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Liquid chromatographic determination of amoxicillin preparations

Interlaboratory validation

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ABSTRACT

A previously published column liquid chromatographic method proposed for the analysis of amoxicillin preparations was subjected to an interlaboratory validation. The method is rigorously defined in terms of performance requirements, yet allows a degree of flexibility to the individual analyst. Nine participating laboratories submitted results for the analysis of four samples in duplicate. Estimates for the repeatability and reproducibility of the method, expressed as relative standard deviations of the results of the analysis of amoxicillin preparations, were found to be less than 0.96% and 6.29%, respectively.

INTRODUCTION

A more specific and reliable method is needed for the assay of amoxicillin preparations because the current official procedures lack specificity [1–4]. 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 amoxicillin and amoxicillin capsule, injection and granule formulations. The protocol for analysis was basically that previously described [5], except that a degree of flexibility was allowed to the individual analyst, while rigorously defining the performance criteria of the method to maintain control.

Control of the method is maintained by specifically defining minimum performance criteria for a system suitability test. 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 the performance criteria.

EXPERIMENTAL

Collaborative study

Each of nine collaborators received a reference standard of amoxicillin sodium, an internal standard of acetaminophen, duplicate samples of bulk material, commercial powdered capsule, injection and granule composites. The collaborators also received a set of instructions regarding the amount of sample to take for analysis, a copy of the method and a report form for recording results. They were also asked to describe specific operational parameters of the instrument system used and to submit their report forms along with their chromatograms. These samples were to be measured against a refer-

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ence amoxicillin sodium sample with a potency of 847.1 μg/mg.

Instrumentation

Each laboratory was asked to use routine LC equipment. Instruments were to be equipped with a 254-nm UV detector and a recording device. In order to obtain a wide 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 allowed.

Reagents

Reference material amoxicillin sodium was an NLFD house standard (National Laboratories of Foods and Drugs, Taiwan). Acetaminophen was a gift from Winthrop Laboratories Taiwan Branch Office, Sterling Products International (Taipei, Taiwan). Methanol was of LC grade. Glacial acetic acid was of analytical-reagent grade. Triply distilled water with a resistivity greater than 15 MΩ was used.

Mobile phase

The mobile phase was methanol–1.25% acetic acid (20:80, v/v). The mobile phase was filtered (0.45-μm Millipore filter) and degassed 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, acetaminophen (40.0 mg), was dissolved in 10.0 ml of methanol and diluted to 1.0 l with water to give the internal standard solution.

Amoxicillin standard solution

To prepare amoxicillin standard solution, internal standard solution was added to an accurately weighed amount of amoxicillin sodium standard equivalent to a 50.0-mg potency of amoxicillin and the volume was adjusted to 50.0 ml.

Sample solution

All solutions of amoxicillin samples were prepared in the same manner as the 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 adjustedto give peaks of satisfactory retention and configuration. The detector sensitivity was adjusted to produce peak heights of 40–90% full-scale detection, with a chart speed of 0.5 mm/min.

System suitability

The column was equilibrated with mobile phase. A minimum of three injections of amoxicillin standard solution were chromatographed. The relative standard deviation for the ratio of peak responses should be $\leqslant 2.0\%$. Injections of 20 μl were suggested for all solutions to be analysed.

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 amoxicillin content as follows: $(P_u\ C_s\ I_s/P_s\ C_u\ I_u) \times 847.1 =$ amoxicillin potency (μg/mg), where P = peak response of amoxicillin, C = concentration of solution, I = peak response of internal standard, u = analyte sample and s = reference material. Calculations and data reduction may be performed manually or with a data processing system. Duplicate injections were run or 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.

All of the collaborators were able to meet the system suitability requirements of the method. 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 means for each sample, showed only one outlier overall. The lowest result for bulk drug, that of laboratory 2, was flagged as an outlier. The data for bulk drug from laboratory 2 were omitted.

TABLE I

INSTRUMENT SYSTEMS USED IN THE COLLABORATIVE STUDY OF THE LIQUID CHROMATOGRAPHIC METHOD FOR AMOXICILLIN

Laboratory	Instrument	Detector	Injector	Mode[a]	Column	Length × I.D. (cm × mm)
1	Waters 600E	W-486	715 WISP	A	Nucleosil C_{18}	30 × 3.9
2	Shimadzu	SPD-6AV	SIL 1A	M	Inertsil 10-ODS	25 × 4.6
3	Waters 510	W-486	712 WISP	A	μBondapak C_{18}	30 × 3.9
4	Spectroflow 400	Spectroflow 757	Micromeritics 725	A	Partisil ODS-3[b]	25 × 4.6
5	Hitachi L-6200	L-4000	Rheodyne	M	Inertsil 10-ODS	30 × 3.9
6	Waters 510	W-481	Shimadzu	M	Inertsil 5-ODS	15 × 4.6
7	Jasco 880PU	L-200	740 WISP	A	LiChrosorb RP-18	25 × 4.5
8	Toyo Soda Cepd	Spectromonitor II 1202	Rheodyne 7125	M	Chemosorb ODS C_{18}	25 × 4.0
9	Gasukuro 576	502U	–	A	μBondapak C_{18}	30 × 3.9

[a] M = manual; A = automatic.

[b] Bulk drug, capsule and injection samples were analysed with a Partisil ODS-3 column and granule sample with an Ultrasphere 5 ODS column.

The statistical terms used are those given by the Association of Official Analytical Chemists [6] and/or commonly used by statisticians. The analysis of variance with each sample is shown in Table II. Results of the analysis of the samples, together with means and relative standard deviations (R.S.D.), are given in Table III. 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 0.59% for bulk drug, 0.80% for capsule, 0.96% for injection and 0.52% for granule and the R.S.D.$_R$ values were 0.86% for bulk drug, 2.38% for capsule, 6.29% for injection and 2.59% for granule (Table III).

The results of standard addition recovery studies of amoxicillin from sample composites of commercial preparations are given in Table IV. The average recoveries were all close to 100.0%. These data fur-

TABLE II

ANALYSIS OF VARIANCE

Source of variation	Sum of squares (1)	Degree of freedom (2)	Mean square (1) (2)
Between laboratories[a]			
Bulk drug	7.62	6	1.270
Capsule	65.69	7	9.384
Injection	407.46	7	58.208
Granule	97.87	7	13.981
Between replicates			
Bulk drug	2.71	7	0.387
Capsule	4.48	8	0.560
Injection	5.42	8	0.677
Granule	2.26	8	0.283

[a] Eight laboratories.

TABLE III

RESULTS FOR THE ANALYSIS OF AMOXICILLIN BULK DRUG AND DOSAGE FORMS

Collaborator	Bulk drug[a] (%)		Capsule[a] (%)		Injection[a] (%)		Granule[b] (%)	
1	106.8	107.5	94.2	94.1	94.3	94.5	108.0	107.6
2	101.7[c]	102.6[c]	91.4	91.1	84.2	84.5	100.2	100.2
3	108.1	108.3	94.8	94.6	78.3	77.8	105.6	105.9
4	106.7	106.0	94.0	94.4	87.9	87.5	105.6	105.9
5	109.3	107.4	97.9	98.2	85.6	86.3	100.6	100.4
6	106.4	107.1	96.2	96.8	93.6	93.7	104.9	105.0
7	107.2	107.2	91.7	93.2	83.7	83.4	103.4	104.8
8	106.1	106.8	94.4	96.9	87.6	90.7	102.6	104.1
9[d]	104.7	105.5	92.5	93.2	79.8	80.8	103.8	103.5
Mean	107.21		94.62		87.08		104.06	
S_r	0.62		0.75		0.82		0.53	
S_R	0.91		2.23		5.43		2.67	
R.S.D.$_r$ (%)	0.59		0.80		0.96		0.52	
R.S.D.$_R$ (%)	0.86		2.38		6.29		2.59	

[a] Compared with reference substance.
[b] Determined as percentage of declared concentration for granule of 100 mg g.
[c] Outlier by Dixon's test.
[d] Data from our laboratory; not included in statistical analysis.

TABLE IV

RECOVERY OF AMOXICILLIN FROM VARIOUS COMMERCIAL COMPOSITES

Product	Manufacturer	Added (mg)	Found (mg)	Average recovery (%)
Capsule, 250 mg	A	11.9	12.04	100.5
	B	12.5	12.86	
	C	12.4	12.62	
	D	11.1	11.17	
	E	13.1	13.00	
	F	11.9	11.82	
	G	10.8	10.83	
	H	11.0	10.96	
Capsule, 500 mg	D	12.4	12.60	100.3
	E	13.4	13.12	
	F	11.4	11.37	
	G	13.3	13.29	
	H	13.8	13.67	
	I	12.2	12.43	
	J	12.0	12.16	
Injection, 250 mg per vial	K	13.9	14.35	103.3
Injection, 500 mg per vial	D	13.4	13.72	102.5
Granule, 200 mg g	H	12.8	12.57	99.2
	L	13.6	13.63	

ther indicate that the proposed LC method is relatively unaffected by the sample matrix.

Collaborators' comments

Most collaborators commented favourably on the method. They used a different brand of packing material than that specified (μBondapak C_{18}) in the method and obtained suitable chromatographic separations.

Collaborator 4 analysed bulk drug, capsule, injection samples with a Partisil ODS-3 column. In order to improve the separation of amoxicillin in granule and internal standard, an Ultrasphere 5 ODS column was used.

Collaborator 6 found that the peak for amoxicillin was sharper when the mobile phase contained phosphate buffer rather than acetic acid. However, the retention times were lengthened for both the amoxicillin and the internal standard. Collaborator 1 considered the method to be superior with respect to specificity to the official method using mercury nitrate titration [1], and to microbiological and iodimetric methods [2–4].

CONCLUSIONS

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

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