Here we detail a preliminary investigation into the potential of LC-MS for analyzing tryptic digests of recombinant α-galactosidase A in human serum. Each sample was analyzed in duplicate. Human serum albumin was specified as the internal standard.

A. In this work we have determined the limit of detection of the enzyme, present in the complex serum background. The identified proteins and their abundance were well-over 100 fmol injections on the columns, which would easily correspond to the clinical concentration of a single protein/mL serum.

A. Finally, we have investigated the potential for relative quantification of the enzyme. Relative quantification of recombinant α-galactosidase A has been shown to alleviate symptoms of the disease and has been used as a long-term enzyme replacement therapy in patients with a confirmed diagnosis of Fabry’s disease.

B. The human serum samples and α-galactosidase A were diluted, and solubilized by incubation at 37°C with 0.01% SDS. Protein samples were fractionated between 5 and 15% SDS-sodium dodecyl sulfate and analyzed using 10% SDS-PAGE. The proteins were then stained with Coomassie blue and destained with 10% acetic acid. The bands were excised from the gel and incubated overnight with a cocktail of trypsin (Promega, Madison, WI) in 50 mM ammonium bicarbonate (pH 8.0) containing 1% TFA.

C. The peptides were then desalted and analyzed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry. Additional samples were subjected to liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a Q-Tof mass spectrometer (Waters Corporation, Milford, MA) for peptide identification.

2. MATERIALS AND METHODS

Sample preparation

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2. RESULTS

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3. DISCUSSION

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