein Separation using a single LC/MS - LC/UV Methodology Kurt Yardley, Paul Rainville, Jeff Mazzeo, Uwe Neue, and Reb Russell II* Waters Corporation, Life Sciences Applications, Milford, MA, USA (*send correspondence: reb_russell@waters.com)



Introduction

HPLC separations on reversed phase columns are an important part of characterizing complex protein samples from basic research and pharmaceuticals. Recent advances in mass spectrometry have allowed for rapid characterization of protein and peptide samples separated buy HPLC, greatly facilitating the identification of target proteins and contaminants. Thus, there is a need for good separation techniques using MS compatible methodology for the separation of proteins by HPLC. We present her the evaluation of peak capacity and recovery in protein separations along with LC/MS.

Our findings resulted in the determination that both Delta-Pak® and Symmetry® 300 are the preferred columns on which

Experimental

Separation Method

Various columns 300Å 5mm 4.6X50mm Column heated to 60°C A: 0.1% Formic acid in Water B: 0.1% Formic acid in Acetonitrile Gradient from 5% B to 70% B in 15 min at 60°C; 0.5 mL/ min, split flow to $\sim 0.2 mL/min$ diverted to ZQ

Waters[®] Alliance[®] HPLC system

2690 or 2790 Waters® 996 PDA Detection at 280 nm

Waters ZQ[™] Mass Detector

Source = ESI(+)Capillary (kV) = 3.3 Cone (V) = 22 Temperature (°C) Source = 150Desolvation = 350 Gas flow (L/Hr) Cone = 50Desolvation = 500Scan Mode m/z = 500-1500

Proteins

RNase A Cytochrome C Horse Myoglobin Carbonic Anhydrase

Protein LC/MS - LC/UV Protein Separation Configuration Allianance[®] (LC), ZQ[®] 4000 (MS), 996 PDA (UV), and Delta-Pak[®] C₁₈ (column)



An Overlay of Protein Separations on Various Reversed Phase Columns viewed at 280nm



The peak capacity of each c	column tested as a function	of each protein and an	average of all four protein

Protein	$\begin{array}{c} \text{Symmetry}^{\$} \\ \text{300 } \text{C}_{18} \end{array}$	Symmetry [®] 300 C ₄	Column A	Delta-Pak [®] C ₁₈	Delta-Pak [®] C_4	Column B	Column C
RNase A	170	114	119	225	255	136	84
Cytochrome C	152	54	76	182	216	115	100
Myoglobin	103	43	53	111	137	74	56
Carbonic Anhydrase	66	20	40	78	107	57	59
Heme	134	71	127	161	103	55	134
Average	100	50	70	1 4 0	1//	0/	70
(except Heme)	122	58	12	148	166	96	15

Peak Capacity = Time of Analysis (min)/ [S peak widths @ 50% height (min)/ number of peaks]

The recovery of each column tested as a function of each protein and an average of all four proteins. Four injections were run for each sample with three blank injections between each protein injection.

Protein	Symmetry [®] 300 C ₁₈	Symmetry [®] 300 C ₄	Column A	Delta-Pak [®] C ₁₈	Delta-Pak [®] C ₄	Column B	Column C
RNase A	92.1	93.9	83.9	100	93.0	86.6	94.9
Cytochrome C	101.0	98.3	98.0	85	90.0	84.9	103.0
Myoglobin	78.9	94.1	77.5	81	89.0	77.6	76.9
Carbonic Anhydrase	97.4	90.6	88.0	95	94.0	88.3	78.8
Heme	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Average (except Heme)	92.3	94.2	86.9	90.3	91.5	84.3	88.4



readily detected by using mass spectrometry.