Journal of Chromatography, 600 (1992) 333–336
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 162

Short Communication

Collaborative study of the determination of cloxacillin by column liquid chromatography

Mei-Chich Hsu* and Weng F. Huang

National Laboratories of Foods and Drugs, Department of Health, Executive Yuan, 161–2, Kuen-Yang St., Nankang, Taipei 11513 (Taiwan)

(First received January 9th, 1992; revised manuscript received March 9th, 1992)

ABSTRACT

A previously published column liquid chromatographic method proposed for the analysis of cloxacillin preparations was subjected to an interlaboratory collaborative study. The method is rigorously defined in terms of performance requirements, yet allows a degree of flexibility to the individual analyst. Eight participating laboratories submitted results for the analysis of three samples in duplicate. The data from one laboratory were rejected because they failed to meet the prescribed performance criteria. Estimates for the repeatability and reproducibility of the method, expressed as relative standard deviations of the results of the analysis of cloxacillin preparations, were found to be less than 0.65% and 1.33%, respectively.

INTRODUCTION

A more specific and reliable method is needed for the assay of cloxacillin preparations because the current official procedures lack specificity [1,2]. This paper reports the results and evaluation of a collaborative study to validate a column liquid chromatographic (LC) method for the determination of the potency of bulk cloxacillin and cloxacillin capsules and injections. The protocol of analysis was basically that described previously [3], except that it was revised for this study by allowing the individual analyst a degree of flexibility while rigorously defining the performance criteria of the method to maintain control.

The control of the method is maintained by specifically defined minimum performance criteria or a system suitability test. The flexibility of the method lies in the discretion given to the analyst to select the specific analytical system (*i.e.*, instrument, injector, detector and column, etc.). The analyst is encouraged to use individual judgement in adjusting the operating conditions to meet those criteria.

EXPERIMENTAL

Collaborative study

Each participating laboratory was provided with the protocol of analysis and duplicate samples of cloxacillin sodium bulk drug, cloxacillin capsules (China Biological and Chemical Laboratories, Taiwan) and cloxacillin injections (Bristol Industries, Taiwan). These samples were to be measured against a reference cloxacillin sodium sample with a potency of 899.7 μg/mg. Analysts were requested to submit all data from duplicate injections for each sample and to report their calculated assay results. They were also asked to describe specific operating parameters of the instrument system used.

0021-9673/92/$05.00

Instrumentation

Each laboratory was asked to use routine LC equipment. This instrument must be equipped with a 254-nm UV detector and a recording device. In order to obtain a wider diversity of systems, analysts were encouraged to use their own columns. However, only microparticulate reversed-phase packing materials that exhibit some degree of polarity, such as hydrocarbon-bonded silicas, were used.

Reagents

Analytical-reagent grade dimethyl phthalate was purchased from E. Merck (Darmstadt, Germany). Reference material cloxacillin sodium was an NLFD house standard (National Laboratories of Foods and Drugs, Taiwan). Methanol was of LC grade. Glacial acetic acid and acetonitrile were of analytical-reagent grade. Triply distilled water with a resistivity greater than 15 MΩ was used.

Mobile phase

The mobile phase was methanol–4% acetic acid (60:40, v/v). The mobile phase was filtered (0.45-μm Millipore filter) and degassed by ultrasonication prior to use. The mobile phase may be sparged with helium through a 2-μm metal filter for the duration of the analysis.

Internal standard solution

The internal standard, dimethyl phthalate (3 g), was dissolved in 100 ml of acetonitrile–water (1:1).

Standard solution

To an accurately weighed amount of cloxacillin sodium standard, equivalent to 50 mg potency of cloxacillin, was added 0.5 ml of internal standard solution and the volume was made up to 50.0 ml with distilled water.

Sample solution

All solutions of cloxacillin samples were prepared in a manner identical with that of reference material.

Conditions for determination

A constant operating temperature (15–30°C) was maintained. The eluent flow-rate, which was not to exceed 2.0 ml/min, was adjusted to give peaks of satisfactory retention and configuration. The detector sensitivity was adjusted to produce peak heights of 40–90% full-scale deflection, with a chart speed of 0.5 mm/min.

System suitability test

The column was equilibrated with mobile phase. A minimum of three injections of cloxacillin standard solution were chromatographed. The relative standard deviation for the ratio of peak responses should be $\leqslant 2.0\%$. Injection volume for all solutions to be analysed was 10 μl.

Assay and calculations

Identical volumes of carefully measured standard and sample solutions were injected sequentially into the chromatograph. The peak response was normalized to the internal standard and compared with that of the reference material to give the cloxacillin content as follows: $(P_u C_s I_s)/(P_s C_u I_u) \cdot 899.7$ = cloxacillin potency (μg/mg), where P = peak response of cloxacillin, C = concentration of solution, I = peak resposne of internal standard, u = analyte sample and s = reference material. Calculations and data reductions may be performed manually or with a data processing system. Duplicate injections were run for each preparation.

RESULTS AND DISCUSSION

Table I shows the diversity of instrument systems used by the collaborators. The adoption of suitability tests can obviate many problems arising from deficiencies in most analytical instrument systems because they demonstrate whether a particular system can perform satisfactorily.

Most of the collaborators were able to meet the system suitability requirements of the method. However, the data from one laboratory were rejected because they failed to meet the prescribed performance criteria. The times required for the collaborators to complete the analysis of the samples in the study varied from one to several days.

The Dixon test for outliers, when applied to laboratory averages for each sample, showed only one outlier overall. The highest result for capsules, that of laboratory 5, was flagged as an outlier. The data for capsules from laboratory 5 were omitted.

The statistical terms used are those given by the Association of Official Analytical Chemists [4] and/or commonly used by statisticians. Results of the analysis of the samples, together with means and

TABLE I

INSTRUMENT SYSTEMS USED IN COLLABORATIVE STUDY OF LIQUID CHROMATOGRAPHIC METHOD FOR CLOXACILLIN

Laboratory	Instrument	Detector	Injector	Mode[a]	Column[b]	Length × I.D. (cm × mm)
1	Waters 6000A	W-440	U6K	A	μBondapak C_{18}	30 × 3.9
2	Waters 6000A	W-450	U6K	M	Partisil ODS	25 × 4.6
3	Waters 600E	W-481	712WISP	A	μBondapak C_{18}	30 × 3.9
4	Waters M45	W-441	U6K	M	Chemcosorb ODS	15 × 4.6
5	Tosoh CCPD	Linear 204	Rheodyne	M	Nucleosil C_{18}	25 × 4.6
6	Hitachi	L-4000	Rheodyne	M	μBondapak C_{18}	30 × 3.9
7	Waters 600E	W-484	715WISP	M	Nucleosil C_{18}	30 × 3.9

[a] M = Manual; A = automatic.
[b] From manufacturer.

relative standard deviation (R.S.D.), are given in Table II. In addition to the mean, a measure of the precision was also calculated for (a) the within-laboratory standard deviation or repeatability (S_r), (b) the between-laboratories standard deviation or reproducibility (S_R), (c) repeatability relative standard deviation (R.S.D.$_r$) and (d) reproducibility relative standard deviation (R.S.D.$_R$). The R.S.D.$_r$ values were 0.28% for the bulk drug, 0.42% for capsules and 0.65% for injection and the R.S.D.$_R$ values were 0.73% for bulk drug, 1.33% for capsules and 1.01% for injection (Table II).

Collaborators' comments

Most collaborators commented favourably on the method. Collaborator 7 found that the capsule preparation was more easily dissolved in methanol than as specified in distilled water. The prescribed sample dissolution in distilled water was found to turn the solution turbid, which might affect the detector responses. To study this aspect, several concentrations of cloxacillin capsules were prepared in two sets by dissolution in distilled water and methanol. The results obtained using the two sets of solution were not significantly different. Collabo-

TABLE II

COLLABORATIVE RESULTS FOR CLOXACILLIN BULK DRUG AND DOSAGE FORMS

Collaborator	Bulk drug[a] (%)	Capsules[a] (%)	Injection[a] (%)
1	98.9, 98.8	96.8, 97.2	104.2, 104.3
2	97.8, 98.0	96.2, 96.3	106.2, 105,7
3	98.9, 98.5	96.5, 96.3	103.7, 103.6
4	98.1, 98.3	95.3, 94.1	105.2, 103.6
5	97.9, 97.7	104.8[b], 105.0[b]	103.2, 103.2
6	98.9, 99.8	96.6, 96.6	103.9, 105.8
7	97.4, 97.4	93.7, 94.2	105.1, 105.3
Mean	98.3	95.8	104.5
S_r	0.28	0.40	0.68
S_R	0.71	1.26	1.04
R.S.D.$_r$ (%)	0.28	0.42	0.65
R.S.D.$_R$ (%)	0.73	1.33	1.01

[a] Compared with reference substance.
[b] Outlier by Dixon's test.

rator 4 considered that the method was superior with respect to specificity to the official US Code of Federal Regulations microbiological and iodometric methods.

CONCLUSIONS

The collaborative study of the reversed-phase column LC method for the determination of cloxacillin in bulk, capsule and injection preparations showed good reproducibility. The method is now under consideration by the Chinese Pharmacopoeia.

ACKNOWLEDGEMENTS

The authors are grateful to the following for their participation in this collaborative study: Department of Pharmaceutics, School of Pharmacy, Taipei Medical College; Eli Lilly (Taiwan); Nang Kuang Pharmaceutical; Pfizer Taiwan; Union Chemical Laboratories, Industrial Technology Research Institute; Upjohn Laboratories Taiwan; Veterans Chemicals, VACRS, Taiwan; and Yunf Shin Pharmaceutical.

The authors also thank Miss Hsiou Chuan Chung for her assistance with the preparation of this manuscript.

This work was supported by the Division of Drug Biology, National Laboratories of Foods and Drugs, Department of Health.

REFERENCES

1 *Code of Federal Regulations, Title 21, Part 440*, US Government Printing Office, Washington, DC, 1988.
2 *Minimum Requirements for Antibiotic Products of Japan*, English Version, Japan Antibiotics Research Association, Tokyo, 1986.
3 M.-C. Hsu and M.-C. Cheng, *J. Chromatogr.*, 549 (1991) 410.
4 W. J. Youden and E. W. Steiner, *Statistical Manual of the AOAC*, Associaton of Official Analytical Chemists, Arlington, VA, 1990.