Efficient and sensitive peptide mapping approach by µPAC columns with ultralow sample loading

Yuan Lin¹, Xuefei Sun¹, Jeff Op de Beeck², Shanhua Lin¹, ¹Thermo Fisher Scientific Sunnyvale, CA, ²Thermo Fisher Scientific, Ghent, Belgium

ABSTRACT

Peptide mapping is an important approach to analyze monoclonal antibodies for the identification of sequences, post-translational modifications and mutations. Traditional packed columns are usually used for peptide mapping at analytical flow rates with large sample loading. For improved separation and sensitivity, low flow chromatography has become the preferred LC method. Microfabricated pillar array columns (µPAC) were introduced as an innovative technology for low flow. Here, peptide mapping is performed with 50 cm µPAC Neo and 5.5 cm High Throughput µPAC Neo columns. With only 20 ng NISTmAb tryptic digest, 96.4% and 98.6% are achieved for heavy chain (HC) and light chain (LC) in 15 mins elution time using 50 cm µPAC Neo column. The same sequence coverages are achieved in 6 mins elution time using 5.5cm High Throughput µPAC Neo column.

INTRODUCTION

Compared with packed bed (and monolithic) column technology microfabricated pillar array columns (µPAC) are an innovative technology that enables high peak capacity separations at moderate LC pump pressures. Through the implementation of lithographic patterning transfer and deep reactive ion etching (DRIE) into silicon wafers, separation channels can be manufactured that contain micrometer sized silicon features. These are perfectly positioned according to a pre-defined design. The introduction of perfectly-ordered separation beds eliminates any eddy dispersion originating from heterogeneous flow paths through the columns and increases column permeability. It also provides high peak capacity separations at low flow rate with enhanced resolution sensitivity.

RESULTS

Figure 1. Sequence coverages vs. Sample Loading and Gradient Time

For both columns, sample loading increasing from 10 ng to 40 ng (b) impact the sequence coverage and resolving the peptides with 15 mins sampling. (c) The FWHM of VEIK in the merged spectrum of 50 cm µPAC Neo, 50 and 15 mins gradient gave the same result, which significant increases the analysis efficiency. For 5.5 cm High Throughput µPAC Neo columns, short gradient is better for low sample loading, like 1.4 ng of sample.

Figure 2. Base peak chromatography of 50cm µPAC Neo Column (15mins vs 30mins)

Figure 3. Chromatography of 5.5cm High Throughput µPAC Neo Column (3min vs 10min)

Figure 4. Sequence coverage for HC and LC for 55cm µPAC Neo Column

For both columns, sample loading increasing from 10 ng to 40 ng (b) impact the sequence coverage and resolving the peptides with 15 mins sampling. (c) The FWHM of VEIK in the merged spectrum of 50 cm µPAC Neo, 50 and 15 mins gradient gave the same result, which significant increases the analysis efficiency. For 5.5 cm High Throughput µPAC Neo columns, short gradient is better for low sample loading, like 1.4 ng of sample.

Figure 5. Sequence coverage for HC and LC for 5.5 cm High Throughput µPAC Neo Column

Figure 6. EIC of signature peptides using 50cm Neo µPAC column

For both columns, sample loading increasing from 10 ng to 40 ng (b) impact the sequence coverage and resolving the peptides with 15 mins sampling. (c) The FWHM of VEIK in the merged spectrum of 50 cm µPAC Neo, 50 and 15 mins gradient gave the same result, which significant increases the analysis efficiency. For 5.5 cm High Throughput µPAC Neo columns, short gradient is better for low sample loading, like 1.4 ng of sample.

Figure 7. EIC of signature peptides using 5.5 cm high through Neo column

For both columns, sample loading increasing from 10 ng to 40 ng (b) impact the sequence coverage and resolving the peptides with 15 mins sampling. (c) The FWHM of VEIK in the merged spectrum of 50 cm µPAC Neo, 50 and 15 mins gradient gave the same result, which significant increases the analysis efficiency. For 5.5 cm High Throughput µPAC Neo columns, short gradient is better for low sample loading, like 1.4 ng of sample.

MATERIALS AND METHODS

Standard NISTmAb tryptic digest was dissolved in water with 0.1% formic acid. Ultimate sample amount of 20 ng was loaded on 50 cm µPAC Neo and 5.5 cm µPAC Neo High Throughput columns. In search of optimal performance, different flow rates and gradient times were investigated. Dissolved peptide mixtures were separated by the Thermo Fisher™ Vesper™ Neo UPLC system from directly analyzed by MS/MS on Thermo Scientific™ Orbitrap Excalibur™ 480 Mass Spectrometers. Raw data were analyzed by Thermo Scientific™ BioPharma Finder™ 3.1 with automatic parameter optimization. Peptide analysis (ppm error) ± 10 ppm, identified only by MS2, MS2 Atlas A=1.33, and MaxC coverage ≥ 2 are selected to calculate sequence coverage. Glycerophosphates were manually confirmed by MS2 spectrum.

Solutions A is a water with 0.1% formic acid (FA), and Solution B is 90% acetonitrile with 0.1% FA. 15 mins method and mass spectrometry parameters are listed as an example.

REFERENCES


TRADEMARKS/LICENSES

© 2023 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others.