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Acetonitrile precipitation of plasma proteins enables targeted (SRM) MS/MS analysis of IGFs

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Overview

Goal: Quantify IGF1, IGF2, IBP2, IBP3 and A2GL proteins in human plasma by nano LC SRM-based analyses

Methods: Precipitation with 60 % acetonitrile, delipidation by solvent extraction, digestion and spiking of heavy-isotope labelled peptides prior to nano LC - SRM MS analysis

Results: Removal of high and medium molecular weight (MW) proteins by precipitation with acetonitrile permits the quantification of the targeted proteins requiring only 7 µl of sample volume with a total analysis time of ~18 min per sample

Introduction

Proteomic analyses of human plasma samples are extremely challenging due to the existing wide differences in protein concentrations. With an estimated dynamic range of more than 12 orders of magnitude, albumin (HSA) and the family of immunoglobulins (Ig) represent the 90 % of the total protein content, including more than 20,000 variants.

Here, we have characterised the depletion of high and medium MW proteins by acetonitrile (ACN) precipitation. Untargeted proteomics analysis have shown the efficiency of the approach by unveiling low abundance proteins otherwise not detected. Based on this depletion, a nano LC SRM-based method has been developed for 5 medium and low abundance proteins of interest in anti-doping and medical fields

PROTOCOL

Delipidation by sample centrifugation
11x diluted plasma
60 % ACN
Benchtop vortex
Transfer supernatant
Evaporate (speed-vac)

Delipidation by solvent extraction
Evaporate (speed-vac)
In-solution digestion
16 h acidification
spiking of heavy-isotope labelled peptides

Sample delipidation Narrower peak widths, less background noise and longer column lifetime with delipidation by solvent extraction

SDS-PAGE of ACN precipitated samples
Higher protein recovery at more diluted plasma

Employing 60 % ACN to precipitate plasma, protein recovery increases at higher sample dilutions

Loading similar protein amounts, depletion efficiency improves at higher sample dilutions

Conclusions

• ACN precipitation is a very simple approach to enrich the LMW fraction exploiting the lower solubility of larger proteins in organic media. The protocol described here effectively removes proteins of MW higher than 60-70 kDa., including the 2 most abundant proteins in plasma (HSA and Ig’s)

• Quantification of the 5 targeted proteins requires only 7 µl of sample employing nano LC chromatography

• Further samples delipidation significantly improves peak shape and sensitivity. Best results are obtained by solvent extraction method

• A short nano LC gradient of 6.5 min with a total analysis time of 18 min allows running up to 80 samples per day, a comparable throughput to standard LC analyses

References


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