IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
August 2016 Vol.:7, Issue:1

Development and Validation of Stability Indicating HPTLC Technique for Determination of Lomefloxacin Hydrochloride in Pharmaceutical Dosage Form

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

[1] Swati Umesh Taksande., [2]Manisha P. Puranik

[1] Dadasaheb Balpande College Of Diploma In Pharmacy,Besa, Nagpur

[2]Institute Of Pharmaceutical Education And Research Borgaon(Meghe)Wardha

Submission: 1 August 2016
Accepted: 7 August 2016
Published: 25 August 2016

Keywords: Lomefloxacin HCl, stability, fluroquinolone antimicrobial, antibiotic

ABSTRACT

Lomefloxacin HCl is a fluroquinolone antimicrobial antibiotic. Used in treatment of respiratory tract, anthrax, biliary tract, and urinary tract infection, gastroenteritis (diarrhea, cholera, and salmonella enteritis).The present study was undertaken with the primary objective to study forced degradation behavior of lomefloxacin HCl by HPTLC and development of validated stability indicating assay method and to make this method accessible to scientific community. In present study, comprehensive stress testing of lomefloxacin HCl was carried out according to the ICH guidelines Q1A(R2). The drug was subjected to acid (0.1N HCl), neutral and alkaline (0.1NNaOH) hydrolytic conditions as well as to oxidative decomposition at room temperature. Photostability study was also carried out. Additionally, the solid drug was subjected to 50^0C for 90 days in the oven and to the combined effect of temperature and humidity at 40^0C/75% RH. The products formed under different stress conditions were investigated by HPTLC. The HPTLC method that could separate all degradation products formed under various stress conditions and a mobile phase consisting of methanol:ammonia [7:3 v/v]. The detection was carried out at 288 nm. The result of analysis of HPTLC method was validated in terms of accuracy, precision, ruggedness, linearity and range, limit of detection and limit of quantification. The methods were found to be sensitive, accurate, reliable, reproducible, rapid and economic. Based on the HPTLC studies it can be concluded that lomefloxacin undergo more degradation in oxidative stress studies and hydrolysis studies specially the acidic and the alkaline hydrolysis studies. The proposed HPTLC method proved to be effective for the determination of lomefloxacin HCl during stability testing of the bulk as well as pharmaceutical dosage form. Forced degradation studies revealed that possible degradation products do not interfere with the determination of lomefloxacin HCl.

INTRODUCTION

Stability studies[12, 13, 14]

Stability of a pharmaceutical preparation can be defined as "the capability of a particular formulation in a specific container/closure system to remain within its physical, chemical, microbiological therapeutic and toxicological specifications throughout its shelf life." The purpose of the stability study of a pharmaceutical product is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, and to establish the shelf life for the drug product and the recommended storage conditions. Forced degradation studies are the part of drug degradation strategy being undertaken to elucidate the intrinsic stability of the drug. Such studies are therefore conducted under more severe and exaggerated conditions than those usually used for long term stability studies. The ICH drug stability test guideline requires that the analysis of stability sample should be done through the use of validated stability indicating analytical method.

TableNo.1:ICH Stability testing guidelines[16]

Topic	Title
Q1A	Stability testing of new drug substances and products
Q1B	Photostability testing of new drug substances and products
Q1C	Stability testing of new dosage forms
Q1D	Bracketing and matrixing designs instability testing of new drug substances and products.
Q1E	Evaluation for stability
Q1F	Stability data package for registration applications in zones III and IV

LITERATURE SURVEY

Greici C. G *et al*[33], **(2005)** published, "Validation of UV Spectrophotometric Method for Determination of Lomefloxacin Pharmaceutical Dosage Form."Beer's law was obeyed in the range 2.0 - 9.0 µg.mL–1 at max 280 nm. Mean correlation coefficient 0.9997. Suhagia B.N *et al*[35], **(2000)** published, "Spectrophotometric estimation of Lomefloxacin hydrochloride in pharmaceutical dosage form."The method was based on the reaction between the drug and the dichlone, in the presence of crotonaldehyde in dimethylsulfoxide, which produces a blue chromogen with absorption maximum at 645 nm.

OBJECTIVE

The present study was undertaken with the primary objective to study forced degradation behavior of Lomefloxacin HCl by HPTLC and development of validated stability indicating assay method and to make this method accessible to scientific community.

EXPERIMENTAL

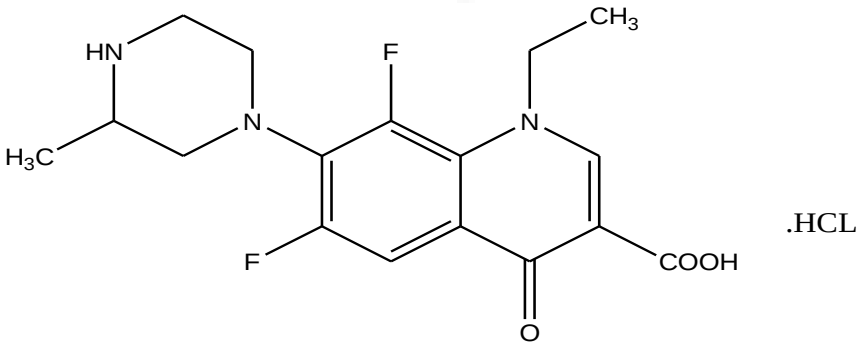
Drug Profile

Instruments

Reagents and Chemical

DRUG PROFILE: [25, 26, 27]

LOMEFLOXACIN HYDROCHLORIDE



.HCL

Chemical Name : 3-quinoline carboxylic acid, (±)-1-ethyl-6,8-difluro-1,4-dihydro-7-(3-methyl-1-piperazinyl)-4-oxo,monohydrochloride.

Molecular Formula : $C_{17}H_{19}F_2N_3O_3 \cdot HCl$

Molecular Weight : 387.81

Description : colorless needles; melts about 295º with decomposition

Solubility : Soluble in water, methanol.

Use : Lomefloxacin is a fluoroquinolones antibacterial. It is used in the treatment of respiratory tract, anthrax, biliary tract, and urinary tract infection, gastroenteritis (diarrhea, cholera, and salmonella enteritis).

Table No.2: Details of Marketed formulation

Drug	Lomefloxacin HCl
Brand	Lomef-400
Label claim	Lomefloxacin HCl – 400mg
Manufactured by	Torrent Pharmaceuticals, Gujarat, India.
Average weight	0.6214 g

Table No.3: Details of procured drugs

Drug	**Procured from**	**Assay result**	**Used as**
LomefloxacinHCl	Dr.Reddy's Lab.Ltd.,Hyderabad, (A.P.) India.	99.81%	Standard
	Nakoda Chemicals Ltd., Hyderabad (A.P.), India.	-	Sample

RESULTS

Estimation of Lomefloxacin Hydrochloride in tablet dosage form by HPTLC method

1. Determination of wavelength for detection of LOM

Preparation of Standard stock solution of Lomefloxacin for scanning

Standard stock solution (S)

An accurately weigh quantity about 100 mg of Lomefloxacin was transferred in a 100 ml volumetric flask, dissolved in sufficient quantity of methanol and volume was made up to the mark with the help of methanol. (1000 µg/ml)

Working standard solution (S1)

A 1.0 ml portion of the above standard solution(S) was diluted up to 10.0 ml with the help of methanol. (10µg/ml).The solution was scanned in the range of 400-200 nm in 1.0 cm cell against reagent blank. The spectrum of LOM is shown in fig.no.1.

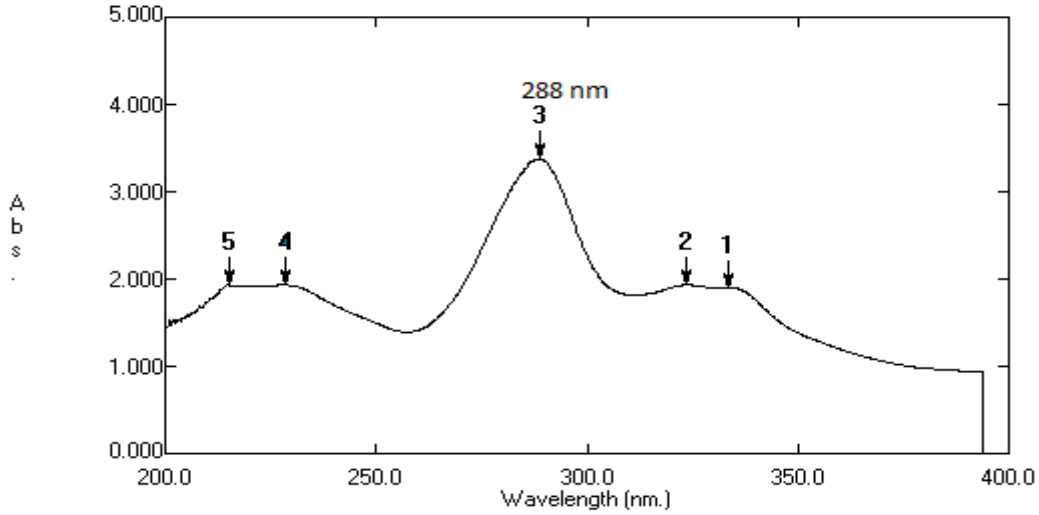


Fig.No.1: Spectra of LOM showing λmax of LOM = 288.0 nm

The suitable wavelength selected for detection of LOM HCl from the spectrum was 288nm.

2. Selection of Mobile Phase

The following mobile phases were tried:

Sr.no	Mobile phase	Remark
1	Chloroform:Methanol:Ammonia(10:7:3v/v/v)	Diffused peak
2	Butanol:Acetic acid: water(8:1:1v/v/v)	Spot not found.
3	Butanol:Methanol:Ethylacetate:6MAmmonia(4:2:3:2v/v/v/v)	Tailing of peak.
4	Butanol: Ammonia(7:3v/v)	Typical peak nature missing.
5	n-propyl alcohol: Ammonia(7:3v/v)	Diffused peak.
6	Methanol: Ammonia(7:1v/v)	Asymmetric peak
7	Methanol: Ammonia(7:3v/v)	Sharp, symmetric peak.

From the various mobile phases tried mobile phase containing methanol: ammonia (7:3v/v) with 30 min time of saturation with filter paper with plate equilibrium was selected and it was used throughout the further experimentation.

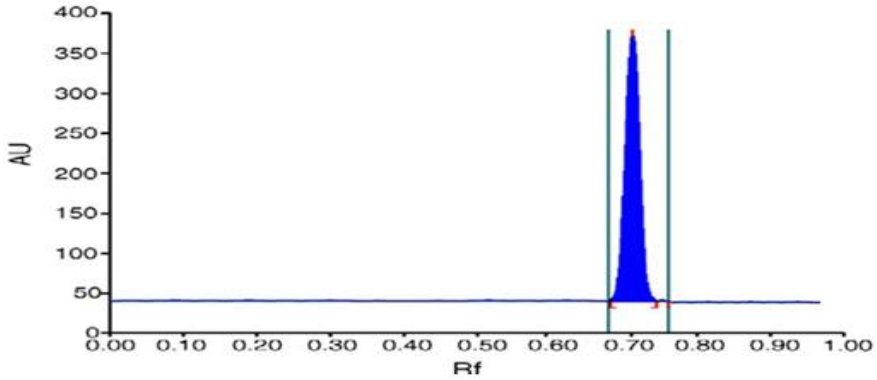


Fig.No.2: Chromatogram of Lomefloxacin with selected mobile phase ethanol: ammonia (7:3v/v).

3. Preparation of standard calibration curve

Standard stock solution:

An accurately weighed quantity of LOM equivalent to 20 mg was dissolved in methanol and volume was made up to 100 ml with methanol (200µg/ml).

Aliquot portions of standard stock solutions of LOM were further diluted with methanol to get 20 µg/ml above solution was applied as bands ranging from 1-10 µl on TLC plate with Linomat V. The plates were developed in Twin trough chamber, already saturated with mobile phase for 30min. After drying it was evaluated densitometrically. The observations are shown in Table No.4.

Table No.4: Observations of standard calibration curve

Sr.no.	Volume applied(µl)	Conc./band(µg)	Peak Area
1	2	0.04	2652.63
2	3	0.06	3991.37
3	5	0.10	5681.64
4	7	0.14	6753.45
5	9	0.18	8198.65
6	10	0.20	8627.97

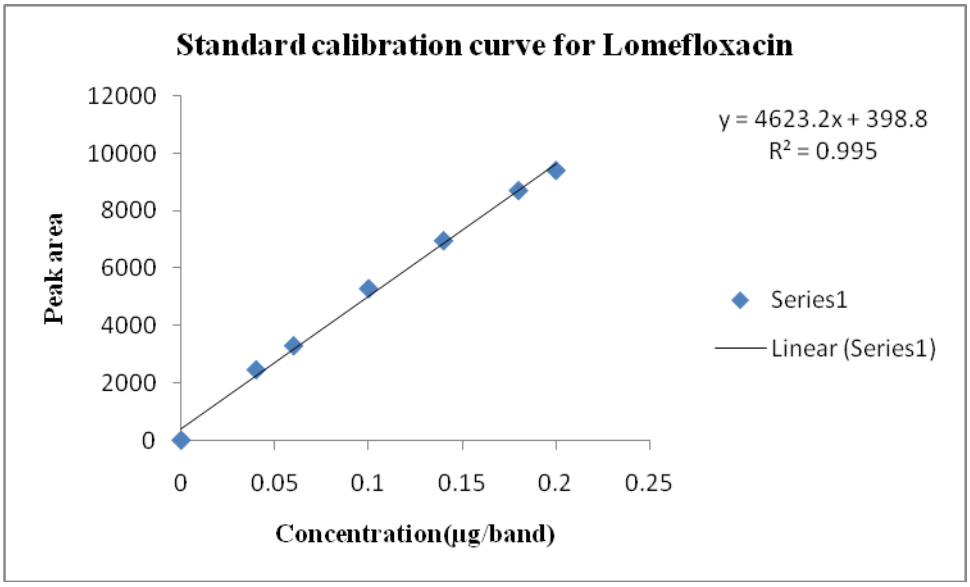


Fig.No.3: Standard calibration curve of Lomefloxacin.

4. System suitability test:

Table No.5: System Suitability Parameters

Sr.no	Asymmetry	Selectivity	Retention Factors	Resolution	Capacity Factor
1	0.8824	1.91	0.69	3.948	4.618
2	0.9109	1.95	0.70	3.623	3.961
3	0.9212	1.98	0.69	2.984	4.608
4	0.8629	1.95	0.69	3.829	4.529
5	0.8631	1.96	0.70	3.686	4.573
Mean	0.8881	1.95	0.694	3.614	4.4578
± S.D.*	0.026963	0.025495	0.005477	0.374148	0.279899
R.S.D.*	0.03036	0.0130740	0.007892	0.10352	0.062789
C.V.*	3.036	1.3074	0.7892	10.352	6.2789

Where, S.D.*=Standard Deviation, R.S.D.*= Relative Standard Deviation, C.V. = Coefficient Variance

5. Analysis of laboratory mixture by proposed method

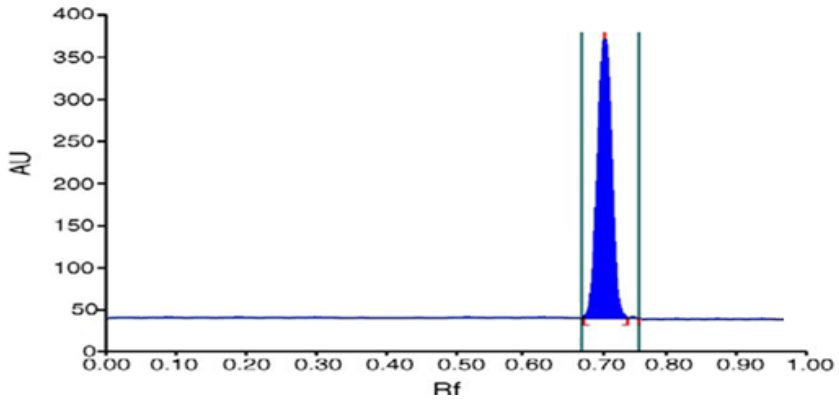


Fig.No.4: Chromatogram of Lomefloxacin (Rf 0.70) in laboratory mixture.

Amount of drug in laboratory mixture was calculated using following formula.no.1

$$\% \text{ Estimated} = \frac{\text{Peak Area}_{(\text{Sample})}}{\text{Peak Area}_{(\text{STD})}} \times \frac{\text{Wt.}_{(\text{STD})}}{\text{Wt.}_{(\text{Sample})}} \times 100 \qquad (1)$$

Table No.6: Result of estimation of LOM in Laboratory mixture

Sr no.	Laboratory mixture	Wt. of LOM taken(gm)	Peak area	% Estimated
1	Standard	0.0201	5683.72	-
2	Sample	0.0203	5698.63	99.27
		0.0207	5841.58	99.79
		0.0209	5901.15	99.85
		0.0204	5778.24	100.17
		0.0202	5672.49	99.81
			Mean	99.778
			±S.D.	0.3233
			R.S.D.	0.00324
			C.V.	0.3240

6. Analysis of marketed formulation by proposed method

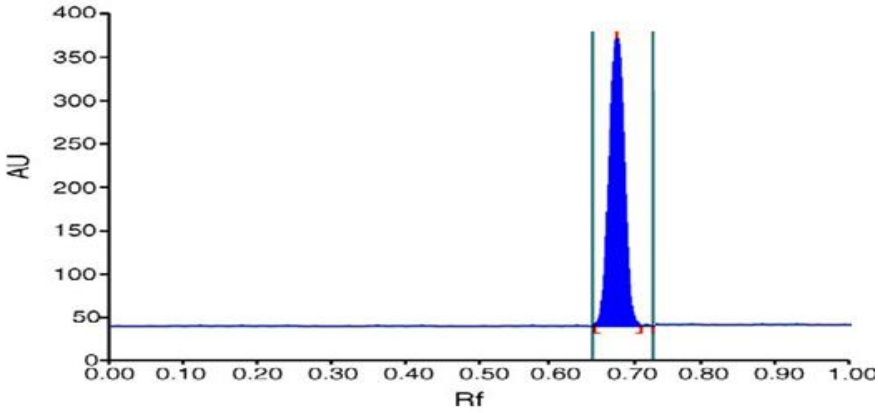


Fig .No.5: Chromatogram of Lomefloxacin (Rf 0.68) in marketed formulation.

The amount of LOM in the tablet formulation was calculated using formula no.2.

$$\%\,\text{Estimated} = \frac{\text{Peak area}_{(\text{Sample})}}{\text{Peak area}_{(\text{Std})}} \times \frac{\text{Wt}_{(\text{Std})}}{\text{Wt}_{(\text{Sample})}} \times \frac{\text{Avg wt}}{\text{Lable claim}} \times 100 \quad (2)$$

Results of estimation of LOM in tablet formulation are shown in Table No.7.

Table No.7: Result of estimation of LOM in tablet formulation

Brand Name: Lomef-400			**Average weight:0.6214 gm**	
Sr. no.	**Sample**	**Wt. of LOM taken(gm)**	**Peak area**	**% Estimated**
1	Standard	0.0201	5683.72	-
2	Tablet	0.0321	5756.68	98.61
		0.0312	5639.57	99.29
		0.0334	5993.25	98.56
		0.0324	5816.38	98.73
		0.0309	5614.21	99.79
			Mean	99.016
			±S.D	0.5199
			R.S.D	0.005251
			C.V	0.5251

7. Validation:

7.1. Accuracy:

Accuracy of an analytical method is the closeness of test results obtained by the method to the true value. It was ascertained on the basis of recovery studies performed by standard addition method.

7.1.1. Recovery study

Table No.8: Result of estimation of recovery study:

Sr. no	Wt of tablet powder (gm)	Level of accuracy	Amt of pure drug added(gm)	% found on preanalysed basis	Peak area	% recovery
1	0.0309	80%	0.016	99.79	9288.56	99.58
2	0.0312	100%	0.020	98.61	9563.79	99.23
3	0.0324	120%	0.024	98.73	9989.54	98.87
					Mean	99.226
					±S.D	0.3550
					R.S.D	0.003578
					C.V	0.3578

7.2. Precision:

Precision of an analytical method is the degree of agreement among individual results when the method is applied repeatedly to multiple readings of a homogeneous sample. It is expressed as S.D. or R.S.D. of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method. Results of precision study are shown in Table No.6.

7.3. Ruggedness:

The studies of ruggedness were carried out under two different conditions:

7.3.1. Different Days

- Interday
- Intraday

7.3.2. Different Analyst.

7.3.1.Different days:

Interday study: The results are shown in Table No.9.

Table No.9: Results of Interday study

Sr.no	Wt.of tablet powder (gm)	Day	Peak area	%label claim
1	0.0324	1	5829.81	99.76
2	0.0312	2	5642.21	99.34
3	0.0321	3	5779.92	98.91
			Mean	99.336
			±S.D.	0.4250
			R.S.D.	0.004278
			C.V	0.4278

Intraday study: The results are shown in Table No. 10.

Table No.10: Results of Intraday study

Sr.no	Wt.of tablet powder (gm)	Time(hrs)	Peak area	%label claim
1	0.0324	0	5831.32	98.52
2	0.0312	3	5639.87	99.49
3	0.0321	6	5781.13	98.92
			Mean	98.976
			±S.D.	0.4875
			R.S.D.	0.004925
			C.V	0.4925

Different analyst: The results are shown in Table No. 11.

Table No. 11: Results of different analysts

Sr.no	Analyst	Wt of tablet powder(gm)	Peak area	% label claim
1	Analyst 1	0.0325	5865.24	99.13
2	Analyst 1	0.0322	5792.39	98.51
			Mean	98.82
			±S.D	0.4384
			R.S.D	0.004436
			C.V	0.4436

7.4. Linearity and range:

The observations are shown in Table No.12.and graphs are depicted in Fig.No. 9.

Table No.12: Observations of linearity and range study

Sr.no	% label claim of LOM	Peak area
1	80%	4524.59
2	90%	5110.42
3	100%	5678.24
4	110%	6136.69
5	120%	6513.88

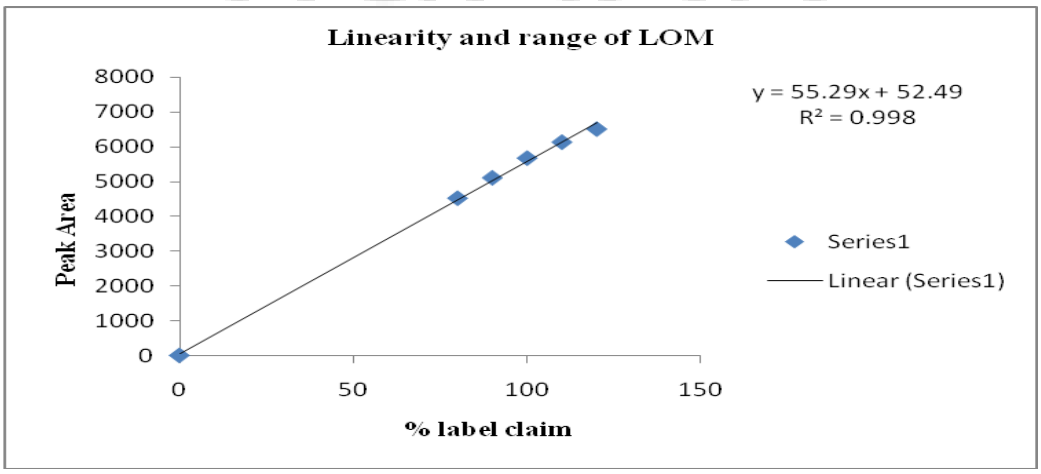


Fig.No.6: Plot of Linearity and range of LOM.

7.5. Limit of Detection (LOD) and Limit of Quantification (LOQ)

Table No.13: Observations of LOD and LOQ study

Parameters	Lomefloxacin
LOD (ng/band)	0.9053
LOQ (ng/band)	2.3852

8. Analysis of stressed samples by HPTLC method:

All stressed samples were analyzed by the proposed method and the % drug to be remained was calculated from Standard calibration curve.

8.1. Hydrolysis study

8.1.1. Acid Hydrolysis

The results of acid hydrolysis study are presented in Table No.14.

Table No.14: Results of Acid hydrolysis study

Study	Sample	Conc. of reactant	Drug conc.	Exposure time	Testing interval (hrs)	%Drug to be remained	
						Bulk	Tablet
Acid Hydrolysis	LOM bulk drug and tablet	0.1N HCl	0.2mg/ml	8 hrs	0	99.38	98.53
					4	84.41	79.06
					8	69.39	65.77

8.1.2. Alkali Hydrolysis:

The results of alkali hydrolysis study are presented in Table No.15.

Table No.15: Results of Alkali hydrolysis study

Study	Sample	Conc. of reactant	Drug conc.	Exposure time	Testing interval (hrs)	%Drug to be remained	
						Bulk	Tablet
Alkali Hydrolysis	LOM bulk drug and tablet	0.1N NaOH	0.20mg/ml	8 Hrs	0	98.65	98.31
					4	85.44	81.44
					8	69.09	63.27

8.1.3. Neutral hydrolysis:

The results of neutral hydrolysis study are presented in Table No.16.

Table No.16: Result of neutral hydrolysis study

Study	Sample	Drug conc.	Exposure time	Testing interval (hrs)	%Drug to be remained	
					Bulk	Tablet
Neutral Hydrolysis	LOM bulk drug and tablet	0.20mg/ml	8 Hrs	0	98.76	98.89
				4	89.46	85.28
				8	76.48	73.47

8.2. Oxidation studies:

Under oxidation studies we perform following tests:

- ✓ Peroxide test
- ✓ Radical initiation test

8.2.1. Peroxide Test:

This test was performed on Lomefloxacin bulk drug as well as on marketed formulation. The reaction was made with 3% hydrogen peroxide solution for 7 days at ambient temperature. The results ofPeroxide Test study are presented in Table No.17.

Table No.17: Resultsof Peroxide Test study

Study	Sample	Conc. of reactant	Exposure time	Testing interval (days)	%Drug to be remained	
					Bulk	Tablet
Oxidation study	LOM bulk drug and tablet	3% H_2O_2	7 day	0	98.73	99.21
				1	80.42	74.45
				7	56.98	36.96

8.2.2. Radical Initiation test:

The typical radical initiator ACN: Water in ratio 0f 50:50 was used for this study. This test is generally performed on bulk drug only.

The results of Radical Initiation test are presented in Table No.18.

Table No.18: Resultsof Radical Initiation test

Study	Sample	Conc. of reactant	Exposure time	Testing interval (Days)	%Drug to be remained
					Bulk
Oxidation study	LOM bulk drug	50:50 v/v	7 days at 40°C	0	99.42
				1	96.17
				7	73.84

8.3. Photostability studies (PH):

Photostability study was followed as per an ICH Q1B guideline that is: Stability Testing: Photostability testing of New Drug Substances and Products.

The results of Photostability study are presented in Table No.19.

Table No.19: Results of Photostability study

Study	Sample	Exposure time	Testing interval (day)	%Drug to be remained	
				Bulk	Tablet
Photostability study	LOM bulk drug and tablet	15 days at ambient temp	0	99.42	99.67
			1	97.87	98.21
			15	62.16	76.58

8.4. Thermal Stability Study (Dry Heat):

The results of Thermal Stability Study are presented in Table No.20.

Table No.20: Results of Thermal Stability Study

Study	Sample	Exposure time	Temp ^{0}C	Humidity % RH	Testing interval (days)	%Drug to be remained	
						Bulk	Tablet
Thermal study	LOM bulk drug and tablet	3 month	40	75	0	98.86	98.79
					90	54.58	47.60

8.5. Humidity study:

The solid drug substances and drug products were subjected to 40^{o}C/75% RH for 3 month in the stability chamber.

The results of Humidity Stability Study are presented in Table No.21.

Table No.21: Resultsof Humidity study

Study	Sample	Exposure time	Humidity % RH	Testing interval (days)	%Drug to be remained	
					Bulk	Tablet
Humidity study	LOM bulk drug and tablet	3 month	75	0	99.53	98.89
				7	98.23	95.93
				45	91.29	86.91
				90	55.21	53.62

DISCUSSION AND CONCLUSION

The present study deals with degradation behavior of lomefloxacin HCl by HPTLC technique and development of validated stability indicating method.

Table.No.22: Statistical summary of HPTLC method

Sr.no	Parameters	Statistical data	HPTLC method
1	Standard laboratory mixture	Mean	99.778
		±S.D	0.3233
		R.S.D.	0.00324
2	Marketed formulation	Mean	99.016
		±S.D	0.5199
		R.S.D.	0.005251
3	Recovery study	Mean	99.226
		±S.D	0.3550
		R.S.D.	0.003578
4	Interday	Mean	99.336
		±S.D	0.4250
		R.S.D	0.004278
5	Intraday	Mean	98.976
		±S.D	0.4874
		R.S.D	0.004925
6	Different analyst	Mean	98.82
		±S.D	0.4384
		R.S.D	0.004436

Stress studies:

HPTLC studies on Lomefloxacin under different stress condition suggested the following degradation behavior.

1. Hydrolysis study:

A. Acidic condition:

In HPTLC method after 8hr 69.39% of pure drug remained and in marketed prepration 65.77 % of drug remained .This shows that drug is less prone to acid hydrolysis. The bulk drug on refluxing in 0.1 N HCl for 8 hrs resulted in the formation of two degradation products with Rf value 0.80 and 0.81 respectively. In case of tablet formulation resulted in the formation of three degradation product with Rf value 0.80, 0.81 and 0.20 respectively.

B. Alkali hydrolysis:

In HPTLC method after 8 hrs 69.09%, while in tablet after 8hrs 63.27 % was remained. In case of pure drug, formation of three degradation products with Rf value 0.32 and 0.43 was observed and in case of tablet formulation two degradation products with Rf value0. 31 and 0.41 was observed.

C. Neutral condition:

The drug and tablet was subjected to neutral hydrolysis for 8 hrs. In case of pure drug two degradation products with Rf value 0.28 and 0.59 respectively were observed and after 8 hrs 76.48% pure drug was remained. While in case of tablet formulation two degradation product Rf values 0.28 and 0.57 were observed. In case of tablet after 8 hrs 73.47% drug was remained.

2. Oxidative condition:

A) Peroxide study:

The drug as well as tablet formulation were subjected to peroxide study for 7 days. After 7 day pure drug shows three minor degradation products with Rf value 0.26, 0.27 and 0.45 respectively. In case of tablet formulation after 7 days two degraded product having Rf value 0.45 and 0.53 was seen. . After 7 days nearly 56.98 % of the pure drug was remained and in

case of tablet 46.96% of drug was remained. The result shows that Lomefloxacin was unstable to 3% H_2O_2 .

B) Radical Initiation test:

In case of radical initiation test for pure drug after 7 day two degradation product having Rf value 0.38, and 0.54 were seen. Around 73.84 % drug was remained.

3. Photostability studies:

After Photostability study pure drug shows two degraded product peak at Rf value 0.27, and 0.50 respectively while in tablet formulation degradation product peak were observed at Rf value 0.27 and 0.48 respectively. After 15 days 62.16 % of pure drug was remained and 76.58 % of drug was remained in case of tablet formulation.

4. Thermal studies:

Pure drug show three degradation peak at Rf value 0.39, 0.40 and 0.83 while in case of tablet formulation three peaks observed at Rf value 0.39, 0.40 and 0.81 respectively. Nearly 54.58 % of pure drug was remained while 47.60 % of drug was remained in case of tablet formulation. More degradation was seen in case of tablet as compared to the pure drug.

5. Humidity studies:

The drug as well as tablet was kept at 40^0 C /75% RH for 3 months 55.21 % of pure drug was remained while 53.62 % of drug was remained in case of tablet formulation. For pure drug two degradation peaks at Rf value 0.22 and 0.23 were observed and for tablet formulation three peaks observed at Rf value 0.22, 0.23 and 0.11 respectively.

CONCLUSION

In this study it was possible to develop a selective and validated stability indicating HPTLC method for Lomefloxacin HCl which could analysed the drug and its degradation products formed under a variety of stress condition under various ICH guidelines.

Based on the the HPTLC studies it can be concluded that lomefloxacin was found to be unstable more in the solution state as compared to the solid state. It can also be concluded that the drug undergo more degradation in oxidative stress studies and hydrolysis studies especially the acidic and the alkaline hydrolysis studies. The proposed HPTLC method

proved to be effective for the determination of lomefloxacin HCl during stability testing of the bulk as well as pharmaceutical dosage form. Forced degradation studies revealed that possible degradation products do not interfere with the determination of Lomefloxacin HCl.

REFERENCES

1. Sharma P. P., (2001). How to practice GMPs, 3rd edition, Vandana publications; p 214.
2. Kasture A. V. Wadodkar, S. G., Mahadik, K. R. and More, N. Pharmaceutical analysis instrumental methods, (2005) 12th Ed., Nirali prakashan, Pune; p 148, 156.
3. Sharma BK. Instrumental methods of chemical analysis**,** 23rd ed., Goel publishing house, Meerat. 2004; p 54-83.
4. Beckett, A.H., Stenlake J.B., Practical Pharmaceutical Chemistry, (1997), 4th edition, CBS Publishers and distributors.vol. II, 1997; p 275-337.
5. Chatwal GR, Anand SK. Instrumental methods of chemical analysis. Mumbai: Himalaya Publishing House; p 2.624.
6. Reich E., Schibli A., High Perormance Thin Layer Chromatography or the analysis of medicinal plant,(2007), 190-227.
7. Willard HH, Merritt LL, Dean JA and Settle EA. Instrumental Methods of Analysis.7th ed. New Delhi: CBS Publishers and distributors.
8. Skoog D. A., West D. M., Holler F. J., Fundamentals of Analytical Chemistry, (1992) Saunders College Publishing, Fort Worth, US.
9. Jeffery G.H., Bassett J., Vogel's textbook of Quantitative Chemical Analysis,5th edition,(1991),217-235.
10. Berry IR., Nash RA, Pharmaceutical Process Validation, Revised and expanded 57, 2nd ed, 1993; p28-42.
11. Dr. Sethi P.D., Quantitative analysis of Pharmaceutical Formulation, 1st Edi., New Delhi: CBS Publishers,(2001),p 742-743.
12. ICH.,Stability Testing of New Drugs Substances and Products:Q1A(R2).,Geneva: International Conference on Harmonization.2003
13. Kulkarni GT. Stability testing of Pharmaceutical Products: An overview.Ind.J.Pharm.Educ.2004;38(4):194
14. Archarya MM. Pharmaceutical Stability Testing and Studies: An Overview. The Eastern Pharmacist, May 1993:1-36
15. KlickS.et al.Toward a generic Approach for stress testing of drug substances and drug products. Pharm. Tech, 2005; 2:48-66.
16. Sarah K. Branch .Guidelines from the International Conference on Harmonisation (ICH).JPharmaceuandBiomedi.Analysis38,2005.p.798805.doi:10.1016/j.jpba.2005.02.037
17. Singh SS., Bakshi M. Development of Validated Stability indicating assay methods critical view Pharmceu.Biomedi.Analysis.2002; 28:1011-1040.
18. FDA .Guidance for Industry: analytical procedure and method validation (Draft guidance), Rockville: Food and Drug Administration, MD.2000.
19. ICH, Stability Testing: Photostability Testing of New Drug Substance and Products.(Q1B).Geneva: International Conference On Hormonisation.2003.
20. Bob Dotter Using Photostability Testing chamber to meet the requirements of the Drug Testing in Accordance with ICH Q1B, 1-7, Online available on www.caronproducts.com (2005).
21. Snyder, R. I., Kirkland. J. J., Glajeh. J. I., Practical HPLC Method Development, (1997), 2nd Edi, Published by John Wiley and Sons Inc, Newyork, p13.
22. Sethi, P. D., (2001), High performance Liquid Chromatography Quantitative Analysis of Pharmaceutical Formulation, 1st Edi., CBS Publishers and Distributors, 11-12,141-142.
23. James, W. M., (2001), Pharmaceutical Analysis Modern Methods, Part- B, International Medical Book Distributors, 16, 51.
24. XU Q.,Trissel L. Stability Indicating HPLC methods for Drug Analysis 2 nd Edition (2008), Published by American Pharmacist Association and Pharmaceutical Press.2003:1.

25. Lippincott Williams, Wilkins, Remington ,The Science and Practice of Pharmacy,33rd ed, B.I.Publication Pvt.Ltd, vol.II, 2005;p1658.
26. Martindale, The complete drug reference, Pharmaceutical Press, 35th ed, 2007; p263
27. Greici CG, Hérida RN. Validation of UV Spectrophotometric Method for Determination of Lomefloxacin in Pharmaceutical Dosage Form.Acta Farm. 2005; 24 (3): 406-8.
28. Tan Feng, Lang Huiyun, Li Yuan. Extraction-Spectrophotometric Determination of Lomefloxacin. Chinese Journal of Analytical Chemistry.2001-2005.
29. Zhu You-zhen,Yan-jun,Xu Li-ying.Uv-Spectrophotometric determination of Lomefloxacin content in drug capsules by the ternary reaction system among Lomefloxacin, alizarin and lanthanum. Physical Testing and Chemical Analysis (PartB: Chemical Analysis).2006-12.
30. Suhagia BN, Shah SA, Rathod IS, Patel HM, Rao YM. Spectrophotometric estimation of Lomefloxacin hydrochloride in pharmaceutical dosage form.IJPS.2006; 68 :(2):247-249.
31. Agrawal H, Kaul N, Paradkar AR, Mahadik KR. Stability indicating HPTLC determination of clopidogrel bisulphate as bulk drug and in pharmaceutical dosage form. Talanta 61 (2003); 581-589.
32. Motwani SK, Khar RK, Ahmad FJ, Chopra S,Talegaonkar S. Application of a validated stability-indicating densitometric thin-layer chromatographic method to stress degradation studies on moxifloxacin. Analytica Chimica Acta 582 (2007); 75–82.
33. Vadera N, Subramanian G, Musmade P. Stability-indicating HPTLC determination of imatinib mesylate in bulk drug and pharmaceutical dosage form.Journal of Pharmaceutical and Biomedical Analysis 43 (2007); 722–726.
34. Patel DB, Patel NJ. Validated stability indicating HPTLC method for the determination of Tamsulosin hydrochloride in pharmaceutical dosage forms.International Journal of ChemTech Research CODEN (USA): IJCRGG ISSN: 0974-429, Vol.2, No.1, (2010): 646-652.
35. Nariman A, Elragehy, Ezzat M, Abdel-Moety, Nagiba Y, Hassan, Mamdouh RR. Stability-indicating determination of meropenem in presence of its degradation product. Talanta. 2008; 77: 28–36.
36. Mohamed H, Abdel-Hay. Stability-indicating derivative Spectrophotometric determination of frusemide. International Journal of Pharmaceutics.1993; 99(2-3):333-336.