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Short communication

High-performance liquid chromatographic separation and determination of small amounts of process impurities of ciprofloxacin in bulk drugs and formulations☆

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Abstract

A simple and rapid high-performance liquid chromatographic method was developed for the separation and determination of small amounts of process impurities such as chlorofluoroaniline, dichlorofluoroacetophenone, cyclopropylacrylate and quinolinic acid in ciprofloxacin. The separation was achieved on a reversed-phase C_{18} column using water–methanol–acetic acid (84:15.9:0.1, v/v/v) as eluent. The method was used not only for quality assurance but also for process development of ciprofloxacin. The mean recovery of ciprofloxacin from authentic samples was 99.57 ± 1.95% and the limit of detection was $5 \cdot 10^{-9}$ g.

1. Introduction

Ciprofloxacin [1-cyclopropyl-6-fluoro-1,4-dihydro - 4 - oxo - 7 - (1-piperazinyl)quinolone - 3 - carboxylic acid] is a broad-spectrum antibiotic used in the treatment of urinary and respiratory tract infections and also gastrointestinal and sexually transmitted diseases [1,2]. It has been used effectively against Gram-positive and Gram-negative bacteria that are resistent to penicillins, cephalosporins, aminoglycosides, β-lactums and tetracyclines. It is produced synthetically in large amounts by cycloarylation of dichlorofluoroacetophenone followed by condensation with piperazine [3,4]. In this process, intermediates, viz., cyclopropyl acrylate and quinolinic acid, are formed at different stages of the reactions and are ultimately converted into ciprofloxacin. It is likely that the unreacted intermediates may be present in small amounts as impurities in ciprofloxacin and reduce its quality. Therefore, the separation and determination of ciprofloxacin and its process components is of great importance not only for quality assurance but also for process development.

Several high-performance liquid chromatographic (HPLC) methods using fluoroscence or ultraviolet detection have been reported [5–7], but these are mainly applicable to the determination of ciprofloxacin and its metabolites in biological fluids. Microbiological assays have been used extensively but suffer from the interference from active metabolites that are generally present in sample matrices [8]. Flow-injection spectrophotometry and differential-pulse polarography have been tried but are not reproducible [9,10]. A thorough literature search

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revealed that no single analytical method for the separation and determination of potential process impurities in ciprofloxacin has been reported. In this paper, we describe a simple and rapid HPLC method for the separation and determination of small amounts of the principal process components of ciprofloxacin using a reversed-phase C_{18} column and methanol–water–acetic acid (84:15.9:0.1, v/v/v) as the eluent at ambient temperature.

2. Experimental

2.1. Materials and reagents

All reagents were of analytical-reagent grade unless stated otherwise. Glass distilled water, HPLC-grade methanol (Spectrochem, Bombay, India) and acetic acid (Qualigens, Bombay, India) were used. Samples of ciprofloxacin and its intermediates were prepared in our laboratory.

2.2. Apparatus

A high-performance liquid chromatograph (Shimadzu, Kyoto, Japan) with a 20-μl loop injector having a six-way high-pressure valve was used. A Shimadzu SPD 6AV variable-wavelength UV–Vis spectrophotometric detector was connected after the column. A reversed-phase C_{18} (Flexit, Pune, India) column (300 mm × 3.5 mm I.D.; particle size 10 μm) was used for separation. The chromatographic and integrated data were recorded with a Chromatopac C-R3A processing system.

2.3. Chromatographic conditions

The mobile phase was methanol–water–acetic acid (84:15.9:0.1, v/v/v). Samples were dissolved in the mobile phase. The analysis was carried out under isocratic conditions at a flow-rate of 1 ml/min and a chart speed of 5 mm/min at room temperature (27°C). Chromatograms were recorded at 254 nm using a UV detector.

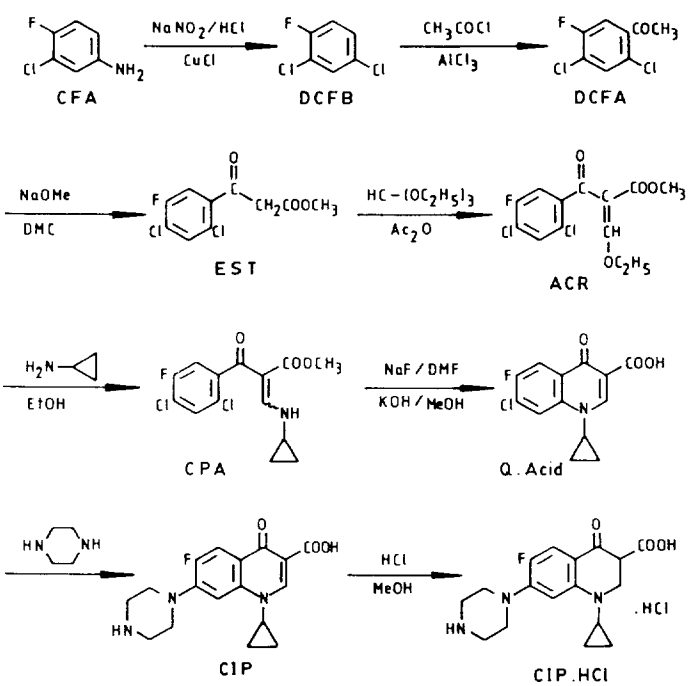

Fig. 1. Reactions involved in the preparation of ciprofloxacin hydrochloride.

2.4. Analytical procedure

Samples (10 mg) were dissolved in the mobile phase (100 ml) and a 20-μl volume of each sample was injected and chromatographed under the above conditions. Synthetic mixtures, bulk drugs and formulations were analysed under identical conditions. The amounts of impurities were calculated from their respective peak areas.

3. Results and discussion

Fig. 1 shows the molecular structures of potential impurities of ciprofloxacin (CIP) produced industrially. The impurities and CIP were subjected to separation by HPLC (Fig. 2). The peaks were identified by injecting the individual

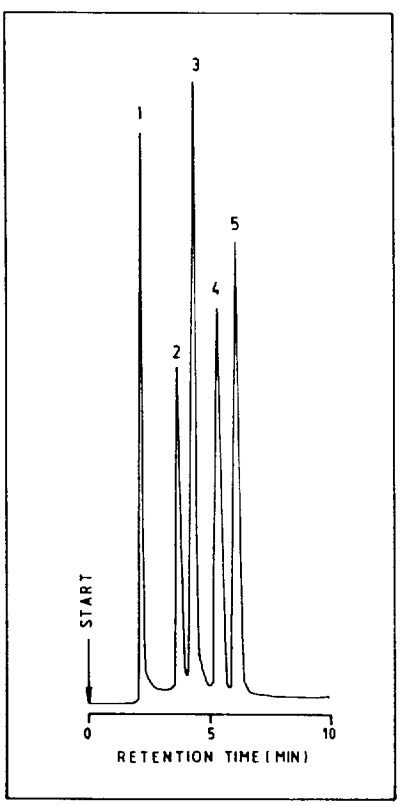

Fig. 2. Typical chromatogram of a mixture containing (1) CIP (21 μg), (2) DCFA (18 μg), (3) Q-acid (21 μg), (4) CPA (20 μg) and (5) CFA (16 μg).

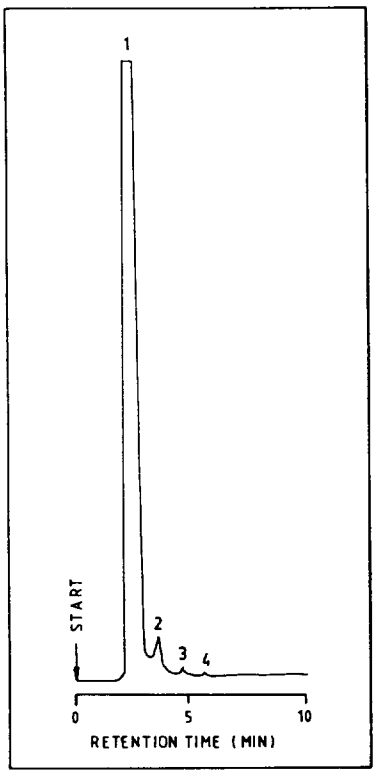

Fig. 3. Chromatogram of a commercial formulation of ciprofloxacin. For identification of peaks, see Fig. 2.

compounds. It can be seen that CIP is well separated from the reactants and intermediates. Aqueous acetic acid (0.1%) was found to improve the separation significantly. Earlier attempts using different columns, i.e. μBondapak C_{18} and Bondclone C_{18}, did not yield the desired separation.

The retention times (t_R) and wavelengths of maximum absorption (λ_{max}) were determined and are given in Table 1. It can be seen that the early elution of CIP ($k' \approx 0.2$) indicates the possibility of overlap of some of the impurities of the process with the CIP band. Therefore, the retention times of all the process impurities were measured systematically and it was confirmed that they do not overlap with the band of CIP. However, the impurity ACR could not be chromatographed as it is unstable and not isolated from the process. The solubility of another impurity, EST, is low in the mobile phase and on

Table 1
Retention data for CIP and potential impurities

Compound	Abbreviation	t_R (min)	λ_{max} (nm)
Ciprofloxacin	CIP	2.25	278
Dichlorofluoro acetophenone	DCFA	3.83	242
Quinolinic acid	Q-acid	4.52	259
Cyclopropyl acrylate	CPA	5.60	308
Chlorofluoroaniline	CFA	6.37	235

examination it was found that at low concentrations it does not interfere with any of the other compounds during the analysis ($t_R = 7.58$ min). The raw material DCFB was also subjected to analysis by HPLC and it was found that it does not overlap with CIP, but interferes with one of the impurities of the process (t_R 3.90 min). DCFB is generally not present in the final product of CIP as it is highly volatile.

The UV detector was set at 254 nm and used for both detection and quantification. Good linearity was found between the mass and integral response for each compound under examination. At 0.001 AUFS the limit of detection for CIP was $5.0 \cdot 10^{-9}$ g with a signal-to-noise ratio of 4.0. The relative response factors for all the compounds are given in Table 2.

Standards containing known amounts of CIP, CPA, CFA, DCFA and Q-acid were prepared and analysed by HPLC. The accuracy of the method was checked by the standard addition technique. Subsequent additions of small amounts were accurately reflected in their peak areas. The measured amounts agreed well (within 1.95%) with the actual values. The results are given in Table 3.

Table 2
Response data

Compound	Concentration range (μg/ml)	Relative response factor	R.S.D. (%)[a]
CIP	950–990	2.33	1.45
DCFA	1–10	1.00	1.97
Q-acid	5–15	7.83	1.08
CPA	3–15	2.34	1.63
CFA	1–10	2.93	1.57

[a] $n = 3$.

Table 3
Analytical data for standard mixtures

Compound	Taken (%)	Found (%)[a]	Error (%)
CIP	95.96	96.09	0.14
DCFA	0.82	0.79	3.65
Q-acid	1.27	1.23	3.14
CPA	1.02	0.99	2.94
CFA	0.93	0.90	3.22

[a] Average of three determinations.

The quality of CIP in several batches of samples obtained commercially was thoroughly checked. The concentrations of various impurities were determined by HPLC and the purity of CIP was calculated. The results are given in Table 4. From these results, it is clear that the method is precise and accurate for the separation and determination of small amounts of process impurities that are present in CIP. The method is suitable not only for process development but also for quality assurance of CIP and related products.

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Table 4
Determination of process impurities in a typical sample of ciprofloxacin

Compound	Concentration (%)	S.D. (%)[a]
DCFA	0.41	1.75
Q-acid	0.26	1.89
CPA	0.03	2.30
CFA	0.02	2.78

[a] $n = 3$.

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