A Comparison of Analytical Methods for the Content and Purity of Cefradine

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Two HPLC methods such as cefadroxil and cefalexin methods were compared in their performance for the quantitative analysis of the content and purity of β-lactamic antibiotic, cefradine, for six bulk drug samples. Between the two methods, the cefadroxil method prescribed by the European Pharmacopoeia (EP) for the determination of impurities in cefradoxil was superior to the cefalexin method prescribed by the EP and by the United States Pharmacopeia (USP) for the determination of cefalexin impurity in cefradine in terms of the greater stability of the chromatogram baselines and the higher precision, *i.e.*, the lower % relative standard deviation (RSD). Based on the comparison of the two HPLC methods, the cefadroxil method was recommended to replace the TLC method, which has been prescribed by the EP as the official method for determination of extraneous impurities in cefradine.

Key Words : Cefradine, Cefalexin, Cefadroxil, Impurity

Introduction

Cefradine is an important cephalosporin antibiotic drug. Cefalexin is a major impurity in cefradine. For the analysis of cefradine and cefalexin, micellar electrokinetic chromatography by the capillary electrophoresis method,[1] liquid chromatography (LC) on poly(styrene-divinylbenzene),[2] a comparative study of two isocratic liquid chromatography methods using a classical column (C_{18}) and poly(styrene-divinylbenzene) as the stationary phase[3] and thin-layer chromatography (TLC)[4] method have been reported.

Cephalosporins are commonly analyzed using LC methods with absorbance detection.[5-17] Official methods to assess antibiotic identity, strength, quality, and purity of cephalosporins are described in the Code of Federal Regulations (CFR Title 21), the United States Pharmacopoeia (USP) and the European Pharmacopoeia (EP).

In the EP[18] and USP,[19] an isocratic HPLC method was developed and validated for determination of cefalexin, the major impurity in cefradine. However, this method does not allow for characterization of any extraneous impurities. The analytical methods prescribed by the EP and USP for analysis of impurities in cefradine and cefadroxil are summarized in Table 1. As shown in Table 1, a TLC method is prescribed by the EP for determination of extraneous impurity levels in cefradine. The TLC method is simple and does not require special equipment. However, it is rarely used to analyze impurities due to its low sensitivity and low reproducibility compared with HPLC method.[20] HPLC method is the most widely used technique for analysis of bulk drugs and their formulations.[21,22]

As shown in Table 2, limitations on the allowable impurity content in cefradine are clearly described by the EP and USP. Even though a rapid and simple HPLC method has been developed to assay antibiotic and impurity levels in bulk drugs,[21] the official method for determination of extraneous impurities in cefradine is TLC method according to the EP and USP as shown in Table 1.

In this study, as an effort to propose more reliable method than TLC method, the content of cefradine and all impurities will be assayed for six cefradine bulk drugs by using the cefalexin method, the official HPLC method prescribed by the EP for analyzing cefalexin, the major impurity in cefradine. In addition, the content of cefradine and all impurities will be assayed by using the cefadroxil method, the official HPLC method prescribed by the EP for all

Table 1. Methods for the analysis of impurities in cefradine and cefadroxil prescribed by the EP and USP

Raw material	Pharmaco-poeia	Impurity	Detector	Mobile phase	Column	Flow rate
Cefradine	EP	Cefalexin	UV 254 nm	Acetate buffer solution with methanol	C_{18}, (4.6 × 250 mm)	1.0 mL/min
		Any extraneous impurity	TLC analytical method			
	USP	Cefalexin	UV 254 nm	Acetate buffer solution with methanol	C_{18}, (4.6 × 250 mm)	1.0 mL/min
Cefadroxil	EP	All impurities	UV 220 nm	Phosphate buffer (pH=5.0) with methanol	C_{18}, (4.6 × 100 mm)	1.5 mL/min (Gradient)
	USP	All impurities	TLC analytical method			

impurities in cefadroxil.[23] Comparison of the two HPLC methods is expected to provide more reliable method than TLC method for determination of the content of cefradine and its all impurities.

Experimental

Chemicals. The standards were USP products. The structures of cefradine, cefalexin and cefadroxil are shown in Figure 1. The Korean Food and Drug Administration (KFDA) donated all of the bulk drugs to the Research Project on the Quality Control of Standard Drugs. Methanol and water, both HPLC grade, were obtained from Merck (Darmstadt, Germany). Glacial acetic acid, sodium acetate and potassium dihydrogen phosphate were purchased from Sigma-Aldrich Chemical, Korea Ltd. (Seoul, Korea). All reagents were analytical-grade.

Preparation of mobile phases and samples. For HPLC analysis of substances related to cefradine, mobile phases and standard samples were prepared according to the "cefalexin method" described in the EP. For the development of the cefradine impurity test, samples were treated according to the cefadroxil sample preparation method described in the EP. Mobile phases were degassed by ultrasonication. The concentrations of the six bulk drug samples were similar to that of the standard solution.

Each of the six bulk drug samples was injected three times to obtain % area and % RSD data. The cefradine impurity test was completed according to the "cefalexin method" described in the EP within 8 hr and 40 min, producing three chromatograms for each bulk drug. The interval between sample injections was approximately 4 hr and 20 min. The cefradine impurity test performed according to the "cefadroxil method" was completed within 10 hr and 20 min, producing three chromatograms for each bulk drug. The sample injection interval was approximately 5 hr and 10 min.

Equipment. The HPLC system was a Waters (Milford, MA, USA) Alliance 2695 separations module system consisting of a 2996 photo diode detector interfaced with a PC data system. Chromatographic data were manipulated using Empower software from Waters Korea Ltd. (Seoul, Korea). HPLC separations were performed with a C_{18} 250 mm × 4.6 mm (UG120 5 μm particle size) column from Shiseido Capcell pak (Tokyo, Japan). The column operating temperature was maintained at 30 °C. Other HPLC conditions are summarized in Table 3. Column length and sample temperature given in the EP were modified slightly in the present study to obtain comparable data.

Results and Discussion

Chromatographic results for the determination of impurities in cefradine with "cefalexin method". The HPLC conditions were those described for the "cefalexin method" in the EP to improve analysis of the impurities in cefradine over the TLC method. The ratio of the major impurity, cefalexin, and any other impurities in cefradine was checked with the "cefalexin method." The method was quite successful in determining the content of all impurities in cefradine. The method facilitated simultaneous determination of cefradine content, which is not possible with TLC method.

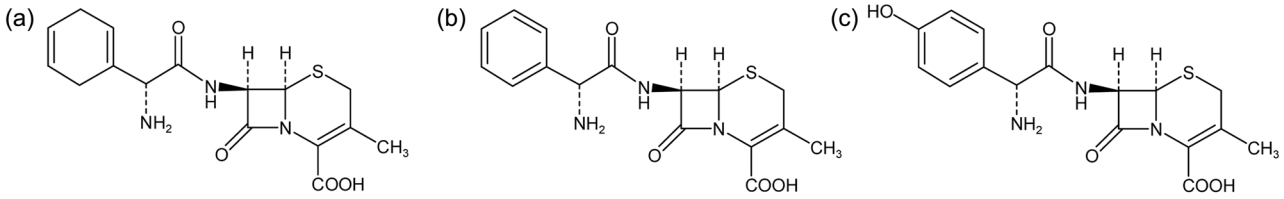

Figure 1. Structures of cefradine (a), cefalexin (b) and cefadroxil (c).

Table 2. Specification of impurities in cefradine prescribed by the EP and USP

Raw material	Impurity	Specification	
		EP	USP
Cefradine	Cefalexin	Not more than 5.0%	Not more than 5.0%
	Any extraneous impurity	Not more than 1.0%	–

Table 3. HPLC conditions for the analysis of cefradine using the "cefalexin" and "cefadroxil" methods

Method	Raw material	Detection wavelength (nm)	Mobile phase	Flow rate
Cefalexin method	Cefradine	254	0.0052 mol/L Sodium acetate solution·Methanol · Acetic acid (800:200:0.12)	1.0 mL/min (Isocratic)
Cefadroxil method	Cefradine	254	− Mobile phase A: Potassium dihydrogen phosphate buffer solution (pH = 5.0) − Mobile phase B: Methanol	1.5 mL/min (Gradient condition is consent with the EP.)
	Cefadroxil	220		

Table 4. Chromatographic results including retention time and % area of each peak obtained with "cefalexin method" for six cefradine bulk drug samples (A~F)

Sa	RT (min)	% Area[a]	Compound	Sa	RT (min)	% Area	Compound
A	3.45	0.02	UN[b]	D	3.43	0.02	UN
	4.00	0.08	UN		3.99	0.08	UN
	10.24	2.34	Cefalexin		5.14	0.10	UN
	15.03	96.63	Cefradine		10.23	2.26	Cefalexin
	23.99	0.93	UN		15.02	96.14	Cefradine
					23.99	1.41	UN
B	3.44	0.05	UN	E	3.44	0.04	UN
	4.01	0.10	UN		3.99	0.03	UN
	5.13	0.09	UN		5.13	0.06	UN
	10.24	2.14	Cefalexin		10.23	2.26	Cefalexin
	15.02	96.20	Cefradine		15.02	96.61	Cefradine
	24.03	1.41	UN		23.95	1.02	UN
C	3.45	0.11	UN	F	3.44	0.03	UN
	4.00	0.08	UN		4.00	0.04	UN
	10.24	3.46	Cefalexin		5.13	0.18	UN
	15.03	95.65	Cefradine		10.24	3.27	Cefalexin
	24.04	0.70	UN		15.01	95.47	Cefradine
					24.02	1.01	UN

[a]Average of % Area to 3 times. [b]Unknown

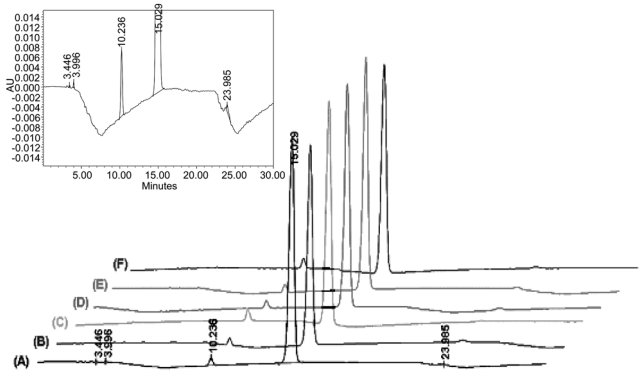

Figure 2. **C**hromatograms of six cefradine bulk drug samples (A~F) and an enlarged chromatogram of sample A for the analysis of impurities in cefradine according to the "cefalexin method" prescribed by the EP.

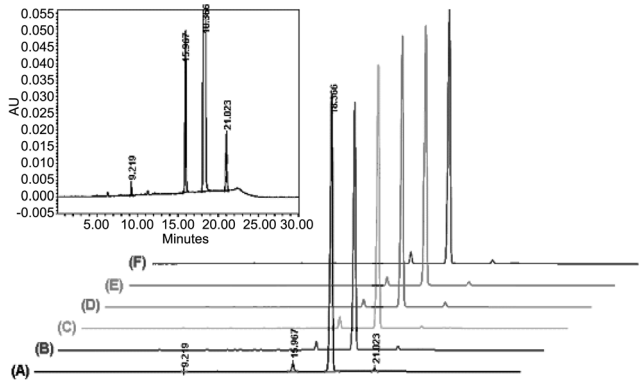

Figure 3. **C**hromatograms of six cefradine bulk drug samples (A~F) and an enlarged chromatogram of sample A for the analysis of impurities in cefradine according to the "cefadroxil method" prescribed by the EP.

HPLC results for retention time and % area for each of the six peaks corresponding to the six (Sa A~F) bulk drugs are summarized in Table 4 and representative chromatograms for each bulk drug are shown in Figure 2. The results of HPLC analysis summarized in Table 4 clearly demonstrate the ratio of cefradine, cefalexin and three or four unknown impurities, although the chromatograph baselines were somewhat unstable.

According to EP specifications, the content of cefradine, when used as an active ingredient, should be not less than 90.0%. In addition, the content of cefalexin as a major impurity should be not more than 5.0%, while the content of any other extraneous impurities should not exceed 1.0%. Therefore, it was concluded that the cefradine bulk drugs, A, B, D, E and F, did not meet EP specifications, because the content of three or four unknown impurities was greater than 1.0%, as shown in Table 4.

Chromatographic results for determination of impurities in cefradine with "cefadroxil method".

Determination of impurities in cefradine: As shown in Figure 1, the structure of cefradine is similar to that of cefadroxil. Consequently, the chromatographic conditions described for the "cefadroxil method" except for the UV detection wavelength can be applied to the determination of impurities in cefradine. The UV detection wavelength for cefradine was set to 254 nm, as shown in Table 3 while the wavelength used for cefadroxil was 220 nm, according to the "cefadroxil method".

Representative chromatograms for the six cefradine (Sa A~F) bulk drugs are shown in Figure 3. The baselines of the chromatograms shown in Figure 3 were much more stable than those obtained with the "cefalexin method" shown in Figure 2. Retention time and % area for each of the six cefradine (Sa A~F) bulk drugs are summarized in Table 5.

Table 5. Chromatographic results including retention time and % area of each peak obtained with the "cefadroxil method" for six cefradine bulk drug samples (A~F)

Sa	RT	%Area[a]	Compound	Sa	RT	%Area	Compound
A	9.18	0.13	UN[b]	D	9.21	0.09	UN
	15.94	2.48	Cefalexin		11.28	0.13	UN
	18.34	96.42	Cefradine		15.95	2.11	Cefalexin
	20.99	0.97	UN		18.35	96.20	Cefradine
B	6.27	0.11	UN		21.00	1.46	UN
	9.20	0.21	UN	E	11.27	0.09	UN
	11.26	0.15	UN		15.94	2.49	Cefalexin
	15.95	2.82	Cefalexin		18.34	96.27	Cefradine
	18.33	95.26	Cefradine		21.00	1.14	UN
	21.00	1.45	UN	F	6.26	0.13	UN
C	6.26	0.15	UN		9.19	0.14	UN
	9.20	0.10	UN		11.27	0.21	UN
	15.94	3.61	Cefalexin		15.95	3.68	Cefalexin
	18.33	95.10	Cefradine		18.33	94.67	Cefradine
	21.00	0.69	UN		21.00	1.17	UN
	22.37	0.17	UN				
	22.77	0.18	UN				

[a]Average of % Area to 3 times. [b]Unknown

Table 6. Comparison of the "cefalexin" and "cefadroxil" methods regarding % area and % RSD of the major ingredient, cefradine, in six cefradine bulk drugs (A~F)

Sample		A	B	C	D	E	F
Cefalexin method	Mean % area	96.63	96.20	95.65	96.14	96.61	95.47
	% RSD	0.20	0.14	0.11	0.17	0.03	0.05
Cefadroxil method	Mean % area	96.42	95.26	95.10	96.20	96.27	94.67
	% RSD	0.02	0.08	0.05	0.06	0.04	0.06

Table 7. Comparison of the "cefalexin" and "cefadroxil" methods regarding % area and % RSD of the major impurity, cefalexin, in six cefradine bulk drugs (A~F)

Sample		A	B	C	D	E	F
Cefalexin method	Mean % area	2.34	2.14	3.46	2.26	2.26	3.27
	% RSD	0.03	0.12	0.04	0.08	0.10	0.02
Cefadroxil method	Mean % area	2.48	2.82	3.61	2.11	2.49	3.68
	% RSD	0.02	0.04	0.03	0.05	0.04	0.04

The retention times of cefalexin and cefradine were approximately 16 and 18 min, respectively, with good reproducibility. The cefalexin content in each of the six cefradine bulk drugs tested was less than 5.0% and was within EP specifications.

According to EP specifications, the content of any extraneous impurity in cefradine must be less than 1.0% (Table 2). However, the impurity content observed at 21 min was greater than 1.0% for cefradine bulk drugs B, D, E and F. In addition, the total extraneous impurity content was more than 1.0% for all six cefradine bulk drugs. In this instance, none of the six cefradine bulk drugs tested, A, B, C, D, E and F, were deemed appropriate for use as drugs.

Comparison of the "cefalexin" and "cefadroxil" methods for the assay of cefradine bulk drugs. The precision of the two HPLC methods for determination of cefradine, cefalexin and any other detectable impurities in cefradine bulk drugs was compared. The % area and % RSD observed with the two methods for cefradine content in six cefradine bulk drugs are summarized in Table 6. The % RSDs for the % areas observed with the "cefadroxil method" (0.02-0.08) were superior to those obtained with the "cefalexin method" (0.05-0.20). The % area and % RSD for the cefalexin content in six cefradine bulk drugs are also summarized in Table 7. As shown in Table 7, the % RSDs for the % areas obtained using the "cefadroxil method" (0.02-0.05) were also superior to those obtained with the "cefalexin method" (0.0-20.12). From the extended chromatograms shown in Figures 2 and 3, it is evident that the stability of the chromatograph baseline was greater with the "cefadroxil method" than with the "cefalexin method." Overall, the "cefadroxil method" is concluded to be quite reliable in determining cefalexin, the major impurity in cefradine, and any other extraneous impurities in cefradine bulk drugs.

Conclusions

In this study, two different HPLC methods were evaluated in their performance for determination of impurities in cefradine bulk drugs. Even though TLC method is prescribed as the official method for determination of extraneous impurities in cefradine by the EP, HPLC method is superior to TLC method in terms of simple sample preparation, greater sensitivity and reproducibility. Between the two HPLC methods, the "cefadroxil method" was found to be superior to the "cefalexin method" due to greater baseline stability and precision. The "cefadroxil method" would be a suitable replacement for TLC method as the official method for determination of impurities in cefradine. In our future study, the "cefadroxil method" will be extended to the quantitative analysis of cefaclor and amoxicillin, which are similar to cefadroxil in their structures.

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