ELSEVIER

Journal of Chromatography A, 692 (1995) 67–72

JOURNAL OF CHROMATOGRAPHY A

High-performance liquid chromatographic method for potency determination of cephalexin in commercial preparations and for stability studies

Mei-Chich Hsu[a,*], Yu-Shan Lin[b], Hsiou-Chuan Chung[b]

[a]*Graduate Institute of Sports Sciences, National College of Physical Education and Sports, 250 Wen-Hwa 1st Road, Kweishan, Taoyuan 33333, Taiwan*

[b]*National Laboratories of Foods and Drugs, Department of Health, Executive Yuan, 161-2, Kuen-Yang Street, Nankang, Taipei 11513, Taiwan*

Abstract

A reversed-phase column liquid chromatographic method was developed for the assay of cephalexin in bulk drugs and pharmaceutical preparations. An equation was derived showing a linear relationship between peak-area ratio of cephalexin to acetaminophen (internal standard) and the cephalexin concentration over a range of 0.2–1.75 mg/ml ($r = 0.9999$). Standard addition recoveries were generally greater than 99.5%. The relative standard deviations were between 0.38 and 0.63% in the within-day assay, and 1.05% in the between-day assay. The column liquid chromatographic assay results were compared with those obtained from a microbiological assay, which indicated that the proposed method is a suitable substitute for the microbiological method for potency assays and stability studies of cephalexin preparations.

1. Introduction

The present official assay method of the *British Pharmacopoeia* [1] for the analysis of potency of cephalexin oral preparations is an iodometric titration. The *US Code of Federal Regulations* [2] described two official methods for potency assay of cephalexin: a microbiological method and iodometric titration. The regulations state that the results obtained from the microbiological method shall be conclusive. The Minimum Requirement for Antibiotic Products [3] for the analysis of potency of cephalexin in bulk drug substance and its preparations indicates microbiological and turbidimetric methods. The greatest disadvantage of the microbiological and chemical methods in current use is their lack of specificity. This deficiency has prompted the search for an alternative method which is fast, simple and selective, e.g. column liquid chromatography (LC). Several LC methods have been applied for separation of cephalexin and other cephalosporins or penicillins [4–10]. Few methods for the determination of cephalexin in pharmaceutical samples have been reported [11–16]. However, the drawback of these procedures is either the tedious sample preparation procedure or lack of comparison with the results of a microbiological method.

In order to establish whether an HPLC meth-

* Corresponding author.

0021-9673/95/$09.50
SSDI 0021-9673(94)00688-1

od would be acceptable, it is important to determine whether it is robust enough for assaying samples kept under extreme conditions. Degradation in the sample should be equally reflected by microbiological and HPLC assays. This paper describes a comparison of a proposed HPLC method with a microbiological assay for the determination of cephalexin in commercial formulations. Further, cephalexin was kept at elevated temperatures as part of an accelerated degradation experiment and assayed by microbiological and HPLC methods.

2. Experimental

2.1. Instruments

A Model 576 LC pump (Gasukuro Kogyo, Tokyo, Japan), a Gasukuro Kogyo Model 502U Spectrodetector and a Gasukuro Kogyo Model 12 Chromatocorder were employed during the study. The mobile phase was pumped through a reversed-phase column (μBondapak C_{18}; 30 cm × 3.9 mm I.D.; particle size, 10 μm; Waters) with an isocratic flow-rate of 1.0 ml/min. The detector was set at 254 nm. Chromatography was performed at room temperature. Injections of 20 μl of all solutions to be analysed were made.

2.2. Reagents and materials

Methanol (LC grade) was supplied by ALPS Chemical Co. (Taipei, Taiwan). Glacial acetic acid (reagent grade) was supplied by E. Merck (Darmstadt, Germany). 7-Aminodesacetoxycephalosporanic acid was purchased from Sigma (St. Louis, MO, USA). Acetaminophen was a gift of Winthrop Labs. Taiwan Branch Office (Sterling Products International, Taipei, Taiwan). Cephalexin monohydrate was USP reference standard (Rockville, MD, USA). Cephalexin bulk drug was supplied by Eli Lilly (Taipei, Taiwan). Capsules, granules and powder for oral solutions were obtained from commercial sources.

2.3. Mobile phase

The mobile phase was methanol–1.25% glacial acetic acid (25:75, v/v). The mobile phase was filtered (0.45-μm pore size Millipore filter) and degassed with an ultrasonic bath prior to use.

2.4. Internal standard solution

Internal standard (acetaminophen, 63.0 mg) was dissolved in 5.0 ml of methanol and diluted to 25.0 ml with water to form the internal standard solution.

2.5. Cephalexin standard solution

To form cephalexin standard solution, 1.0 ml of internal standard solution was added to an accurately weighed amount of cephalexin standard equivalent to a 25.0-mg potency of cephalexin and the volume was brought up to 50.0 ml.

2.6. Sample preparations

To form sample preparations, 1.0 ml of internal standard solution was added to an accurately weighed amount of bulk drugs, homogeneous capsule contents, granule or powder for oral solution formulations equivalent to a 25.0-mg potency of cephalexin and the volume was brought up to 50.0 ml with water.

2.7. Solution for linearity response

Eight concentrations of cephalexin (0.2, 0.3, 0.5, 0.75, 1.0, 1.25, 1.5 and 1.75 mg/ml) were prepared. Each concentration was chromatographed six times.

2.8. Solution for recovery studies

To an accurately weighed 25.0-mg potency of sample composites of commercial preparations were added different amounts of cephalexin standard and 1.0 ml of internal standard solution. Each solution was made up to 50.0 ml with water and was chromatographed in triplicate.

2.9. Microbiological assay procedure

The protocol of analysis was basically that described in Minimum Requirements for Antibiotics Products of Japan [3]. *Bacillus subtilis* (Culture Collection and Research Center, Hsinchu, Taiwan) was used in the microbiological assay. According to the cup plate method, standards and test drugs were diluted to 1.0 mg/ml (potency) concentrated solution with 1% phosphate buffer solution (pH 6.0) and then diluted to 20.0 and 5.0 μg/ml with 1% phosphate buffer solution (pH 6.0) on the day of analysis. Five petri dishes of 9.0 cm inside diameter were used for each sample. After incubation for 16 to 18 h, the inhibition zone diameter was measured by a zone analyzer (ZA-F; Toyo, Tokyo, Japan).

3. Results and discussion

The linearity of the peak-area ratio (cephalexin versus internal standard) was verified by injection of eight solutions containing cephalexin in a concentration range of 0.2 to 1.75 mg/ml. A straight line with a correlation coefficient of 0.9999 ($y = 5.5536x + 0.0178$) was obtained when the ratios of the area counts of the cephalexin divided by the area counts of the internal standard were plotted against concentration of cephalexin.

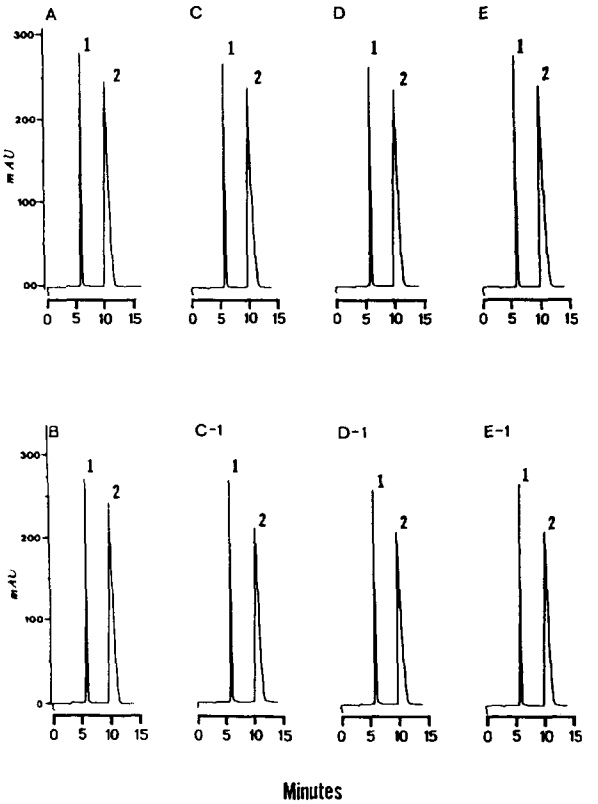

Fig. 1. Chromatograms of cephalexin preparations. (A) USP standard; (B) bulk drug substance; (C) 500-mg capsule; (C-1) degraded 500-mg capsule; (D) 500 mg/g granule; (D-1) degraded 500 mg/g granule; (E) 500 mg/g powder for oral solution; (E-1) degraded 500 mg/g powder for oral solution. Peaks: 1 = acetaminophen; 2 = cephalexin.

Table 1
Recovery of cephalexin from various commercial composites

Formulation	Added (mg)	Found (mg)	Recovered (%)	Average recovered (%)
Capsule				
250 mg	10.0	9.95	99.5	99.6
	15.0	14.97	99.8	
	20.0	19.90	99.5	
500 mg	10.0	10.01	100.1	100.2
	15.0	15.02	100.1	
	20.0	20.09	100.4	
Granule, 500 mg/g	10.0	9.96	99.6	99.7
	15.0	14.98	99.9	
	20.0	19.94	99.7	

Reproducibilities for both the within-day assay and the between-day assay were evaluated. The relative standard deviations (R.S.D.s), on the basis of peak-area ratios for six replicate injections in the within-day assay, were between 0.38 and 0.63% at the concentration of 0.5 mg/ml. The R.S.D. in the between-day assay ($n = 6$) was 1.05% at the same amount.

The results of standard addition recovery studies of cephalexin from sample composites of commercial preparations are shown in Table 1. The average recovery was greater than 99.6%.

Typical chromatograms of the cephalexin commercial dosage forms are shown in Fig. 1. The retention time was about 6.0 min for the internal standard and 11.3 min for cephalexin. Excipients from commercial formulations did not interfere. Furthermore, the HPLC method can detect a compound related to the cephalexin, i.e., 7-aminodesacetoxycephalosporanic acid, which was eluted prior to cephalexin (Fig. 2).

When sample solutions of capsule, granule and powder for oral solution were heat degraded, the resulting mixtures yielded chromatograms containing additional peaks, none of which interfered with the interpretation and measurement of the chromatographic peaks for cephalexin and acetaminophen. Chromatograms of 2-, 3- and 5-h degraded solutions of granule samples at 100°C showed cephalexin disappearance and degradation product accumulation with increasing incubation time (Fig. 3). Several additional peaks were found when the detection sensitivity was increased. It is obvious that decomposition products elute before the intact drug. Same results were found when samples decomposed by using 0.1 *M* sodium hydroxide. To examine the purity of the cephalexin peak in the heat-degraded samples, a UV photodiode array detector was used. The evaluation of chromatographic peak homogeneity was performed by absorbance ratios and a three-dimensional spectrochromatogram. The results presented good confirmation of the cephalexin peak identity (data not shown).

Studies were initiated to ascertain the suitability of the proposed method for stability studies.

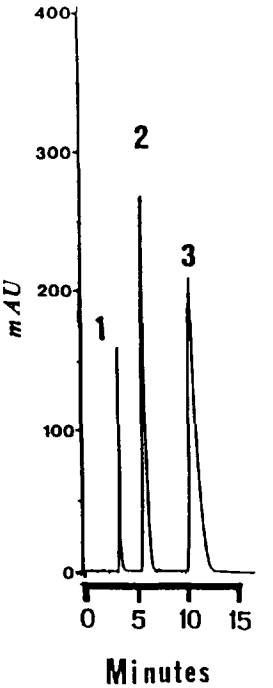

Fig. 2. Chromatogram of a mixture preparation. Peaks: 1 = 7-aminodesacetoxycephalosporanic acid; 2 = acetaminophen; 3 = cephalexin.

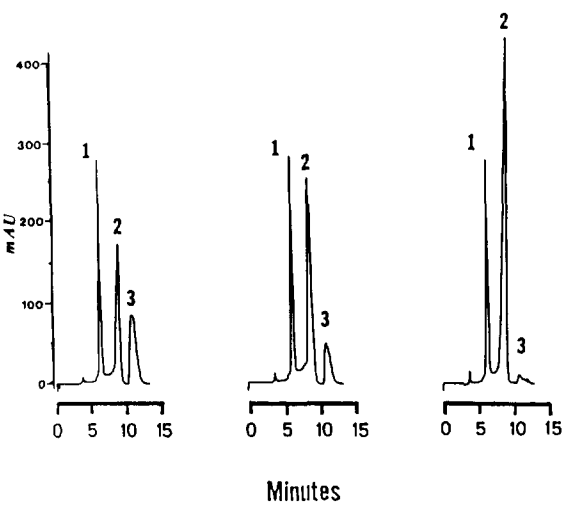

Fig. 3. Chromatograms of 2-, 3- and 5-h degraded solutions (from left to right) of granule samples at 100°C showing cephalexin (peak 3) disappearance and degradation product (peak 2) accumulation with increasing incubation time. Peak 1 is acetaminophen.

For the degraded solutions, the solutions of capsule, granule and powder for oral solution formulations containing 1.0 mg/ml cephalexin were prepared by water. The solutions were degraded at 100°C. For the degraded powdered composites, the capsule, granule and powder for oral solution formulations were directly stored in temperature-controlled cabinets (100°C). Degraded solutions and degraded powdered composites were taken from the cabinets periodically for microbiological and HPLC assays. The assay values, expressed as a percentage of the level claim, are given in Tables 2 and 3. The nine paired values in Table 2 have a correlation coefficient of 0.9934. The 21 paired values in Table 3 have a correlation coefficient of 0.9364. Hence, no significant difference in the assay values obtained by the two analytical methods was found for degraded or non-degraded samples. A number of samples of bulk drug substance and commercial preparations of eight brands were analyzed for cephalexin content by HPLC. These samples were also assayed by the microbiological method. The results are shown in Table 4. A *t*-test was applied to the data; analysis showed no significant difference at the 95% confidence level for any of the preparations when assayed by the microbiological or HPLC methods.

This study demonstrates the applicability of the proposed HPLC method for the potency determination of cephalexin in bulk drug and capsule and granule formulations. The method can be successfully used for routine quality control and stability assays and offers advantages in speed, simplicity and reliability.

Table 2
Comparison of percent potency of cephalexin and its degraded solutions in capsule, granule and powder for oral solution formulations as dertermined by microbiological and HPLC methods

Formulation	Degradation time (h)	% of declared concentration by	
		Microbiological method	HPLC method
Capsule	0	110.6	111.6
	1	92.7	94.4
	2	50.5	48.5
Granule	0	111.3	109.5
	1	98.5	96.1
	2	49.7	48.4
Powder for oral solution	0	106.3	108.6
	1	94.1	92.9
	2	21.0	31.8

Table 3
Comparison of percent potency of cephalexin and its degraded powdered composites in capsule, granule and powder for oral solution formulations as determined by microbiological and HPLC methods

Formulation	Degradation time (h)	% of declared concentration by	
		Microbiological method	HPLC method
Capsule	0	113.5	110.5
	3	108.6	104.2
	5	103.5	102.8
	7	99.6	101.6
	9	98.4	101.1
	11	96.9	101.0
	42	86.6	84.8
Granule	0	111.1	108.5
	3	104.4	102.0
	5	102.8	101.2
	7	101.0	102.2
	9	100.4	98.4
	11	95.8	98.1
	42	85.2	84.3
Powder for oral solution	0	110.8	107.7
	3	96.7	100.8
	5	94.4	98.0
	7	93.1	98.4
	9	94.3	97.3
	11	92.6	97.1
	42	83.7	86.0

Table 4
Comparison of microbiological and HPLC assays for cephalexin

Sample	Potency[a] by	
	Microbiological method	HPLC method
Bulk drug		
USP standard	950.0	950.0
Brand A	939.2	936.8
Dosage form, declared		
Brand A, 250 mg/capsule	105.8	104.6
Brand A, 500 mg/capsule	109.8	107.0
Brand B, 250 mg/capsule	106.4	104.4
Brand C, 500 mg/capsule	106.5	103.1
Brand D, 250 mg/capsule	105.3	108.2
Brand D, 500 mg/g granules	105.7	107.8
Brand E, 500 mg/capsule	103.5	104.2
Brand F, 500 mg/capsule	107.7	106.5
Brand G, 250 mg/capsule	106.2	101.5
Brand H, 500 mg/g powder for oral solution	106.5	104.5

[a] The potency was determined as μg/mg for bulk drug and as a percentage of the declared amount for dosage forms. Values for the microbiological assay are averages of five determinations; values for HPLC are averages of triplicate determinations.

Acknowledgements

We thank Winthrop Laboratories Taiwan Branch Office, Sterling Products International, Taipei, Taiwan, for their generous gifts of acetaminophen for the study.

This work was supported by grant DOH 82-22 from the Department of Health, Executive Yuan, Taipei, Taiwan.

References

[1] *British Pharmacopoeia*, Her Majesty's Stationary Office, London, 1988.
[2] *Code of Federal Regulations*, Title 21, Part 440, US Government Printing Office, Washington, DC, 1990.
[3] *Minimum Requirements for Antibiotic Products of Japan*, English version, Japan Antibiotics Research Association, Tokyo, 1986.
[4] E.R. White, M.A. Carroll, J.E. Zarembo and A.D. Bender, *J. Antibiot.*, 38 (1975) 205.
[5] M.A. Carroll, E.R. White, Z. Jancsik and J.E. Zarembo, *J. Antibiot.*, 30 (1977) 397.
[6] E. Crombez, G.A. Bens, G. Van der Weken, W. Van den Bossche and P. De Moerloose, *Chromatographia*, 11 (1978) 653.
[7] F. Salto, *J. Chromatogr.*, 161 (1978) 379.
[8] A. Brunetta, L. Mosconi, S. Pongiluppi, U. Scagnolari and G. Zambonin, *Boll. Chim. Farm.*, 120 (1981) 335.
[9] M.C. Nahata, *J. Chromatogr.*, 228 (1982) 429.
[10] N. Kovacic-Bosnjak, Z. Mandic and M. Kovacevic, *Chromatographia*, 23 (1987) 350.
[11] L.J. Lorenz, F.N. Bashore and B.A. Olsen, *J. Chromatogr. Sci.*, 30 (1992) 211.
[12] V. Das Gupta and J. Parasrampuria, *Drug Dev. Ind. Pharm.*, 13 (1987) 2231.
[13] J. Marincel, N. Bosnjak and M. Lamut, *Acta Pharm. Jugosl.*, 38 (1988) 35.
[14] *United States Pharmacopeia XXII, National Formulary XVII*, United States Pharmacopeial Convention, Rockville, MD, 1990.
[15] D.P. Wang and M.K. Yeh, *Chin. Pharm. J. (Chunh-Hua Yao Hsueh Tsa Chih)*, 42 (1990) 349.
[16] Y. Zhao, X. Qian and Z. Li, *Sepu*, 10 (1992) 183.