Sustainable Medical Technologies

Harnessing the power of electrophoresis and chromatography: Offline coupling of reverse phase liquid chromatography-capillary zone electrophoresis-tandem mass spectrometry for peptide mapping for monoclonal antibodies

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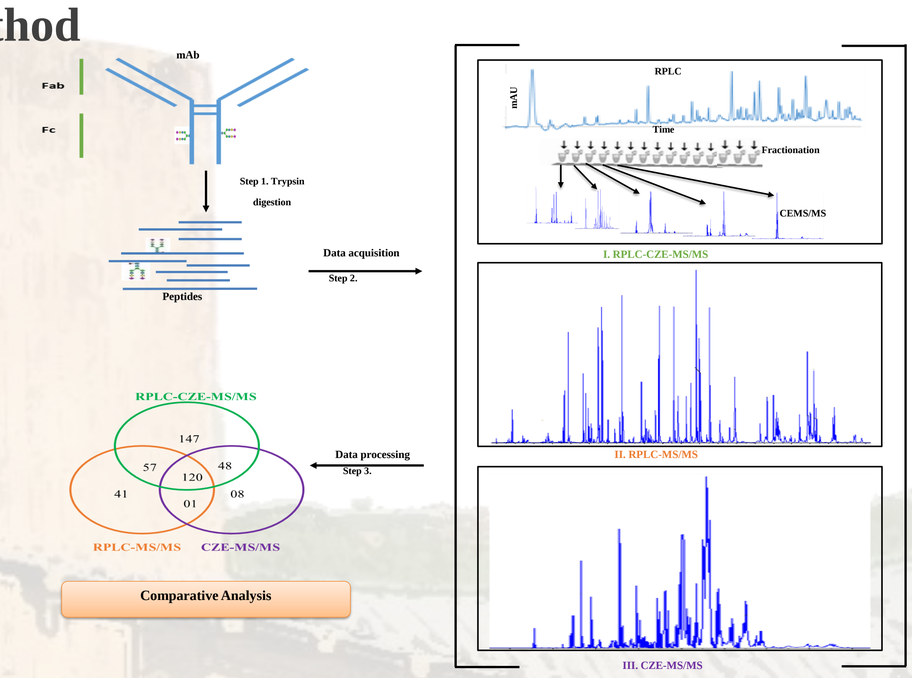
Abstract

CZE-MS/MS is an orthogonal alternative to routine RPLC-MS/MS, which has limited peak capacity and is difficult for detection of small and hydrophilic peptides. In the present work, for the first time, offline coupling of an RPLC-CZE-MS/MS has been demonstrated for peptide mapping of a monoclonal antibody-based therapeutic product.

Introduction

Reversed-phase liquid chromatography (RPLC) coupled with electrospray ionization– tandem mass spectrometry (ESI-MS/MS) is routinely used for peptide mapping of monoclonal antibodies (mAbs) but has a poor retention of small and hydrophilic peptides and limited peak capacity[1-2]. Capillary zone electrophoresis (CZE) has long been offered as an orthogonal alternative to RPLC[3]. Currently, both RPLC and CZE are used for characterization of mAbs[4]. In this study, we have combined both the techniques for peptide mapping of a mAb.

Method



Results

Table 1. Sequence coverage analysis. HC: heavy chain; LC: light chain.

Technique	HC (%)	LC (%)	Total (%)
RPLC-MS/MS	89.76	92.06	90.91
RPLC-CZE-MS/MS	99.55	98.6	99.1
CZE-MS/MS	94.21	95.79	95

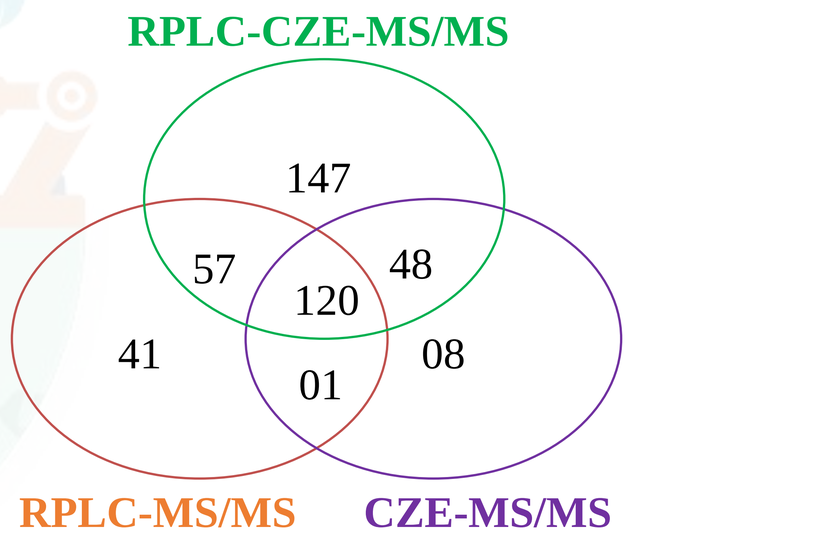


Figure 2. Comparative analysis of identified peptides.

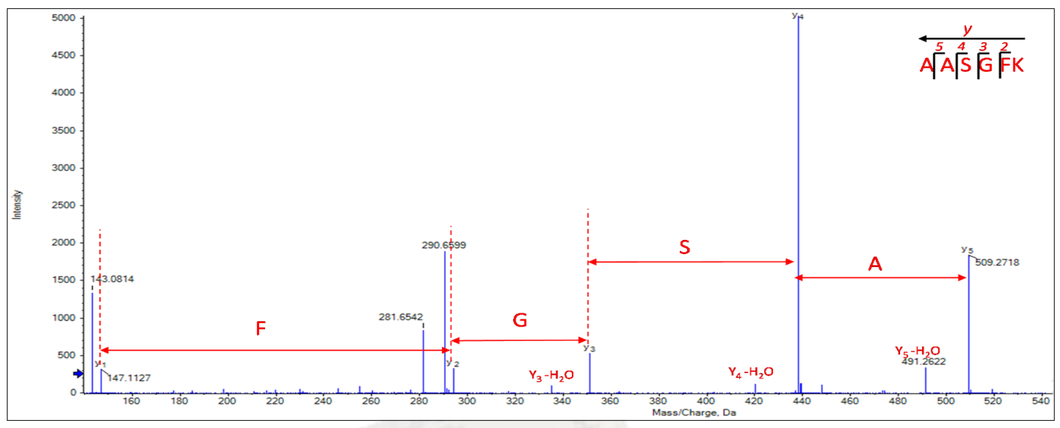


Figure 3. MS/MS spectra of "AASGFK" peptide. The spectra were analyzed with PeakView 2.0 software. Amino acids were labeled according to the mass difference between adjacent y ions.

Conclusions

1. A novel RPLC-CZE-MS/MS platform has been established for mAb characterization.
2. One-dimensional RPLC-MS/MS and CZE-MS/MS-based platforms may not be enough for characterization of complex molecules such as mAbs.

Industrial Significance

The proposed approach should be an essential addition to the analytical toolkit for in-depth primary structural characterization of mAbs.

Technology Readiness Level: Ready

References

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Chemical Engineering