Waters Lab Highlights

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NO. 221 A SIMPLIFIED APPROACH TO PEPTIDE MAPPING USING GRADIENT HPLC AND MULTI-ABSORBANCE RATIOS

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Traditionally, peptide mapping has been used as a means of locating unique peptides. The physicochemical properties of each peptide allow it to be mapped with two-dimensional chromatogrpahy, electrophoresis, or HPLC. In this study of the separation of adrenocorticotrophic hormone (ACTH) and several of its peptide fragments (1-10, 4-10, 1-24, 11-24), a state-of-the art programmable UV/visible absorbance detector, the Waters 490, allows the chromatographer to monitor multiple wavelengths and absorbance ratios simultaneously. The information from a single analysis provides qualitative information which is useful for characterizing peptides. Although the UV spectra of the ACTH peptides were not very different, when the appropriate wavelengths were chosen for absorbance ratios, peptides can be distinguished. The separation was accomplished by means of a Waters μBondapak™ C_{18} column using an acetonitrile/TFA gradient. By combining the retention time data and the absorbance ratio data, peak identity can be obtained for each peptide. The use of more than one absorbance ratio increases the level of confidence in the identification of each peak. In addition to the ratio value, the shape of ratioplots can be used to determine homogeneity of each fragment. The four programmable channels on the Waters 490, can provide four times as much real time information (both qualitative and quantitative) about a sample in a single chromatographic run than a simple UV detector can provide. The multitasking aspects of the 490 UV detector will also be shown to improve productivity and be invaluable in sample limited applications.