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Liquid chromatographic determination of cephalexin preparations: interlaboratory validation

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Abstract

A previously published column liquid chromatographic method proposed for the analysis of cephalexin preparations was subjected to an interlaboratory validation. The method was rigorously defined in terms of performance requirements, yet allowed a degree of flexibility to the individual analyst. Nine participating laboratories submitted results for the analysis of bulk drug substance, capsules and powder for oral suspension. Estimates of the within-laboratory standard deviation, the between-laboratories standard deviation, repeatability relative standard deviation and reproducibility relative standard deviation of the results of the analysis of cephalexin preparations were found to be 0.34, 2.01, 0.32 and 1.94%, respectively.

Keywords: Interlaboratory validation; Cephalexin; Acetaminophen; Antibiotics

1. Introduction

A more specific and reliable method is needed for the assay of cephalexin 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 cephalexin and cephalexin capsules and powder for oral suspension formulations. The protocol for analysis was basically that described previously [3], 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 was maintained by specifically defining minimum performance criteria for a system suitability test. The suitability test is a repeatability test, not a resolution test. The main purpose of carrying out this collaborative test on an analytical method is to determine the precision of the method when an analyst carries out measurements in replicate. Flexibility of the method lies in the discretion given to the analyst to select the specific analytical system

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(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.

2. Experimental

2.1. Collaborative study

Each of nine collaborators received a reference standard of cephalexin sodium, an internal standard acetaminophen and eight samples of cephalexin. For cephalexin, samples A and B were blind duplicate bulk material; C and D were blind duplicate samples of 250-mg commercial capsules; E and F were blind duplicate samples of 500-mg commercial capsules; and G and H were blind duplicate samples of powder for oral suspension. 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 reference cephalexin sodium sample with a potency of 939.2 μg/mg.

2.2. 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-phased packing materials that exhibit some degree of polarity, such as hydrocarbon-bonded silicas, alumina, magnesia and Florisil, were allowed.

2.3. Reagents

Reference material cephalexin 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 and glacial acetic acid was of analytical-reagent grade. Triply distilled water with a resistivity greater than 15 mΩ was prescribed.

2.4. Mobile phase

The mobile phase was methanol–1.25% acetic acid (25:75, 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.

2.5. Internal standard solution

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

2.6. Cephalexin standard solution

To prepare cephalexin standard solution, 1.0 ml of internal standard solution was added to an accurately weighed amount of cephalexin sodium standard equivalent to a 25.0-mg potency of cephalexin and the volume was adjusted to 50.0 ml with water.

2.7. Sample solution

All solutions of cephalexin samples were prepared in the same manner as the reference material.

2.8. 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.

2.9. System suitability

The column was equilibrated with mobile phase. A minimum of three injections of cephalexin standard solution were chromatographed. The relative standard deviation for the ratio of peak responses should be ≤2.0%. The resolution for cephalexin and internal standard should be ≥1.25. Injections of 20 μl were suggested for all solutions to be analysed.

2.10. Assay and calculation

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 cephalexin content as follows: $(P_s C_r I_r)/(P_r C_s I_s) \cdot 939.2$ = cephalexin potency (μg/mg), where P = peak response of cephalexin, C = concentration of solution, I = peak response of internal standard, s = analyte sample and r = reference material. Calculations and data reduction may be performed manually or with a data processing system. Duplicate injections were run for each preparation.

3. Results and discussion

The cephalexin materials consisted of two samples of bulk material, two samples of 250-mg capsules, two samples of 500-mg capsules and two samples of powder for oral suspension. All dosage forms were commercial formulations.

Table 1 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.

A typical chromatogram of cephalexin is 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.

The data were examined to see whether any laboratory showed consistently high or low values. The results showed that there were no consistent systematic errors for any laboratory (more than one in twenty times). All the laboratories admitted to experience in using the method. The data were next examined to see whether there was any outlying result which

Table 1
Instrument systems used in the collaborative study of the liquid chromatographic method for cephalexin

Laboratory	Instrument	Detector	Injector	Mode[a]	Column	Dimensions (cm × mm I.D.)
1	Spectroflow 400	Spectroflow 757	Micromeritics 725	A	μBondapak C_{18}	30 × 3.9
2	Waters 501	Waters 486	Waters 712	A	μBondapak C_{18}	30 × 3.9
3	Jasco 880-PU	Jasco 870-UV	Rheodyne 7125	M	Inertsil 10-ODS	25 × 4.6
4	Waters	Waters 440	U6K	M	Beckman C_8	25 × 4.6
5	Shimadzu LC-6A	SPD-6AV	SIL 1A	M	Vercopak 5-ODS	25 × 4.6
6	Waters 501	Abi 1000S	Rheodyne 7125	M	Inertsil 10-ODS	15 × 4.6
7	Jasco 880-PU	Jasco 875-UV	Rheodyne 7000	A	Licrocart RP-18e	12.5 × 4.0
8	Waters 600E	Lambda-Max 481	Waters 712 Wisp	A	μBondapak C_{18}	30 × 3.9
9	Waters 600E	Lambda-Max 481	Waters 712 Wisp	A	μBondapak C_{18}	30 × 3.9
10	Shimadzu LC-6A	SPD-6AV	SIL 6A	A	μBondapak C_{18}	30 × 3.9

[a] M = manual; A = automatic.

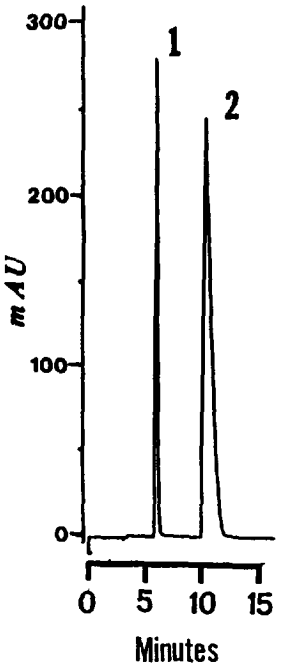

Fig. 1. Chromatogram of a cephalexin preparation. Peaks: 1 = acetaminophen; 2 = cephalexin.

differed from the rest of the data by a greater amount than could be reasonably expected by chance (one in twenty times). Dixon's test was applied. The data obtained by each laboratory on each sample were ranked. The results showed that all the laboratories were satisfying the criterion.

Before the statistical analysis was undertaken, the data were examined to see whether the within-sample and within-laboratory variances were sufficiently homogeneous. The results showed that there were no significant differences in variances. The variances may be considered sufficiently homogeneous between laboratories and between replicates.

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 relative standard deviations (R.S.D.s), are given in Table 2. 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

Table 2
Results for the analysis of cephalexin bulk drug and dosage forms

Collaborator	Bulk drug (%)		250-mg capsule (%)		500-mg capsule (%)		Powder for oral suspension (%)	
	A	B	C	D	E	F	I	J
1	104.0	103.6	104.0	103.7	108.6	108.4	106.8	100.9
2	103.2	103.4	104.0	103.9	108.1	107.8	103.6	102.3
3	103.6	103.3	104.1	104.3	106.9	108.6	102.0	102.4
4	105.3	106.6	106.0	106.0	105.9	109.4	102.4	102.4
5	101.7	103.1	108.1	108.7	108.5	108.2	106.4	103.3
6	103.2	104.8	103.9	105.6	108.6	106.2	106.4	110.1
7	101.7	101.7	103.3	103.4	107.2	107.7	104.6	108.4
8	101.1	101.9	102.1	101.5	106.5	106.7	107.1	106.5
9	102.4	101.6	101.4	102.1	106.0	107.4	110.4	107.4
10	100.4	103.9	104.9	103.9	110.6	107.8	105.3	112.4
Mean					105.07			
S_r					0.34			
S_R					2.01			
R.S.D.$_r$ (%)					0.32			
R.S.D.$_R$ (%)					1.94			

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 values found were $S_r = 0.34\%$, $S_R = 2.01\%$, R.S.D.$_r = 0.32\%$ and R.S.D.$_R = 1.94\%$.

The analysis of variance with each sample is shown in Table 3. The variance ratios in the final column provide a means of assessing whether the between-laboratory variance and laboratory–sample interaction are significant. The result showed that the between-laboratory variance ratio is not significant (value of the ratio for significance with 8 and 56 degrees of freedom ≈ 3.0). However, the interaction variance ratio is significant (at the 5% level, value for significance with 56 and 72 degrees of freedom ≈ 1.5). Because the interaction variance ratio is significant, the analytical method showed greater variation when carried out by different laboratories than within one laboratory.

3.1. Collaborators comments

No difficulty with the method was encountered by any of the collaborators. 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 ($R_s \geq 1.25$).

Collaborators 4 analysed the samples using a Beckman C_8 column. Methanol–1.25% acetic acid (30:70, v/v) was used as the mobile phase instead of the ratio of 25:75 to shorten the retention times for both the cephalexin and the internal standard.

Collaborators 6 analysed bulk drug and capsule samples using an Inertsil ODS C_{18} column. The data reported in Table 2 were obtained with methanol–1.25% acetic acid (25:75, v/v) as mobile phase. However, collaborators 6 found that the peak shape of cephalexin in suspension samples was broad. When the mobile phase was modified to methanol–0.05 *M* potassium acetate–acetic acid (25:73:2, v/v), the peak for cephalexin was sharper.

4. Conclusions

The collaborative study of the reversed-phase column LC method for the determination of cephalexin in bulk, 250- and 500-mg capsules and powder for oral suspension preparations showed good reproducibility between laboratories. The method also showed greater variation when carried out by different laboratories than within one laboratory, but evidently no consistent laboratory bias existed.

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Table 3
Analysis of variance

Source of variation	Sum of squares	Degree of freedom	Mean square	Variance ratio
Between laboratories	52.27	8	6.534	0.80
Between samples	6.14	7	–	
Laboratory–sample interaction	459.39	56	8.203	72.87
Between replicates	8.11	72	0.113	

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