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Author: J. Pesek Th. Krüger B. Tautkus H. Rhode

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Colorful quality control of chromatographic sample preparation

J. Pesek, Th. Krüger, B. Tautkus, H. Rhode

Institute of Biochemistry I, Universitätsklinikum Jena, Nonnenplan 2, 07740 Jena

Author to whom correspondence should be addressed:
PD Dr. Heidrun Rhode, Department of Biochemistry I, Universitätsklinikum Jena, D-07740 Jena, Germany, Heidrun.Rhode@mti.uni-jena.de,
Phone: +49-3641-938620
Fax: +49-3641-9396302

Abbreviations:
SEC, size exclusion chromatography; AEC, anion exchange chromatography; CPS, colored protein standards

Abstract
Multidimensional chromatographic separation for proteomic biomarker search generates sets of several hundred homologous fractions, which have to be compared. Due to the high number of sequential steps, deviations between samples may be produced randomly by slight processing differences. These deviations may falsify proteomic results. In order to overcome this problem, we tested the applicability of quality control by colored phycobilins as internal standards. The elution of the used protein standards themselves shows a high reproducibility since their main peak location is practically constant under proper performance of size exclusion and anion exchange chromatography. This applies to runs of one phycobilin alone, combined with another phycobilin, or combined with plasma proteins. Thus, these protein standards do not disturb sample processing. Characteristic peak shifts of phycobilins allow easy observation of deviations caused by typical failures in the elution protocol (aberrant step number, buffer permutation). Mass spectrometric analysis is not influenced by their presence since protein coverage, peptide numbers, and protein numbers are not altered. Thus, colored protein standards may be used for quality control and evaluation of robustness of various chromatographic applications.

1 Introduction

For proteomic biomarker search, we recently developed a native multidimensional and chromatographic method for serum and plasma fractionation. This method includes 1-D size exclusion chromatography (SEC), which is followed by free-flow 2-D anion exchange