ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
14 (1996) 641-654

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

High-pressure liquid chromatographic methods for ciprofloxacin hydrochloride and related compounds in raw materials

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Received for review 7 February 1995; revised manuscript received 4 October 1995

Abstract

High-pressure liquid chromatographic (HPLC) methods were developed for the analysis of ciprofloxacin hydrochloride raw materials. Method A is for drug content and the determination of related compounds eluting before the drug, including the ethylenediamine analog of ciprofloxacin. Method B may be used for the determination of fluoroquinolonic acid and other related compounds eluting after the drug. Both methods require a 5 μm Inertsil ODS2 column (150 × 4.6 mm), a mobile phase containing tetrahydrofuran, acetonitrile and buffer (0.005 M 1-hexane-sulphonic acid sodium adjusted to pH 3.0 with 0.1 M phosphoric acid); 10:5:85 (v/v/v) for method A and 25:15:60 (v/v/v) for method B, and a flow rate of 1 ml min^{-1}. Detection for method A is at 254 nm; a programmable variable wavelength detector is required for method B: 254 nm for 12 min, then 220 nm for 23 min. The limit of quantitation of the related compounds was 0.05% or less. The precision of the assay method was lower than 1.0%. Drug content in four raw material samples ranged from 98.7% to 101.6% calculated with reference to the anhydrous substance. The water content in these samples ranged from 5.9% to 7.8%. Total impurity levels were 1.0% or lower. Levels of ethylenediamine analog and fluoroquinolonic acid were below 0.4%. A second analyst, using a different HPLC system and a column from a different supplier, repeated the analysis of two raw materials samples and obtained similar results.

Keywords: Ciprofloxacin hydrochloride; Assay; Impurities; HPLC

1. Introduction

Ciprofloxacin hydrochloride (**I**), 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid, monohydrochloride, monohydrate, is a broad-spectrum quinolone antibacterial agent used against a wide range of infections. Ciprofloxacin was originally prepared by Grohe et al. [1]. Routes of synthesis have been reviewed by Zhang [2] and by Chu and Fernandes [3]. Structures for this drug and several related compounds are presented in Fig. 1; of these, compounds **V**, **VI**, **VIII**, **X**, **XVI** and **XXI** were not available to us during method development.

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SSDI 0731-7085(95)01667-8

Compounds **II–XVI** are starting materials, reagents or synthetic intermediates, and compounds **XVII–XXI** are potential impurities identified by manufacturers. Compound **XX** is a possible degradation product resulting from the decarboxylation of the drug, **XVII** is a potential photolysis product, **XVIII** and **XIX** are by-products of the synthesis. Ciprofloxacin hydrochloride is fairly stable in the solid state and in aqueous solutions [4,5] but undergoes degradation in dilute aqueous solutions when exposed to light [6].

Spectrophotometric [7–10] and HPLC [11,12] methods for the quantitation of ciprofloxacin in tablets, either as the base or hydrochloride, have been reported in the literature. Ciprofloxacin may also be assayed by polarographic [13,14] or voltammetric [15] methods. Several papers have compared HPLC and bioassays for the determination of ciprofloxacin in serum and urine [16–18].

Ciprofloxacin hydrochloride is official in the United States Pharmacopeia (USP) [19] and European Pharmacopoeia (EP) [20]. These monographs contain essentially identical TLC methods for fluoroquinolonic acid (**XIII**) and similar HPLC methods for the assay and determination of other related compounds. Assay limits are 98.0–102.0%, and the limit on **XIII** is 0.2% in both monographs. The USP places a limit of 0.4% on ciprofloxacin ethylenediamine analog (**XVII**), 0.2% on other individual impurities and 0.7% on total impurities, excluding **XIII**. The EP limits are 0.2% **XVII**, 0.2% **XVIII** and 0.5% on total impurities.

The purpose of this work was to develop a single HPLC method for the assay of ciprofloxacin hydrochloride and the determination of all related compounds, including fluoroquinolonic acid. However, because of great differences in polarity among the various related compounds it was necessary to use two methods requiring the same column but different mobile phases. Although these methods could probably be modified to a single gradient method it was felt that the two methods would offer better reproducibility and be suitable to laboratories which do not have access to gradient HPLC systems.

2. Experimental

2.1. Chemicals

Acetonitrile (J.T. Baker, Phillipsburg, NJ), phosphoric acid (Fisher, Fair Lawn, NJ) and inhibitor-free tetrahydrofuran (Aldrich, Milwaukee, WI and Caledon Laboratories, Georgetown, Ont.), all HPLC grade, hexanesulphonic acid sodium salt (Aldrich, Milwaukee, WI) and hydrochloric acid (Anachemia, Champlain, NY) were used. The water used was distilled then deionized in a Barnstead Nanopure II system. For Karl Fischer moisture determination the following were used: Hydranal Solvent, Hydranal Titrant 5, Hydranal Standard Methanol, all from Riedel-de Haen (Seelze, Germany), and nitrogen gas (Matheson, Ottawa, Ont.). Samples of related compounds **II**, **III**, **IV**, **IX** and **XIV** were obtained from Aldrich (Milwaukee, WI), **VII**, **XI**, **XII**, **XIII** and **XV** from Cipla (Bombay, India), and **XIII**, **XVII**, **XVIII**, **XIX** and **XX** from Uquifa (Barcelona, Spain). The following USP reference standards were also used: **I** (Lots F and F-1), **XIII** (Lot F) and **XVII** (Lot F). The infrared, proton magnetic resonance and mass spectra of the related compounds used as standards during method development were consistent with their respective structures. Raw materials were obtained directly from manufacturers.

2.2. Apparatus

The HPLC system (Varian Vista 5560) was equipped with a programmable variable wavelength detector (Varian UV 200) set at 254 nm for method A, and initially set at 254 nm then switched to 220 nm at approximately 12 min for method B; an autosampler (Varian 8085) and a data processor (Varian Vista 402). The HPLC system was operated at ambient temperature with a flow rate of 1 ml min^{-1}. Two columns were used: both were Keystone Inertsil 5-μm ODS2 (15 cm × 4.6 mm, # 099215, Chromatographic Sciences Co., Montreal, Que. and # 102051P (Lot SQS-906), Keystone Scientific, Bellefonte, PA). The first was used for method development and sample analysis by the first analyst; the second

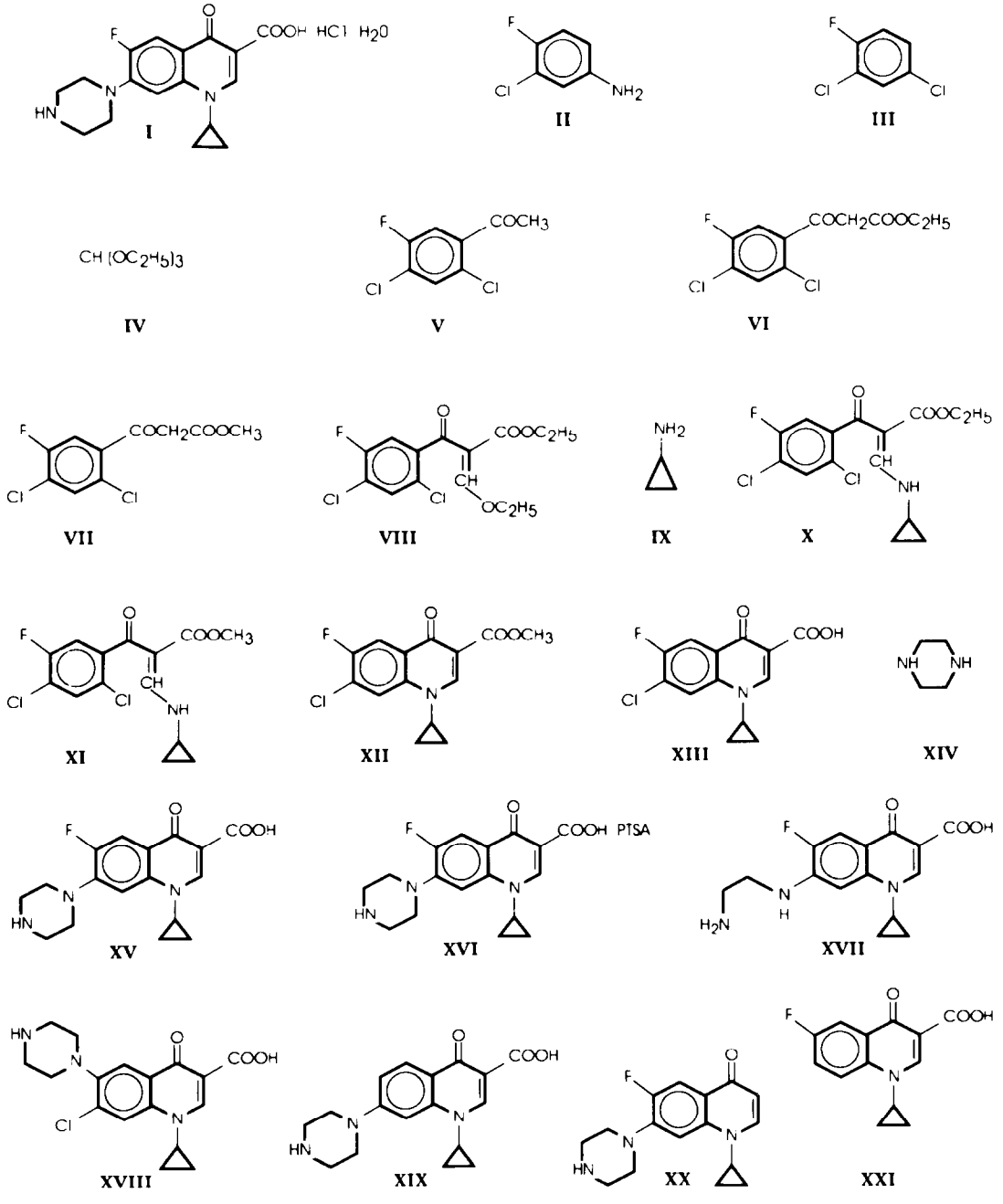

Fig. 1. Chemical structures of ciprofloxacin hydrochloride and related compounds: (**I**) ciprofloxacin hydrochloride, 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quino-linecarboxylic acid, monohydrochloride, monohydrate; (**II**) 3-chloro-4-fluoroaniline; (**III**) 1,3-dichloro-4-fluorobenzene; (**IV**) triethyl orthoformate; (**V**) 2,4-dichloro-5-fluoroacetophenone; (**VI**) ethyl 2,4-dichloro-5-fluorbenzoylacetate; (**VII**) methyl 2,4-dichloro-5-fluorobenzoylacetate; (**VIII**) ethyl 2-(2,4-dichloro-5-fluorobenzoyl)-3-ethoxyacrylate; (**IX**) cyclopropylamine; (**X**) ethyl 2-(2,4-dichloro-5-fluorobenzoyl)-3-cyclopropylaminoacrylate; (**XI**) methyl 2-(2,4-dichloro-5-fluorobenzoyl)-3-cyclopropylaminoacrylate; (**XII**) 7-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid methyl ester; (**XIII**) fluoroquinolonic acid, 7-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid; (**XIV**) piperazine; (**XV**) ciprofloxacin base; (**XVI**) ciprofloxacin *p*-toluenesulfonic acid salt; (**XVII**) ethylenediamine analog, 7-[(2-aminoethyl)-amino]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid; (**XVIII**) by-product A, 7-chloro-1-cyclopropyl-1,4-dihydro-4-oxo-6-(1-piperazinyl)-3-quinolinecarboxylic acid; (**XIX**) 1-cyclopropyl-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid; (**XX**) 1-cyclopropyl-6-fluro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline; (**XXI**) 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid.

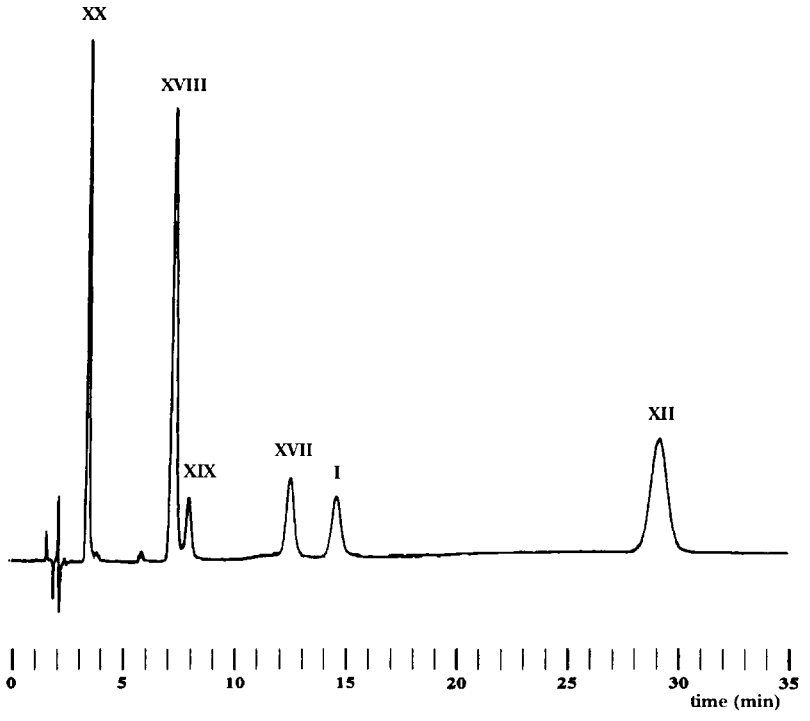

Fig. 2. Chromatogram of ciprofloxacin hydrochloride and related compounds at about 0.2% (Method A).

was used by the first analyst to check ruggedness, and by a second analyst for the evaluation of the method. Other equipment included a pH meter (Fisher Accumet 915), a UV–VIS spectrophotometer (Varian DMS 90) connected to a computer (Hewlett-Packard HP-85) and plotter (Hewlett-Packard HP-7470A). A Mettler DL18 Karl Fischer titrator was used for the water determination.

3. Method A: Assay of ciprofloxacin hydrochloride and related compounds eluting before the drug

3.1. Mobile phase

Tetrahydrofuran: acetonitrile: 1-hexanesulphonic acid sodium (0.005 M, pH 3.0 with 0.1 M phosphoric acid) (10:5:85, v/v/v).

3.2. Solutions

The following solutions were prepared in the mobile phase and sonication was used, if necessary, for dissolution:

(1) resolution solution for system A: 0.004 mg ml^{-1} (accurately known) ciprofloxacin hydrochloride and 0.004 mg ml^{-1} ethylenediamine analog (**XVII**);

(2) assay standard solution: 2 mg ml^{-1} (accurately known) ciprofloxacin hydrochloride reference standard;

(3) test solution: 2 mg ml^{-1} (accurately known) ciprofloxacin hydrochloride.

3.3. System suitability

For the determination of related compounds, six 10 μl aliquots of the resolution solution were injected into the chromatograph. The system was deemed to be suitable for use if the efficiency of the column, calculated using the ciprofloxacin peak, was not less than 24 000 plates m^{-1}, the relative standard deviation of the peak responses due to ciprofloxacin was not more than 5.0%, and the resolution between **XVII** and ciprofloxacin was not less than 2.0. The retention time of

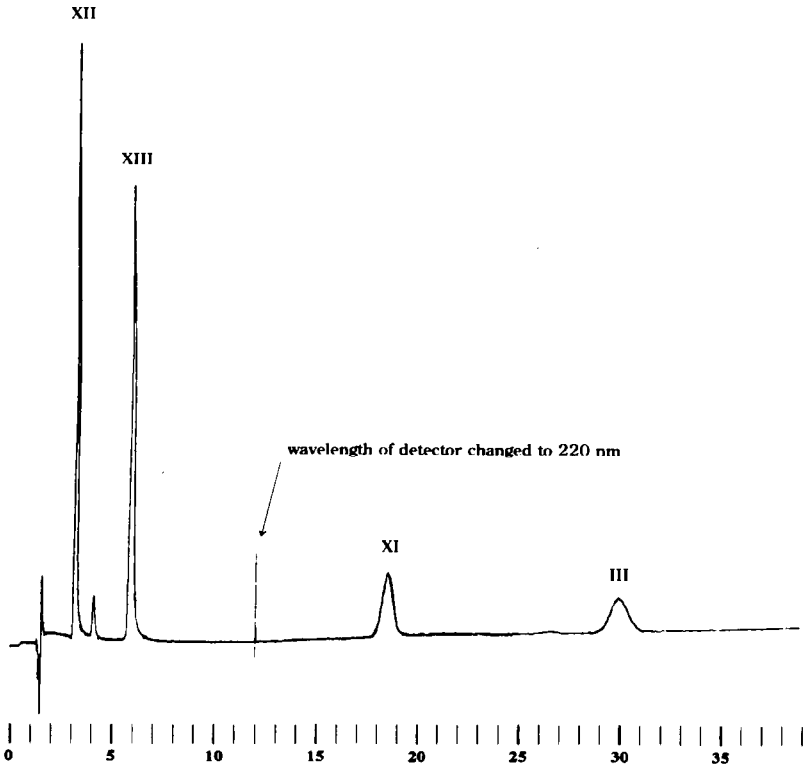

Fig. 3. Chromatogram of ciprofloxacin related compounds at about 0.2% (Method B).

ciprofloxacin was within the range 11.5–15.0 min and the relative retention time of **XVII** was about 0.86. For the assay method, two injections of the resolution solution were required to demonstrate that the system met the efficiency and resolution requirements listed above, then six 10 μl aliquots of the assay standard solution were made and the system was deemed suitable if the relative standard deviation of the peak responses due to ciprofloxacin in this latter solution was not more than 1.0%.

3.4. Procedure for related compounds

Individual 10 μl aliquots of the resolution and test solutions were injected into the chromatograph and run for 35 min. The amount of each impurity in the test solution eluting before the drug peak as a percentage of the total amount of drug was calculated from 100 (A_u/A_s) (C_s/C_u), where A_u is the peak area due to the individual impurity, A_s is the area of the peak due to ciprofloxacin hydrochloride in the resolution solution, and C_s and C_u are the concentrations of ciprofloxacin hydrochloride in the resolution and test solutions respectively.

3.5. Assay

Separate 10 μl quantities of the standard and test solution were injected into the chromatograph and run for 35 min. The amount of ciprofloxacin hydrochloride in the test solution was calculated using the formula 100 (A_u/A_s) (C_s/C_u), where A_u and A_s are the areas of the peak due to ciprofloxacin hydrochloride in the test and standard solutions respectively, and C_s and C_u are the concentrations of ciprofloxacin hydrochloride in the standard and test solutions respectively, calculated with reference to the anhydrous substance.

Table 1
UV characteristics of ciprofloxacin related compounds

Compound	Concn. (mg ml^{-1})	Maxima (nm)	Absorbance at 254 nm (AU)	Relative[a] absorbance at 254 nm	Absorbance at 278 nm (AU)	Relative absorbance at 278 nm
Solvent: water						
I	0.0060	273	0.270	1.00	0.58	1.00
XVII	0.0046	272	0.213	1.03	0.593	1.33
Solvent: 0.1 N HCl						
I	0.0058	276	0.186	1.00	0.589	1.00
XV	0.0054	275	0.191	1.10	0.809	1.48
Solvent: water/acetonitrile 1:1 (v/v)						
I	0.00524	279	0.128	1.00	0.551	1.00
II	0.00477	205	0.000	—	0.014	0.03
III	0.01273	206	0.000	—	0.026	0.02
IV	0.174	—	0.003	—	0.004	—
VII	0.00477	212, 252	0.164	1.41	0.060	0.12
IX	0.0482	—	0.012	0.01	0.008	
XI	0.00534	242, 309	0.204	1.56	0.162	0.29
XII	0.00424	245, 307	0.238	2.30	0.196	0.44
XIII	0.00502	216, 229, 254, 262	0.461	3.76	0.030	0.06
XIV	0.0536	—	0.003	—	0.001	—
XV	0.00530	278	0.189	1.46	0.630	1.13
XVIII	0.00492	224, 254, 320	0.329	2.74	0.269	0.52
XIX	0.00569	283	0.101	0.73	0.777	1.30
XX	0.00492	231, 265, 322	0.332	2.76	0.079	0.15
			Absorbance at 220 nm (AU)	Relative Absorbance at 220 nm		
I	0.00524	279	0.146	1.00		
II	0.00477	205	0.188	1.41		
III	0.01273	206	0.279	0.79		
VII	0.00477	212, 252	0.298	2.24		

[a] Relative to ciprofloxacin hydrochloride in the same solvent.

4. Method B: Related compounds eluting after ciprofloxacin

4.1. Mobile phase

Tetrahydrofuran: acetonitrile: 1-hexanesulphonic acid sodium (0.005 M, pH 3.0 with 0.1 M phosphoric acid) (25:15:60, v/v/v).

4.2. Solutions

The following solutions were prepared, using sonication if necessary to dissolve samples:

(1) resolution solution for system B: 0.004 mg ml^{-1} fluoroquinolonic acid (accurately known) and 0.004 mg ml^{-1} impurity **III**, prepared by separately dissolving 20 mg of each in 200 ml mobile phase for method B then diluting 4 ml of each solution to 100 ml in mobile phase for method A;

(2) test solution: 2 mg ml^{-1} (accurately known) ciprofloxacin hydrochloride dissolved in mobile phase for method A.

Table 2
Response data for ciprofloxacin related compounds

Compound	RRT	Slope (counts ng^{-1})	Intercept (counts)	R^2	Relative response[a]	LOQ (%)[b]	RSD at 0.2% level (%)
Method A							
I	1.00	485	−1651	0.9994	1.00	0.05	2.4
XVII	0.86	561	−1758	0.9998	1.16	0.05	2.7
XVIII	0.50	1081	−1121	0.9990	2.23	0.01	0.7
XIX	0.55	332	−1078	0.9976	0.68	0.05	1.8
XX	0.24	1199	392	0.9910	2.47	0.005	1.0
Method B							
I	1.00						
XIII	3.53	1730	−2857	0.9950	1.00	0.01	0.4
II	17.3	592	761	0.9997	0.34	0.05	5.1
III	17.3	602	96	0.9998	0.35	0.05	3.5
VII[c]	12.3	2633	18073	0.9977	0.48	0.2	12.3
XI	10.7	689	−817	0.9989	0.40	0.05	1.7
XII	1.93	1270	−617	1.0000	0.73	0.01	1.0

[a] Responses for Method A are relative to ciprofloxacin hydrochloride (**I**) and those for method B are relative to **XIII**.
[b] Limit of quantitation, defined as 4 × noise.
[c] Linearity for this compound was done using a different HPLC system (Waters system).

4.3. System preparation

Initially, the detector was programmed to change wavelength from 254 nm to 220 nm after approximately 12 min. A 10 μl aliquot of the resolution solution was injected into the system to determine the retention times of **XIII** and **III**. The change in wavelength from 254 nm to 220 nm was set at about one-third of the distance between **XIII** and **III**.

4.4. System suitability

Six 10 μl aliquots of the resolution solution for system B were injected into the chromatograph. The system was deemed suitable if the efficiency of the column, calculated using the fluoroquinolonic acid peak, was not less than 20 000 plates m^{-1}, the relative standard deviation of the peak responses due to fluoroquinolonic acid was not more than 5.0%, and the resolution between **XIII** and **III** was not less than 20. The retention time of fluoroquinolonic acid was about 5.6 min and the relative retention time of **III** was about 4.9.

4.5. Procedure

Separate 10 μl amounts of the resolution solution for system B and the test solution were injected into the chromatograph and run for 35 min. The amount of each impurity in the test solution eluting after the drug as a percentage of the total amount of drug was calculated using the formula $100\ (A_u/A_s)\ (C_s/C_u)$, where A_u is the peak area due to the individual impurity, A_s is the area of the peak due to fluoroquinolonic acid in the resolution solution, C_s is the concentration of fluoroquinolonic acid in the resolution solution and C_u is the concentration of ciprofloxacin hydrochloride in the test solution.

5. UV spectra

The UV spectra of ciprofloxacin and related compounds were measured in various solvents.

Table 3
System suitability test results

Method A

Date	Retention time (min)		Resolution	Efficiency (plates m^{-1})	RSD[a] (%) Resolution solution	RSD (%) Assay std. solution
	I	XVII				
Column No. 099215						
29/10/92	12.57	10.86	2.22	24507	1.02	
03/11/92	13.66	11.74	2.73	33667	3.47	
05/11/92	14.37	12.29	2.67	29587	1.89	
02/12/92	14.69	12.56	2.81	35507	4.85	0.28
03/12/92	12.59	10.92	2.75	42653	9.80[b]	0.65
09/12/92	14.67	12.55	2.99	40373	3.50	0.25
10/12/92	14.61	12.54	3.25	41227	2.79	0.38
16/12/92	14.31	12.25	3.12	43160	1.39	0.33
29/12/92	13.13	11.29	3.01	42707	1.37	0.20
05/01/93	13.24	11.41	2.80	36260	1.50	0.12
15/02/93	11.69	10.11	2.87	39320	2.23	0.14
17/02/93	11.92	10.32	2.78	40287	1.75	0.18
Column No. 102051P						
31/03/93	14.40	12.29	3.01	40273	1.12	
01/04/93	13.94	11.97	2.89	39507	1.61	0.56
29/09/94	13.93	12.04	2.87	41287	0.92	0.20

Method B

Date	Retention Time (min)		Resolution	Efficiency (plates m^{-1})	RSD (%) Resolution solution
	XIII	III			
Column No. 099215					
17/11/92	5.52	27.18	22.0	29700	0.86
18/11/92	5.53	27.34	25.2	29973	0.32
25/11/92	5.61	27.90	23.2	34073	0.63
30/11/92	5.57	27.62	24.6	35853	0.86
17/12/92	5.57	27.58	26.5	41773	1.36
21/12/92	5.89	30.35	27.0	41693	0.70
05/01/93	5.43	26.44	25.2	38387	2.29
11/01/93	5.64	27.73	23.5	40047	1.07
14/01/93	5.44	26.58	22.2	39420	1.61
08/02/93	5.90	30.34	25.2	40173	1.31
09/02/93	5.52	27.26	21.4	38980	1.59
15/02/93	5.37	26.00	23.1	42873	0.60
01/03/93	5.76	28.98	22.6	29773	3.40
Column No. 102051P					
31/03/93	5.92	30.47	25.9	38667	2.25
01/04/93	5.87	30.17	25.6	38040	1.09
26/09/94	6.51	31.65	28.7	35480	1.35

[a] RSD of peak responses.
[b] RSD unacceptably high, baseline noise high and response to ciprofloxacin low, therefore the lamp was changed.

6. Results and discussion

6.1. Chromatography

Separation of all available ciprofloxacin related compounds by a single isocratic HPLC method could not be achieved because of large differences in polarity. Therefore two methods, A and B, requiring the same type of column but different mobile phases were developed. Several C-18 columns were investigated, however a completely end-capped Keystone Inertsil ODS2 column provided the best peak shape for compound **XIII** (fluoroquinolonic acid). Method A is used for the determination of drug content and impurities eluting before ciprofloxacin. Method B is for the determination of impurities eluting after the drug. A programmable UV detector is required for Method B for the detection of late eluting impurities (**VII**, **II** and **III**) at 220 nm. With this method, compounds **II** and **III** were resolved from the drug but unresolved from each other. All other available related compounds were resolved from each other and the drug using the appropriate method (A or B) with detection at 254 nm. Chromatograms showing the resolution of ciprofloxacin hydrochloride and its related compounds by these two methods are presented in Figs. 2 and 3.

Table 4
Results of assay and water determination (%)

Sample code	Assay [a] (%)	% Water
A1	99.2 ($n = 3$, RSD = 0.67%)	6.6 ($n = 3$, RSD = 1.8%)
B1	101.6 ($n = 3$, RSD = 0.88%)	7.8 ($n = 2$, RSD = 8.1%)
C1	100.7 ($n = 3$, RSD = 1.45%)	6.6 ($n = 3$, RSD = 8.6%)
C2	98.7 ($n = 3$, RSD = 0.36%)	5.9 ($n = 5$, RSD = 5.4%)
D		6.9 ($n = 1$)

[a] Samples were assayed using A1 as standard. A1 was assayed against D, the USP standard. Assay values were calculated with reference to the anhydrous substance.

6.2. UV and HPLC characteristics

The UV absorbance of ciprofloxacin hydrochloride and the available related compounds is given in Table 1. Based on duplicate weighings with multiple dilutions, the response of the HPLC systems to ciprofloxacin and the related compounds was linear from the minimum quantifiable amount to 2.0%. Response data can be found in Table 2. The response of the system for method A to ciprofloxacin hydrochloride at concentrations ranging from 50% to 150% of the assay concentration (1–3 mg ml^{-1}) was also linear ($R^2 = 0.9993$, slope = 473 counts ng^{-1} on column, calculated on the anhydrous basis).

6.3. Precision

The precision of the response of the related substances methods at the 0.2% level relative to the drug was determined by making six replicate injections of a solution of drug or related compound at this concentration and calculating the relative standard deviation (RSD) of the peak area responses (Table 2). RSDs of the peak area responses of six injections of the assay standard solution determined on 10 different days are given in Table 3. Except for the day when the UV lamp was defective, RSDs were below 0.78% and therefore the system precision was suitable for a method where the assay acceptance range is 98.0–102.0% and samples are analyzed in duplicate or triplicate [21]. The precision of the assay method was determined by analyzing raw material sample B1 in triplicate on six different days. Results in percent, with RSDs in parentheses, were: 101.6 (0.88), 101.2 (0.20), 101.9 (0.17), 100.2 (0.69), 101.1 (0.42), 100.4 (0.10).

6.4. Stability of solutions

Test solutions of ciprofloxacin hydrochloride stored at room temperature showed no evidence of decomposition over a period of 17 h.

Table 5
Impurities in ciprofloxacin hydrochloride raw materials (%)

RRT [a]	Sample code			
	A1	B1	C1	C2
Method A				
0.12		0.02		
0.14	0.01	0.02		
0.16	0.03	0.02		
0.17	0.01			
0.18		0.01		
0.21				0.01
0.25[b]	0.02	0.30		
0.27				0.01
0.28			0.01	
0.29	0.02			
0.35		0.02		
0.46	0.01			
0.53[c]	0.45	0.03		
0.55[c]			0.22	0.15
0.60	0.01			
0.62			0.06	0.03
0.67			0.11	
0.69				0.05
0.74	0.04	0.01		
0.92[d]	0.06	0.03	0.23	
0.96	0.26			
Method B				
1.34[e]		0.04		
1.48		tr		
2.05[e]			0.02	
2.08[e]	0.01			
2.10[e]				0.01
2.31	0.04			
3.28		0.01		
3.48[f]		tr	tr	
3.57[f]	0.03			
3.64[f]				0.01
5.40	tr			
23.1		0.04		
Total Method A	0.92	0.46	0.63	0.25
Total Method B	0.08	0.09	0.02	0.02
Total impurities	1.00	0.55	0.65	0.27

[a] Retention time relative to ciprofloxacin hydrochloride at about 12.3 min for Method A and 1.6 min for Method B.
[b] Retention time consistent with compound **XX**.
[c] Retention time consistent with compound **XVIII**.
[d] Retention time consistent with compound **XVII**.
[e] Retention time consistent with compound **XII**.
[f] Retention time consistent with compound **XIII**.

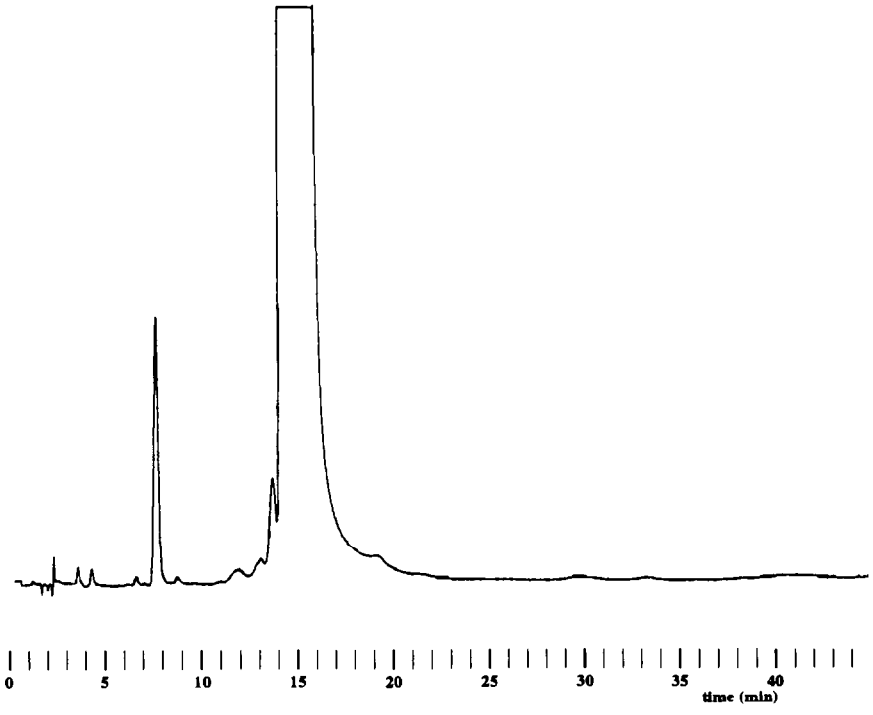

Fig. 4. Chromatogram of Sample A1 at 2.0604 mg ml^{-1} (Method A).

6.5. System suitability parameters

The compounds chosen for the resolution solution in Method A were the ethylenediamine analog, a signal impurity, and ciprofloxacin hydrochloride, which is used as the external standard for quantitation. The resolution solution for Method B contains fluoroquinolonic acid, a signal impurity which also serves as external standard, and compound **III**, which is commercially available and useful for verifying that the run time is long enough. A number of parameters suitable for the definition of a system suitability test were monitored during method development and are given in Table 3. Efficiency was calculated using width at half height. The system suitability requirements could be met on two columns from different manufacturers.

6.6. Ruggedness

Retention times for the related compounds were reproducible on a second column from a different manufacturer. An increase in the percentage of buffer in the mobile phase caused an increase in retention times and broadening of peaks. If the concentration of aqueous buffer remained at 85% v/v but the concentration of acetonitrile was slightly increased or decreased, some of the related compounds were unresolved from each other.

6.7. Water determination and assay of raw materials

Four samples of ciprofloxacin were available at the time this work was done. Results of the assays and water determinations are summarized in Table 4. All samples met the USP and EP assay requirements of 98.0–102.0%. Water content was determined by direct titration in a system that was continuously purged with dry nitrogen. Sample B1 and the USP standard (D) did not meet the USP and EP requirements for water content: 4.7–6.7%. The USP is aware of the high water content in its ciprofloxacin hydrochloride standard [22].

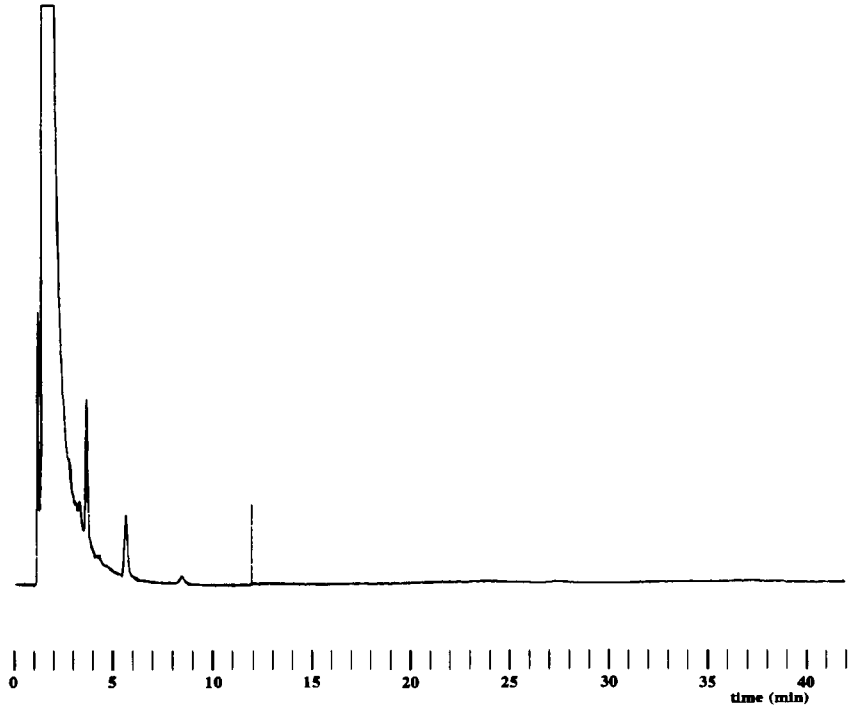

Fig. 5. Chromatogram of Sample A1 at 2.0720 mg ml^{-1} (Method B).

Sample B1 was received in a plastic bag and water may have permeated through the plastic during storage. The limits for water content are probably suitable if the raw material is properly stored in tightly closed containers.

6.8. Impurities in raw materials

Results of the impurity levels for these samples are presented in Table 5. Total impurities ranged from 0.3% to 1.0%. Levels of ethylenediamine analog and fluoroquinolonic acid were 0.2% or lower. Typical chromatograms for Sample A1 are shown in Figs. 4 and 5. Sample A1 contained two impurities at levels above 0.2%: the first had a retention time consistent with an impurity termed "by-product A" in the EP (**XVIII**) (RRT = 0.53); the second eluted between compound **XVII** (ethylenediamine analog) and the drug and was present at a level of about 0.3%. These samples would meet the USP and EP requirements for ethylenediamine analog and fluoroquinolonic acid. Although sample A1 appears to exceed the EP limit on by-product A and the USP limit on individual impurities, impurity levels could be considered satisfactory when differences in response factors at 254 nm vs. 278 nm are taken into account (see Table 2).

6.9. Reproducibility

The methods were evaluated by a second analyst who had not been involved in method development. Two samples were analyzed, and the results are summarized in Table 6. There was good agreement between the results of the first and second analyst. The impurity profiles were essentially the same, except that impurities which had eluted as two peaks between 0.60 and 0.69 on the first column were unresolved on the second column. Assay results obtained by the second analyst were the same for sample C1 and slightly

higher for C2, although both samples were still within the range 98.0–102.0%. The second analyst suggested that the run time for the two methods should be increased from 30 to 35 min, because late eluting impurities on his system had longer retention times. In addition, retention times generally increased over time. This may be due in part to the fact that his system continuously purges the mobile phase with helium, thus promoting the evaporation of the more volatile components in the mobile phase. This change has been incorporated into the methods.

Table 6
Results for the analysis of ciprofloxacin hydrochloride raw materials by the second analyst

RRT [a]	Sample C1	Sample C2
	Impurities (%)	
Method A		
0.25	tr[b]	
0.26		tr
0.50	0.18	
0.52		0.12
0.59	0.13	
0.60		0.12
0.89	0.29	
Method B		
1.92		0.01
1.93	0.03	
2.07	tr	
2.18		tr
3.21		0.01
3.28	tr	
5.04		tr
5.34	tr	
Total Method A	0.60	0.24
Total Method B	0.03	0.02
Total impurities	0.63	0.26
Assay Results (%)		
Assay (mean of 3)	100.7	101.7
RSD	0.63	0.76

[a] Retention time relative to ciprofloxacin hydrochloride at about 15 min for method A and 2.1 min for method B.
[b] Trace (i.e. below the minimum quantifiable amount).

Acknowledgements

Thanks are expressed to the late H. Beckstead for IR spectra, T.D. Cyr and J.-C. Ethier for mass spectra, and to B.A. Dawson and D. B. Black for NMR spectra. We also thank M. Podnar and D. Long of the Bureau of Biologics for the Karl Fischer moisture determinations.

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