I: Investigation of the effect of column temperature in capillary reversed phase high performance liquid chromatography of proteins.II: Fractionation and separation of basic plasma proteins using two dimensional liquid chromatography [

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Master thesis

Monografía

Abstract part I: Investigation of the effect of column temperature in capillary reversed phase high performance liquid chromatography of proteins The effect of column temperature on separation of proteins in reversed phases capillary LC has been investigated in the range of 25ºC-125ºC. Five proteins have been used as models to study changes in retention time and recovery using a 150 x 0.3 mm PLRP-S column and a water/Acn mobile phase gradient. Generally, the retention time decreases as the temperature increases. No significant reduction in recovery was found below 100ºC. Furthermore, the proteins were heated prior to injection to cause denaturation, and the effect of denaturation was measured. All model proteins but one showed reduced recovery after being heated to 75ºC and 100ºC for one hour. In no case could renaturation be observed after cooling the proteins for 24 hours. Instead, the recovery of some proteins tended to decrease even more after this "storage". It has also been investigated how protein denaturation is influenced by the time period the proteins are exposed to high temperature (residence time). Increased column residence time did not result in more denaturation below 100ºC during a measurement period of 60 minutes. At 100ºC, a significant decrease in recovery was observed for most proteins when exceeding 30 minutes additional residence time on the column. Additionally, separation of two and three proteins using temperature gradient as an alternative to mobile phase gradient has been investigated. Satisfying separation was accomplished using both mobile phase and temperature gradient without any significant difference in recovery between the modes. Furthermore, the use of temperature to aid

separation of complex mixtures has been investigated on a monolithical 60 x 0.180 mm column. A mobile phase gradient that almost separated a mixture of ten proteins at 30ºC was carried out under different temperature conditions, flow rates, and with or

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