A REVIEW OF ANALYTICAL METHODS FOR THE ESTIMATION OF LEVOFLOXACIN INPHARMACEUTICAL PREPARATION

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ABSTRACT

Levofloxacin also called L-ofloxacin is a fluroquinolone antibiotic. It is a broad spectrum antimicrobial agent. Levofloxacin is used to treat bacterial infections including acute bacterial sinusitis, pneumonia, urinary tract infections, chronic prostatitis and some types of gastro enteritis. Techniques like UV-visible spectrophotometry, High Performance Liquid Chromatography, High performance Thin Layer Chromatography, Spectrofluorimetry, Bio analytical methods etc have been used for analysis.UV-Visible spectroscopy HPLC methods have been used more widely.

Key words: Levofloxacin, HPLC, HPTLC, UPLC, UV-Spectrophotometry.

No: of Tables: 4 **No: of References: 19**

INTRODUCTION

Levofloxacin{ (2s)_7fluro-2-methyle-6-)4-methyle piperazine-1-yl)-10-0-1 azatricyclo-11-carboxylic acid}.is afluoroquinolone antibiotic also called L-ofloxacine.It is abroad spectrum antibiotic. It is a second generation antibacterial agent and are greatly effective against gram positive and negative bacteria. It acts by inhibiting bacterial DNA gyrase which is required for DNA replication and thus causes bacterial lysis. Levofloxacin (C18 H20 F N3 O4) have 6-fluoro and 7-piperazinyl groups.It greatly increases antibacterial activity. Levofloxacin is used to treat bacterial infections including acute bacterial sinusitis,pneumonia, urinary tract infection, chronic prostatitis etc.Common side effects include nausea, diarrhoea and sleep trouble. Mechanism of action is by inhibiting bacterial type 2 top isomerises 4, DNAgyrase.Levofloxacin like other fluoroquinolone inhibits the subunits of DNAgyrase,two subunits super coiling and rescaling DNA replication and transcription is inhibited. Various analytical methods have been reported for the estimation of Levofloxacin in dosage forms or in biological samples. A review of various analytical methods reported for the estimation of levofloxacin is presented here. Mainly UV Spectrophotometric methods. [1,2,3], HPLC methods [7,8,9], HPTLC methods[10], fluorimetric methods[11,12,13],bio analytical methods[14,15,16], and UPLCmethod[18] have been reported. Apart from this bio analytical methods for the estimation of Levofloxacin in blood, a stability indicating analytical method and visible spectrometric method also been reported.

ANALYTICAL METHODS FOR ESTIMATION OF LEVOFLOXACIN

UV Visible spectrophotometry

Levofloxacin is a Fluroquinoloneantibiotic.Soluble in water PH 6.7 and it have two pka values, 5.59 and 7.94.Water can be used as solvent in UV Spectrophotometry as it is soluble in water. Hence many of the published methods used water as solvents[1,2,3].However in one method [6] 0.1M hydrochloric acid is used as solvent. Perhaps it may be due to the fact that Pka values of Levofloxacin is 5.59 and 7.94 and it is preferable to keep Pka value two units away from Pkavalue.When water is used as solvent Levofloxacin exists in the mixture of ionised and unionised forms. In one publication[1] mixture of acetonitrile: methanol: water has been used as the solvent. In the reported methods linearity of the response is appropriate 2-12 µ/ml.Visible spectrophotometric method has also been reported which is based upon the reaction between 2,4–dinitrophenylhydrazine with the carbonyl group of Levofloxacin to give a condensation product. The resulting product exhibited absorption maximum of 510nm.Visible Spectrophotometric method based on reaction between Levofloxacin and 2,4-dinitrophenol to give resulting compound exhibits absorption maximum of 510nm.[1,2,3]

Solvent	Water:methanol: acetonitrile	Distilled water	Water	Water	Distilled water	0.5M Hcl
Λmax	292 nm	291.2	286.4	288nm	289nm	290nm
Linearity	1-12µg/ml	2-10µg/ml	4-10µg/ml	2-10µg/ml	0.5-8µg/ml	0.25-12µg/ml
Reference	1	2	3	4	5	6

Table 1: Comparative account of UV Spectrophotometric method for the estimation of Levofloxacin

CHROMATOGRAPHIC METHODS

High Performance Liquid Chromatography (HPLC)

HPLC is an advanced form of liquid chromatography used for separating the complex mixture of molecules in chemical or biological systems which is very useful in quantify, separate and detect the drug,its various impurities and drug related degrades that can form on storage or synthesis. A number of chromatographic parameters like mobile phase, column, column temperature, detector wavelength and flow rate are to be optimised.[19]

Table 2: Comparative account of HPLC methods for the estimation of Levofloxacin

Column	C18	C18	C18
Mobile Phase	Buffer :Acetonitril 85:15	Methanol : Water (70:30 v/v)	Water: Acetonitrile:Phosphoric acid(0.025M)pH adjusted to 3 by using Triethylamine(60:20:20 v/v/v)
Buffer	0.05M citric acid 84ml 1ml ammonium acetate PH adjusted 2.9 with KOH solution	-	-
Flow Rate	1ml/min	1ml/min	
Temperature	Room temperature	Room temperature	Room temperature
Detector Wavelength	293nm	294nm	UV Detector-294nm UV Visible detector-202nm
Retention time	15.7min	2.1min	-
Reference	7	8	9

High Performance Thin Layer Chromatography (HPTLC)

It is the advanced technique used for drug analysis. HPTLC is a fast separation technique. It is very flexible to analyse a wide variety of samples. This technique is very simple to handle and requires a short time to analyse. It is suitable for both quantitative and qualitative analysis.HPTLC is used for analysing Levofloxacin.[19] Chromatographic method is summarised in table 3:

Table 3: HPTLC method for the estimation of Levofloxacin

Column	Mobile Phase	Detector Wavelength	Reference
Merck TLC aluminium sheets of Silica gel 607_{254}	n-butanol:Methanol:Ammonia (5:1:1.5v/v/v)	298 nm	10

Spectrofluorimetry

Spectrofluorimetry is one of the important analytical methods, more sensitive and selective than UV-spectrophotometry and the sensitivity is almost equal to that of chromatography. It is based on the principle of absorption of UV/ Visible radiation causes transition of electrons from singlet ground state to singlet excited state. As this state is not stable, it emits energy in the form of UV /visible radiation and returns to singlet ground state. This study or measurement of this emitted radiation is called Fluorimetry .240-370nm is the excitation range obtained for all spectral excitation emission matrices(EEM) and 380-550nm is the emission range. Two spectrofluorimetric methods are used to the determination of levofloxacin in spiked human urine and tablets.The first method permits the determination of levofloxacin in aqueous solution using zero order caliberation.The parallel factor analysis with standard additions is the second method used for the determination of levofloxacin in urine. Using linear regression and the standard additions method levofloxacin human urine is quantified.The presence of interferences makes the spectrofluorimetric method difficult. Various studies have been conducted in previous years to overcome this problem by merging spectrofluorimetric data and three way chemometric tools, mainly parallel factor analysis, so that complicated steps can be eliminated by replacing physical separation of interferences by a mathematical separation of their signals. It is used for the minimal sample manipulation.[11,12,13]

Spectroflourimetric methods are summarised in table given below.

SOLVENT	Chloranilic acid	Methanol,ethanol,acetonitrile, Isopropanol,acetone,chloroform	Distilled water
Excitation wavelength	285-330nm	275-290nm	330nm
Emission wavelength	445-492nm	450-470nm	-
Linearity	20-1000ng/ml	0.02-3.1µgm/ml	0.04~4.0µgm/ml
Reference	11	12	13

Bio-analytical methods

Comparative account of Bio Analytical Methods for the estimation of Levofloxacin in biological samples is given in the table 4:

Table 4: Comparative account of Bio Analytical Methods for the estimation of Levofloxacin in biological samples.

Parameters/Attributes	1	2	3	4
Column	C18	C18	C18	C18
Sample Preparation	Liquid-Liquid extaction	Sloid-liquid extraction	Sloid-liquid extraction	Liquid-Liquid extaction
Mobile Phase	Acetonitrile:water PH 3.5 orthophosphoric acid(80:20v/v)	80:20v/v Phosphate buffer PH 2.5,acetonitrile	Acetonitril gradient of 5-75% with 10mM monobasic potassium phosphate of PH 3.5	Methonol:water 70:30(v/v)
Buffer	-	-		-
Flow rate	1.4ml/min	1ml/min	1ml/min	1ml/min
Temperature	Ambient	Room temperature	30c	Ambient
Detector wavelength	296nm	235nm	Levofloxacin – 295nm PHN-260nm	294nm

Retention time	Plasma-1.382 Saliva-1.384	Levofloxacin:5.9 (+/-)0.05min Internal standard: 10.1(+/-) 0.03min -	6.199min	2.1min
Reference	14	15	16	17

UPLC

UPLC method has been reported by Himanshu Gupta etal in a single reverse phase UPLC method for quantification of levofloxacin in aqueous humour and pharmaceutical dosage forms.It is a method made to develop a single specific and sensitive gradient reversed phase ultra performance liquid chromatographic method for quantitative analysis of levofloxacin.This single method developed is applied for the quantification of levofloxacin both in aqueous humour as well as pharmaceutical dosage forms. Chromatographic separation of levofloxacin was achieved on a Waters Aquity HSS T-3column(100x2.1mm,1.8µ) within a short run time of 5 min.Mobile phase used is mixture of 0.1% aqueous trifluroacetic acid and acetonitrile.Flow rate is 0.45 ml/min.Column temperature is maintained at 50°C.Volume injected is 2µl.Linearity was found to be 70-130% v/v.Stress studies are carried out by acid degradation,oxidative degradation and reductive degradation by using 1N hydrochloric acid,1N sodium hydroxide 30%hydrogenperoxide and 10% sodium thiosulphate respectively. Analysis was performed on pure samples bulk samples and aqueous humour samples of levofloxacin and the method is validated[18].

CONCLUSION

The present review highlights on various analytical methods reported on levofloxacin HPLC, HPTLC, UV Spectrophotometry, Fluorimetry, UPLC and bioanalytical methods etc. were used for the analysis of the same. Among these HPLC-UV methods were found to be most widely used.HPLC method is frequently used because of high sensitivity, specificity and better separation efficiency. These chromatographic methods are rapid and far more economical. The presented information is useful for the researchers.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Department of Pharmaceutical Analysis, St. James College of Pharmaceutical Sciences, Chalakudy ,Kerala, India for providing necessary facilities to carry our review work

CONFLICT OF INTEREST

We declare we have no conflict of interest

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