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A comparative analysis of UV-visible spectroscopic method vs HPLC method for determining content assay of ciprofloxacin 500 MG tablets

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Abstract

Ciprofloxacin tablets are usually prescribed in GIT infections particularly in typhoid. In Pakistan there are several brands of ciprofloxacin available with different trade names in local market. The standard method of determining the amount of active ingredient present in tablets described by US Pharmacopeia is HPLC method. This study was conducted to compare the results of analysis of UV-visible spectroscopic method and HPLC method to determine the amount of ciprofloxacin (active) present in different brands. Content assay was performed by these two methods. Other tests like weight variation, hardness, disintegration and dissolution test were performed on all brands under study. For this study eight brands of ciprofloxacin were purchased from local market of Islamabad and evaluated on the basis of above mentioned tests according to compendial methods. Results showed that all brands complied with USP specifications for all parameters. UV-visible spectroscopic method showed reliable and comparable results to that of HPLC method so this can provide a reliable method for assay of the drug as that of compendial method HPLC.

Keywords: Ciprofloxacin, Fluoroquinolones, UV-visible spectroscopy, Content assay, HPLC.

Introduction

The quinolones are antimicrobial agents which are synthetic in nature, bactericidal in action and used in the treatment of various infections especially urinary tract infections[1]. Quinolones were not widely used antimicrobial until the early 1980s, when a second generation of this class was developed. These newer drugs were ciprofloxacin, norfloxacin, and ofloxacin, displayed remarkably improved activity against gyrase and showed penetration into Gram-positive organism's cell wall and enhanced pharmacodynamics and pharmacokinetics. The most important change to the quinolone basic structure was the introduction of a fluorine at position C6 and a major ring substituent (piperazine or methyl-piperazine) at C7. Due to this substitution of fluorine, quinolones are often termed as "fluoroquinolones"[2,3]. Quinolones exert their mode of action by inhibition of bacterial replication. Fluoroquinolones primarily target bacterial enzyme DNA gyrase or topoisomerase II[4].

Due to the development of bacterial resistance, in 1980 fluorine atom was inserted in the quinolone ring which broadened the spectrum of this class to gram positive and also enhanced action against gram negative. Ciprofloxacin was made by these molecular changes. Ciprofloxacin is fluoroquinolone with fluorine at position 6 of naphthyridinering[5]. It is most effective against gram negative bacteria and widely used to treat respiratory and Urinary tract infections[6]. Its molecular structure is related to other quinolones for example nalidixic acid, norfloxacin, ofloxacin and enoxacin, but it differs from them in having a cyclopropyl residue at the position number 1 of the quinolone nucleus[7].

Several analytical methods for the quantitative determination of fluoroquinolones in pharmaceutical formulations were reported in scientific literature like UV spectrophotometry, titration and high performance liquid chromatography (HPLC), which is often used for quantification of ciprofloxacin in medicines[8-11].

Methodology

For comparing the method of UV-visible spectroscopy to that of HPLC method for determining the amount of ciprofloxacin present in marketed brands, eight brands were purchased from local market of Islamabad. All the physical parameters were also assessed during the assessment which included weight variation, hardness, disintegration test, dissolution test along with content assay.

The brands were given codes which were used further to represent them in this study. Ciprofloxacin due to addition of ferric Chloride shifted to spectral band of lower frequencies/longer wavelengths and this shift is known as bathochromic shift. This shift made analysis of Ciprofloxacin possible at the wavelength of 438nm by using UV-visible spectroscopy[12].

List of brands along with codes and manufacturer name is shown in Table-1.

Table-1: Samples of Ciprofloxacin Tablets.

Brand Code	Brand Name	Company Name	Exp. Date
C-1	Cyrocin	Highnoon pharma	9/2018
C-2	Cipesta	Getz pharma	01/2020
C-3	Gavil	Tabros pharma	4/2019
C-4	Inoquin	Barrett Hodgson	02/2020
C-5	Veprox	Siza pharma	02/2017
C-6	Cipoxcin	Nenza pharmaceuticals	12/2018
C-7	Cialox	Global pharmaceuticals	07/2019
C-8	CipiZef	Zafa Pharmaceuticals	02/2021

Weight variation test: From each brand, twenty tablets were individually weighed and average weight was calculated. According to USP 2013, tablets weighing more than 500mg should not deviate from ±5% limit.

Hardness test: Monsanto hardness tester was used to assess hardness of tablets. Ten tablets from each brand were randomly selected and then average hardness was calculated. The limit for tablet hardness is 5-10kg/cm^2.

Disintegration test: According to USP 2013, the disintegration time for film coated tablets should be not more than 30 minutes. For determination, 0.1N HCl solution was made and poured in the assembly. Temperature was maintained at 37˚C±2. Six tablets from each brand were placed in six tubes and their disintegration time was determined. Average disintegration time was calculated for each brand[13].

Dissolution test: Dissolution apparatus II was used. 900mL of 0.01N HCl solution was poured in each vessel. Then six tablets of each brand were individually placed in each vessel. The paddle was rotated at the speed of 50 revolutions per minute (rpm) for a time period of thirty minutes. After thirty minutes 0.5mL of the sample was taken and diluted up to 25mL with the dissolution medium. By using UV spectrophotometer the percentage drug released after thirty minutes was determined by measuring the absorbance at 277nm on filtered portions of the solution under test. Not less than 80% of the labeled amount dissolved in 30 minutes[13].

Content assay by UV-visible spectroscopic method: To determine content assay of ciprofloxacin by UV Visible spectroscopy, fresh solution of 1%w/v of ferric chloride was prepared along with another solution of 100mcg/mL of pure ciprofloxacin. Five tablets from each brand were crushed and 100mg of the powdered samples were weighed, dissolved in 100mL of 0.1N hydrochloric acid and further dilution was made up to 100mcg/mL for each brand. 1mL of ferric chloride was added to 5mL of each brand and pure sample and made up to 50mL with 0.1N HCl. The absorbance of each sample was calculated at 438nm against blank reagent with an ultraviolet spectrophotometer. The percentage content was calculated for each brand[14].

Content assay by HPLC method: The official method for determining content assay of ciprofloxacin in USP is HPLC technique coupled with UV- detector. Mobile phase consisted of triethlyamine and acetonitrile (87:13). Standard solution of ciprofloxacin RS and test solution of all brand were prepared as mentioned in USP 2013.

Flow rate was adjusted at 1.5mL per minute. Then 10µl of each ciprofloxacin standard and samples were injected into the chromatograph, chromatograms, major peaks and area under curves were recorded and quantity was calculated in mg for each sample. Ciprofloxacin tablets should contain ciprofloxacin hydrochloride equivalent to not less than 90% and not more than 110% of the labeled amount of ciprofloxacin[13].

Results and discussion

Results for weight variation test: The results for hardness test showed that all the brands were within the specified limit that is ±5%. C-1 had the maximum average weight that was 893mg while the minimum average weight was that of C-4 which was 719mg. Table-2 summarizes results for weight variation test.

Table-2: Results for Weight variation Test.

Brands Code	Average weight (mg)	% Deviation
C-1	893	-0.336
C-2	729	-0.273
C-3	816	-0.366
C-4	719	-0.138
C-5	722	0.258
C-6	776	-0.129
C-7	840	-0.834
C-8	802	0.250

Results for hardness test: It was found from the results that all the brands complied with specification limit of hardness test that is 5-10kg/cm^2. C-5 had the highest value for hardness which was 9.3kg/cm^2 while C-7 had the lowest value which was 7.1kg/cm^2.

Results for hardness test of all the eight brands mentioned in Table-3.

Table-3: Results for Hardness test.

Brands Code	Average Hardness (kg/cm^2)
C-1	7.4
C-2	7.9
C-3	8.8
C-4	9.0
C-5	9.3
C-6	8.7
C-7	7.1
C-8	8.2

Results for disintegration test: All the eight brands showed disintegration time with in the specified limit for film coated tablets according to USP. The maximum time taken to disintegrate was taken by C-6 which was 5 minutes while the least time taken was by C-2 which was 1.8 minutes. Results for disintegration test are given in Table-4.

Table-4: Results for Disintegration Test.

Brands Code	Average Disintegration Time (minutes)
C-1	2.5
C-2	1.8
C-3	4
C-4	2.8
C-5	3.1
C-6	5
C-7	2.1
C-8	3.2

Results for dissolution test: Dissolution test was performed on all the eight brands. The amount of drug released after 30 minutes was determined by UV-visible spectroscopic method at 277nm. The maximum amount of drug released was shown by C-4 that was 98.4%. The minimum amount of drug released was shown by C-5 that was 88.7 %.

Results for percentage of drug released after 30 minutes are given in Table-5.

Results for content assay by UV-spectroscopic method: The USP 2013 states that the drug content for ciprofloxacin tablets 500mg should range from 90 to 110%. The results for UV-visible spectroscopic method showed that all the brands had content greater than 90% and they fulfill the stated claim as mentioned on the label. The maximum amount of drug content was shown by a multinational brand C-4 which was 99.07% while C-7 had the lowest drug content that was 91.7% which was a local brand. Table-6 shows the drug content of all brands by UV-visible spectroscopic method.

Table-5: Results for Dissolution Test.

Brands Code	Percentage of drug released after 30 minutes
C-1	96.7
C-2	94.2
C-3	97.5
C-4	98.4
C-5	88.7
C-6	95.3
C-7	97.7
C-8	95.8

Table-6: Results for Drug Content Assay by Uv-visible spectroscopic method.

Brands code	Absorbance of tablets	Absorbance of standard	Drug Content (mg)	% Drug Content	% Drug Content Deviation
C-1	0.0894	0.0969	461.3	92.3	- 7.7
C-2	0.0915		472.13	94.4	- 5.6
C-3	0.0931		480.4	96.0	- 3.93
C-4	0.0960		495.5	99.0	- 0.93
C-5	0.0904		466.5	93.3	- 6.7
C-6	0.0954		492.5	98.5	- 1.5
C-7	0.0889		458.7	91.7	- 8.3
C-8	0.0934		481.9	96.3	3.7

Results for content assay by HPLC method: To confirm the results from UV-visible spectroscopic analysis by content assay of ciprofloxacin tablets 500mg, official method of HPLC was adopted. The retention time for ciprofloxacin HCl is from 6.4 to 10.8 minutes. From the results of HPLC technique it can be seen that all the brands had content assay >90%. The highest amount of drug was shown by C-4 which was 99.8%. The minimum amount was shown by C-7 which was 92.1%. Results of content assay from HPLC method of all the brands are mentioned in Table-7.

Conclusion

The Basic aim of the study was to evaluate the Uv-visible method for determining the content assay of ciprofloxacin and compare results of assay of UV-visible method with assay result conducted by using HPLC method. HPLC is standard method for assessing content assay, but it requires high expertise and is time consuming. Eight brands of ciprofloxacin were used in this study Cyrocin, Cipesta, Gavil, Inoquin, Veprox, Cipoxcin, Cialox and CipiZef. Manufactured by Highnoon pharma, Getz pharma, Tabros pharma, Barrett Hodgson, Siza pharma, Nenza Pharmaceuticals, Global Pharmaceuticals and Zafa Pharmaceuticals respectively. These brands were given code names C-1, C-2, C-3, C-4, C-5, C-6, C-7 and C-8 respectively.

All of brands were subjected to Hardness testing, Weight variation testing, Disintegration testing and Dissolution testing according to compendia. All tests complied with acceptable limits of these test. None showed unacceptable deviation from standard values. Local brands were complying with specification as well as multinational brand (C-4).

It can be concluded from the results that there is only slight variation between the amounts of drug measured by UV-visible method to that of HPLC method. The value of content assay for C-1 was 92.3 as determined by UV-visible spectroscopy and 93.0 by HPLC method. C-2 value of content assay was 94.4 as determined by UV-visible spectroscopy and 94.7 as determined by HPLC Method. Value of C-3 for content assay as determined by UV-visible spectroscopy was 96.0 and 97.2 as determined by HPLC method. Value of C-4 for content assay determined by UV-visible Spectroscopy as 99.0 and 99.8 as determined by HPLC method. Value of content assay for C-5 was 93.3 as determined by UV-visible spectroscopy and 94.8 as determined by HPLC Method. Value of content assay for C-6 as determined by UV-visible spectroscopy was 98.5 and 97.7 as determined by HPLC method. Value of content assay for C-7 was 91.7 as determined by UV- visible spectroscopy and 92.1 as determined by HPLC method. Value of content assay for C-8 as determined by UV-visible spectroscopy was 96.3 and 96.9 as determined by HPLC method. Comparison of data of content assay of eight brands of Ciprofloxacin by UV-visible spectroscopic and HPLC method showed in Figure-1.

From the results of this study, it can be concluded that UV-visible spectroscopic analysis for measuring the content assay of ciprofloxacin can be adopted for accurate analysis. It is also concluded that all the pharmaceutical companies of the eight brands are manufacturing the tablets according to the specifications of USP 2013, and can be used alternatively in GIT infections. Table-8 summarizes the comparison between the two techniques.

Table-7: Results for Drug Content Assay by HPLC method.

Brands Code	Retention time	Area under curve of sample	Area under curve of Standard	Drug Content (mg)	% Drug Content	Limit
C-1	8.550	4295	4618	465.0	93.0	90 to 110%
C-2	9.456	4375		473.6	94.7	
C-3	7.159	4487		485.8	97.2	
C-4	10.007	4610		499.1	99.8	
C-5	9.321	4378		474.0	94.8	
C-6	9.666	4515		488.8	97.7	
C-7	8.369	4256		4608	92.1	
C-8	7.512	4475		484.5	96.9	
Ciprofloxacin Standard	10.066	-		-		

Table-8: Comparison of content assay of eight brands of ciprofloxacin by Uv-visible spectroscopic and HPLC method.

Brands Code	Content Assay by UV-visible spectroscopy	Content Assay by HPLC method
C-1	92.3	93.0
C-2	94.4	94.7
C-3	96.0	97.2
C-4	99.0	99.8
C-5	93.3	94.8
C-6	98.5	97.7
C-7	91.7	92.1
C-8	96.3	96.9

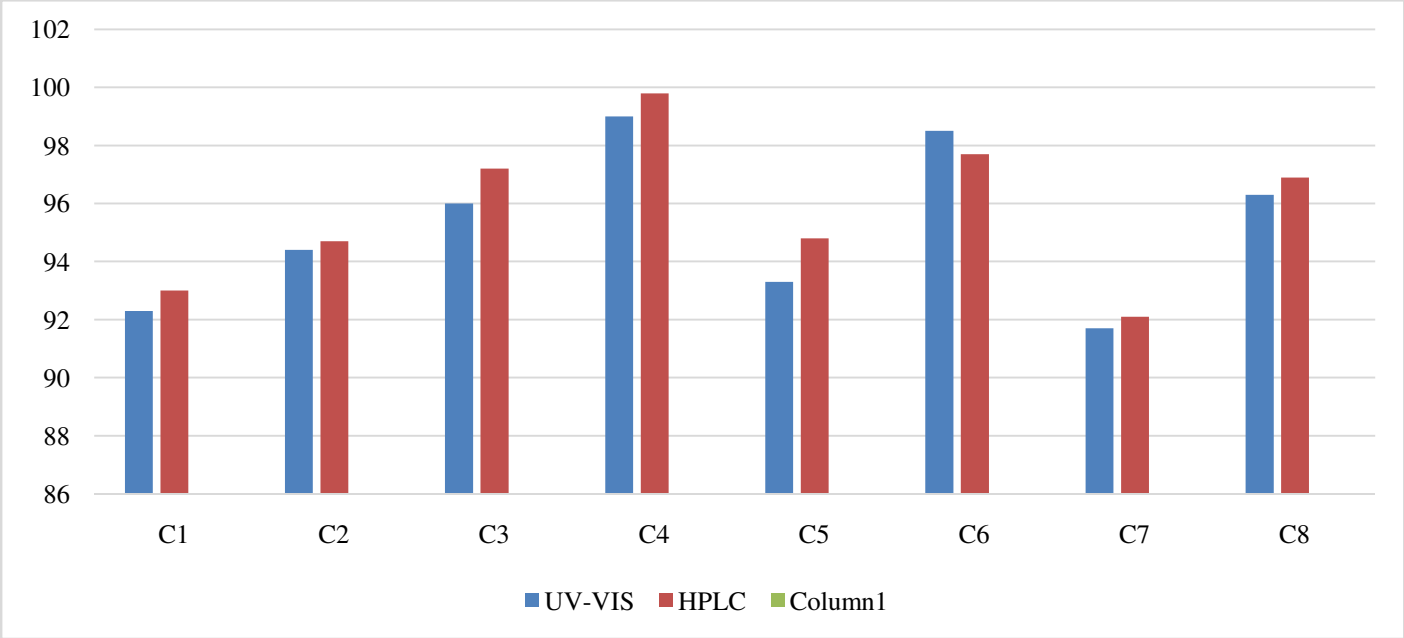

Figure-1: Comparison of content assay of eight brands of ciprofloxacin by Uv-visible spectroscopic and HPLC method.

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