# Determination of Lysine on the Automated Amino Acid Analyzer by a Triplicate-Sample Method[1]

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**ABSTRACT**

A triplicate-sample method was developed for determination of lysine by ion-exchange chromatography on an automated amino acid analyzer. In this method, three samples of cereal hydrolysate are applied to the short column of the analyzer, and then the lysine from each sample is resolved with pH 5.10 sodium citrate buffer (0.20*M* in Na) and recorded as a group of three lysine peaks. The resin is next regenerated with 0.2*N* sodium hydroxide and then equilibrated with citrate buffer for the next triplicate analysis. Accuracy of the lysine analysis by the triplicate-sample method is good. The number of lysine analyses that could be run per day is estimated to be 15 with one column fitted to the analyzer, 24 with two columns, and 36 with three columns. The triplicate-sample method has been used for lysine determination of numerous samples of several cereals.

For many years the proteins of cereals have been known to be nutritionally deficient in the essential amino acid lysine. The first major breakthrough in improvement of the nutritional value of cereal protein was made by Mertz, Bates, and Nelson in 1964 (1) when they discovered a mutant gene in corn that increases the lysine and tryptophan contents of corn seed protein. Later reports at the 1966 High Lysine Corn Conference (2) indicated that the corn seed protein was made nutritionally equivalent to the protein of milk.

If high-lysine genes exist in other cereals, a rapid and accurate method of analysis for lysine would greatly facilitate their discovery, since many hundreds of samples might have to be examined. For this reason we have developed a more rapid method for analyzing for lysine on the automated amino acid analyzer. The replicate-sample method first used by Stefanye and Spero (3) in 1964 for analysis of mixtures of acidic and neutral amino acids has been developed for lysine analysis on the short column of an amino acid analyzer.

## MATERIALS AND METHODS

D,L-Histidine and D,L-lysine used in early work were purchased from Nutritional Biochemicals Corporation. An amino acid calibration mixture was from Beckman Instruments, Inc. L-Lysine monohydrochloride of high purity, which was used as a standard for the lysine analysis, was M. A. grade from Mann Research Laboratories, Inc.

The pH 5.28 sodium citrate buffer of 0.35*N* sodium concentration was prepared as described by Benson and Patterson (4). Other sodium citrate buffers which differed in ionic strength and/or pH were made the same way, except that the amounts of sodium citrate and/or hydrochloric acid were changed to give the desired ionic strength and pH.

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A Beckman model 120B analyzer was used for the amino acid analysis. Beckman spherical custom resin PA-35 was used in the short column (15 cm.) for the lysine analysis by the Benson and Patterson accelerated method (4) and our new triplicate-sample method.

## RESULTS AND DISCUSSION

In the replicate-samples method of Stefanye and Spero (3), samples are applied to the column with short intervals of pumping buffer between samples. After development of the chromatogram, each amino acid in the samples emerges from the column as a group of peaks—such as three lysine peaks coming from samples 1, 2, and 3.

The Benson and Patterson accelerated method for basic amino acids (4) did not leave enough room for additional lysine peaks between lysine (first peak) and histidine (second peak), as would be required in a replicate-samples analysis. The pH and ionic strength of the buffer and the column temperature were changed to make the position of the peaks suitable for a triplicate-sample analysis.

The table below lists the conditions found for successful lysine determination by a triplicate-sample method on a mixture of lysine and histidine, an amino acid calibration mixture, or cereal hydrolysates. For comparison,

| | *Methods of Analysis* | |
|---|---|---|
| | *Triplicate* | *Accelerated* |
| pH | 5.10 | 5.28 |
| Sodium concentration | 0.20*N* | 0.35*N* |
| Column temperature | 40°C. | 55°C. |
| Resin | PA-35 | AA-27 |
| Resin height | 9 cm. | 6 cm. |
| Buffer flow rate | 68 ml./hr. | 68 ml./hr. |

the conditions used by Benson and Patterson for their accelerated method on a single sample are also given. For triplicate-sample analysis, the pH of the sodium citrate buffer was lowered to 5.10, the sodium concentration to 0.20*N*, and the column temperature to 40°C. Each change of condition helped to increase the space between the lysine and histidine peak on the analysis of a single sample. To obtain sharper peaks, the Beckman PA-35 resin was used in place of the AA-27 resin originally supplied in our short column. The resin bed height was also increased to 9 cm. to overcome incomplete resolution of triplicate lysine peaks with cereal hydrolysates.

Under the above conditions for triplicate analyses but with a 6-cm. resin bed height, good resolution of lysine from three replicate samples was obtained from a mixture of lysine and histidine and an amino acid calibration mixture. Figure 1 is the chromatogram obtained from three 1-ml. aliquots of the amino acid calibration mixture with lysine peak 1 from aliquot 1, peak 2 from aliquot 2, and peak 3 from aliquot 3.

When triplicate-sample analysis was tried on three aliquots of a wheat hydrolysate on the 6-cm.-height resin bed, the lysine from each aliquot was not completely resolved from the other amino acid or ammonia. Since the lysine in the amino acid calibration mixture was resolved, some of the very

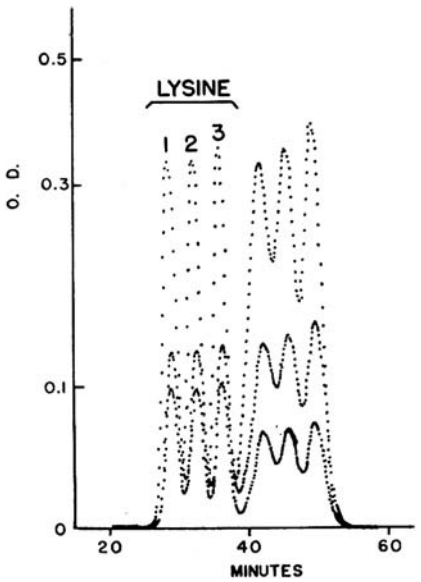


Fig. 1 (left). Triplicate-sample analysis of three 1-ml. aliquots of an amino acid calibration mixture that contains 0.25 μmole/ml. of each amino acid. Buffer flow rate was 68 ml./hr.

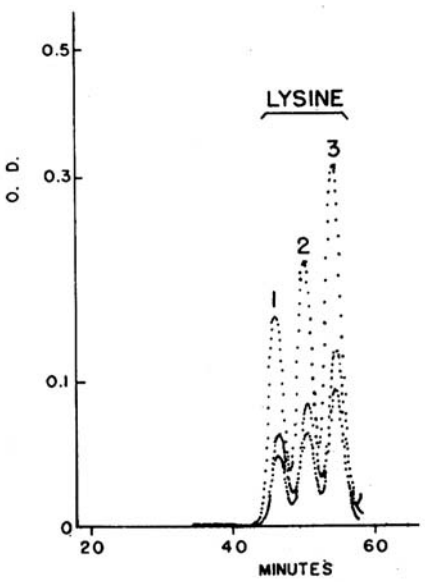


Fig. 2 (right). Triplicate-sample analysis of three 1-ml. samples that contain 0.5, 0.67, and 1 ml. of a whole wheat hydrolysate. Buffer flow rate was 68 ml./hr.

large amount of ammonia in wheat hydrolysates is suspected to have moved in under lysine peaks 2 and 3 to cause their incomplete resolution. To obtain complete resolution of the lysine from triplicate samples of wheat hydrolysate, the resin bed was increased from 6 to 9 cm. Figure 2 shows the chromatogram obtained from three different amounts of a wheat hydrolysate. This chromatogram is typical of the many others that we have obtained with cereal hydrolysates.

The accuracy of the replicate-samples method was checked by analyzing different amounts of a whole-wheat hydrolysate which were all contained in a volume of 1 ml. by appropriate dilution with pH 2.2 sodium citrate buffer when necessary. The results are in the table below, and the chromatogram of

| *Amount of Hydrolysate* ml. | *Number of Replicate* | *Calculated Lysine* μmoles | *Lysine Measured* μmoles |
|---|---|---|---|
| 0.500 | 1st | 0.137 | 0.133 |
| 0.667 | 2nd | 0.182 | 0.183 |
| 1.00 | 3rd | 0.274 | 0.274 |

this analysis is shown in Fig. 2. The amount of lysine that should have been in each replicate was calculated from the lysine value obtained for 1 ml. of hydrolysate analyzed by the Benson and Patterson accelerated method for basic amino acids. The amount of lysine found in each sample in the triplicate-sample analysis was from 97 to 100% of the calculated value.

Good accuracy by the triplicate-sample method was also obtained on whole-wheat hydrolysate to which different amounts of pure lysine (as 0.5 $\mu M$/ml. solution) were added. The results are given in the table below. The

| *Lysine Added* | *Lysine in Hydrolysate* | *Total Lysine* | *Lysine Measured* |
|---|---|---|---|
| *μmoles* | *μmoles* | *μmoles* | *μmoles* |
| 0.250 | 0.098 | 0.348 | 0.338 |
| 0.100 | 0.098 | 0.198 | 0.197 |
| 0.150 | 0.098 | 0.248 | 0.238 |

total lysine contained in each sample was that calculated from an analysis of 1 ml. of the hydrolysate by the accelerated method, plus the lysine added. The amount of lysine found in each sample was from 96 to 99% of the expected value.

The procedure we use at present for triplicate analysis for lysine at a buffer flow rate of 68 ml./hr. is as follows: Aliquots (1-ml.) of three hydrolysates to be analyzed for lysine are applied to the column. For each sample about 8.5 min. total time is required to add the sample. This includes pushing it into the resin with nitrogen pressure (30 p.s.i.) and washing the column walls thrice with pH 5.10 buffer. Sodium citrate buffer of pH 5.10 is pumped through the column for 3.6 min. between application of samples 1 and 2, and 2 and 3. The lysine in each sample is resolved as separate peaks by pumping pH 5.10 buffer through the column for about 57 min. The first lysine peak begins after 44 to 45 min. Immediately after the last lysine peak the analysis is stopped; then the resin is regenerated by pushing 0.2$N$ sodium hydroxide through the column for 7 min. with 30 p.s.i. nitrogen pressure. Finally, the column is equilibrated for the next analysis by pumping pH 5.10 buffer through the column for 25 min.

The analysis time on protein hydrolysates with the triplicate-sample method can be shortened by increasing the buffer flow rate from 68 ml./hr. to 80 with no loss in accuracy or resolution of the lysine peaks. The number of lysine analyses per day (8 A.M. to 5 P.M.) at the higher buffer flow rate with our amino acid analyzer has been estimated by the multiple activity charts shown in Fig. 3. A complete analysis cycle is given at the bottom of this figure. An automatic shut-down timer is used to complete the equilibration of resin or development of chromatogram on one column after 12 noon and 5 P.M. On analyzers equipped with two or three columns. effluents of columns from which lysine peaks are not being recorded are directed around the reaction coil and colorimeter to a drain bucket (indicated by drain on bar graph). With one column run continuously, 15 lysine analyses can be completed. This is illustrated by the left bar of Fig. 3. With two columns and two buffer pumps (bars 1 and 2), 24 lysine analyses can be run with the operator's lunch hour free. With three columns and three buffer pumps (bars 1, 2, and 3), 36 analyses can be done with the noon hour still free.

When one desires to analyze cereals for lysine alone on the automated

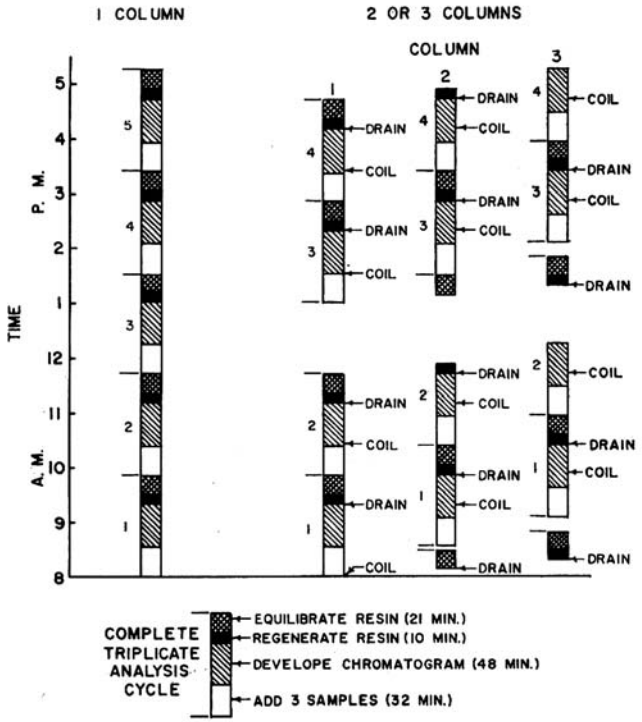


Fig. 3. Multiple activity charts for triplicate-sample analysis on an amino acid analyzer containing one, two, or three columns. Buffer flow rate was 80 ml./hr. and work day was from 8 A.M. to 5 P.M.

amino acid analyzer, the triplicate-sample method described here can save time, chart paper, ninhydrin reagent, and citrate buffer. We have found this method for lysine analysis to be very useful in the analysis of numerous samples of wheat, rye, and triticale.

**Acknowledgment**

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