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Research Article

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Estimation and evaluation of the effect of pH on ciprofloxacin in drug formulations

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ABSTRACT

Qualitative and quantitative analysis of ciprofloxacin in drug formulations using RP-HPLC treated and maintained at various physiological pH conditions to evaluate the effect of pH on ciprofloxacin active ingredient on different mode of drug administration. The result in this study gave a clear evidence that there is an effect of pH on drug compound or active ingredient ciprofloxacin. The effect is in both ways qualitative by degradation and quantitative by reduced level of concentration.

Key words: quantitative, ciprofloxacin, RP-HPLC, physiological pH.

INTRODUCTION

Ciprofloxacin is the most widely prescribed quinolone antibiotics in Europe. (1)It is a synthetic antibiotic of the fluoroquinolone drug class (2) (3) It is a second-generation fluoroquinolone antibacterial. It kills bacteria by interfering with the enzymes that Ciprofloxacin cause DNA to rewind after being copied, which stops synthesis of DNAand of protein. Ciprofloxacin is 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid (4). Its empirical formula is $C_{17}H_{18}FN_3O_3$ and its molecular weight is 331.4 g/mol. It is a faintly yellowish to light yellow crystalline substance. Ciprofloxacin hydrochloride is the monohydrochloride monohydrate salt of ciprofloxacin. It is a faintly yellowish to light yellow crystalline substance with a molecular weight of 385.8 g/mol. Its empirical formula is $C_{17}H_{18}FN_3O_3HCl{\bullet}H_2O$. Medical uses are Chronic bacterial prostatitis (recommended as a first-line antibiotic choice) (5). Lower respiratory tract infections (6) (7), Skin and skin structure infections, Bone and joint infections, and Infectious diarrhea. (8).Science its inception in the late 1960's HPLC has made significant practical impaction on the areas of pharmaceutical, clinical, forensic environmental and industrial research development analysis. Several methods have been reported for the analysis of ciprofloxacin in biological fluids (9.10, 11) and also determination of ciprofloxacin,cloxacillin and ipuprufen drugs in human urine samples (12) HPLC method applied for quantitative determination of second generation quinolones such as ciprofloxacin and norfloxacin in pharmaceutical formulations(13) Urvish H.Desai(et al) applied PR- HPLC method to the simultaneous determination ofciprofloxacin in eye/ear drops.(14)also pharmaceutical products with different trade names having ciprofloxacin as active ingredient was assayed under similar conditions for active ingredient applying HPLC techniques(15).

The HPLC method using the optimal conditions can be tested for selectivity, linearity, precision, and accuracy, limit of quantitation and limit of detection. The applicability of the suggested method was, as well, tested on the stability of ciprofloxacin in pharmaceutical preparations (tablets and infusion solution) under stress stability data. (16)

For some separations the effect of changing pH is minimal. However for acids and bases, the effect of even a very small change in pH is very significant. Changing in the pH will effect on the degree of ionisation of molecules in solution. It thus affects their polarity, and as a consequence it changes their retention times in an HPLC separation. (17)

Since it is said to be ciprofloxacin have relatively high levels of bioavailability, it is used in versatile routs of drug administration and its modes of action is expected in respective physiological conditions. pHis primary physiological factor which has its effect on dissociation of the drug and its biological and physiological activity in the system. Various body fluids in human body show different and specific pH. These different physiological pH are provided in table1

Table 1. pH conditions of various human body fluids

S. No.	Body fluid	Physiological pH
1	Blood	7.2 to 7.5
2	Gastric juice	0.7 to 2
3	Cerebrospinal fluid	7.2-7.5
4	Pancreatic juice	8.0 to 9
5	Saliva	7.0
6	Urine	5.0 to 6.2
7	Semen	7.5

The objective of this research was quantitative and qualitative analysis of ciprofloxacin in drug formulation treated and maintained at various physiological pH conditions to evaluate the effect of physiological pH on drug by using RP-HPLC.

EXPERIMENTAL SECTION

Qualitative and Quantitative analysis by HPLC:
Four different kinds of buffers are prepared to suit each of the pH conditions of body fluids because ciprofloxacin is popularly used in gastro intestinal, urinary track and systemic infections. In oral administration saliva is the first body fluid that get interacted with the active ingredient Ciprofloxacin. In parenteral administrations the drug Ciprofloxacin is getting injected directly into blood stream through intra-venal injection. In certain system infections like Meningitis is also clinical practice to inject the ciprofloxacin in the spinal fluids.

So the test procedure designed based on all these facts and a representative buffers are prepared as shown in the table2.

Table 2. Buffers used in the experiment and their representative body fluids

S. No	Ph	Representation of body fluid	Buffer used
1	2	Gastric juice	Glycine- HCl Buffer
2	5	Urine	Citrate phosphate buffer
3	7	Saliva, Blood, Cerebrospinal fluid and semen	Phosphate buffer
4	8	Pancreatic juice	Tris- HCl buffer

Preparation of Buffers:
1. Glycine- HCl Buffer:
It is prepared by adding 44ml of 0.2M HCl to 50 ml of 0.2M solution of Glycine anddilute it to form 200ml final volume in water.

2. Citrate- Phosphate Buffer:
Prepared by adding 24.3 ml of 0.1M solution of Citric acid (19.21g/ Lt) and 25.7ml of 0.2M solution of dibasic sodium phosphate (Add 53.65g of Na2HPO$_4$7H2O and 71.7g of $Na_2HPO_4$12H_2O) and dilute it to 100ml in water.

3. Phosphate Buffer:
Prepared by adding 50ml of 0.1M KH_2PO_4 (13.6g/L) to 44.5ml of 0.1M NaOH, diluted to 100ml in water.

4. Tris- HCl Buffer:
Prepared by adding 50ml of 0.2M solution of Tris (Hydroxy methyl) amino methane (24.2 g/Lt to 26.8ml of 0.2M HCl and diluted it to 200ml in water.

Sample: Drug formulation from manufacturer CIPLA brand named as CIPRON containing the formulation is selected for the analysis and evaluation at different set pH conditions with HPLC. The total weight of the sample is weighed and recorded is about 750 mg, in order to compare the percentage assay values to the concentration of active compound in a single tablet.

Formulation: Ciprofloxacin Hydrochloride 650mg, Tinidazole 75mg and other excipients in Quantitative base.

Reference Standard: Purified form of Ciprofloxacin hydrochloride procured from HIMEDIA chemicals and micro biologics Ltd. With assay of 98% label claim is taken as a Reference standard (RS) for the study.

Chromatographic system selected for the study:
1. Operation of the HPLC - General Procedure
2. Cartridge: 250X 4.6 mm- Bondapak C18 (a reversed phase column from YMC)
3. Mobile phase preparation:
3a) Take 870 ml of buffer and make it to 1000ml by adding 130 ml of acetonitrile (CAN).
3b) Buffer preparation: Take 0.025M H_3PO_4 and add it to 1000 ml of water. Adjust the PH to 3.0± 0.1 by adding TEA.
4. Wave length: 278 nm
5. Temperature: 30^o C (ambient)
6. Injection volume: 10 µl
7. Run time: 30 min
8. Flow rate: 1.5 µl
9. Sample preparation: 0.1g of the sample and RS (pure Ciprofloxacin) weighed and the sample is dissolved in 25 ml of respective buffers whereas RS is supplied with the mobile phase solution.

The greatest enemy of HPLC is fine particulate matter, which can damage the pumping system and irreversibly block the column. Therefore, all solvents have been filtered through fine membranes (0.4-0.5 micron) and all solutions to be injected MUST be prepared either with filtered solvent, or filtered. The mobile phase prepared as well as the sample solution also rendered to a through filtration through 0.2 micron membrane filter. Also the test solution and the mobile must be sonicated by using Ultra sonication cabinet to remove dissolved gaseous impurities.

The Waters instrument comprises three main components:
1. The injector (located on the top right-hand corner of the console)
2. The solvent delivery system
3. The UV/ Vis. Detector.

As this is quantification based on the reference sample run, a Reference Standard (RS) which is a pure chemical of Ciprofloxacin with known concentration is obtained (make: MERCK, Conc: 99%) and the calculation of concentration of unknown samples (soft drinks) been performed by comparing the areas of principle peaks identified based on the RT (retention times).

The calculation of percentage concentration (Assay) is based on the following standard formula.

$$\% \text{ Conc. of eluted compound} = \frac{\text{PA of sample X Weight of the sample taken X 100}}{\text{PA of the Standard X Weight of the sample taken}}$$

RESULTS AND DISCUSSION

In each set up of the experiment a reference standard (RS is run for qualitative analysis to assess the error by standard deviation in retention times (RT). Subsequently the crude samples are also rendered to run in HPLC under ideal conditions and appropriate dilutions. The RT of the Reference Standard is set to match with that of average RT of the each of the samples of different pH treatments by incubating the sample preparation in the respective pH

buffer stated in the method. Based on the RT comparison with the RS sample run the treatment samples are fixed in integration of the principal peaks of *Ciprofloxacin* in data processing by using EMPOWER-II software.

Here in the current experiment RS samples are run two times, referred by RS-A and RS-B in triplicates as the column is regenerated in the middle of the experiment session to clear and wash the column stationary phase. The treatment samples are compared with the respective RS samples based on the retention time (RT) match.

Then sample is prepared as per the method, filtered and sonicated for quantitative analysis. Quantitative analysis is based on the ratio of peak areas between RS (Reference Standard) and sample which are injected thrice each so as to obtain the results in triplicates.

The averages in terms of Mean values are taken for the calculation in to the formula according to the method mentioned in the previous section. The mean values, Standard Deviation and Relative Standard deviation are taken to assess the degree of error in results.

Analysis of data

1. Use standard Ciprofloxacin samples to identify the Ciprofloxacin peak and record the retention time of Ciprofloxacin
2. Use the retention time to determine if Ciprofloxacin is present in the soft drink sample
3. To quantitatively determine the amount of Ciprofloxacin in the sample, measure the Ciprofloxacin peaks of the standard as well as the sample peaks for their peak areas
4. Measure the Ciprofloxacin peak in the sample chromatogram, and use the concentration to peak area relationship to determine the concentration of Ciprofloxacin in percentages and then after convert the values in terms of milligrams (mg). The quantitative data obtained is provided in the table 3.

Tab.3 Quantitative data of critical parameters obtained from the chromatograms
(Presented in Appendices),

S. No.	Name of the sample	*RT	#PA (µV X (sec)	^PH (µV)	~PW (sec)
1	RS-A-1	9.205	255607124	2523503	174
2	RS-A-2	9.377	172332449	2588167	182
3	RS-A-3	11.113	171088091	2213744	196
4	pH-2-1	9.405	117289083	2614595	178
5	pH-2-2	9.656	149007001	2581242	201
6	pH-2-3	9.656	149007001	2581242	182
7	RS-B-1	16.424	73139610	595282	195
8	RS-B-2	14.723	223106399	2524860	180
9	RS-B-3	15.012	174335452	2318526	185
10	pH-5-1	15.872	200344900	1950777	175
11	pH-5-2	15.281	171076643	1801660	180
12	pH-5-3	15.246	179664846	1879862	192
13	pH-7-1	15.812	12974287	445405	201
14	pH-7-2	15.994	12840433	410381	220
15	pH-7-3	15.958	12863148	411912	178
16	pH-8-1	16.295	160125312	2329499	186
17	pH-8-2	16.907	130574893	2176939	195
18	pH-8-3	17.096	156737780	2213251	172

*Note: *RT- Retention Time; #PA- Peak Area; ^PH- Peak Height; ~PW- Peak Width*

The statistical analysis data for the mean, standard deviation (SD) and relative standard deviation (RSD) values acquired from the data are provided in the table 4.

The assay values of each of the four different pH treatments (pH-2. pH-5. pH-7 and pH-8) are calculated according to the formula and their respective assay values are recorded. Actual weight of the ciprofloxacin tablet taken is 750 mg; hence the concentrations from each of the samples given with pH treatment are recorded by mathematical calculation (cross multiplication). The assay values are calculated based on the peak areas integrated in chromatograms, sample weights and standard assay of RS. Comparisons made considering the assay values of RS with treatments, the concentration values are recorded thereof with different pH treatments. The comparative figures are presented in the table 5.

Tab. 4 Statistical analysis of data from the chromatograms

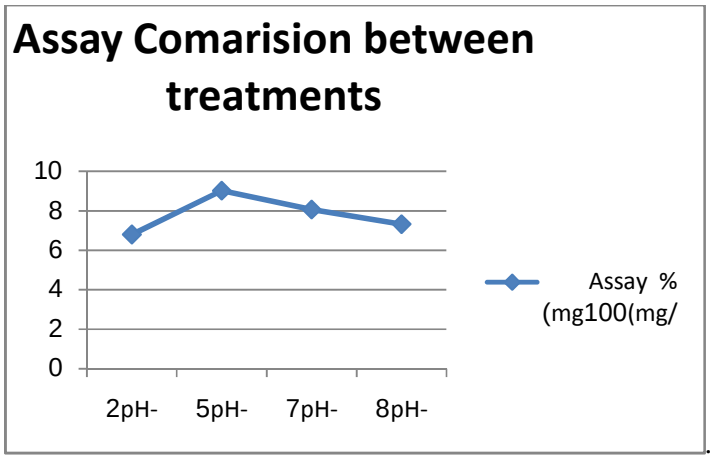
S. No	Name of the sample	Mean	*SD	#RSD
For RT				
1	RS-A	9.8983333	1.055442	10.66282308
2	RS-B	15.386333	0.910189	5.915569062
3	pH-2	9.5723333	0.922236	9.634389421
4	pH-5	15.466333	0.439932	2.844450474
5	pH-7	15.921333	0.096381	0.60535877
6	pH-8	16.766	0.418702	2.497325327
For PA				
1	RS-A	199675888	48441867	24.26024868
2	RS-B	156860487	76495361	48.76649464
3	pH-2	138434362	18312348	13.22818141
4	pH-5	183695463	15044671	8.190006785
5	pH-7	12892623	71629.54	0.555585446
6	pH-8	149145995	16171989	10.84305925
For PH				
1	RS-A	2441804.7	200135.2	8.196201337
2	RS-B	1812889.3	1059514	58.44337081
3	pH-2	2592359.7	19256.36	0.742812187
4	pH-5	1877433	74588.17	3.97288047
5	pH-7	422566	19793.96	4.684229679
6	pH-8	2239896.3	79693.91	3.557928345
For PW				
1	RS-A	184	11.13553	6.051917786
2	RS-B	186.66667	7.637626	4.091585442
3	pH-2	187	12.28821	6.571233009
4	pH-5	191	8.736895	4.574290549
5	pH-7	199.66667	21.03172	10.53341673
6	pH-8	184.33333	14.29452	7.754713071

RS- Reference Standard, RT: Retention Time; PA: Peak Area
*PH: Peak Height; * Std. Dev: Standard deviation*
#R. Std dev: Relative Standard deviation

Table 5. Concentration comparison between pH treatments of Ciprofloxacin

S. No	Name of the sample	% Assay (mg/100mg)	Concentration in a tablet (mg/750mg)	Label claim (mg) of the tablet
1	pH-2	6.794	509.55	650
2	pH-5	9.015	676.125	650
3	pH-7	8.054	604.05	650
4	pH-8	7.32	549	650

Fig. 1 Comparison between the different pH treatments in terms of assay



Note: On X-axis- treatments
On Y-axis- Assay values (mg/100mg of sample)

A line diagram to offer the graphical representation depicting above facts and figures has been drawn by which one can easily understand the effect of pH treatments over different treatment in terms of concentrations of ciprofloxacin active ingredient. The same is presented in the graph (Figure 1).

The mean Retention time values generated by different peaks in the chromatograms are compared to the deviation among them by calculating their Standard Deviations (SD). The values are presented in the table6.

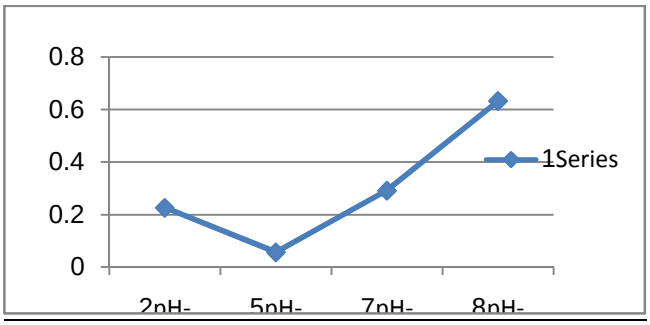
Table 6. RT comparison between pH treatments of Ciprofloxacin

S. No.	Treatment	Mean of RT	*SD
1	RS-A	9.89	--
2	RS-B	15.38	--
3	pH-2	9.57	0.226
4	pH-5	15.46	0.0565
5	pH-7	15.92	0.2914
6	pH-8	16.76	0.633

**SD- Standard deviation*

Another line diagram to offer the graphical representation depicting average or mean values of peak retention times in chromatograms (Presented in Appendences), a qualitative parameter has also been drawn by which one can easily understand the effect of pH treatments over different treatment in terms of qualitative identification or degeneration of the drug substance ciprofloxacin in samples. The graph (figure 2) is presented below.

Fig. 2 Comparison between the different pH treatments in terms of peak RT



Note: On X-axis- treatments
On Y-axis- standard deviation between retention times

CONCLUSION

To meet the objective of the study a drug material (tablet) having Ciprofloxacin as active ingredient is taken for the experiment and rendered to both qualitative and quantitative analysis by HPLC method. The results are obtained from the data generated in the form of peaks in chromatogram by HPLC analysis of four different treatments treated with pH-2, pH-5, pH-7 and pH-8 representing the various conditions of body fluids like Gastrointestinal juice, Urine, saliva and blood, pancreatic juice respectively.

The results generated in this study gave the clear evidence that there is definitely the effect of pH on drug compound or active ingredient Ciprofloxacin. The effect is in both ways qualitative by degradation and quantitative by reduced levels of concentration.

When chromatograms of pH treatments are compared the deviation in the RT values between pH-2, pH-5 and pH-7 are varied around a specific average levels but the treatment with pH-8 is significantly deviated from rest of the three. Though the there is some standard deviation between each and every pH treatment is found it is evident with pH-8.

When the concentrations or assay values (quantitative parameters) generated from the chromatograms are compared it`s again pH-8 that is showing significant reduced levels of concentrations of active drug Ciprofloxacin. However

the reduced levels of Ciprofloxacin can be related to its qualitative test status of high levels of generation represented by the deviation in Retention Times (RT) from the standard retention times. So we can infer the quantitative parameters of analysis of ciprofloxacin is proportional to the quantitative parameters (concentrations),

The results obtained by HPLC in this set of experiments are reproducible and error is averaged by triplicate experiment design. The conclusions and remarks from this study states that effect of pH on Ciprofloxacin in drug formulation is evident but it is insignificant with pH-2, pH-5 and pH-7 but it's is significant with pH-8. So the drug preparation targeting the infections of systemic organs where the pancreatic secretions form the medium may need

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