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ACCELERATED AMINO ACID ANALYSIS USING HPLC WITH POST-COLUMN DERIVATIZATION

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BACKGROUND

The traditional, and to date most accurate, method of detecting free amino acids in solution is the use of ion-exchange separation followed by post-column derivatization with Ninhydrin. No other technique has been shown to match the reproducibility of the analysis from simple solutions to the most challenging matrices.

Nevertheless, with run times of typically 1-2hrs, this method can be very time-consuming in terms of laboratory throughput. While the sample preparation is very and the instrumentation relatively simple for the analyst, the long turn around time in todays busy laboratory environment can be viewed as a disadvantage. If a laboratory can guarantee faster turn around, but maintain accurate results, this will enable them to remain competitive in the market. For this reason, we have been working to accelerate Amino Acid Analysis while at the same time maintaining the high reproducibility and sensitivity that the industry expects from Ninhydrin derivatization.

Here we present new Accelerated Amino Acids Analysis methods for both Physiological and Protein Hydrolysate samples. Our method utilizes a combination of temperature and eluant gradients followed by post-column derivatization.

Lithium Amino Acids (typically Physiologic Fluids) can be analyzed in 88 minutes inject-to-inject and Sodium Amino Acids (typically Protein Hydrolysates) can be analyzed in 39 minutes inject-to-inject.

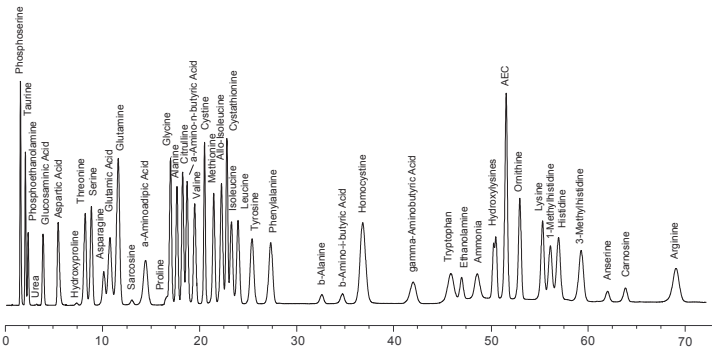
METHODS

Each chromatogram was performed using a Linear Citrate-Buffer Gradient (Lithium Citrate for Physiologic samples, Sodium Citrate for Hydrolysate samples) along with a Column Temperature Gradient to effect the elution. The derivatization of the amino acids was performed using Post-Column Derivatization with Trione® Ninhydrin Reagent at 130° C and with a reactor volume of 0.5ml.

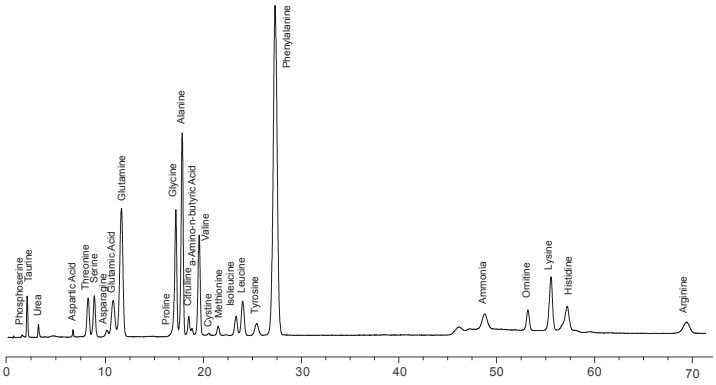
All chromatograms were generated using an HPLC pump, autosampler, Pickering Pinnacle PCX post-column derivatization instrument, and HPLC UV/Vis detector*.

All reagents, columns, and standards are produced by Pickering Laboratories, Inc.

Real samples were provided by independent laboratories.



Amino Acid standard for physiological fluids, containing 2 internal standards – Glucosaminic Acid and 2-Aminoethyl-L-Cysteine (AEC). Eluants: 1700-1125, Li365, Li375, RG003, column 0354675T (Pickering Laboratories, Inc), temperature gradient from 32 °C to 70 °C



A plasma sample of patient with PKU. Eluants: 1700-1125, Li365, Li375, RG003, column 0354675T (Pickering Laboratories, Inc), temperature gradient from 32 °C to 70 °C

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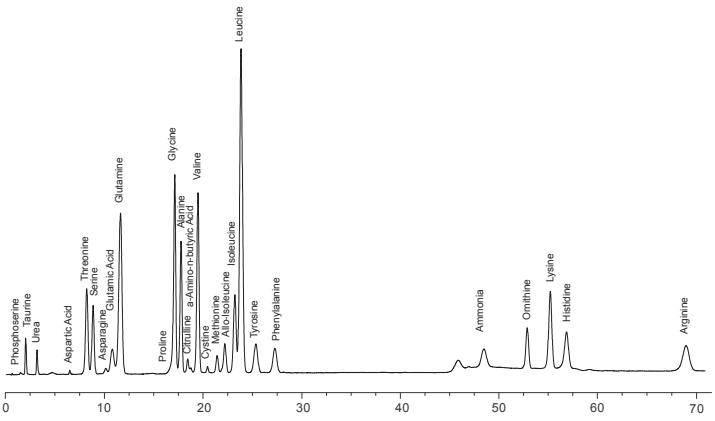
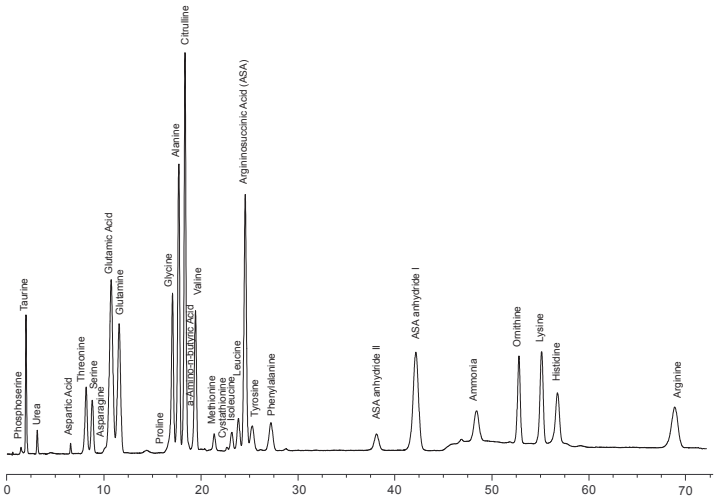
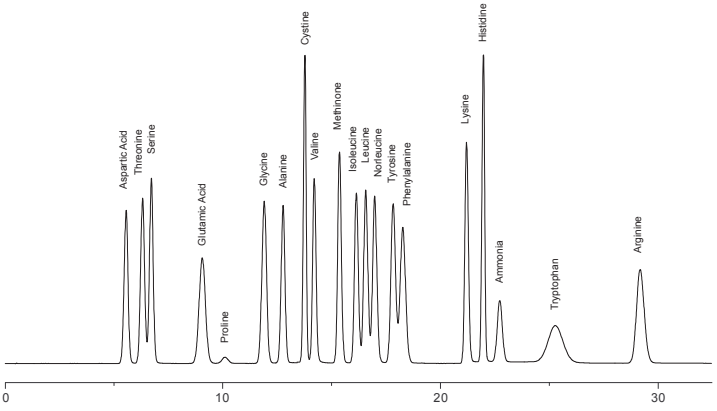


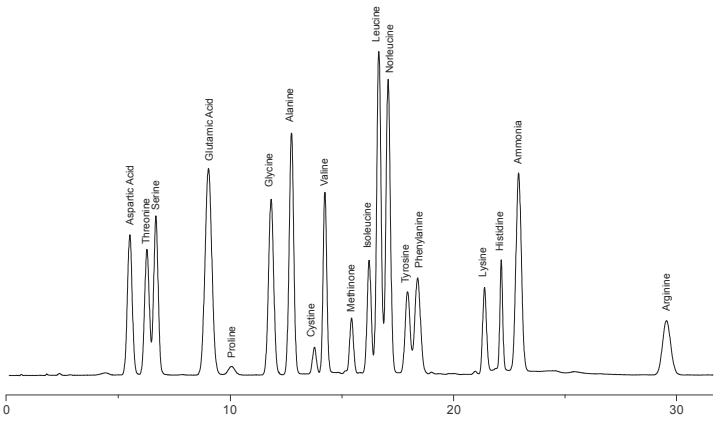
A plasma sample of patient with MSUD. Eluants: 1700-1125, Li365, Li375, RG003, column 0354675T (Pickering Laboratories, Inc), temperature gradient from 32 °C to 70 °C



A plasma sample of patient with Argininosuccinic aciduria (ASA). Eluants: 1700-1125, Li365, Li375, RG003, column 0354675T (Pickering Laboratories, Inc), temperature gradient from 32 °C to 70 °C



Amino Acid standard for protein hydrolysate, containing Norleucine as internal standard. Eluants: Na315, Na740, RG011, column 1154110T (Pickering Laboratories, Inc), temperature gradient from 46 °C to 70 °C



A feed sample, hydrolyzed according to AOAC Method 994.12, containing Norleucine as internal standard. Eluants: Na315, Na740, RG011, column 1154110T (Pickering Laboratories, Inc), temperature gradient from 46 °C to 70 °C

RESULTS AND DISCUSSION

When the speed of the amino acid run was increased, it was determined that the current guard model at the time was not adequate to match the speed and separation requirements of the chromatogram. In other words, the traditional ion-exchange guards were creating band-spreading which had a deleterious effect on the separation.

For this reason, we looked to other guard possibilities and created the New GARD™ Column Protection System. With the new GARD™, we are able to achieve a fast, reliable separation; all the while protecting our analytical column without the loss of peak resolution.

Our Pinnacle PCX post-column derivatization system provides a unique opportunity which allows analysts to combine eluant gradient capabilities of modern HPLC instruments with column temperature gradients.

Our protocols are utilized in laboratories worldwide for detecting disease, monitoring nutritional levels in food and beverages, and ensuring quality control in pharmaceuticals and biotechnology products.

**This method can be used with virtually ANY manufacturer's HPLC system when used in conjunction with Pinnacle PCX*

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