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Short communication

Quantitative determination of gatifloxacin, levofloxacin, lomefloxacin and pefloxacin fluoroquinolonic antibiotics in pharmaceutical preparations by high-performance liquid chromatography

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Abstract

The objective of this research was to develop and validate analytical methods for quantitative determination of fluoroquinolones of third generation. Simple and rapid chromatographic method was developed and validated for quantitative determination of four quinolone antibiotics in tablets and injection preparations. The fluoroquinolones studied were gatifloxacin (GAT), levofloxacin (LEV), lomefloxacin (LOM) and pefloxacin (PEF). The quinolones were analyzed by using a LiChrospher® 100 RP-18 column (5 μm, 125 mm × 4 mm) and a mobile phase constituted of water:acetonitrile (80:20, v/v) with 0.3% of triethylamine and pH adjusted to 3.3 with phosphoric acid. The flow rate was 1.0 mL/min and the analyses were performed using UV detector with wavelengths varying from 279 to 295 nm. The analyses were performed at room temperature (24 ± 2 °C). All fluoroquinolones were separated within 5 min. The calibration curves were linear ($r \geq 0.9999$) over a concentration range from 4.0 to 24.0 μg/mL. The relative standard deviation (R.S.D.) was <1.0% and average recovery was above 99.54%.

Keywords: Fluoroquinolones; HPLC; Pharmaceutical preparations

1. Introduction

The quinolones comprise a series of broad-spectrum synthetic antibacterial agents derived from nalidixic acid. They were discovered casually in 1962 and since then are essentially used in the treatment of wide range of infectious diseases [1–4,7].

The fluoroquinolones are quinolones with fluorine at position 6 of naphthyridine ring. The chemical structures of fluoroquinolones are shown in Fig. 1. Published structure–activity data show that fluorine atom help broadens their activity spectrum against both Gram-negative and Gram-positive pathogens [2–4,5–7].

Several analytical methods for quantitative determination of fluoroquinolones in pharmaceutical formulations are described in scientific literature, like capillary electrophoresis [9–11], UV spectrophotometry [12,13], titrimetry [14,15] and high-performance liquid chromatography (HPLC) [6,8,15–17] amongst others.

Majority of these HPLC methods were applied in determination of fluoroquinolones in biological fluids, edible animal products, feeds and to a lesser extent, in pharmaceutical formulations. Most of the reported methods involve troublesome mobile phase (buffers) and difficult detection methods (fluorescence or mass detectors) [6,8,15–17]. The objective of this research was to develop and validate rapid, economical and sensitive method for quantitative determination of four fluoroquinolones: gatifloxacin (GAT), levofloxacin (LEV), lomefloxacin (LOM) and pefloxacin (PEF) in tablets and injectable preparations. The major advantage of the proposed methods is that four flourquinolones can be determined on a single chromatographic system with minor modification in detection wavelengths.

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2. Experimental

2.1. Material

2.1.1. Reagents and chemicals

All solvents were of HPLC grade and all reagents were analytical grade. Acetonitrile and phosphoric acid were obtained from Merck®. Analytical grade triethylamine was purchased from Sigma®. Water was purified with Milli-Q® Plus, Millipore System. All solvents and solutions were filtered through membrane filter or filtration units (Millipore® Millex-HV filter units, Durapore-PVDF, polyethylene, 0.45 μm pore size) and degassed before use.

Raw material GAT (99.6%), LEV (100.0%), LOM (100.2%) and PEF (100.6%) were kindly donated by local pharmaceutical industries and were used as reference standards without further purification.

2.1.2. Samples

The samples used in this research are presented in Table 1.

2.1.3. Instrumentation

HPLC system: High-pressure liquid chromatograph model Shimadzu® LC-9A, equipped with UV–vis detector model SPP-6A, controlling system SCL-6B, connected to microcomputer with "Chemstation" Shimadzu® Class LC-10 Version 1:62 is used for integration and processing of chromatograms.

Column: The analytical column was a reversed phase LiChrospher® 100 RP-18 (125 mm × 4 mm, 5 μm) (Merck®).

2.2. Methods

2.2.1. Chromatographic conditions

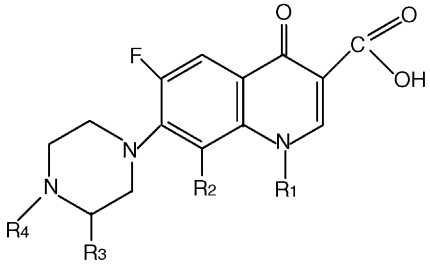
All analysis were done at ambient temperature (24 ± 2 °C) under isocratic conditions. The mobile phase consisted of a mixture of water:acetonitrile (80:20, v/v) with 0.3% of triethylamine and pH adjusted to 3.3 with phosphoric acid. The flow rate was 1.0 mL/min and volume of injection was 20 μL. All solutions, including mobile phase, were sonicated during 25 min before use. The UV detection was made at 293 nm for GAT, at 295 nm for LEV, at 288 nm for LOM and at 279 nm for PEF.

2.2.2. Standard solutions preparation

Accurately weighed amounts of standards of GAT, LEV, LOM and PEF equivalent to 20.0 mg of free base were transferred to 100 mL volumetric flask, separately. The volumes were completed with mobile phase. The resulting solutions were sonicated during 25 min and filtered through membrane filter. Final concentrations were 200.0 μg/mL. Aliquots of each solution were accordingly diluted with mobile phase in order to obtain solutions with final concentration of 50.0 μg/mL. All solutions were prepared fresh each day.

2.2.3. Calibration curves

Six different concentration levels (4.0, 8.0, 12.0, 16.0, 20.0 and 24.0 μg/mL) were obtained of each standard solution, conveniently diluted with mobile phase. Each solution was injected in the chromatographic system ($n = 3$) and mean values of peak areas were plotted against concentrations. The



General structure of fluoroquinolones

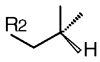
Drugs	R1	R2	R3	R4
Gatifloxacin		OCH3	CH3	N
Levofloxacin		O—	---	CH3
Lomefloxacin	C2H5	F	CH3	N
Pefloxacin	C2H5	---	---	CH3

Fig. 1. Chemical structures of gatifloxacin, levofloxacin, lomefloxacin and pefloxacin.

Table 1
Commercial pharmaceutical dosage form of fluoroquinolonic antibiotics used as samples in the research

Sample number	Pharmaceutical industries[a]	Drug	Pharmaceutical dosage form
1	A	Gatifloxacin (freebase)	Tablets (400 mg)
2	B	Levofloxacin (freebase)	Tablets (250 mg)
3	B	Levofloxacin (freebase)	Injection 100 mL (5 mg/mL)
4	C	Lomefloxacin hydrochloride	Tablets (400 mg)
5	D	Lomefloxacin hydrochloride	Tablets (400 mg)
6	E	Pefloxacin mesilate	Tablets (400 mg)
7	E	Pefloxacin mesilate	Ampoule 5 mL (80 mg/mL)

[a] Pharmaceutical industries are identified by letters and samples by numbers.

curves were adjusted by linear regression with least mean squares method [18,19].

2.2.4. Linearity

The linearity of the proposed method was evaluated by using calibration curves to calculate coefficient of correlation and intercept values.

2.2.5. Sample preparation

2.2.5.1. Tablets. Twenty tablets of each sample were individually weighed and triturated to obtain homogeneous mixture. An amount of powder equivalent to 100.0 mg of freebase was transferred to 100 mL volumetric flask. The volumes were completed with mobile phase. The resulting solutions were sonicated during 25 min to facilitate proper solubilization. Aliquots of each solution were accordingly diluted with mobile phase, in order to obtain solutions with final concentration of 50.0 μg/mL. All sample and standard solutions were filtered through Millex-HV® filter unit before injection into the system.

2.2.5.2. Ampoules and injection. A pool of three ampoules of PEF and 5.0 mL of injection formulation were used for sample solution preparation. The procedure adopted for the preparation of injectable samples (ampoules and injection) was similar to that described for tablets. Appropriate dilutions were made with mobile phase to final solutions containing 10.0 μg/mL of each drug as freebase.

2.2.6. Selectivity and specificity

The selectivity and specificity of proposed method was evaluated through possible interference due to excipients presented in the pharmaceutical formulations. For that, placebo of each tablet sample was prepared by mixing respective excipients and solutions were prepared following procedure described in Section 2.2.5.1. Following excipients were used in the preparation of placebo (without active substance): starch, monohydrated lactose, carboxymethylcellulose, magnesium stearate, hydroxypropylcellulose, polyoxyl stearate, hydroxypropylmethylcellulose, polyethylenglycol 400, aerosol, croscarmelose sodium, dicalcium phosphate and neutral talc.

2.2.7. Accuracy

To evaluate the accuracy of the proposed method, recovery tests were carried out with all samples. Recovery tests were performed by adding known amounts of standard solutions to sample followed by analysis using proposed method. Aliquots of standard and samples solutions were transferred to 25 mL volumetric flasks and final volumes were completed with mobile phase. The percentage of recovery (*R*%) was calculated as indicated by Association of Official Analytical Chemists International [17,20].

2.2.8. Precision

The precision of proposed method was evaluated through intra-day repeatability of responses of sample solutions presented in Table 1. All solutions were prepared fresh and responses were determined after replicate ($n = 10$) injection of sample solutions (5.0 μg/mL). The precision is expressed as relative standard deviation (R.S.D.) amongst responses in each case.

2.2.9. Robustness

The robustness was evaluated by intentional minor modifications in the composition of the mobile phase used in the proposed method.

2.2.10. Detection limit (DL) and quantification limit (QL)

The DL and QL were calculated using Eqs. (1) and (2), respectively. The theoretically determined values of detection and quantitation limits were crossed checked by actual analysis of these concentrations using proposed methods:

$$\mathrm{DL} = \frac{\mathrm{S.D.}}{\alpha} \times 3 \quad (1)$$

$$\mathrm{QL} = \frac{\mathrm{S.D.}}{\alpha} \times 10 \quad (2)$$

where S.D. is the standard deviation of curve and α is the slope of curve.

3. Results and discussion

In order to validate an efficient method for analysis of drug in pharmaceutical formulations, preliminary tests were per-

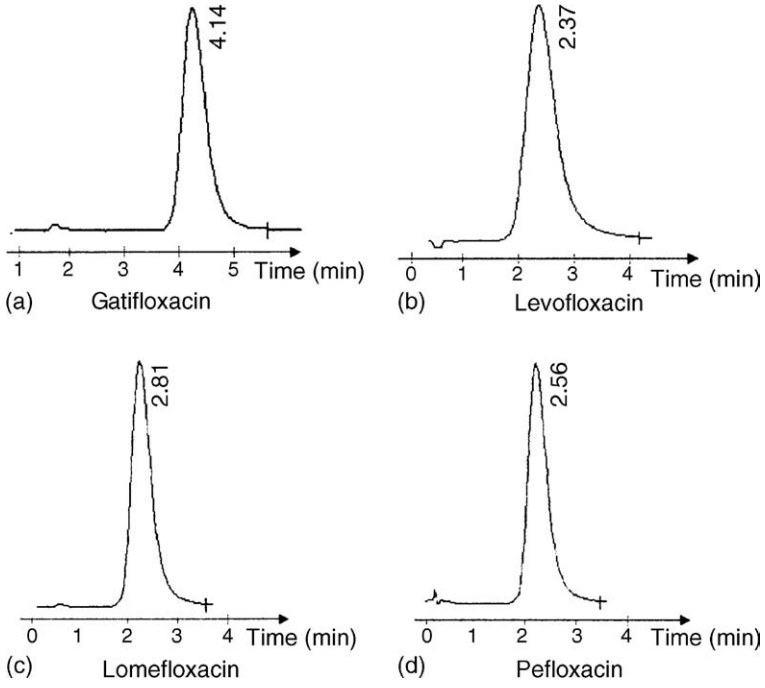


Fig. 2. Representative chromatograms of samples: (a) gatifloxacin; (b) levofloxacin; (c) lomefloxacin; (d) pefloxacin (15.0 μg/mL). *Conditions:* LiChrospher® RP-18 column (125 mm × 4 mm, 5 μm), mobile phase, H_2O:MeCN:TEA (80:20:0.3, v/v/v); pH adjusted to 3.3 with phosphoric acid; flow rate, 1.0 mL/min; UV detection of GAT, LEV, LOM and PEF at 293, 295, 288 and 279 nm, respectively, and ambient temperature (24 ± 2 °C).

Table 2
Statistical results of linear regression analysis in the determination of GAT, LEV, LOM and PEF by proposed method

Statistical parameters	GAT	LEV	LOM	PEF
Slope of curve	101108	103072	103196	62631
Intercept of curve	5882	11799	2406	2931
Standard error of estimate (S_e)	5882	9636	6261	3032
Linear correlation coefficient (r)	0.9999	0.9999	0.9999	0.9999

$n = 3$.

formed with the objective to select adequate and optimum conditions. Parameters, such as detection wavelength, ideal mobile phase and their proportions, optimum pH and concentration of the standard solutions were exhaustively studied.

Several binary or ternary eluents were tested using different proportions of solvents, such as acetonitrile, methanol and water. Some ion-pair reagents were also studied. A flow rate of 1.0 mL/min was selected after preliminary tests.

The proposed method is simple and do not involve laborious time-consuming sample preparation. The method was statistically evaluated for their accuracy and precision.

The chromatograms of sample containing GAT, LEV, LOM and PEF can be observed in Fig. 2.

All calibration curves showed linearity over a concentration range from 4.0 to 24.0 μg/mL. The correlation coefficients obtained with linear regression of curve were above 0.9999. Linearity data show concentration interval of studied fluoroquinolones in which the intensity of the detector response is proportional to the concentration of the analyzed substance. The DL and QL of GAT, LEV, LOM and PEF were 0.13, 0.39; 0.15, 0.46; 0.17, 0.52 and 0.08, 0.25 μg/mL, respectively. The theoretically determined values of quantitation limits were crossed checked by actual analysis of these concentrations using proposed methods. The consequent R.S.D. amongst these values are within acceptable

Table 3
Statistical data obtained in the analysis of samples using the proposed method

Sample number	Declared theoretical concentration (μg/mL)	Found experimental concentration (μg/mL)[a]	R.S.D. (%)	Content (%) confidence interval[b]
1	15.00	15.88	0.17	105.89 ± 0.02
2	15.00	15.30	0.65	101.99 ± 0.07
3	15.00	15.15	0.56	100.98 ± 0.06
4	15.00	14.68	0.37	97.85 ± 0.04
5	15.00	14.82	0.65	98.80 ± 0.07
6	15.00	14.91	0.51	99.37 ± 0.05
7	15.00	14.06	0.92	93.75 ± 0.09

[a] Average of 10 determinations.
[b] 95.0% confidence level.

Table 4
Recovery data of standard solutions added to the samples analyzed by using the proposed HPLC method

Sample number	Added amount (μg/mL)	Found amount[a] (μg/mL)	Recovery (%)	
			Result	Average
1	12.00	12.33	99.15	99.68
	14.00	14.40	100.22	
	16.00	16.35	99.68	
2	12.00	12.06	97.37	98.29
	14.00	14.19	99.60	
	16.00	16.01	97.89	
3	12.00	11.95	96.86	99.25
	14.00	14.20	100.81	
	16.00	16.14	100.08	
4	12.00	11.86	98.52	98.90
	14.00	13.89	99.34	
	16.00	15.83	98.85	
5	12.00	11.81	98.65	99.15
	14.00	13.83	99.22	
	16.00	15.85	99.57	
6	12.00	11.86	100.20	99.70
	14.00	13.83	99.27	
	16.00	15.76	99.13	
7	12.00	11.79	103.57	101.87
	14.00	13.71	101.67	
	16.00	15.61	100.36	

[a] Average of three determinations.

limits (<2%). Table 2 shows the statistical treated linear regression data of GAT, LEV, LOM and PEF.

The standard deviation amongst replicate responses and relative standard deviation values were less than 1.0%, indicating precision of the method. The statistical data results obtained in the analysis of commercially available samples are shown in Table 3. The recovery values obtained were between 96.86 and 103.57%, confirming accuracy of proposed method. The percentage of recovery results is presented in Table 4. The excipients present in pharmaceutical dosage forms do not interfere in the analysis. The results prove specificity of the proposed methods. However, close retention times of drugs do not permits concomitant and selective determination of these fluorquinolones.

When mobile phase components were intentionally altered as much as 5 mL each, practically, no effect was observed in the chromatogram. There was minor delay in the retention time when pH of the mobile phase varies in order of 0.5 units. These observations confirm the robustness of the method.

4. Conclusion

The proposed HPLC method enables quantitative determination of third generation fluoroquinolones, such as GAT, LEV, LOM and PEF in pharmaceutical formulations. Preparation of samples is easy and efficient. UV detection at 279, 288, 293 and 295 nm were found to be suitable without any interference from tablets or injectable solution excipients or solvents. All calibration curves were found to be linear with correlation coefficients above 0.9999. The R.S.D.s were less than 1.0%. Analytical results of samples were in accordance with those of standard solution in the same concentrations. The proposed HPLC method is fast, precise, accurate, sensitive and efficient and can be used in routine analysis in quality control laboratories.

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