ELSEVIER

Journal of Chromatography A, 697 (1995) 397–405

JOURNAL OF CHROMATOGRAPHY A

Development of an isocratic high-performance liquid chromatographic method for monitoring of ciprofloxacin photodegradation

Kirsti Torniainen[a,*], Eila Mäki[b]

[a]*Turku University Hospital, Hospital Pharmacy, FIN-20520 Turku, Finland*

[b]*Department of Pharmacy, Pharmaceutical Chemistry Division, P.O. Box 15, University of Helsinki, FIN-00014 Helsinki, Finland*

Abstract

An isocratic reversed-phase ion-pair high-performance liquid chromatographic method was developed for monitoring the photodegradation of ciprofloxacin in aqueous solutions. Changes in the chromatographic behaviour of ciprofloxacin and its degradation products under the influence of organic modifiers, anionic and cationic ion-pair reagents and pH were investigated. Baseline separation was achieved with use of an eluent of acetonitrile–phosphoric acid (20 m*M*, pH 2.3) containing 2.5 m*M* 1-heptanesulphonic acid sodium salt. The method was applied to the quantification of ciprofloxacin in irradiated solutions. The calibration graphs were linear from 5 to 150 μg/ml ($r > 0.9999$). Within the same range, the accuracy was 99–102% of the expected values. The intra-assay repeatability of the peak areas and retention times was good (R.S.D. $\leq 0.9\%$). Likewise the intra-day reproducibility of the method was good: R.S.D. values ($n = 6$) were $\leq 2.3\%$ and 1.8% for the lowest and highest concentrations, respectively. The inter-day precision gave a mean R.S.D. of 2.4% for peak areas and R.S.D. of 3.3% for retention times during a working period of three months.

1. Introduction

Ciprofloxacin (1 - cyclopropyl - 6 - fluoro - 1,4-dihydro - 4 - oxo - 7 - (1 - piperazinyl) - 3 - quinolone carboxylic acid) (Fig. 1), a synthetic fluoroquinolone antibiotic with nalidixic acid as progenitor, has a broad spectrum Gram-negative and Gram-positive antibacterial activity [1]. Commercial dosage forms are tablets and infusion fluids containing ciprofloxacin as hydrochloride and lactate salt, respectively.

Ciprofloxacin is a relatively stable molecule, but storage protected from light is recommended [2,3]. A wavelength-dependent loss of antibiotic activity with maximal effect around 320 nm has been observed in irradiated ciprofloxacin solutions [4]. Very few studies have been made on the photochemical stability of fluoroquinolones,

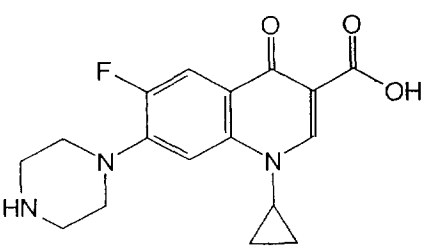

Fig. 1. Structure of ciprofloxacin.

* Corresponding author.

0021-9673/95/$09.50
SSDI 0021-9673(94)00831-0

and most of these have dealt with quinolone drugs in solid state. Ciprofloxacin tablet powder turned yellowish brown after exposure to sunlight for seven days. However, no degradation products were detected by UV, IR or thin-layer chromatography (TLC) [5]. Powdered enoxacin tablets illuminated with fluorescent light at room temperature changed colour, and degradation products were observed [6]. Two pathways have been suggested for the degradation of temafloxacin hydrochloride powder exposed to a high-intensity UV lamp: ring opening followed by cleavage of the piperazine ring, and oxidation of the secondary and tertiary amino groups [7]. Irradiation of ciprofloxacin solutions, with a high-pressure mercury lamp caused degradation of the parent compound, with the product formation depending on the pH of the solutions [8]. In a recent study on the photodegradation of quinolones including ciprofloxacin in UVA-irradiated solutions, Tiefenbacher et al. [9] detected several degradation products by HPLC.

The aim of our study was to develop an isocratic reversed-phase high-performance liquid chromatographic (RP-HPLC) method allowing adequate separation of the degradation products from each other and from ciprofloxacin, and accurate quantification of the parent compound in the presence of its photodegradation products.

2. Experimental

2.1. Chemicals

The identity and purity of ciprofloxacin hydrochloride (Bayer, Leverkusen, Germany) were verified by TLC, by measuring the melting point (Electrothermal digital melting point apparatus, Southend, UK) and by UV and IR spectrometry (Philips PU 8740 UV–Vis spectrometer and Unicam SP3-200 IR spectrometer, both from Pye Unicam, Cambridge, UK).

All chemicals and solvents were of analytical or HPLC grade. Disodium hydrogenphosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$), methanol (MeOH), orthophosphoric acid (H_3PO_4) and acetic acid (CH_3COOH) were purchased from E. Merck (Darmstadt, Germany). Acetonitrile (ACN) was obtained from J.T. Baker (Deventer, Netherlands), tetrahydrofuran (THF) from Rathburn (Walkerburn, UK) and triethylamine (TEA) from Riedel-de Haen (Seelze, Germany). 1-Heptanesulphonic acid sodium salt (Na-HSA) and tetrabutylammonium phosphate (TBAP) were from Sigma (St. Louis, MO, USA). Water was processed with Finn-Aqua H75 Santasalo-Sohlberg (Espoo, Finland).

2.2. Irradiation experiments

Solutions were irradiated with two different high-pressure mercury lamps, TQ 150 and TQ 718, both equipped with a quartz glass cooling mantle (Hanau, Germany). For qualitative experiments, a 1 mg/ml ciprofloxacin solution as hydrochloride salt was prepared in ethanol–0.1 *M* hydrochloric acid (1:1). Aliquots of 2 ml in 10-ml clear glass vials were irradiated for 2 h at a distance of 2 cm from the lamp TQ 150 ($\lambda > 300$ nm).

For quantitative purposes, ciprofloxacin (1 mg/ml) hydrochloride solutions were prepared in 0.001, 0.01 and 0.1 *M* hydrochloric acid and in phosphate buffer pH 3.1. Aliquots of 3 ml were irradiated at ambient temperature in 1 cm I.D. glass cuvettes at a distance of 10 cm from the lamp (TQ 718 at 500 W). A Corning CS-7-54 filter was used to cut off wavelengths longer than 400 nm. During the irradiation the solutions were magnetically stirred (Thermolyne Nuova II stirrer, Dubuque, IA, USA). The lamp was switched off when the samples were taken and restarted after 5 min. At appropriate time intervals, 0.5-ml aliquots of the test solutions were diluted with water to give a concentration of 0.1 mg/ml. Before HPLC runs the samples were filtered through Acrodisc LC 25, Ø 0.22-μm (Gelman, Ann Arbor, MI, USA). The follow up time for the photodegradation was 4 h and all experiments were made in triplicate. A reference sample of ciprofloxacin (1 mg/ml) with the appropriate solvent was prepared for each determination. All the samples were stored in dark after irradiation and after preparation for HPLC runs.

2.3. HPLC systems

The following apparatus from Waters (Milford, MA, USA) was used for the method development: a Model 501 solvent-delivery pump coupled to a 20-μl Rheodyne 7125 manual injector, a Model 484 variable-wavelength UV detector and a Model 741 data module printer. Chromatographic separations were performed at room temperature using a stainless-steel Nova-Pak C_{18} column (4 μm, 15 × 0.39 cm I.D.). The mobile phases (Table 1.) were vacuum filtered with a Waters filtering kit. Helium served for degassing before pumping of the mobile phase at 1.5 ml/min, and the stabilization period after the eluent was changed was 30 min. Detection was at 278 nm. The hold-up time (t_0) for the column was determined with sodium nitrate.

Other Waters equipment was used for the peak purity control: two 501 pumps coupled to an automated gradient controller with the same kind of injector as noted above, a Model 991 diode-array detector with NEC PowerMate 386/25 computer and photodiode array (PDA) software combined with a 5200 printer/plotter. The UV spectra were recorded in the range 210–350 nm. The column was the same as for the first apparatus. The mobile phase for the runs was ACN–phosphoric acid (20 m*M*, pH 2.3) (15:85, v/v) + 2.5 m*M* Na-HSA used at a flow-rate of 1.5 ml/min.

Duplicate injections were used throughout the study.

3. Results and discussion

3.1. Development of the HPLC method

Antimicrobial fluoroquinolones are ampholytic compounds. The pK_a value of the carboxylic group of ciprofloxacin is 6.09 and that of the nitrogen on the piperazinyl ring 8.74 [10]. Near neutral pH, ciprofloxacin is in zwitterionic form and its solubility is at minimum level. The majority of published HPLC applications for ciprofloxacin deal with its separation from other fluoroquinolones [11–13] or its metabolites in body fluids and tissues [14–16]. An HPLC method based on gradient elution with THF–phosphoric acid has been proposed for quantitative determination of ciprofloxacin in irradiated solutions [17]. Gradient elution requires special equipment however, and the total time of analysis may be long due to the need to re-equilibrate the column. With the purpose of developing a simple isocratic method for monitoring of ciprofloxacin photodegradation, we investigated the effect of organic modifiers, ion-pairing agents and pH of the aqueous phase on the retention factor (k) and the peak resolution (R_s).

Effect of organic modifiers

In qualitatively irradiated ciprofloxacin solutions two major degradation products were formed and several minor ones. Method development was started with mixtures of ACN and phosphoric acid (Table 1, eluent 1), which

Table 1
Mobile phases used in the development of an LC method for photodegradation studies on ciprofloxacin

No.	Components	Organic modifier (%, v/v)
1	ACN–H_3PO_4 (20 m*M*, pH 2.3)	10–50
2	MeOH–H_3PO_4 (20 m*M*, pH 2.3)	22–30
3	THF–H_3PO_4 (20 m*M*, pH 2.3)	4–12
4	ACN–H_3PO_4 (20 m*M*, pH 2.3) + Na-HSA (1.0–4.0 m*M*)	15
5	ACN–H_3PO_4 (20 m*M*, pH 2.3) + Na-HSA (2.5 m*M*) + TBAP (0.05–0.25 m*M*)	15
6	THF–H_3PO_4 (20 m*M*, pH 2.3) + Na-HSA (0.125–2.0 m*M*)	6
7	ACN–Na_2HPO_4 (20 m*M*, pH 2.3–7.0, adjusted with H_3PO_4)	15
8	ACN–Na_2HPO_4 (20 m*M*, pH 6.0) + TBAP (0.5–3.0 m*M*)	15

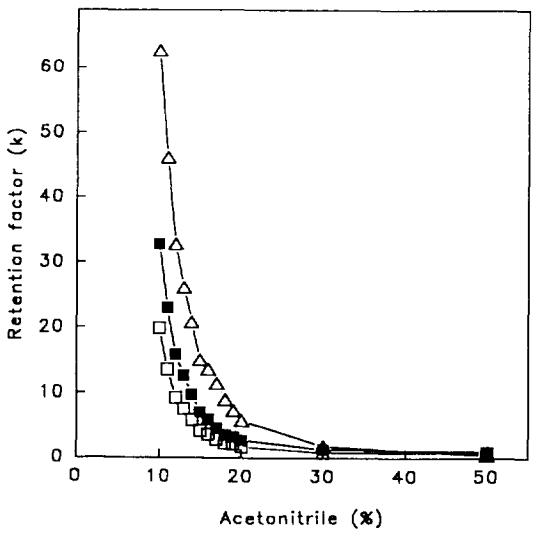

Fig. 2. Effect of the proportion of acetonitrile in the mobile phase (20 m*M* phosphoric acid, pH 2.3) on the retention factor of ciprofloxacin (■), compound I (□) and compound II (△). LC conditions: column, Nova-Pak C_{18} (4 μm, 15 × 0.39 cm I.D.); flow-rate, 1.5 ml/min; detection, UV at 278 nm.

are typical components in HPLC of fluoroquinolones [12–16]. Fig. 2 shows the retention factor of the parent drug and the two major compounds, tentatively named compounds I and II, as a function of the percentage of ACN. When the content of organic modifier was greater than 20%, the compounds eluted partly together with retention time <4.5 min. With less than 12% ACN, the analysis time for compound II was >45 min. Best separation was achieved with ACN and 20 m*M* phosphoric acid (pH 2.3) (15:85, v/v), the retention factors being 4.24, 6.98 and 14.99 for compound I, ciprofloxacin and compound II, respectively. A problem arose in the separation of the medium-sized degradation product Ia, which at all ACN concentrations eluted close to compound I (maximum $R_s = 1.0$) without baseline separation, partly due to the peak tailing. Addition of TEA improved the shape of the first peaks, but the resolution between compounds I and Ia then became worse. Amine modifier had no effect on compound II. Addition of acetic acid, which should reduce the interaction of acidic compounds with the silica surface, had similar effects as TEA on the elution of ciprofloxacin and compounds I and Ia. The retention of compound II was strongly reduced, however, suggesting it to have predominantly an acidic character.

MeOH and THF were tested as organic modifiers in place of ACN (Table 1, eluents 2 and 3). Solvent strength equivalent to 15% ACN was approximated by using the nomograph described by Snyder et al. [18]. MeOH gave the worst resolutions. The retention times were increased, but compounds I and Ia were virtually unseparated and eluted partly together with the parent compound in the MeOH concentration range 22–30% (v/v). THF suppressed the peak tailing and strongly decreased the retention of the first eluting peaks. Compounds I and Ia eluted in reversed order relative to the order in ACN and MeOH, but the resolution remained poor.

Effect of ion-pair reagents

Since ciprofloxacin and presumably also some of its degradation products are amphoteric, we studied the effect of both anionic and cationic ion-pair reagents on the retention. Addition of Na-HSA (Table 1, eluent 4) strongly increased the retention of ciprofloxacin and compounds I and Ia, indicating an ion-pair formation (Fig. 3). The increase was most pronounced for ciprofloxacin, which was now the last eluting compound. Na-HSA had no effect on the retention of compound II. The chromatographic behaviour of compound II suggested that it had lost the basic character of the parent drug and the ability for ion-pair formation with an anionic ion-pair reagent. A baseline separation ($R_s = 1.4$) for the poorly resolved peaks of I and Ia was obtained with ACN–20 m*M* phosphoric acid (pH 2.3) (15:85, v/v) containing 2.5 m*M* Na-HSA (Fig. 4). A cationic ion-pair reagent TBAP (Table 1, eluent 5) added to this eluent exerted a similar deteriorating effect as TEA on the retention and resolution of ciprofloxacin and compounds I and Ia. The retention of compound II was not affected.

In a mobile phase containing THF (Table 1, eluent 6), the addition of Na-HSA had a similar effect as in eluents containing ACN. The re-

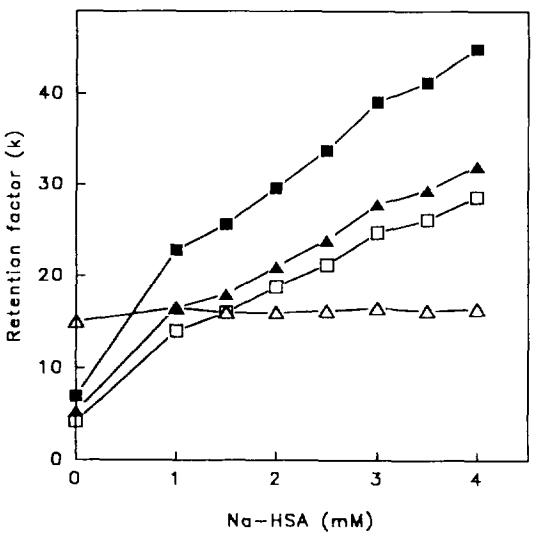

Fig. 3. Effect of the concentration of 1-heptanesulphonic acid sodium salt on the retention factor of ciprofloxacin (■), compound I (□), compound Ia (▲) and compound II (△). LC conditions: mobile phase, acetonitrile–phosphoric acid (20 m*M*, pH 2.3) (15:85, v/v). Other conditions as in Fig. 2.

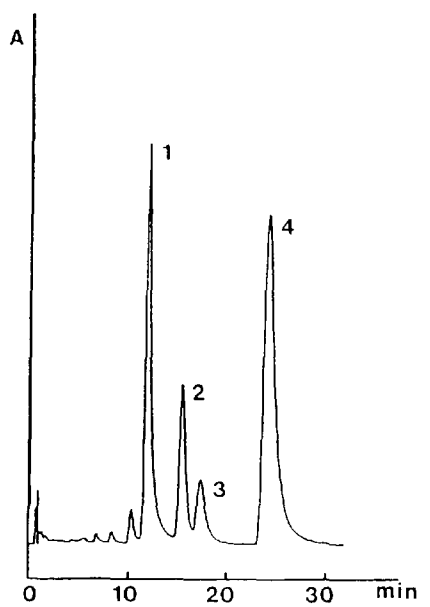

Fig. 4. Chromatogram of a ciprofloxacin hydrochloride solution (ethanol–0.1 *M* hydrochloric acid, 1:1) exposed to Hg lamp TQ 150 for 2 h. LC conditions: mobile phase, acetonitrile–phosphoric acid (20 m*M*, pH 2.3) (15:85, v/v) containing 2.5 m*M* 1-heptanesulphonic acid sodium salt. Other conditions as in Fig. 2. Peaks: 1 = compound II; 2 = compound I; 3 = compound Ia; 4 = ciprofloxacin.

tention of ciprofloxacin and compounds I and Ia increased, while the retention of compound II was unchanged. The peaks were sharp, but satisfactory separation of all compounds was not achieved within a reasonable time. For quantification of ciprofloxacin in irradiated solutions in which compound II was absent, a mixture of THF and 20 m*M* phosphoric acid (pH 2.3) (6:94, v/v) containing 0.25 m*M* Na-HSA provided a good assay method, the retention time of ciprofloxacin being 11.5 min.

Effect of pH

The effect of pH on the retention was tested using a constant proportion of ACN (15%) and phosphate buffer in the range pH 2.3–7 (Table 1, eluent 7). Fig. 5 shows the dependence of the k values on the pH of the aqueous phase. There was a marked decrease in the retention of compound II at pH higher than 5, in agreement with the assumption that compound II has only acidic properties. The ionization of the COOH group should become marked near pH 6 if the pK_a value of the degradation product is similar to that of the parent compound ($pK_a = 6.09$).

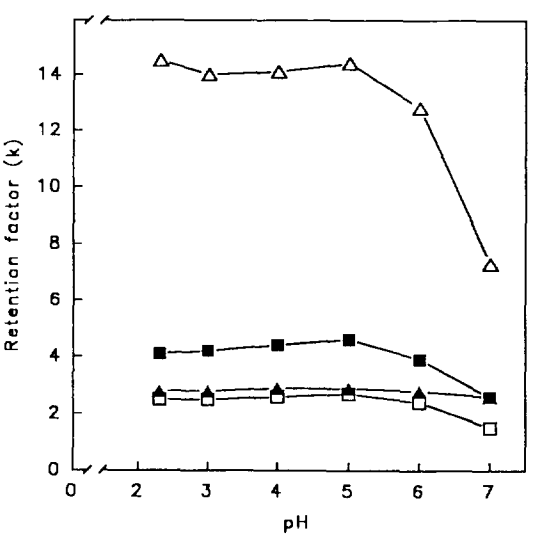

Fig. 5. Effect of the pH of the aqueous phase on the retention factor of ciprofloxacin (■), compound I (□), compound Ia (▲) and compound II (△). LC conditions: mobile phase, acetonitrile–20 m*M* disodium hydrogenphosphate (15:85, v/v). Other conditions as in Fig. 2.

The pH effect was smaller for ciprofloxacin, and also for degradation products I and Ia, which probably have retained their amphoteric character and therefore are in ionized form over a wide pH range. With all mobile phases containing phosphate buffer, the peaks tended to broaden, which further reduced the resolution between amphoteric compounds. Addition of TBAP to the eluent at pH 6 (Table 1, eluent 8), where the carboxyl groups are approximately half ionized, did not improve the separation. A slight increase was observed in the retention of compound II, and a decrease in the retention of the other compounds. Apparently, the TBAP concentrations were too low for a true ion-pair formation.

3.2. Validation of the method

For quantitative analysis of ciprofloxacin, the linearity was tested using two different eluents: ACN–20 m*M* phosphoric acid (pH 2.3) (15:85, v/v) with 2.5 m*M* Na-HSA (eluent A) and THF–20 m*M* phosphoric acid (pH 2.3) (6:94, v/v) with 0.25 m*M* Na-HSA (eluent B). The calibration graphs were constructed by plotting peak areas versus ciprofloxacin concentration, and the linearities were verified on seven (eluent A) and three (eluent B) separate days. The data collected in Table 2 show excellent linearity over the concentration range (5–150 μg/ml) studied.

The equipment with diode-array detector was used to perform the UV spectral analyses and to test the peak purity of ciprofloxacin and its degradation products (Fig. 6). The similarities in spectra of the parent compound and the major degradation products indicated the presence of an unchanged chromophoric system in all compounds. The maximum absorbance of compound II was slightly shifted to lower wavelengths (274 nm) relative to that of the parent compound (278 nm).

The repeatability of the chromatographic systems (eluents A and B) was determined by six replicate injections of solutions containing 5 and 150 μg/ml of ciprofloxacin and six injections of a ciprofloxacin solution that had been irradiated for 2 h (initial concentration 100 μg/ml). At minimum, six replicate samples were used to assess the precision of the whole procedure. Intra-day repeatability was very good, with a mean R.S.D. ≤ 0.9% for both the peak areas and the retention times. Mean R.S.D. values for inter-day determinations were slightly higher: ≤ 1.6% for peak areas and ≤ 3.3% for retention times. The intra-day precision of the whole method was good with R.S.D. values of ≤ 2.3% and 1.8% for concentrations of 5 and 150 μg/ml, respectively. Measurement of the day-to-day precision gave a mean R.S.D. of 2.4% for peak areas and R.S.D. of 3.3% for retention times during a period of three months ($n = 13$, 100 μg/ml, eluent A).

The accuracy of the method was determined with six separate ciprofloxacin solutions at three concentration levels (5, 100 and 150 μg/ml). Samples were diluted using two separate stock solutions (2 and 1.5 mg/ml). The accuracy expressed as percentages of the nominal concentration was 99–102% (R.S.D. ≤ 1.7%, $n = 6$) with eluent A and 102% (R.S.D. = 1.4%, $n = 6$) with eluent B.

Table 2
Calibration graphs for ciprofloxacin

Eluent	Slope (b), $\times 10^7$	Intercept (a), $\times 10^5$	r
ACN–H_3PO_4	9.5235	−1.1813	0.9999
(20 m*M*, pH 2.3)	9.5213	−1.2940	0.9999
+ 2.5 m*M*	9.4410	−1.0127	0.9999
Na-HSA	9.4134	−1.3175	0.9999
	9.4647	−1.3573	0.9999
	9.2068	−0.8482	0.9999
	9.2795	−0.3819	0.9999
THF–H_3PO_4	8.8449	0.4793	0.9999
(20 m*M*, pH 2.3)	8.7661	0.5061	0.9999
+ 0.25 m*M*	8.7595	0.5106	0.9999
Na-HSA	8.7823	0.4007	0.9999
	8.7801	0.4345	0.9999
	8.7727	0.3865	0.9999
	9.1240	−0.0554	0.9999

Ciprofloxacin concentration range 5–150 μg/ml. $y = bx + a$, where x = concentration of the injected substance (μg/ml) and y = peak area of ciprofloxacin. r = correlation coefficient.

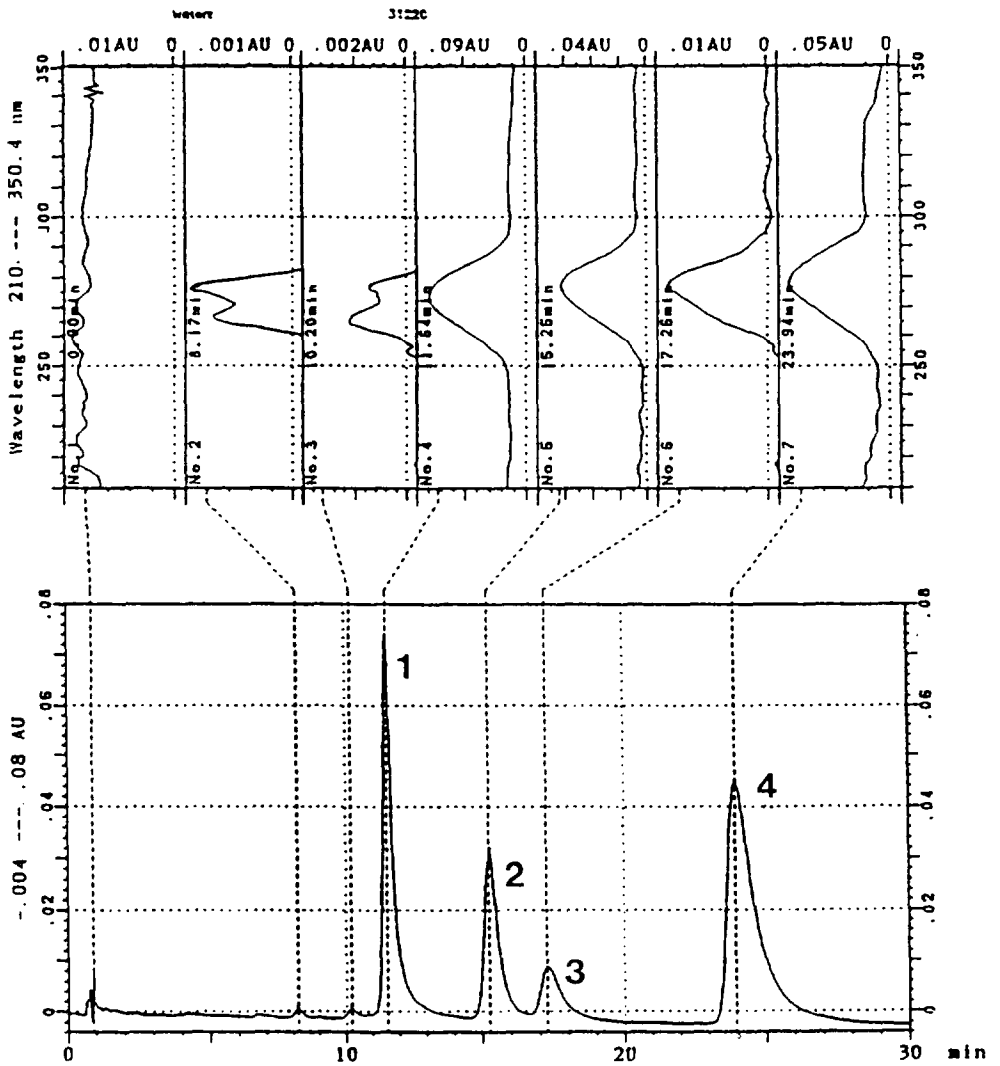

Fig. 6. Chromatogram of a ciprofloxacin hydrochloride solution (ethanol–0.1 *M* hydrochloric acid, 1:1) exposed to Hg lamp TQ 150 for 2 h and UV spectra of ciprofloxacin and its degradation products. LC conditions: photodiode array detector. Other conditions and peak identification as in Fig. 4.

3.3. Application to quantification

The applicability of the method (eluent A) for the quantification of ciprofloxacin was demonstrated by following the disappearance of the drug in solutions irradiated with a high-pressure mercury lamp (TQ 718). The degradation proceeded fastest in 0.1 *M* hydrochloric acid (pH 1.2). After exposure of the solutions for 4 h, about 40% of the initial ciprofloxacin content was left (Fig. 7). Compound II was clearly formed only in this solution. The reaction rate decreased with decreasing concentration of hydrochloric acid. During an irradiation period of 4 h, the ciprofloxacin content decreased about 20 and 10% from the initial value in 0.01 *M* (pH 2.2) and 0.001 *M* (pH 3.1) hydrochloric acid, respectively. It is noteworthy that the degradation proceeded at approximately the same rate in phosphate buffer of pH 3.1 as in 0.01 *M*

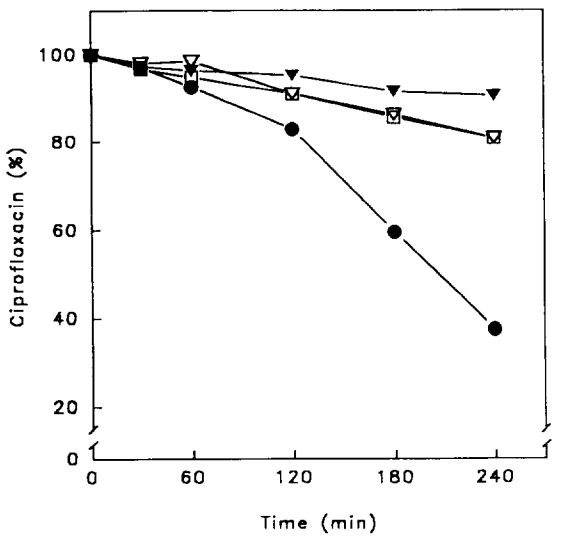

Fig. 7. Time course for the photodegradation of ciprofloxacin hydrochloride in 0.001 *M* hydrochloric acid (pH 3.1) (▼), 0.01 *M* hydrochloric acid (pH 2.2) (▽), 0.1 *M* hydrochloric acid (pH 1.2) (●) and phosphate buffer (pH 3.1) (□). Radiation source: Hg lamp TQ 718.

hydrochloric acid. The possible accelerating effect of buffer species on the reaction remains to be studied. The radiation source had apparently no influence on the product formation. The same photodegradation products were formed in solutions artificially irradiated and exposed to normal daylight. No degradation of ciprofloxacin was observed in solutions kept in the dark, indicating that the reaction was light-induced.

For comparison, the ciprofloxacin concentrations in a photodegraded solution prepared in 0.1 *M* hydrochloric acid were determined by high-performance TLC (HPTLC) with subsequent densitometric measurement [19]. The two methods correlated well. A straight line with a slope 1.05 ($r = 0.998$, $n = 10$) was obtained when ciprofloxacin concentrations (percentages from the initial content) obtained in HPLC were plotted against those obtained in HPTLC.

4. Conclusions

An isocratic HPLC method was developed for monitoring the photodegradation of ciprofloxacin in aqueous solutions. The procedure enables investigations with the kind of basic equipment available in hospital pharmacies, where stability studies of drugs are essential.

Ciprofloxacin and some of its photodegradation products showed very similar chromatographic behaviour. To obtain a baseline separation for the parent compound and its major degradation products in an isocratic run, some peak tailing and a relatively high retention factor of ciprofloxacin ($k = 33.8$) had to be accepted. The method was accurate and precise, and suitable for further studies on the photodegradation kinetics of ciprofloxacin.

Acknowledgement

K.T. is grateful for financial support received from the Finnish Culture Foundation, which made this work possible.

References

[1] S. Norris and G.L. Mandell, in V.T. Andriole (Editor), *The Quinolones*, Academic Press, London, 1988, p. 4.
[2] *United States Pharmacopeia/National Formulary (USP XXII/NFXVII)*, Suppl. 4, USP Convention, Rockville, MD, 1991, p. 2458.
[3] J.E.F. Reynolds (Editor), *Martindale: The Extra Pharmacopoeia*, Pharmaceutical Press, London, 30th ed., 1993, p. 145.
[4] G. Phillips, B.E. Johnson and J. Ferguson, *J. Antimicrob. Chemother.*, 26 (1990) 783.
[5] Y. Hiraki, T. Nomaguchi, T. Kawazoe, Y. Kumamoto, T. Shinohara, K. Kusaka and Y. Syouji, *J. Jpn. Soc. Hosp. Pharm.*, 25 (1989) 1375; *C.A.*, 112 (1989) 42442g.
[6] N. Mizushima, F. Ohuchi and T. Fujii, *Byoin Yakugaku*, 16 (1990) 198; *C.A.*, 114 (1990) 12063h.
[7] L. Elrod, C.L. Linton, B.P. Shelat and C.F. Wong, *J. Chromatogr.*, 519 (1990) 125.
[8] S. Tammilehto, H. Salomies and K. Torniainen, *Abstracts of the XII Helsinki University Course in Drug Research, 17–18 June 1993*, Finnish Pharmaceutical Society, Helsinki, 1993, p. 33.
[9] E.-M. Tiefenbacher, E. Haen, B. Pryzbilla and H. Kurz, *J. Pharm. Sci.*, 83 (1994) 463.
[10] D.L. Ross and C.M. Riley, *Int. J. Pharm.*, 63 (1990) 237.

[11] Zs. Budvari-Barany, Gy. Szasz, K. Takacs-Novak, I. Hermecz and A. Lore, *J. Liq. Chromatogr.*, 14 (1991) 3411.
[12] E. Brunt, J. Limberg and H. Derendorf, *J. Pharm. Biomed. Anal.*, 8 (1990) 67.
[13] K. Tyczkowska, K.M. Hedeen, D.P. Aucoin and A.L. Aronson, *J. Chromatogr.*, 493 (1989) 337.
[14] W.M. Awni, J. Clarkson and D.R.P. Guay, *J. Chromatogr.*, 419 (1987) 414.
[15] C.M. Myers and J.L. Blumer, *J. Chromatogr.*, 422 (1987) 153.
[16] F. Kees, W. Raasch and H. Grobecker, *Arzneim. Forsch.*, 42 (1992) 570.
[17] K. Linden and S. Tammilehto, *Abstracts of the XII Helsinki University Course in Drug Research, 17–18 June 1993*, Finnish Pharmaceutical Society, Helsinki, 1993, p. 34.
[18] L.R. Snyder, J.L. Glajch and J.J. Kirkland, *Practical HPLC Method Development*, Wiley, New York, 1988, p. 32.
[19] S. Tammilehto, H. Salomies and K. Torniainen, *J. Planar Chromatogr.*, in press.