INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

DOI: https://dx.doi.org/10.33289/IJRPC.12.2.2022.12(24)

A NEW CHROMATOGRAPHY SEPERATION TECHNIQUE FOR ESTIMATION OF CIPROFLOXACIN IN ITS BULK AND DOSAGE FORM

Gadi Vijaya Lakshmi*, Medaboyina Baby Krishna Priyanka, Murapaka Vasavi, Parameswari Bhavani, Nakka Mounika, Pallala Radhika Pavani and Palleti Alekhya

Department of Pharmaceutics, Vj's College of Pharmacy, Diwancheruvu, Rajahmundry, Andhra Pradesh, India.

ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Ciprofloxacin in Tablet dosage form. Chromatogram was run through Symmetry (4.6 x 150mm, 5µm). Mobile phase containing Methanol and 0.1% TEA in the ratio of 40:60 was pumped through column at a flow rate of 1.0 ml/min. Temperature was maintained at 30°C. Optimized wavelength for Ciprofloxacin was 275nm. Retention time of Ciprofloxacin were found to be 2.254 min. %RSD of the Ciprofloxacin were found to be 0.4. %Recover was Obtained as % for Ciprofloxacin. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Keywords: Ciprofloxacin, RP-HPLC, Chromatography and retention time.

INTRODUCTION

Importance of drug analysis

'Health is wealth'. It is vital fact that a healthy body is desire of every human being. Good health is first condition to enjoy the life and all other things which mankind is having. Nowadays peoples are more concentrating towards health. Even governmental bodies of different countries and World health organization (WHO) are also focusing for health of human being.

HPLC was commonly used for the separation of chemical compounds. New techniques improved separation, identification, purification and quantification far above the previous techniques. Computers and automation added to the convenience of HPLC. Improvements in type of columns and thus reproducibility were made as such terms as micro column, affinity columns, and Fast HPLC began to immerge.

Today's HPLC requires very special apparatus which includes the following.

1. Extremely precise gradient mixers.
2. HPLC high pressure pumps with very constant flow.
3. Unique high accuracy, low dispersion, HPLC sample valves.
4. Very high efficiency HPLC columns with inert packing materials.
5. High sensitivity low dispersion HPLC detectors.
6. High speed data acquisition systems.
7. Low dispersion connecting tubes for valve to column and column to detector.

HPLC Gradient mixtures

HPLC gradient mixers must provide a very precise control of solvent composition to maintain a reproducible gradient profile. This can be complicated in HPLC by the small elution volumes required by many systems. It is much more difficult to produce a constant gradient when mixing small volumes then when mixing large volumes. For low pressure systems this requires great precision in the operation of the miniature mixing General Introduction valves used and low dispersion flows throughout the mixer. For multi-pump high pressure systems it requires a very precise control of the flow rate while making very small changes of the flow rate.

HPLC Sample Valves

Since sample valves come between the pump and the column it follows that HPLC sample valves must also tolerate pressures up to 10,000 psi. For analytical HPLC, the sample volume should be selectable from sub micro liter to a few micro liters, whereas in preparative HPLC the sample volume may be even greater than 10 ml. To maintain system efficiency the sample valve must be designed to have very low dispersion characteristics, this is true not only for flow dispersion but also for the less obvious problems of dispersion caused by sample adsorption/desorption on valve surfaces and diffusion of sample into and out of the mating surfaces between valve moving parts. It goes without saying that the valves must deliver a very constant sample size but this is usually attached.

HPLC Columns

HPLC columns are packed with very fine particles (usually a few microns in diameter). The very fine particles are required to attain the low dispersion that give the high plate counts expected of modern HPLC. Plate counts in excess of 25,000 plates per column are possible with modern columns, however, these very high efficiencies are very rarely found with real samples because of the dispersion associated with injection valves, detectors, data acquisition systems and the dispersion due to the higher molecular weight of real samples as opposed to the common test samples. Packing these small particles into the column is a difficult technical problem but even with good packing a great amount of care must be given to the column end fittings .The main consideration with HPLC Sis the much wider variety of solvents and packing materials that can be utilized because of the much lower quantities of both which are required.

In particular very expensive optically pure compounds can be used to make Chiral HPLC stationary phases and may even be used as (disposable) HPLC solvents.

DRUG PROFILE

Ciprofloxacin Description

Ciprofloxacin is a broad-spectrum antimicrobial carboxyfluoroquinoline. The bactericidal action of ciprofloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, strand supercoiling repair, and recombination.

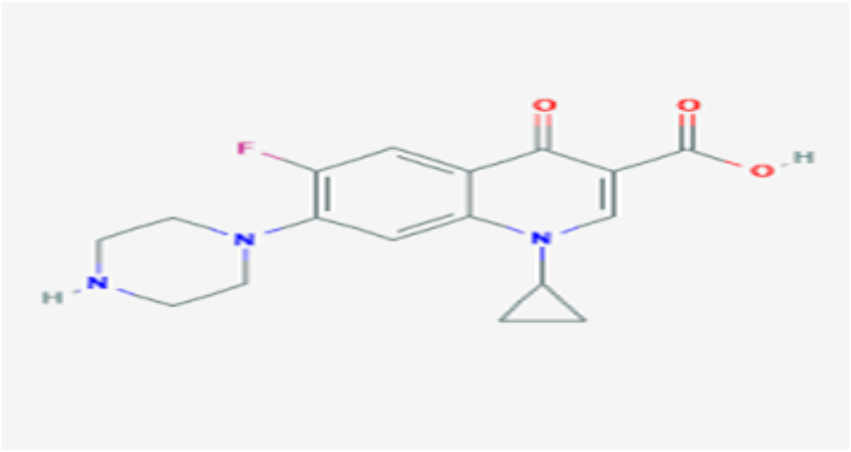

Fig. 1: Structure

Systematic (IUPAC)name

1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid

PHYSICOCHEMICAL DATA

Formula : C17H18FN3O3

Molecular weight : 331.341

Pharmacodynamics

Ciprofloxacin is a broad-spectrum antiinfective agent of the fluoroquinolone class. Ciprofloxacin has *in vitro* activity against a wide range of gram- negative and gram-positive microorganisms. The mechanism of action of quinolones, including ciprofloxacin, is different from that of other antimicrobial agents such as beta-lactams, macrolides, tetracyclines, or aminoglycosides; therefore, organisms resistant to these drugs may be susceptible to ciprofloxacin. There is no known cross-resistance between ciprofloxacin and other classes of antimicrobials. Notably the drug has 100 times higher affinity for bacterial DNA gyrase than for mammalian.

Mechanism of action

The bactericidal action of ciprofloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, strand super coiling repair, and recombination.

Pharmacokinetic characters

Absorption

Rapidly and well absorbed from the gastrointestinal tract after oral administration. The absolute bioavailability is approximately 70% with no substantial loss by first pass metabolism.

Metabolism

Hepatic. Four metabolites have been identified in human urine which together account for approximately 15% of an oral dose. The metabolites have antimicrobial activity, but are less active than unchanged ciprofloxacin.

Half-life

4 hours

Affected organisms

Humans and other mammals

EXPERIMENTAL METHOD

Table 1: Instruments used

SL. NO	INSTRUMENT	MODEL
1	HPLC	WATERS, software: Empower, 2695 separation module.2487 UV detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000^{+}
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table 2: Chemicals used

SL. No	Chemical	Company Name
1	Ciprofloxacin	PHARMATRAIN
2	Water for HPLC	FINER chemical LTD
3	Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	MOLYCHEM
5	Trifluoro acetic Acid	MERCK
6	NaOH	FINER chemical LTD

HPLC METHOD DEVELOPMENT
PREPARATION OF BUFFER AND MOBILE PHASE
Preparation of 0.1% TFA buffer
Take 1 ml of TFA in 1000 ml of HPLC water, pH was adjusted with NaOH up to 3.0. Final solution was filtered through 0.45 µ Membrane filter and sonicate it for 10 mins.

Preparation of mobile phase
Accurately measured 400 ml of Methanol(40%) and 600 ml of above buffer (60%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation
The Mobile phase was used as the diluent.

ASSAY
Standard Solution Preparation
Accurately weigh and transfer 50 mg of Ciprofloxacin working standard into a 100 ml clean dry volumetric flask add about 50 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 2.5 ml of the above stock solutions into a 25 ml volumetric flask and dilute up to the mark with diluent. Further pipette 3 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent. (15 ppm Ciprofloxacin)

Sample Solution Preparation
Accurately weigh 10 tablets crush in motor and pestle and transfer equivalent to 50 mg (65 mg of tablet power) of Ciprofloxacin sample into a 100 ml clean dry volumetric flask add about 50 mL of diluent and sonicate it up to 30 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.45 micron injection filter. (Stock solution) Further pipette 2.5 ml of the above stock solutions into a 25 ml volumetric flask and dilute up to the mark with diluent. Further pipette 3 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent. (15 ppm Ciprofloxacin)

Procedure
Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Ciprofloxacin peaks and calculate the %Assay by using the formulae.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used	:	Waters HPLC with auto sampler and UV detector.
Temperature	:	Ambient
Column	:	Symmetry (4.6 x 150mm, 5µm)
Buffer	:	0.1% TFA
pH	:	3.0
Mobile phase	:	Methanol: 0.1% TFA (40:60)
Flow rate	:	1 ml per min
Wavelength	:	275 nm
Injection volume	:	20 µl
Run time	:	8 min.

RESULTS AND DISCUSSION

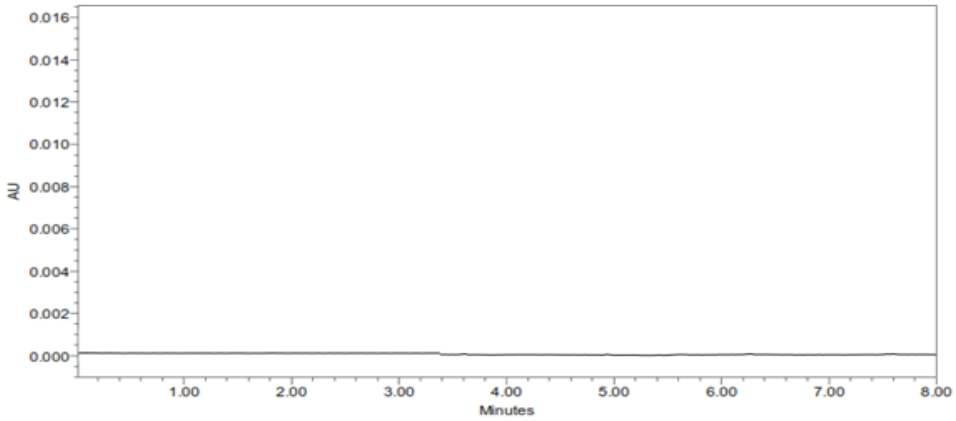

Fig. 2: Chromatogram for Blank

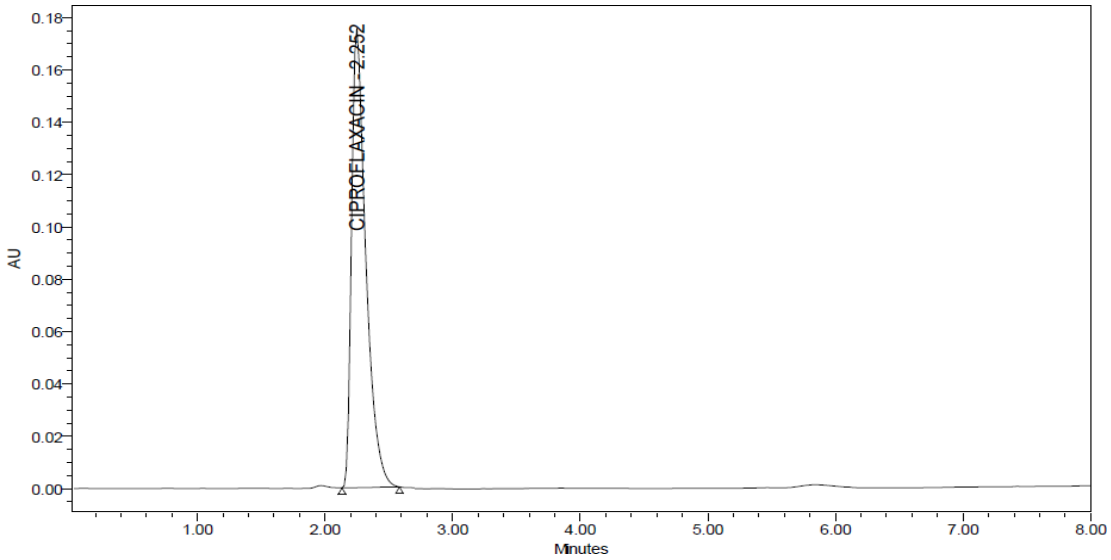

Fig. 3: Chromatogram for ciprofloxacin

Table 3: Results of system ciprofloxacin

S. No.	Name	RT(min)	Area (µV sec)	Height (µV)	USP tailing	USP plate count
1	Ciprofloxacin	2.252	5286677	175131	1.23	3672.48

.

Table 4: Results of Assay for Ciprofloxacin

	Label Claim (mg)	% Assay
Ciprofloxacin	150	100.09

VALIDATION PARAMETERS

1. LINEARITY

The linearity range was found to lie from 5µg/ml to 250µg/ml of Ciprofloxacin and chromatograms are shown below.

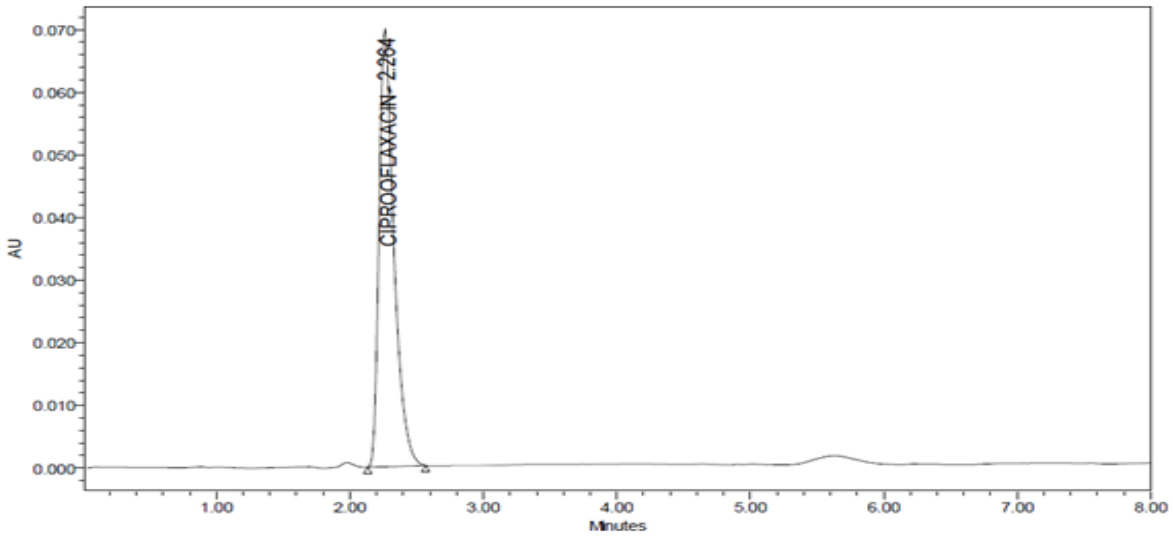

Fig. 4: Chromatogram for Linearity

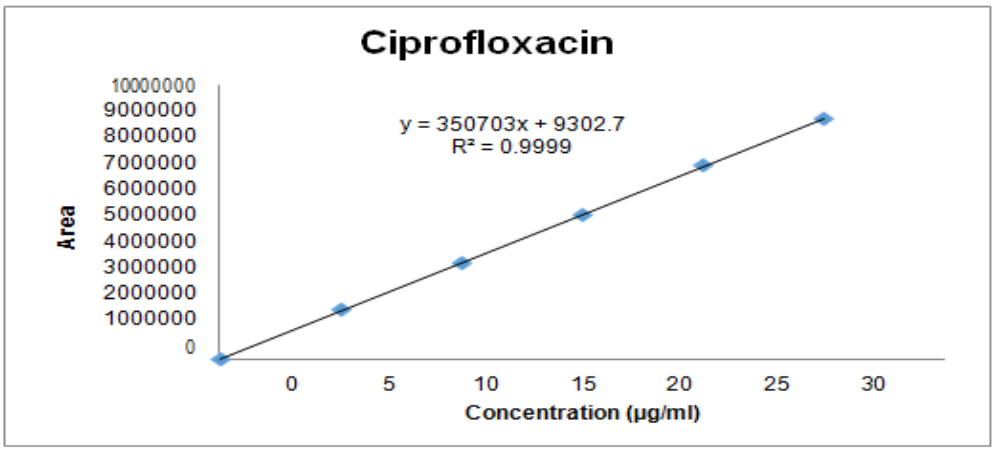

Fig. 5: Calibration graph for Ciprofloxacin

Table 5: Analytical performance parameters of Ciprofloxacin

Parameters	Ciprofloxacin
Slope (m)	58450
Intercept (c)	9302.7
Correlation coefficient (R^2)	0.999

2. PRECISION

Precision of the method was carried out for both sample solutions as described under experimental work. The corresponding chromatograms and results are shown below.

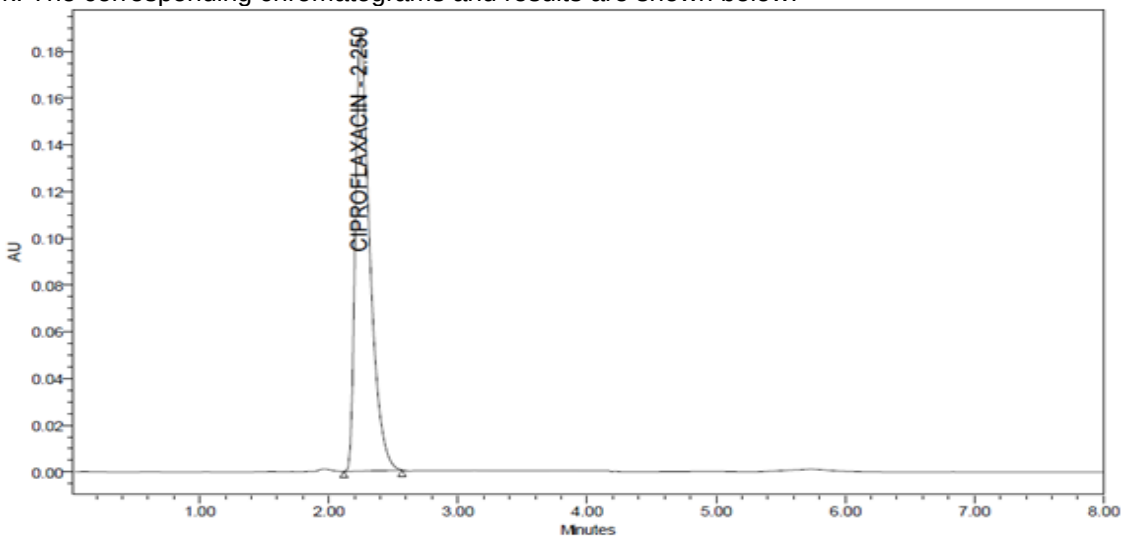

Fig. 6: Chromatogram for Precision -1

Table 6: Results of Precision for Ciprofloxacin

Injection	Area
Injection-1	5298687
Injection-2	5249785
Injection-3	5286744
Injection-4	5249078
Injection-5	5286744
Injection-6	5297463
Average	5278083.5
Standard Deviation	22769.3
%RSD	0.4

3. ACCURACY

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

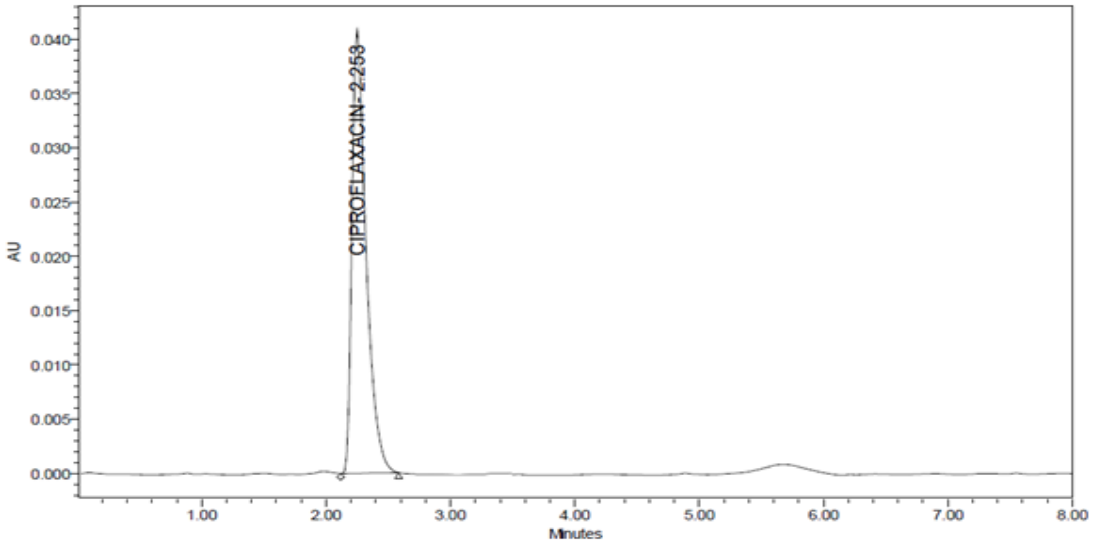

Fig. 7: Chromatogram for Accuracy 50%-1

Table 7: Accuracy (recovery) data for Ciprofloxacin

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	2640915.0	25	25.07	100.28	
100%	5271408.3	50	50.04	100.08	
150%	7952508.7	75	75.49	100.66	100.34

*Average of three determinations

SUMMARY AND CONCLUSION

The estimation of Ciprofloxacin was done by RP-HPLC. The assay of Ciprofloxacin was performed with tablets and the % assay was found to be 100.09 which show that the method is useful for routine analysis. The linearity of Ciprofloxacin was found to be linear with a correlation coefficient of 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.4 for Ciprofloxacin which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.4 for Ciprofloxacin which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 98.0% - 102.0%. The total recovery was found to be 100.34% for Ciprofloxacin. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

BIBLIOGRAPHY

1. Brahmankar DM and Sunil B Jaiswal, Biopharmaceutics and Pharmakokinetics, A Treatise, 1st ed, Vallabh Prakasan, Delhi. 2005;27:5-6.
2. James Swarbrick and James C Boylan., Encyclopedia of Pharmaceutical Technology. 2nd edition,1:8.
3. Christian Leuner and Jennifer Dressmann. Improving drug solubility for oral delivery using solid dispersions. European Journal of Pharmaceutics and Biopharmaceutics. 2000;50:47-48.
4. Leon Shargel and Andrew BC. Applied Biopharmaceutics and Pharmacokinetics, Appleton- Century-Crofts. 4th Ed, 1985;134.
5. Gare Kani HA, Sadeghi F, Badiee A, Mostafa SA and Rajabisiahboomi AR. Crystal habit modifications of Ibuprofen and their Physicochemical Characteristics. Drug Development and Industrial Pharmacy. 2001;27(8):803-809.
6. Leon Shargel and Andrew BC. Applied Biopharmaceutics and Pharmacokinetics, Appleton- Century-Crofts. 4th Ed, 1985;135.
7. Brahmankar DM and Sunil B Jaiswal. Biopharmaceutics and Pharmakokinetics, A Treatise, 1st ed, Vallabh Prakasan, Delhi. 2005;27:29-30.
8. Nandita G Das and Sudip K Das. Formulation of Poorly Soluble Drugs. Drug Delivery Report Spring/Summer. 2006;52-55.
9. Chiou WL and Riegelman SJ. J Pharm Sci. 1971;60:1283-1297.
10. James Swarbrick and James C Boylan. Encyclopedia of Pharmaceutical Technology. 2nd ed,1:641-647.
11. Christian Leuner and Jennifer Dressman. Improving Drug solubility for oral delivery using solid dispersions. European Journal of Pharmaceutics and Biopharmaceutics. 2000;50:48-51.
12. Modi A and Tayade P. Enhancement of dissolution profile of solid dispersion (Kneading) technique. AAPS Pharm Sci Tech. 2006;7(3):1-13.
13. Devarajan, Padma V and Gore SP. Melt-in-mouth tablets- innovative oral drug delivery systems. Express Pharma Pulse. 2000;7(1):16-18.
14. Reddy LH, Ghosh B and Rajneesh. Fast dissolving drug delivery systems: A Review of Literature. Indian J Pharm Sci. 2002;64(4):331-36.
15. Kuchekar BS, Bhise SB and Armugam V. Design of fast dissolving tablets. Ind J Pharm Edu. 2001;35(4):150-152.
16. Tetsuya O. Design of rapidly disintegrating oral tablets using acid-treated yeast cell wall: A Technical Note. AAPS PharmSci Tech. 2003;4(4).
17. Beatrice P. Formulation design of carbamazepine fast-release tablets prepared by melt granulation technique. Int J Pharma. 2003;256:53–63.
18. Abdelbary G. The preparation of orally disintegrating tablets using a hydrophilic waxy

binder. Int J Pharma. 2004;278:423–433.
19. Okuda Y. A new formulation for orally disintegrating tablets using a suspension spray-coating method. Int J of Pharma. 2009;382:80–87.
20. Sastry SV, Nyshadham JR and Joseph AF. Recent technological advances in oral drug delivery a review. PSTT. 2000;3(4).
21. Bandari S, Mittapalli RK, Gannu R and Rao YM. Orodispersible tablets: an overview. Asian J Pharm 2008 Jan [Cited 2008 Nov 9]. Available from URL: http://www.asiapharmaceutics.info.
22. Christinat R and Zulliger HW. Stability indicating HPLC-method for the determination of Ciprofloxacin in cream and lotion formulations, Arzneimittelforschung. 1984;34(5):551-3.
23. Asmaa A El-Zaher, Ehab F Elkady and Amaal A Al-Tahami. RP-HPLC-UV Detection Method For The Simultaneous Determinat Ion Of Ciprofloxacin, Triamcinolone Acetonide And Benzoic Acid In Ternary Mixture, Bentham Science Publishers. 2019;15(7):738-744.