DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 13(15), 2771-2787 (1987)

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS AND DISSOLUTION OF AMPICILLIN AND CLOXACILLIN IN CAPSULE FORMULATION

Mutaz A. Sheikh Salem*
and
Hanan N. Alkaysi

College of Pharmacy
Yarmouk University
Irbid, Jordan.

ABSTRACT

A reversed phase high performance liquid chromatographic method was developed for the quantitation of ampicillin and cloxacillin in capsule using a mobile phase consisting of acetonitrile (45.3%) in phosphate buffer. Sample preparation before analysis was minimal. The method is precise and adaptable for quality control purposes. The use of the analytical method for studying capsule dissolution is described.

INTRODUCTION

Ampicillin (I) and cloxacillin (II) are among the most widely used penicillin derivatives. Combination of both drugs are commercially available in different

dosage forms such as capsules, injections and powders for suspension. Various methods are used for the analysis of both drugs individually. The USP recommends non-aqueous titration for ampicillin and spectrophotometric determination for cloxacillin (1). Other methods include iodometric and colorimetric determination of hydrolysis products (2,3). The difficulty arises when a simultaneous quantitative determination of I and II is required. Interference is expected and separation between the components becomes important. A chromatographic method to separate several penicillin derivatives on reverse phase thin layer chromatography was reported (4). High performance liquid chromatographic methods were reported for either antibiotic alone or in mixture with other penicillin derivatives (5-8).

This report describes a reverse phase high pressure liquid chromatographic method for the quantitative determination of both ampicillin and cloxacillin in capsule dosage form. The assay was applied successfully to four different commercial brands and proved to be free of interference from excipients normally used in formulations. The method was also utilized for a comparative study of dissolution rates of capsules. The assay is simple fast and does not require sample manipulation.

EXPERIMENTAL

Apparatus: A Beckman chromatographic system was used, consisting of solvent delivery pumps 114 A; and injection head with 10µl loop and a variable wave length detector 165 which was connected to spectraphysics integrator 4270. The column used was 5 micron Altex Ultrasphere^{R} C-18, provided with a guard column.

Chemicals and Reagents: Ampicillin trihydrate, cloxacillin sodium and sulfamethoxazole were of pharmacopeial standards, provided by Jordanian Pharmaceutical Manufacturing Company. Acetonitrile was HPLC grade (J.T. Baker U.S.A.), water was glass distilled and demineralized. Potassium dihydrogen phosphate was analar grade (BDH, pool, Dorset U.K.).

Chromatographic Conditions: The mobile phase used consisted of 0.05M potassium dihydrogen phosphate buffer (pH 4.5, unadjusted) mixed with acetonitrile (45.3%) flowing at a rate of 1.5 ml/min. Injection volume was 10µl, and detection was carried at 254 nm. Mobile phase and samples were filtered before use. Sulfamethoxazole solution in methanol (1 mg/ml) was prepared and stored in a tightly covered flask and 100µl aliqoutes were added to assay solutions before injection.

Calibration curves for concentration vs. response were performed by preparing a stock solution of ampicillin trihydrate and cloxacillin sodium standards in the

mobile phase at a concentration of 1 mg/ml. A series of dilutions were prepared by measuring 0.5-6ml of the stock solution, adding 100µl of internal standard solution and diluting up to 10 ml with mobile phase. The concentration range was 0.05-0.6 mg/ml.

Dosage Forms: Four commercially available brands of capsules containing 250mg of ampicillin B.P. and 250 mg cloxacillin sodium B.P. according to the label were used. The four brands were designated as A,B,C and D respectively.

Reference Standards: Standards were obtained by successive crystallization of ampicillin trihydrate and cloxacillin sodium using water and alcohol-chloroform solution respectively. Purity was checked by TLC, HPLC and melting point. Sulfamethoxazole used as internal standard was recrystallized before use.

Sample Preparation:

a. Content uniformity: Individual capsules were emptied, weighed and powdered. The equivalent of 20 mg was weighed accurately. The powder would therefore contain 10 mg of each component (I and II). Dissolution in 10 ml of mobile phase was affected in an ultrasonic bath. 2 ml aliquote was pippetted and placed in 10 ml volumetric flask. 100µl of the internal solution were added and the volume was completed to the mark with the mobile phase. All samples and standard solutions were freshly prepared before injection.

b. Average: The contents of ten capsules were weighed and the average weight of one capsule was determined. The equivalent of 20 mg was weighed accurately and the same procedure described under content uniformity was followed.

c. Recovery Experiments: The contents of 10 capsules were emptied, weighed and the average weight of a capsule was calculated. The equivalent of 20 mg of I and II was weighed. To this powder 50 mg of ampicillin trihydrate reference standard and 10 mg of cloxacillin sodium reference standard were added. The mixture was dissolved in 10 ml of mobile phase and 1 ml of the solution was placed in 10 ml volumetric flask and completed to volume, after adding 100µl of the internal standard solution. The concentration of I would be 0.6 mg/ml and of II 0.2 mg/ml. 10µl of this solution was injected. A similar experiment was conducted by adding 50 mg of cloxacillin sodium and 10 mg of ampicillin trihydrate. While a third sample was prepared by adding 10 mg of each component.

Determination of Dissolution Rate: The conditions specified by the USP XXI for the determination of dissolution rate of ampicillin and cloxacillin capsules by the basket method were followed. The dissolution vessel was filled with 900 ml of water and the capsules were placed in the basket. 10 ml aliquotes of dissolu-

tion medium were withdrawn at 0,5,10,15,20,30,40,50 and 60 minutes. The water was replaced. 5 ml samples were filtered and diluted up to 10 ml with mobile phase. 100µl of internal standard solution was added prior to dilution.

Calculations: The results were calculated by using the following formula

$$\frac{(RR)_a}{(RR)_s} \times 100 = \text{Percent of the label claim}$$

where $(RR)_a$ is the ratio of peak areas (I or II/ internal standard) of the assay solution, and $(RR)_s$ is the ratio calculated for standard solution of identical concentration injected sequentially.

The same calculation formula was used for dissolution rate determination, the standard solution was prepared by dissolving the label amounts of drug ingredients of one capsule in 900 ml of water and was subjected to identical analytical conditions.

RESULTS AND DISCUSSION

The major aim of the work was to develope a quantitative HPLC analysis for dosage forms containing ampicillin and cloxacillin. The importance of such a method would be in its utilization for quality control purposes, thus it was necessary to achieve good resolution between components, minimum sample manipulation, short time of analysis and precision.

The exact composition of the mobile phase that seemed to fulfill the above requirements was 45.3% acetonitrile in 0.05 M phosphate buffer, this percentage was realized in the course of studying the effect of acetonitrile on the retention time and peak shape of both ampicillin and cloxacillin. It was found that ampicillin peak shape and retention time was independent of the concentration of acetonitrile in the mobile phase. The retention time was almost constant over the entire range of acetonitrile percentage used (figure 1-a). Cloxacillin, on the other hand, showed broad peaks with appreciable tailing at lower precentage of acetonitrile in the mobile phase (20-35%), the retention time was too long for practical analytical purposes. As the concentration of acetonitrile increases, cloxacillin peak becomes sharper, with increased absorbance and a shorter retention time (figure 1-b). The choice of mobile phase used for the analytical method provided sharp peaks and reasonable resolution (Rt = 0.85-1.00 min).

A typical chromatogram is shown in figure 2. The selection of sulfamethoxazole as internal standard was based on its reproducible sharp peak under the chromatographic conditions.

The quantitative aspects of the analytical method were studied, the response ratios (expressed in peak

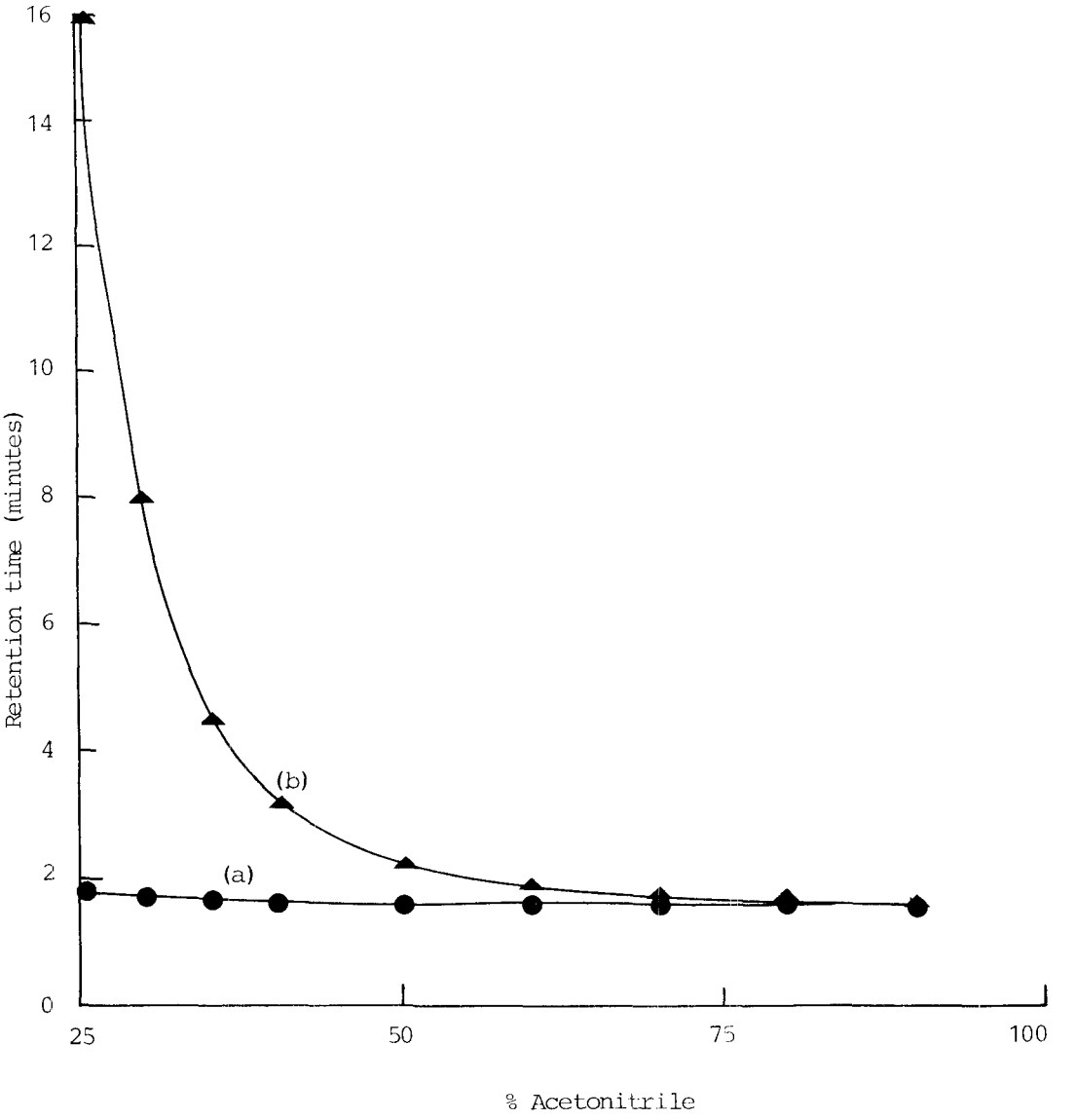

FIGURE 1

Effect of mobile phase composition (% acetonitrile) on the retention time of a-ampicillin (●) and b-cloxacillin (▲).

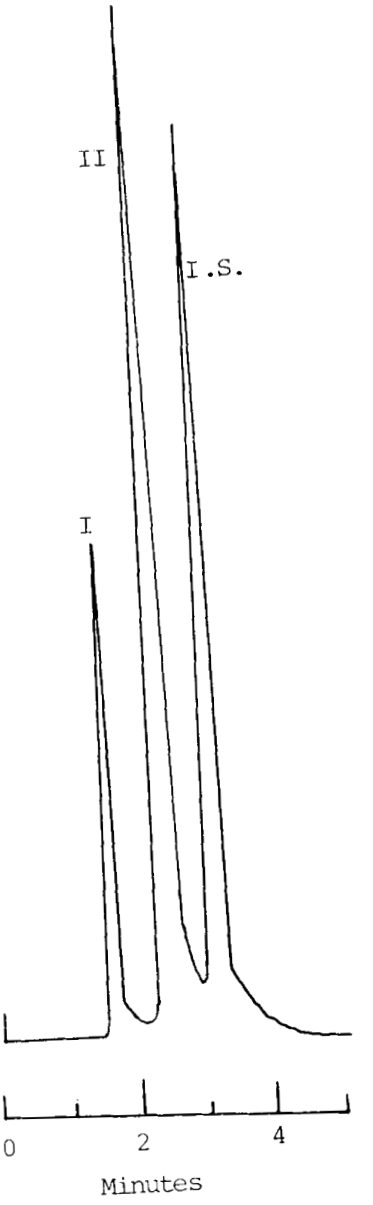

FIGURE 2

A typical chromatogram of the dosage form solution (10µl) representing 2µg of each : ampicillin (Rt = 1.55 min), cloxacillin (Rt = 2.5 min). I.S.Sulfamethoxazole (Rt = 3.5 min).

TABLE 1

Statistical Data for the Calibration Curves of Ampicillin (I) and Cloxacillin (II)

	Correlation Coefficient	Slope	Intercept	Standard Error of Estimate
I	0.997	1.613	0.005	0.019
II	0.999	6.201	0.017	0.045

area) were linear for both components I and II over the concentration range 0.05-0.6 mg/ml (n = 6). The statistical data for the calibration curves are listed in table 1. Although the method was sensetive to lower concentrations, the range used for calibration was adequate for the requirements of the analytical methods.

The applicability of the method for the analysis of capsules containing ampicillin and cloxacillin was investigated. The content uniformity of ten individual capsules (of the four different brands) was assayed. The results are listed in tables 2 and 3. Additionally samples corresponding to average weight of capsules calculated from the gross weight of ten capsules were analyzed and the percentage recoveries of label claim are presented in table 4.

TABLE 2

Content Uniformity of Ampicillin in Different Brands, Expressed as % of Label Claim

Capsule No.	A	B	C	D
1	100.84	102.30	100.13	100.99
2	100.52	102.90	99.96	100.46
3	101.10	103.10	100.26	101.02
4	100.32	103.60	102.52	100.54
5	102.43	102.32	101.01	102.62
6	100.91	103.20	100.22	101.08
7	101.21	101.72	101.12	100.84
8	100.73	102.35	101.58	99.89
9	100.33	103.12	100.05	103.72
10	100.01	103.02	99.98	101.10

The accuracy of the analytical method was tested by calculating the recovery of known amounts of I and II added to samples representing the average weight of the dosage form. To illucidate whether variable concentrations of I and II would affect the assay method, different ratios of ampicillin and cloxacillin were added. Ratios of I : II used were 1:5, 5:1 and 1:1

TABLE 3

Content Uniformity of Cloxacillin in Different Brands, Expressed as % of Label Claim

Capsule No.	A	B	C	D
1	100.21	103.72	99.77	102.07
2	102.30	102.65	100.32	101.72
3	101.81	104.01	100.97	100.97
4	101.56	102.93	101.02	101.09
5	102.43	101.61	101.08	101.86
6	103.02	102.35	101.16	101.08
7	100.95	103.05	102.07	100.96
8	101.89	103.30	100.99	103.10
9	100.58	102.88	100.85	101.06
10	99.96	101.98	100.59	100.66

respectively. The above experiments were performed on two brands only. The results, shown in table 5, indicate the low value of the relative standard deviation (0.260-1.083) which is an evidence for the precision of the analytical method. The results also exclude the possibility of interference between the two components at any concentration.

TABLE 4

Analysis of Ampicillin (I) and Cloxacillin (II) in Average Weight

Brand		*Mean (% Label Claim)	± S.D.
A	I	101.65	± 1.0406
	II	100.55	± 0.3447
B	I	102.85	± 0.7317
	II	102.82	± 0.6337
C	I	100.79	± 0.9805
	II	100.64	± 0.5289
D	I	101.38	± 0.9856
	II	101.08	± 0.4986

*An average of 6 determinations

TABLE 5

Recovery of Ampicillin and Cloxacillin from Spiked Samples (Two Brands A & C)

	Amount added (mg)	% Recovery (a)	
		A	C
I	50	100.590 ± 0.4706	100.864 ± 0.6929
II	10	101.140 ± 0.5226	101.264 ± 0.5034
I	10	101.516 ± 1.0830	101.004 ± 0.8640
II	50	100.935 ± 0.4471	101.256 ± 0.6433
I	10	100.914 ± 0.2602	101.258 ± 0.6320
II	10	101.030 ± 0.6868	100.672 ± 0.7360

(a) Mean ± RSD for 5 determinations.

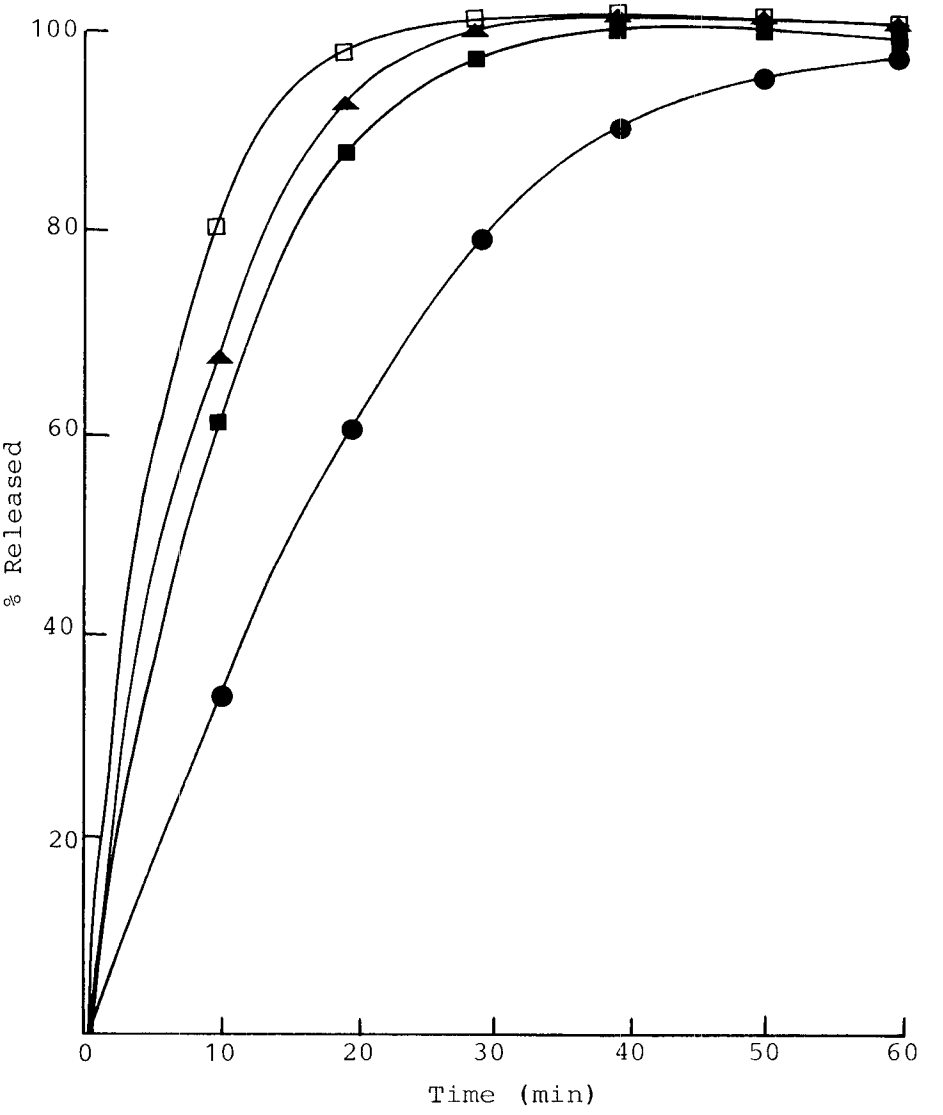

FIGURE 3

Dissolution rates of ampicillin in water at 37°C. Brand A (▲), B (■), C (●) and D (□).

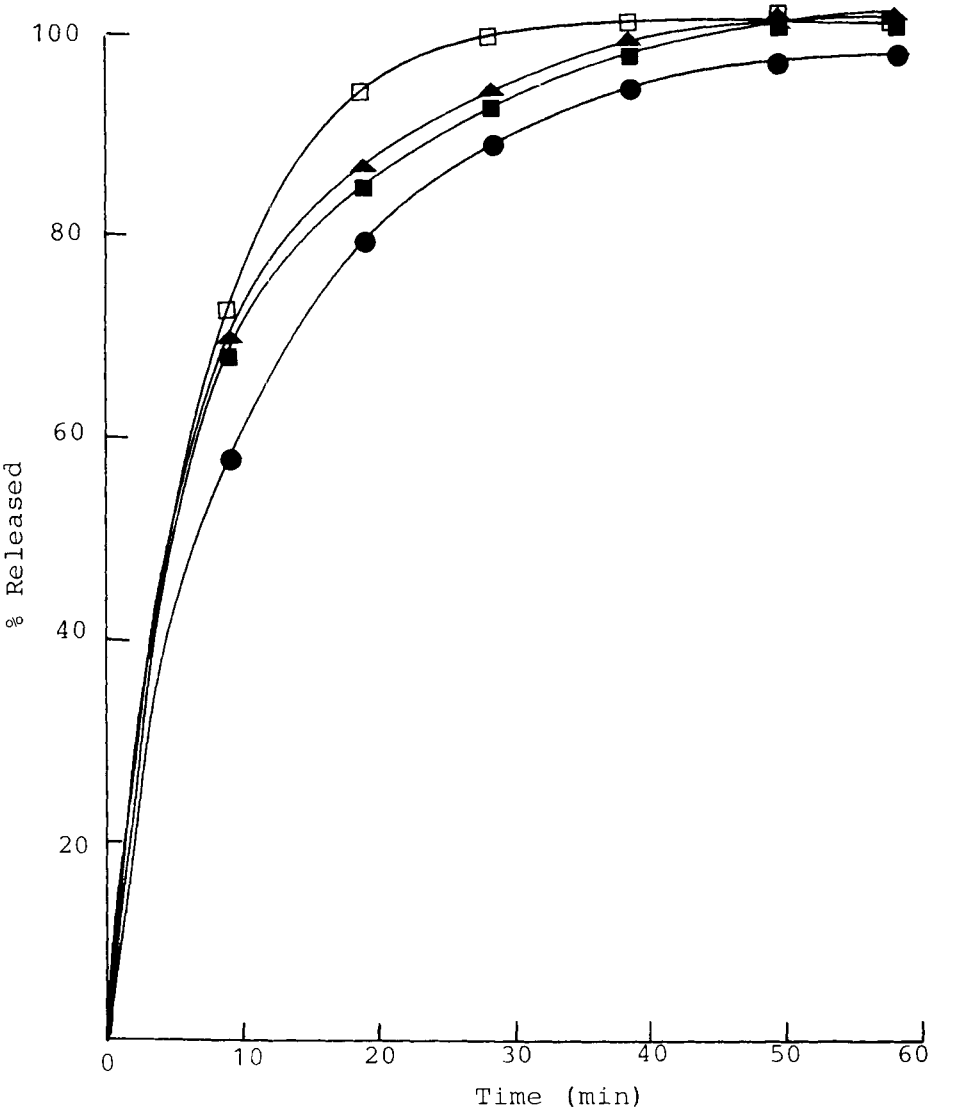

FIGURE 4

Dissolution rates of cloxacillin water at 37°C.
Brand A (▲), B (■), C (●) and D (□).

A preliminary qualitative analysis was performed on other dosage forms (injections, powder for suspension) containing ampicillin and cloxacillin. The chromatogram showed no interference with additives or excipients.

The analytical method was adapted for the assay of ampicillin and cloxacillin in dissolution medium. The dissolution rates for the four commercially available brands of capsules are shown in figures 3 and 4. The slow release of ampicillin from brand (C) could be explained in part by the filling procedure of the capsules. This was concluded upon emptying capsule content which was in the form of slugs rather than powder as in the other brands.

In conclusion, the analytical method presented for the simultaneous determination of ampicillin and cloxacillin could be utilized readily for routine quality control of pharmaceuticals, since it offers a simple system and short analytical time coupled with reproducibility and accuracy.

ACKNOWLEDGEMENT

The financial support provided by Yarmouk University and Jordanian Ministry of Planning, is gratefully acknowledged. The authors would like to thank the Jordanian Pharmaceutical Manufacturing Co., Naor, Jordan for providing chemicals and reagents.

REFERENCES

1. The United States Pharmacopeia 21st ed. Mack Publishing Co., Easton, Pa. PP.59,237 (1985).
2. Code of Federal Regulations, Title 21, Part 440, U.S. Government Printing Office, Washington, D.C. (1981).
3. H. Bundgaard and K. Ilver, J. Pharm. Pharmacol., 24, 790 (1972).
4. A. Aboul Khier, G. Blaschke and ElSadek M., Analytical Letters, 17(B 14), 1659 (1984).
5. A. Aboul Khier, G. Blaschke and ElSadek M., Ibid. P.1667.
6. N. Margosis, J. Chromatogr., 236, 469 (1982).
7. H.H.W. Thijssen, J. Chromatogr, 183, 339 (1980).
8. G. Lauriault, M.J. LeBelle and A. Vilim, J.Chromatogr., 240, 157 (1980).