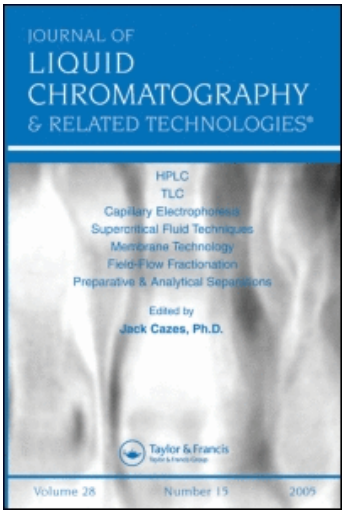

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QUANTIFICATION OF LYSINE IN DIETARY SUPPLEMENT TABLETS AND CAPSULES BY REVERSED PHASE HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY WITH VISIBLE MODE DENSITOMETRY

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ABSTRACT

A quantitative method using chemically bonded C-18 silica gel HPTLC plates, automated bandwise sample application, detection with ninhydrin chromogenic reagent solution, and automated visible mode densitometry has been developed for the determination of L-lysine hydrochloride in nutrition supplements. Three products containing 500 mg of lysine and additional ingredients were analyzed. Accuracy was validated by analysis of spiked blank and standard addition samples and precision by performing replicated analyses. Accuracy was found to be within 3% of the theoretical values, and precision was 2% relative standard deviation.

Key Words: Thin layer chromatography; Densitometry; Amino acid; Lysine; Nutrition supplements

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INTRODUCTION

Lysine is an essential amino acid that aids proper growth and tissue synthesis, processing of fatty acids, absorption of calcium, formation of collagen, synthesis of carnitine, and maintenance of good nitrogen balance. It is prescribed by doctors for a variety of purposes, including treatment of genital herpes and cold sores and prevention and reversal of atherosclerosis. Because it cannot be synthesized by the human body, dietary or nutritional supplements in the form of L-lysine are widely consumed, and methods are needed for analysis of these products by manufacturers and government regulators.

The standard method for determination of lysine in dietary supplements involves the use of an amino acid analyzer.[1] In earlier studies, high performance thin layer chromatography (HPTLC) methods were developed for the quantitative determination of amino acids in snails[2] and snail conditioned water[3] and for assay of vitamins[4] and creatine[5] in nutritional supplements. A computer-assisted search of the literature through Chemical Abstracts and the Web of Science found no previous papers on the TLC or HPTLC analysis of amino acid dietary supplements. Therefore, in this research, the HPTLC methods described previously for biological samples were adapted for analysis of lysine supplements using a chemically bonded reversed phase C-18 silica gel layer, detection with ninhydrin spray reagent, and automated bandwise sample application and densitometric scanning. The new method was validated for sensitivity, linearity, accuracy, and precision and applied to three different supplement brands.

EXPERIMENTAL

Preparation of Standard and Sample Solutions

A stock standard solution of L-lysine hydrochloride (#L8662, Sigma, St. Louis, MO, USA; CAS Registry No. 657-27-2) was prepared at a concentration of 100 mg mL^{-1} in ethanol–deionized water (7 : 3). The TLC standard was prepared at 0.100 mg mL^{-1} in the same solvent by direct weighing or appropriate dilution of the stock solution.

Three different brands of dietary supplement products with label specifications of 500 mg of L-lysine hydrochloride were purchased from local pharmacies, two in the form of tablets, and one capsules. A sample stock solution of each was prepared by grinding one tablet or the contents of one capsule to a fine powder using a mortar and pestle and transferring the powder through a funnel into a 100 mL volumetric flask by washing with ca. 70 mL of deionized

water. The solution was stirred magnetically for 1 hr to completely dissolve the lysine, after which the stirbar was removed. The solution was diluted to the line with deionized water and shaken, and the flask was allowed to stand for 30 min. The theoretical lysine concentration of each solution was 5.00 mg mL^{-1}, based on the label declaration. TLC test solutions for capsules with a theoretical concentration of 0.0500 mg mL^{-1} were prepared by pipetting 1.00 mL of the clear sample stock solution into a 100 mL volumetric flask, and diluting to the line with ethanol–deionized water (7 : 3). Tablet solutions did not become completely clear during the standing period and required filtering of unsettled solid by vacuum filtration through filter paper before removing the 1.00 mL aliquot for dilution.

Thin Layer Chromatographic Analysis

Analyses were performed on 10×20 cm high performance reversed phase RP-18 F_{254S} plates with concentrating zone (Art. 15 498, EM Science, Gibbstown, NJ, USA, an affiliate of Merck KGaA, Darmstadt, Germany). The plates were precleaned by development to the top with dichloromethane–methanol (1 : 1) and dried before use. Standard and sample solutions were applied to the preadsorbent area of the plate by means of a Camag (Wilmington, NC USA) Linomat IV automated spray-on band applicator equipped with a 100 μL syringe and operated with the following settings: band length 6 mm, application rate 10 s μL^{-1}, table speed 10 mm s^{-1}, distance between bands 4 mm, distance from the side edge 0.7 cm, and distance from the bottom, 1.5 cm. The volumes applied for each analysis were 2.00 μL, duplicate 4.00 μL, and 8.00 μL of the standard (0.200–0.800 μg of lysine) and duplicate 8.00 μL of the sample solutions (0.400 μg theoretical lysine content).

Plates were developed to a distance 6 cm beyond the origin (preadsorbent-silica gel interface) with the mobile phase consisting of 1-butanol–glacial acetic acid–deionized water (3 : 1 : 1) in a vapor-equilibrated Camag (Wilmington, NC, USA) twin-trough chamber containing a saturation pad (Analtech, Newark, DE, USA). The development time was ca. 1.5 hr.

After development, the mobile phase was evaporated from the plate by drying in a fume hood for 2 hr with a hair dryer and then sprayed heavily and evenly with ninhydrin reagent (0.3 g of ninhydrin dissolved in 100 mL of 1-butanol plus 3 mL of glacial acetic acid). The plate was dried in a fume hood for 30 min and then heated on a Camag plate heater at 110°C for 15 min to produce purple zones of lysine on a white layer background. The zones of standards and samples were measured by linear scanning at 610 nm by use of a Camag TLC Scanner II with the tungsten source, slit length 4, slit

width 4, and scanning rate 4.0 mm s^{-1}. The CATS-3 software controlling the densitometer produced a calibration curve by polynomial regression of the weights and areas of the standard zone scans and automatically interpolated the weights of the sample zones from their scan areas. For each analysis, recovery (%) was calculated by dividing the average experimental weight of the duplicate samples by the theoretical weight predicted by the label declaration and multiplying by 100.

Validation

The accuracy of the new method was validated by two spiking analyses and one standard addition analysis. Other amino acid supplement capsules were chosen as blanks for the spiking experiments. One contained L-tyrosine (500 mg), calcium (134 mg), and phosphorus (105 mg) as active ingredients, and the other contained L-methionine (500 mg). These were appropriate blanks because the active ingredients were separated from lysine on the layer and, together, they contained eight inactive ingredients present in the three lysine products analyzed, including gelatin, cellulose, magnesium stearate, MCT, silica, stearic acid, and dicalcium phosphate. Additional inactive ingredients were present in the blanks that may be in different brands of lysine products that were not analyzed.

To prepare the blank solutions, methionine and tyrosine capsules test solutions were prepared as described above, but 50.0 μL of the 100 mg/mL lysine stock solution was added after the 1 : 100 dilution to each of the final 100 mL volumetric flasks before dilution to the line to simulate lysine tablet solutions containing exactly the label amount. Unspiked solutions of each blank capsule were prepared similarly. The spiked and unspiked solutions were diluted 1 : 100 and analyzed as described above. Recovery was calculated by comparing the analytical results for the spiked blank solutions with the theoretical value based on the weight of lysine added.

The standard addition experiment was performed as follows: A lysine tablet test solution was prepared as described above, and a 1.00 mL aliquot was placed in a vial and mixed with 50.0 μL of the 100 mg/mL stock solution. The solutions were analyzed on the same plate by applying the usual standard aliquots, duplicate 8.00 μL aliquots of the test solution, and duplicate 4.00 μL aliquots of the spiked solution. The mean result of the spiked sample was compared to the theoretical result (the mean result of the unspiked sample plus the added weight) to calculate the recovery.

The precision of the method was evaluated by analyzing one sample six times on one plate as described above and calculating the relative standard deviation of the recovery results.

RESULTS AND DISCUSSION

Water was found to be the best solvent for dissolving the samples, but fully aqueous solutions could not be spotted in compact initial zones using the Linomat. Therefore, the 1 : 100 dilution required to bring the sample zone concentration within the calibration range of the standards was made with ethanol–water (7 : 3), which can be spotted efficiently with the Linomat.

Lysine formed a narrow, flat, band-shaped zone with an R_f value of 0.22 on the C-18 layer developed with 1-butanol–acetic acid–water. The detection and quantifications limits were about 100 ng per zone. The scanning wavelength of 610 nm was the absorption maximum of the in situ spectrum of a lysine standard zone measured with the spectral mode of the Camag scanner. No additional zones were detected in sample chromatograms, except for the amino acids present in the blanks used in the validation studies. Preparation of the calibration curves using polynomial regression led to superior analytical results, compared to linear regression. The value of r (correlation coefficient) for polynomial regression of the calibration curves (scan areas vs weights for the four standards) was 0.999 in all analyses.

Table 1 shows the results of a survey of the two tablet and one capsule commercial lysine supplements, carried out using the new HPTLC method. The values are generally higher than the 90–110% recovery usually specified for pharmaceutical dosage forms in the USP[6] and found in our earlier surveys of commercial pharmaceutical dosage forms (e.g., Ref.[7]). Comparable published ranges of acceptable active ingredient content are not available for amino acid or other nutritional supplements.

Accuracy was validated by preparing and analyzing spiked blank and standard addition samples as described above. Recoveries of added lysine from the spiked tyrosine and methionine capsule solutions were 100% and 103%, respectively, which represented 0.00% and 3.00% errors. Analysis of unspiked

Table 1. Recoveries of Lysine Nutrition Supplements Relative to the 500 mg Label Value

Sample Number	Tablet 1	Tablet 2	Capsule 1
1	113	113	118
2	99.0	94.4	121
3	89.2	99.6	124
4	95.3	107	94.4
5	109		95.1
6	104		
Average	102	104	110

blank solutions showed that no interferences occurred at the R_f value of lysine and no correction of the scan areas of the spiked blank solutions was required. In the standard addition validation using a tablet one (Table 1) sample, the tablet solution initially assayed at 111% recovery, compared to the label declaration, and the recovery was within 1.72% of the amount of lysine added to this solution.

Reproducibility was determined by spotting six 8.00 μL aliquots of sample solutions prepared from the capsule product and tablet 1 (Table 1). Average recoveries, relative to the label value, were 110% and 107% and relative standard deviations were 1.95% and 2.14%, respectively. As another measure of precision, percent difference values of the areas of duplicate samples and standards spotted in the analyses averaged 2.1% with a range of 0.048–4.8%. These precision values are excellent, considering that application of a detection reagent by spraying was a step in the method.

It has been demonstrated above that accuracy and precision of the new method meet the guidelines set by the International Conference on Harmonization (ICH) for assay of pharmaceutical products[8] and are similar to values reported regularly in the literature for HPTLC and HPLC analysis of pharmaceutical products and nutritional supplements. It will be valuable for use by manufacturing companies in qualitative or quantitative quality assurance analysis and by government laboratories for survey and regulation of products being sold. The method will be useful, directly, for analyzing other commercial brands of lysine supplements and can be adapted for analysis of supplements containing mixtures of two or more amino acids if they can be separated by HPTLC. We have published R_f data for numerous amino acids on silica gel, cellulose, reversed phase, and ion exchange layers that will aid analysts in choosing appropriate systems for analysis of these mixed formulations.[2,3,8,9] Compared to the amino acid analyzer (column ion exchange chromatography), the method is easier to perform and has the ability to analyze multiple samples against standards chromatographed on the same plate, leading to increased accuracy, speed, and sample throughput and lower cost and solvent usage per sample.

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REFERENCES

1. Helrich, K., Ed. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th Ed.; AOAC, 1990; 1100–1101.

2. Norfolk, E.; Khan, S.H.; Fried, B.; Sherma, J. Comparison of Amino Acid Separations on High Performance Silica Gel, Cellulose, and C-18 Reversed Phase Layers and Application of HPTLC to the Determination of Amino Acids in *Biomphalaria glabrata* Snails. J. Liq. Chromatogr. **1994**, *26* (6), 1317–1326.
3. Steiner, R.A.; Fried, B.; Sherma, J. HPTLC Determination of Amino Acids in Snail-Conditioned Water from *Biomphalaria glabrata*, Two Strains of *Helisoma trivolvis*, and *Lymnaea elodes.* J. Liq. Chromatogr. and Rel. Technol. **1998**, *21* (3), 427–432.
4. Sherma, J.; Ervin, M. Quantification of Niacin and Niacinamide in Vitamin Preparations by Densitometric Thin Layer Chromatography. J. Liq. Chromatogr. **1986**, *9* (16), 3423–3431.
5. Wagner, S.D.; Kaufer, S.W.; Sherma, J. Quantification of Creatine in Nutrition Supplements by Thin Layer Chromatography-Densitometry with Thermochemical Activation of Fluorescence Quenching. J. Liq. Chromatogr. and Rel. Technol. **2001**, *24* (16), 2525–2530.
6. *The United States Pharmacopeia/The National Formulary (USP 24/NF 19)*; United States Pharmacopeial Convention, Inc.: Rockville, MD, 2000.
7. Wagner, S.D.; Sherma, J. Analysis of the Active Ingredient Cimetidine in Acid Reduction Tablets by High Performance Thin Layer Chromatography with Ultraviolet Absorption Densitometry. Chromatography **2001**, *22* (2), 97–99.
8. Sleckman, B.P.; Sherma, J. A Comparison of Amino Acid Separations on Silica Gel, Cellulose, and Ion Exchange Thin Layers. J. Liq. Chromatogr. **1982**, *5* (6), 1051–1068.
9. Wagner, S.D.; Pachuski, J.; Fried, B.; Sherma, J. Thin Layer Chromatographic Analysis of Carbohydrates and Amino Acids in *Schistosoma mansoni Cercariae.* Acta Chromatogr. **2002**, *12*, 159–169.

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