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# Simultaneous Determination of Cephalexin and Lysine in Their Salt Using High-Performance Liquid Chromatography of Derivatives

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Received November 2, 1979, from the *Analytical Department, Laboratorios Almirall, C/Cardoner, 68-74, Barcelona 24, Spain.* Accepted for publication February 29, 1980.

**Abstract** □ A sensitive and useful high-performance liquid chromatographic method using derivatization was developed for the simultaneous determination of intact cephalexin and lysine in their salt. This method is rapid and reliable, and its inherent specificity makes it an improvement over the common wet chemical methods for stability studies.

**Keyphrases** □ Cephalexin—simultaneous high-performance liquid chromatographic determination with lysine in their salt using derivatization □ Lysine—simultaneous high-performance liquid chromatographic determination with cephalexin in their salt using derivatization □ High-performance liquid chromatography—analysis, cephalexin and lysine in their salt, derivatization

The sodium salt of cephalexin is absorbed poorly after intramuscular injection (1, 2). Similar observations were made with a related compound, cephradine (3). Investigations with other soluble salts of cephalexin showed that some basic amino acids increased the absorption and bioavailability of cephalexin (4). The extensive use of the lysine salt of cephalexin necessitated the development of methods for the simultaneous determination of both components in this salt. Alkalimetric determination of carboxylic groups and nonaqueous titration of $\alpha$-amino groups cannot be used because these two groups are present in both components. For the same reason, the Folin reaction with sodium $\beta$-naphthoquinone-4-sulfonate or hypobromite and $\alpha$-naphthol (5) and the Saxena method (6) for microtitration of amino acid mixtures are unsatisfactory, and the selective titration of the $\epsilon$-amino group of lysine in aqueous media with sulfuric acid is insufficiently accurate.

A recent report described the intramolecular aminolysis of some cephalosporins, caused by the nucleophilic attack of an $\alpha$-amino group in the C-7 side chain in the $\beta$-lactam nucleus, which yields piperazine-2,5-dione derivatives (7). Estimation of the rate of disappearance of free amino groups was carried out by the 2,4,6-trinitrobenzenesulfonic acid (I) assay of Satake *et al.* (8) in a modified form. Applications of this technique to cephalexin lysinate was not possible due to interference by lysine.

A high-performance liquid chromatographic (HPLC) procedure for the analysis of lysine and cephalexin trinitrophenyl derivatives (II and III, respectively) is presented here. It is a rapid, sensitive, and specific method for the simultaneous determination of these two substances.

## EXPERIMENTAL

**Apparatus**—Absorption spectra were obtained with a double-beam spectrophotometer[1] with a 1.0-cm cell. HPLC assays were performed with a liquid chromatograph[2] equipped with a variable-wavelength detector[3] and a 1-$\mu$l loop injection valve[4]. A stainless steel column (25 × 0.46 cm) loaded with alkylamine[5] (13 $\mu$m) was used.

**Reagents**—Cephalexin monohydrate[6], lysine hydrochloride[7], the lysine salt of cephalexin[8], and trinitrobenzenesulfonic acid[9] were used. The buffer solutions of pH values up to 7.6 were McIlvaine's buffers (9), the pH 7.6 buffer was prepared according to the method of Bundgaard (7), and the buffer solutions of higher pH were those of Clark and Lubs (10). All other reagents were analytical reagent grade.

**General HPLC Procedure**—An aqueous solution containing the lysine salt of cephalexin (2.0 ml) ($5 \times 10^{-4}$–$2 \times 10^{-3}$ *M*) was mixed in a 100-ml volumetric flask with buffer solution (pH 10, 2.0 ml) and I (0.4% in water, 5.0 ml). The mixture was shaken and kept in the dark for 1 hr. Acetate buffer (pH 4.8, 20.0 ml) and aqueous 0.1% *o*-nitrophenol (2.0 ml as the internal standard) were added, and the solution was brought to volume with water.

A sample of this solution (1 $\mu$l) was injected into the liquid chromatograph and developed using an eluent system of 1% citric acid in a methanol–water mixture (5:40 v/v) with an isocratic flow rate of 0.75

[1] Perkin-Elmer 323.
[2] Perkin Elmer 601.
[3] Perkin-Elmer LC-55.
[4] Chrompack.
[5] Amino Sil-X-I.
[6] Lilly.
[7] Merck.
[8] Laboratorios Almirall.
[9] Aldrich.



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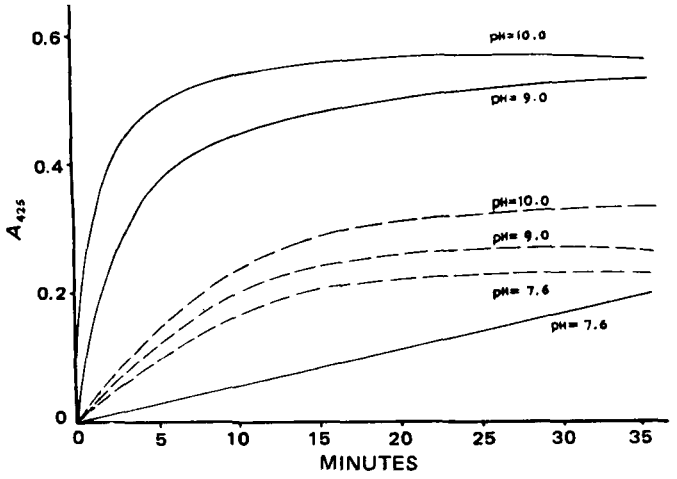


**Figure 1**—*Time effect on reaction of cephalexin* (- -) *and lysine* (—) *at different pH values.*

ml/min and detection at 425 nm. Good chromatographic performance was achieved only when the column temperature was 50°.

Any significant degradation products were detected by TLC after the initial reaction at pH 10.

**Procedure in Basic Degradation Study**—Solutions ($\sim 10^{-3}$ *M*) of cephalexin and lysine in 0.5 *N* NaOH were prepared. The assay was carried out on samples taken after standing for 20 and 60 min, respectively, at room temperature. The pH was adjusted to pH 4.8 with 2 *N* HCl, and HPLC of II and III was carried out as described.

**Procedure in Acid Degradation Study**—Solutions of cephalexin and lysine ($\sim 10^{-3}$ *M*) in 1 *N* HCl were maintained at 60° for 1 hr. These solutions were brought to pH 4.8 with 1 *N* NaOH, and HPLC of II and III was carried out as described.

## RESULTS AND DISCUSSION

The simple spectrophotometric method of Bundgaard (7) could not be used for the simultaneous analysis of lysine and cephalexin because both derivatives show identical absorption maxima (425 nm). The velocity constants of the reaction of I with the primary amino compounds is known to be related exponentially to the pH of the reaction medium (11). Consequently, a study was made to find conditions for the direct spectrophotometric assay of cephalexin and lysine in mixtures of these two components. Cephalexin and lysine do not react with I at an appreciable rate below pH 6. Both II and III are stable over long periods and, at other pH values, the reaction obeys pseudo-first-order kinetics, except the reaction of lysine and I at pH 7.6, which is linear with time (Fig. 1).

The different reaction rates of the $\alpha$- and $\epsilon$-amino groups of lysine with

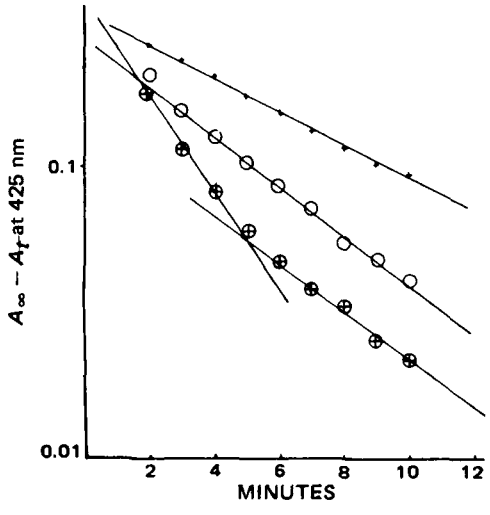


**Figure 2**—*Reaction kinetics of I at pH 10.0. Key:* ×, *cephalexin;* ○, *salt; and* ⊕, *lysine.*

**Table I—Absolute Retention Times (Minutes) on Amino Sil-X-I Using Different Eluents**

| Compound | Eluent[a] A | B | C | D |
|---|---|---|---|---|
| I | 3.3 | >60 | 7.6 | 7.6 |
| II | 6.7 | 4.2 | 3.8 | 3.9 |
| III | >60 | >60 | 7.3 | 11.6 |
| *o*-Nitrophenol | — | — | — | 5.5 |

[a] Eluent A is methanol, Eluent B is 1% acetic acid in methanol–water (5:40 v/v), Eluent C is methanol–0.01 *M* sulfuric acid (5:40 v/v), and Eluent D is 1% citric acid in methanol–water (5:40 v/v).

**Table II—Parameters of Calibration Curves**

| Equation[a] | Compound | $a_0$, mg/ml | Correlation Coefficient | *n* |
|---|---|---|---|---|
| $c(\text{mg/ml}) = a_0 + a_1 \frac{h}{h_s}$ | II | 0.0477 | 0.9909 | 8 |
| | III | 0.7501 | 0.9873 | 8 |
| $c(\text{mg/ml}) = a_0 + a_1 h(\text{mm})$ | II | 0.0177 | 0.9916 | 15 |
| | III | 0.0833 | 0.9977 | 15 |

[a] $h$ and $h_s$ are the heights of the sample and internal standard chromatographic peaks, respectively.

I are apparent from a change in the slope of the rate curve (Fig. 2). The slope of the absorbance–time curve for lysine at pH 7.6 is proportional to the concentration of lysine and is linear over the concentration range of 0.5–1.3 × $10^{-3}$ *M*. By measuring the change of absorbance with time at pH 7.6, it is possible to measure the concentration of both cephalexin and lysine, but there are only small changes in the slope of the line with large changes in the lysine concentration, leading to low sensitivity.

For these reasons, the liquid chromatography of derivatives of I was selected since these derivatives are stable (12), they behave well on alkylamine columns (13), and lysine does not absorb appreciably in the visible or UV regions.

Quantitative formation of II and III occurred most rapidly when the reaction medium was at pH 10 (Fig. 1). However, Amino Sil-X-I columns are not stable at this pH, and the pH of the medium after formation of these derivatives was reduced to pH 4.8. The cephalexin derivative is stable for up to 20 hr at this pH and the lysine derivative is stable for 2 hr, after which it slowly decomposes. The retention times of II and III using different eluents are shown in Table I.

Lysine and especially cephalexin (14) are degraded in both alkaline and acidic solutions. Many of the degradation products contain primary amino groups and are a potential source of interference in the specific analysis of lysine and cephalexin, particularly in pharmaceutical products undergoing stability studies.

Pure solutions of lysine and cephalexin were degraded under acidic and alkaline conditions for different times, and the chromatograms of II and III were studied. Under alkaline conditions, the original peaks of unchanged lysine and cephalexin derivatives (retention times of 3.9 and 11.6 min, respectively) disappeared, and well-defined peaks of degradation products appeared (at 8.8 min from II and at 1.8, 2.4, and 9.4 min from III). However, none of these peaks interfered with the main peaks

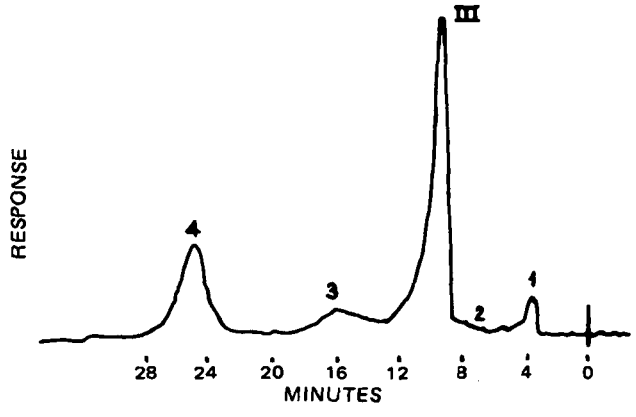


**Figure 3**—*Chromatogram of III and acid-degraded cephalexin derivative mixture.*

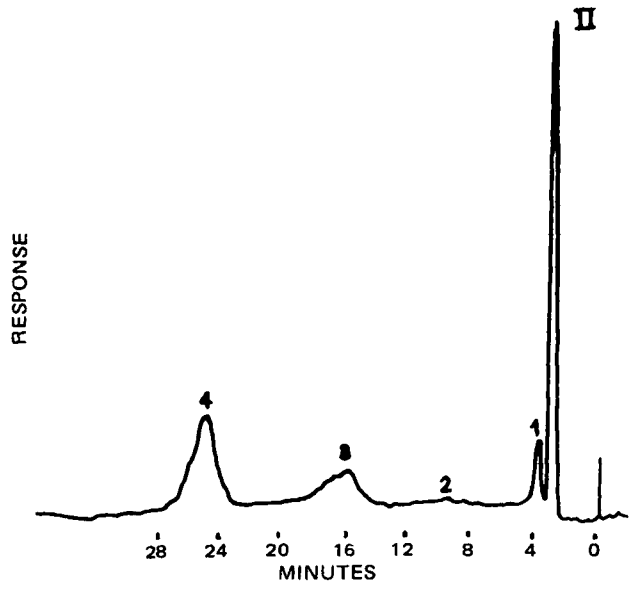


**Figure 4**—*Chromatogram of II and acid-degraded cephalexin derivative mixture.*

used in the assay of lysine and cephalexin. Cephalexin solutions stored under acidic conditions at 60° yielded four secondary peaks (retention times of 4.1, 11.0, 15.2, and 27.2 min), which could interfere with the chromatographic assay. Trinitrophenyl derivatives were prepared from mixtures containing 50% of acid-hydrolyzed cephalexin solutions and 50% of a pure cephalexin or pure lysine solution (Figs. 3 and 4), respectively. It is apparent that the peak of II was not affected by the acid decomposition products of cephalexin and that the peak of III was affected only minimally. The chromatogram of II after acid hydrolysis (1 hr) showed only two small additional peaks at 16.4 and 26.0 min.

The calibration factors used for the quantitative analysis of cephalexin and lysine are given in Table II. The low values of the intercept at the origin ($a_0$), when operating with the loop-injection technique but without the internal standard, allow use of this technique. Table II shows that similar calibration parameters were obtained by both techniques (with and without an internal standard), corresponding to the listed straight-line equations. The average response factors for II and III were 110.94 and 36.92 mm/mg with standard deviations of 0.76 and 1.05, respectively, for a set of 45 chromatograms. Sensitivity was three times higher for lysine than for cephalexin, but the sensitivity of the instrument allows lower detection limits of 0.1 and 0.3 μg/ml for lysine and cephalexin, respectively.

The high reproducibility and sensitivity of the HPLC method described here recommend it as a suitable procedure for the simultaneous analysis of cephalexin and lysine in mixtures of these two products for quality control and stability studies of pharmaceutical formulations containing cephalexin lysinate.

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# Determination of Dextromethorphan Hydrobromide by High-Performance Liquid Chromatography Using Ion-Pair Formation

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**Abstract** □ The chromatographic retention behavior of dextromethorphan hydrobromide on an octadecylsilane column was investigated as a function of the pairing ion and the mobile phase composition. Dramatic increases in the capacity factor were observed with pairing ions containing more than eight carbons and with decreasing organic modifier (acetonitrile) concentration. Several pharmaceutically important amines exhibited similar behavior with respect to acetonitrile concentration. An analytical method was developed for dextromethorphan hydrobromide bulk drug and syrups and was applied to commercial preparations.

**Keyphrases** □ Dextromethorphan hydrobromide—high-performance liquid chromatographic analysis using ion-pair formation □ High-performance liquid chromatography—analysis, dextromethorphan hydrobromide, ion-pair formation □ Antitussives—dextromethorphan hydrobromide, high-performance liquid chromatographic analysis using ion-pair formation

Dextromethorphan hydrobromide [(+)-3-methoxy-17-methyl-9α,13α,14α-morphinan hydrobromide, I] is a centrally active antitussive which, unlike codeine, is devoid of analgesic properties and exhibits little or no depression of the central nervous system. Because of its lack of addiction potential, it is widely used in nonprescription



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