

IDD Publication #10

Presentation at scientific meetings

Piotrowski M, Haigh, B, and Litchfield J: (1998) **ESI-LC/MS analysis of a non-enzymatically glycosylated albumin peptide: A novel glycation assay.** Journal American Society for Mass Spectrometry 9, 4, 383

An ESI-LC/MS assay was developed that offers structural information and enables quantitation of the non-enzymatic glycation of an albumin peptide without hydrolysis or chemical derivatization, both of which can result in artifacts due to side reactions. The synthetic peptide (KQTAL), used as a substrate, mimics the preferentially glycosylated site (lysine 525) of in vivo generated glycoalbumin. Incubation of the peptide with carbohydrate was followed by direct injection onto a Waters DeltaPak (2x150mm) C-18 column for desalting, followed by ESI-LC/MS SIM analysis (PE Sciex API 100) of both native and glycosylated protonated peptide. The extent of glycation of peptide incubated with 125mM glucose in 200mM phosphate buffer, pH 7.4, or bicarbonate buffer, pH 8.3, increased with time over 7 days to 5% and 9% respectively. Ribose glycosylated the peptide more efficiently, reaching greater than 20% after 7 days at 37°C. The peptide exhibited a concentration dependent increase in glycation by glucose and ribose. The removal of chemical manipulation steps in this assay accelerated the measurement of non-enzymatic peptide glycation and provides more accurate results by eliminating interfering side reactions. Quantitatively measuring the extent of glycation can be useful in determining the effect of inhibitors on the Maillard reaction.