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Multisegment Injections Improve Peptide Identification Rates in Capillary Zone Electrophoresis-based Bottom-up Proteomics

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Highlights:

- Multisegment injection was applied to bottom-up analysis of the yeast proteome.
- Reducing time between injections to 30 minutes resulted in a doubling of the peptide identification rate.
- Peak width was not degraded by this protocol.
- There was a 20% decrease in total numbers of identifications.
- Multisegment injection provides an outstanding approach to maximize identifications in bottom-up proteomics.

Abstract. While capillary zone electrophoresis (CZE) provides dramatically improved numbers of peptide identifications compared with reversed-phase chromatography for bottom-up proteomics of mass limited samples, CZE inevitably produces lower numbers of peptide identifications than RPLC for larger samples. One reason for this poorer performance is the dead time between injection of samples and subsequent appearance of the fastest moving component. This dead time is typically 25% of the separation window in CZE, but is only 5% of the separation window in gradient elution RPLC. This dead time can be eliminated in CZE by use of a multisegment injection mode where a series of samples is analyzed by injecting each sample while the preceding sample is still being separated. In this paper, we demonstrate that capillary

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