Influence of different sample preparation techniques on the simultaneous quantification of different insulins in serum using cap-HPLC/MS^2

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New insulin analogs with altered pharmacokinetic (PK) properties have been developed over the past few years for improving glucose control in diabetes [1]. In order to study and compare their PK profiles, it is necessary to quantify all analog insulins in human blood samples at physiological levels. Immunoassays are commonly used for this purpose but do have certain drawbacks (laborious development of specific antibodies for each insulin, susceptibility to cross reactivity). An LC/MS approach capable of simultaneously quantifying different insulins is thus a promising alternative to immunoassays. Sample preparation for removal of interfering proteins such as human albumin, without losing insulin from serum samples, is a challenging task. Different sample preparation procedures (SPE, protein precipitation and ultrafiltration) and their effect on a cap-LC/MS^2 method [2], capable of distinguishing between 7 different insulins (human, porcine and bovine insulin; aspart, glulisine, lispro and glargine) in the fmol-range (8-500 fmol on column), will be shown.

[1] J. Brange et al, Advanced Drug Delivery Reviews 35, 307 (1999)
[2] C. Magnes et al, “Strategies for Quantification of Insulin and Insulin Analogues by LC MS/MS”, ASMS Meeting, Nashville, TN, 2004