Ion exchange chromatography of acidic amino acids by using a weakly basic anion exchange resin

A chromatographic procedure for the quantitative separation of acidic amino acids such as α-aminoadipic, glutamic, aspartic acids, and isovalthine[1,2] has been described. On an analytical scale, a 0.9 × 15 cm column of Amberlite CG 45 Type II in the acetate form was used. Acetic acid of the following concentration, 0.05, 0.15, 0.25 and 2.0 *M*, was employed as eluent, and a satisfactory resolving power and quantitative recoveries were obtained by this method.

Preparation of column

Amberlite CG 45 Type II, freed of fine particles, was converted to the acetate form after washing with 2 *N* sodium hydroxide and water, and was packed into 0.9 × 15 cm column. The column was equilibrated with 100 ml of 0.05 *M* acetic acid before use.

Fractionation

An authentic sample mixture containing about 2 micromoles of each amino acid dissolved in 3 ml of 0.05 *M* acetic acid was placed on the top of the column and washed in two 0.5 ml portions of the same acetic acid. Then the amino acids were eluted stepwise with 0.05, 0.15, 0.25 and 2.0 *M* acetic acid. Each ml of the eluate was collected with an automatic fraction collector. A flow rate up to 20 ml per hour could be used without difficulty. The neutral and the basic amino acids passed through the column. α-Aminoadipic acid and glutamic acid were eluted with 0.15 *M* acetic acid, aspartic acid was eluted with 0.25 *M*, and isovalthine with 2.0 *M* acetic acid (Fig. 1). Even

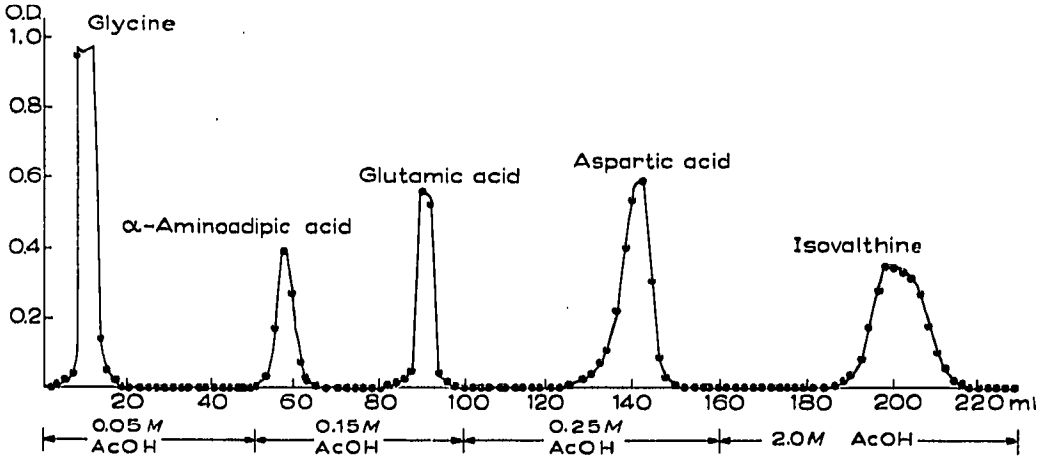

Fig. 1. Separation of the acidic amino acids on a 0.9 × 15 cm column of Amberlite CG 45 Type II. The column was operated in the acetate form at 20° with the sequence of acetic acid concentrations as indicated. Flow rate was 8–10 ml/h.

numbered fractions were used for the photometric determination by the modified ninhydrin method of MOORE AND STEIN[3]. Sufficient colour development could never be attained unless the fractions eluted with 2.0 *M* acetic acid were neutralized before the addition of ninhydrin reagent. Odd numbered fractions were dried under reduced pressure and used for identification by paper chromatography. Each fraction of

acidic amino acids was found to contain essentially one compound. The recoveries of α-aminoadipic, glutamic, aspartic acids, and isovalthine were 103, 98, 97, and 101 %, respectively. In order to analyze acidic amino acids in urine, it is necessary to desalt the urine sample by appropriate procedures. In the analysis of isovalthine in urine it is desirable to concentrate the isovalthine fraction by the procedure described above, except that a column with larger cross section is used first, because the concentration of isovalthine in urine is very low.

Gradient elution

The separation of acidic amino acids by gradient elution chromatography was possible with the same column and a mixing chamber of 235 ml. The column was equilibrated with 0.05 *M* acetic acid. After the neutral amino acids had been eluted with 25 ml of 0.05 *M* acetic acid, a gradual increase in the concentration of eluent was effected with 2.0 *M* acetic acid. Glutamic and aspartic acids were well resolved by this procedure. When the concentration of eluent was increased by 7.0 *M* acetic acid, isovalthine was eluted as a separate peak from aspartic acid.

Effect of temperature, sodium chloride and urea

Changes of the temperature of the column in the range from 5 to 35°, and the presence in 3 to 5 ml solution of sodium chloride and urea in physiological concentration, caused little effect on the elution curve.

Another eluent

When formic acid was employed as eluent in a stepwise procedure, α-aminoadipic acid was eluted with 0.05 *M* acid, glutamic acid with 0.15 *M* acid, and aspartic acid with 0.25 *M* acid, respectively.

Preparative scale

5 g of casein hydrolyzate (CASAMINO ACID. DIFCO Lab. Inc.) was dissolved in 0.05 *M* acetic acid and applied on a 6.5 × 13 cm column of Amberlite CG 45 Type II, equilibrated with 0.05 *M* acetic acid, and analyzed. The basic and the neutral amino acids were eluted with 1300 ml of 0.05 *M* acetic acid, and successive elutions with 2000 ml of 0.15 *M* acetic acid and 2000 ml of 0.25 *M* acetic acid yielded glutamic and aspartic acids, respectively. The yields were 682 and 218 mg.

In the case of human urine small portions of two unknown ninhydrin positive substances which resisted hydrolysis partially overlapped with isovalthine in the chromatograms obtained by the stepwise or the gradient elution procedures. Further studies on these substances are necessary.

The author wishes to acknowledge the technical assistance of MISS NORIE SUGINO of his laboratory in the course of these experiments.

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Received July 15th, 1963