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Application of RP-HPLC method for the simultaneous determination of cetirizine in the presence of quinolones

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Abstract

Background: Present work describes a fast, simple, and sensitive procedure for the simultaneous determination of cetirizine in the presence of quinolones using diclofenac sodium as an internal standard. The present work was designed to analyze these compounds in pharmaceutical and clinical labs being economical for use.

Results: The mobile phase consisted of the simple composition of methanol, acetonitrile, and water in a ratio of 50: 20:30 with a pH adjusted to 3.1 at a flow rate of 1 mL min^{-1}. The UV detection was performed at 225 nm. The linearity was assessed over the range of 2.5–50 μg mL^{-1} for all drugs. The parameters such as accuracy, precision, linearity (>0.999), and sensitivity were satisfactory.

Conclusion: The method was equally applicable for formulation and human serum with recovery values between 95 and 105%. The results of the method were validated statistically according to ICH guidelines.

Keywords: Quinolones, Cetirizine, HPLC method, UV detection

Background

Cetirizine (Fig. 1), an H1-receptor antagonist, is co-administered with quinolones in several cases; however, drug-induced urticaria has been reported with a wide range of drugs and vaccines. NSAIDs and antibiotics are most commonly associated with urticaria, although reliable data from prospectively controlled studies are scarce [1, 2]. Quinolone antibiotics induce nonspecific histamine release [3]. Several reports of anaphylactic reactions with ciprofloxacin, many with a first known exposure, strongly suggest non-immune-mediated histamine release [4]. It has also been reported that most patients with multiple drug allergy syndrome and more than one-third of subjects with a history of hypersensitivity to a single antibacterial drug were characterized by circulating histamine-releasing factors, which might play a role in drug-induced adverse reactions observed in these patients [5].

Moreover, the administration of several drugs (six H_1-receptor blockers, seven beta-adrenergic antagonists, four analgesics, ten diuretics, and five quinolones) which modulate the function of P-glycoprotein to patients may adversely affect the natural process of this efflux pump. It may cause drug-drug interactions induced side effects [6]. One study confirmed the potential fertility hazards of commonly used drugs such as H_1 receptor antagonists, antiepileptics, and antibiotics [7]. Patients taking ciprofloxacin are usually advised to protect their skin from direct sunlight [8]. From these clinical manifestations, it became apparent to develop a method wherein simultaneous quantification of cetirizine in the presence of quinolones could be accomplished.

On this basis, it became apparent to develop and validate for the first time a method for simultaneous determination of cetirizine in the presence of quinolones. Many analytical methods of these drugs were reported. Marika et al. [9] developed a simple, sensitive RP-HPLC

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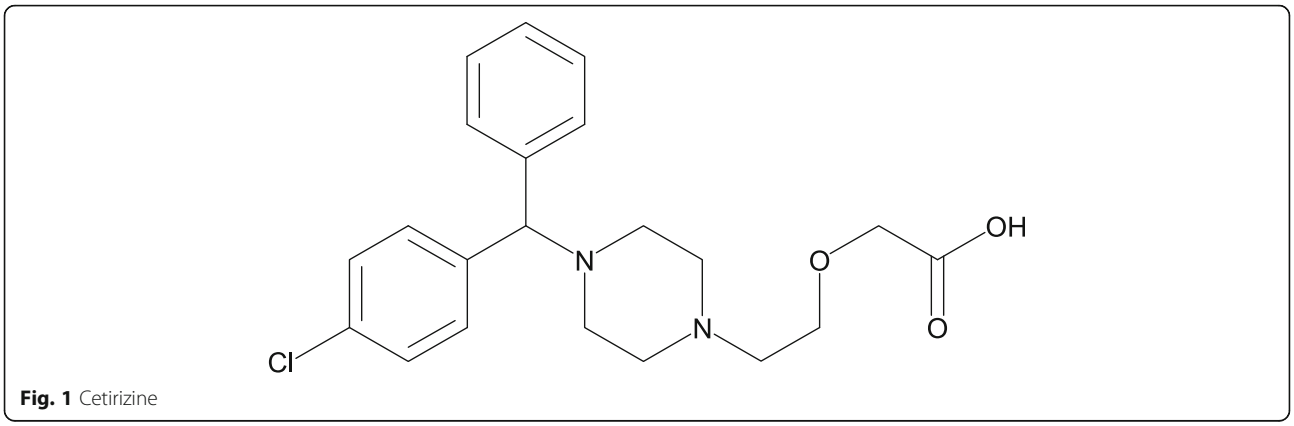

Fig. 1 Cetirizine

isocratic method for quantifying ciprofloxacin in plasma and urine with ultraviolet detection. The quantification limit was 0.01 mg mL^{-1} in plasma and 0.5 mg L^{-1} in the urine. This method was sufficiently sensitive for pharmacokinetic studies. Amini et al. [10] developed an isocratic method for the ofloxacin analysis and the determination of pharmacokinetics and dose control in veterinary and humans. Hermann et al. [11] developed a method for the determination of norfloxacin in human plasma and urine and was used to support human pharmacokinetic studies. Branislava et al. [12] developed a gradient RP-HPLC method to detect norfloxacin, and its major impurities in pharmaceuticals with the quantitation limit were found 0.12–0.47 μg mL^{-1}. Hérida et al. [13] developed an HPLC method for the assay of gatifloxacin in raw materials and tablets. Brian et al. [14] developed and validated HPLC method for determining gatifloxacin concentrations in serum and urine at 293 nm. A simultaneous HPLC method of four quinolones using multi-wavelength calibration was also reported [15].

Similarly, the number of HPLC methods has been reported for the determination of cetirizine in the presence of several other drugs as pseudoephedrine [16], chloroquine and pyrimethamine [17], hydroxyzine [18], H_2 receptor antagonists [19], statins [20], with different antihistamine [21], calcium-channel blockers [22], and NSAIDs [23]. However, no method for the simultaneous determination of cetirizine in the presence of quinolones was reported. Hence in the present work, a method was developed and validated.

Table 1 Chromatographic conditions

Parameter	Optimized conditions
Mobile phase	Methanol to acetonitrile to water 50:20:30
pH	3.1
Wavelength	225 nm (isosbestic point)
Temperature	25± °C

Method

Materials

Pure sample

The pharmaceutical grade (purity, ≥97%) of all the actives was obtained from different local pharmaceutical industries.

Formulations

The tablets of all actives were purchased from the pharmacy.

Chemicals and reagents

HPLC grade methanol was purchased from Merck Germany. Orthophosphoric acid and other chemicals used were of analytical grade.

Instrumentation

Shimadzu HPLC system equipped with LC-10 AT *VP* pump, SPD-10A *VP* UV–*vis* detector utilizing a Purospher® STAR RP-18 end-capped (5 μm, 25 × 0.46 cm) column was used. The integrated chromatographic data were recorded using Shimadzu Class-GC 10 software (version 2) for data acquisition and mathematical calculations.

Mobile phase preparations

The mobile phase consisted of a mixture of methanol, acetonitrile, and water in a ratio of 50:20:30 with a pH adjusted to 3.1 using orthophosphoric acid.

Table 2 Regression statistics and sensitivity of the method

Drugs	r^2	LOQ μg mL^{-1}	LOD μg mL^{-1}
Ofloxacin	0.999	0.110	0.03
Levofloxacin	0.999	0.270	0.08
Ciprofloxacin	0.995	0.140	0.04
Enoxacin	0.999	0.001	0.0003
Sparfloxacin	0.999	0.860	0.26
Norfloxacin	0.999	0.160	0.04
Cetirizine	0.999	0.035	0.01

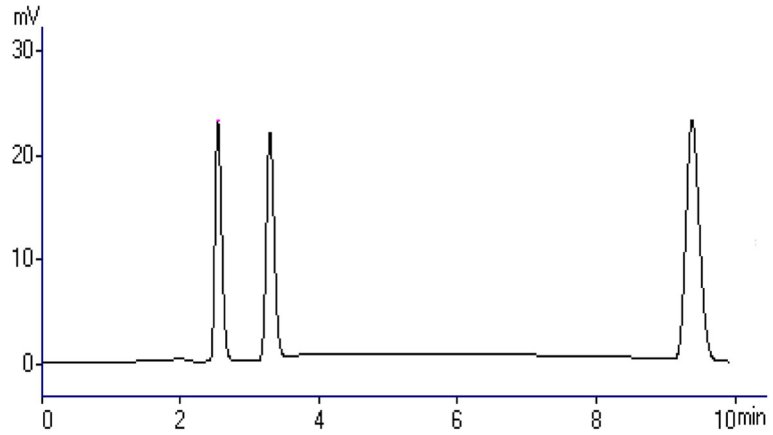

Fig. 2 Representative chromatogram of quinolone, cetirizine, and internal standard

Preparation of solutions

Solutions of cetirizine and quinolones (ofloxacin, levofloxacin, ciprofloxacin, enoxacin, sparfloxacin, and norfloxacin) were prepared by accurately weighing 10 mg of standards and transferred to 100-mL volumetric flask, separately, and volumes were completed with the unbuffered mobile phase. The resulting solutions of 100 μg mL^{-1} were sonicated and then filtered. From the stock solutions, different dilutions, i.e., 2.5, 5, 15, 20, 25, and 50 mL, were pipetted out in different 100-mL volumetric flasks and made up to the mark with the same solvent to have the required concentrations of 2.5, 5, 15, 20, 25, and 50, respectively.

Linearity studies

Linearity studies were performed by preparing a solution at six different concentration levels, i.e., 2.5, 5, 15, 20, 25, and 50 μg mL^{-1} for all the drugs assayed. The standard calibration curves were evaluated by linear regression.

Precision

The method's precision was performed by injecting four representative samples on each of the 2 days (n=18).

Accuracy

The method's accuracy was performed at three different concentrations, i.e., 8, 10, 12 μg mL^{-1} (n=6), by adding known quantities of the analyte to the drug product.

Procedure for formulations

Twenty tablets of each drug were individually weighed and triturated to obtain a homogeneous mixture. Amount of powder equivalent to 10 mg of drug was transferred to a 100-mL volumetric flask. The flask's content was shaken, and volumes were completed with the mobile phase. This solution was filtered and diluted to obtain five different concentration levels in the range of 2.5–25 μg mL^{-1}. The % recovery and %RSD were calculated in each case.

System suitability requirement

The %RSD of the standard peak area should be less than 2, and the tailing factor of the standard peak should not be more than 2. The number of theoretical plates should not be less than 2000.

Table 3 Accuracy

Drugs	%Conc	%RSD	%Recovery
Ofloxacin	80%	1.39	97.49
	100%	0.49	99.43
	120%	1.19	99.81
Levofloxacin	80%	0.42	100.44
	100%	0.67	100.01
	120%	0.46	100.26
Ciprofloxacin	80%	0.98	99.52
	100%	0.26	99.71
	120%	0.92	100.36
Enoxacin	80%	1.30	99.35
	100%	1.20	99.93
	120%	0.96	96.92
Sparfloxacin	80%	0.39	98.8
	100%	0.85	99.92
	120%	0.75	101.22
Norfloxacin	80%	0.60	99.62
	100%	1.52	101.34
	120%	1.08	101.69
Cetirizine	80%	0.22	99.85
	100%	1.14	97.59
	120%	1.13	101.1

Table 4 Intermediate precision of the method

Conc. (μg mL^{-1})	% RSD	2.5	5	15	20	25	50
Ofloxacin	Interday	0.29	0.89	0.11	0.75	1.21	0.75
	Intraday	0.38	0.59	0.56	0.96	1.56	0.62
Levofloxacin	Interday	0.55	0.19	1.09	0.72	0.8	0.99
	Intraday	0.67	0.28	1.16	0.89	0.98	1.03
Ciprofloxacin	Interday	0.54	0.13	0.11	1.04	0.89	0.96
	Intraday	0.74	0.45	0.55	1.21	1.31	1.32
Enoxacin	Interday	0.008	0.1	0.007	0.005	1.2	0.69
	Intraday	0.12	0.16	0.25	0.18	1.5	0.89
Sparfloxacin	Interday	0.458	0.11	0.4	0.87	0.03	0.85
	Intraday	0.72	0.54	0.67	0.92	1.2	1.5
Norfloxacin	Interday	1.27	0.37	0.89	0.96	0.75	0.22
	Intraday	2.03	0.88	0.96	1.23	1.07	1.09
Cetirizine	Interday	0.51	0.26	0.74	0.95	0.82	0.71
	Intraday	0.72	0.74	0.92	1.03	1.05	1.78

Serum drug analysis

The stated chromatographic conditions determined cetirizine and quinolones' recoveries in human serum. Blood was deproteinated by acetonitrile. The supernatant obtained was filtered through a 0.45-μm pore size membrane filter. The serum thus obtained was mixed to different aliquots of the stock standard solution to produce desired concentrations. These were then stored at − 20 °C, and 10 μL volume of each sample was injected and chromatographed.

Results

Initially, different compositions of the mobile phase were tried for the separation of cetirizine in the presence of quinolones. It was observed that the mixture of two polar organic solvents was necessary for good resolution of peaks since any ratio of methanol: H_2O did not give good symmetry of peaks. Due to the hydrophobic character in water and a mixture of polar solvents as acetonitrile/methanol, peaks were separated with suitable retention time and good peak symmetry. The optimized mobile phase consisted of a combination of methanol, acetonitrile, and water in a ratio of 50:20:30 with a pH adjusted to 3.1, giving an excellent resolution of peaks. The wavelength selected for this purpose was 225 nm (isosbestic point) (Table 1). The method's linearity was assessed within the concentration range of 2.5–50 μg mL^{-1}, with the following regression equations.

Ofloxacin $y = 35513x + 113198$

Levofloxacin $y = 26760x + 46066$

Ciprofloxacin $y = 19631x + 3187.7$

Enoxacin $y = 43660x–42680$

Sparfloxacin $y = 16775x + 279617$

Norfloxacin $y = 20760x–14247$

Cetirizine $y = 19349x + 21761$

Retention time observed for quinolones, cetirizine, and diclofenac sodium in each case were found to be 2.5, 3.5, and 9.5 min, respectively. The correlation coefficients and sensitivity of the method are presented in Table 2.

Table 5 %Recoveries in formulations

Conc. (μg mL^{-1})		2.5	5	15	20	25
Ofloxacin	%Recovered	106.28	100.09	100.92	102.47	94.51
	Found (μg mL^{-1})	2.65	5.00	15.14	20.49	23.63
Levofloxacin	%Recovered	101.23	104.55	108.22	101.74	95.36
	Found (μg mL^{-1})	2.53	5.23	16.23	20.35	23.84
Ciprofloxacin	%Recovered	100.25	101.54	102.75	100.87	100.12
	Found (μg mL^{-1})	2.51	5.08	15.41	20.17	25.03
Enoxacin	%Recovered	97.54	96.45	100.21	99.45	99.09
	Found (μg mL^{-1})	2.44	4.82	15.03	19.89	24.77
Sparfloxacin	%Recovered	98.55	99.75	100.02	100.33	100.45
	Found (μg mL^{-1})	2.46	4.99	15	20.07	25.11
Norfloxacin	%Recovered	99.88	97.12	96.25	100.04	101.57
	Found (μg mL^{-1})	2.49	4.86	14.44	20.01	25.39
Cetirizine	%Recovered	97.12	98.57	99.62	100.26	101.45
	Found (μg mL^{-1})	2.43	4.93	14.94	20.05	25.36

Discussion

Due to the possible co-administration of these drugs, there is a need to develop an analytical method which is robust and less time consuming. Many methods already reported for estimations of cetirizine with many other drugs [16–23] and no method has been reported so far with any quinolones. The present work is validated according to ICH guidelines [24].

The specificity and robustness of the method were also established. It was found that the proposed method passed the test for robustness and specificity [24]. The respective chromatogram demonstrated that the method was specific. The chromatogram obtained from the standard solution was identical to that obtained from the spiked solution.

The representative chromatograms (Fig. 2) showed no other peaks on assayed drugs' retention time, and the retention times did not change. Accordingly, the proposed method can be considered selective.

The method's robustness and ruggedness were established as the %deviation from the mean assay value [24]. Method robustness concerning variation in wavelength and temperature (±1 °C) was studied. Peak areas and retention time changes were observed. Results indicated that peak area values were influenced less, up to 2% for all the drugs assayed for wavelength change, but no change is observed in result when temperature of system changed [24]. Moreover, the separation was found to be sufficient. To evaluate the proposed method's accuracy, recovery tests were carried out with all samples by adding known amounts of standard solutions to the sample, followed by analysis using the proposed method [24]. The accuracy and precision of the method are presented in Table 3. To evaluate the intra-day precision, assaying samples were prepared at six levels of concentration on different days. Each day's relative standard deviation was calculated and found acceptable within the given range (Table 4). The method was successfully applied to determine pharmaceutical formulations (Table 5) and human serum (Table 6).

Table 6 %Recoveries in serum

Conc. (μg mL^{-1})		2.5	5	15	20	25
Ofloxacin	%Recovered	102.11	100.06	98.56	100.33	100.72
Levofloxacin	%Recovered	100.33	100.85	99.61	99.76	101.82
Ciprofloxacin	%Recovered	98.87	98.63	100.88	98.23	104.19
Enoxacin	%Recovered	99.23	99.14	102.54	100.74	100.99
Sparfloxacin	%Recovered	97.62	99.25	101.77	100.33	102.4
Norfloxacin	%Recovered	98.22	100.63	100.11	101.36	100.69
Cetirizine	%Recovered	96.13	100.78	103.66	95.63	99.82

Conclusion

It was observed that the method passed the test for all purposes. The present method was very simple and rapid, having a run time of 10 min. This method could be easily applied to determine cetirizine, any of the quinolones, and even diclofenac sodium. The procedure was very simple and did not involve expensive instrumentation or excessive use of expensive solvents. It could be easily applied in the pharmaceutical sector for routine analysis of drugs saving much of the time and chemicals by single preparation of samples. Moreover, it could be very useful for therapeutic purposes and clinical labs. The method employed commercially readily available columns and internal standards. The sample preparation was effortless and could easily be operated.

Abbreviations

RP-HPLC: Reverse phase high-performance chromatography;
ICH: International Conference on Harmonization; LOQ: Limit of detection

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Authors' contributions

HS: design, perform experiment, writing, and supervise the project. AZM: help in writing and supervise the project. All authors have read and approved the manuscript.

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Availability of data and materials

Data and materials are available upon request.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not Applicable

Competing interests

No competing interests have been reported for the manuscript.

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