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Formulation and evaluation of ofloxacin aqueous injection

Y. S. Nagaraja[1], T. S. Nagaraja[2], D. R. Bharathi[2] and T. O. Manjunatha[2]
S.J.M. College of Pharmacy, Chitradurga, (Karnataka) - India

Abstract

Ofloxacin is a synthetic fluoroquinolone broad spectrum anti microbial agent used in the treatment of bacterial infections and it is presently available in the market only as tablet dosage form. It is preferred in the treatment of adults with community acquired pneumonia and acute bacterial exacerbations of chronic bronchitis caused by susceptible organism. The present study was undertaken with an intention to develop a stable and effective parenteral formulation, containing the drug Ofloxacin. Ofloxacin is a light sensitive and water soluble drug but unstable at higher temperature in water. So the effects of various co solvents in the solubility of Ofloxacin have been evaluated. Ofloxacin was tried with co solvents such as PEG-200, Span 20 and Glycerin. The drug was made into injection formulation for administered as a SVP. Various batches of Ofloxacin injection formulation were prepared in order to assess the influence of heat, light, atmospheric oxygen and antioxidant on the stability of the drug and the formulations were also subjected to accelerated stability test. Out of all trials, formulation containing 45% of PEG-200 was found to be more stable and passed all tests satisfactorily.

Key-Words: Ofloxacin, Fluoroquinolone, Parenteral formulation, SVP, Accelerated stability

Introduction

Injections include a wide variety of therapeutic agents, e.g., for the treatment of cancer, infections, cardiovascular diseases, arthritis, inflammatory diseases, diabetes, hormonal deficiencies and many other disease states including life threatening emergency conditions. There are more than 400 injections products listed in the USP and, because of the huge number of biotechnology molecules in clinical study, this number will continue to grow rapidly over the next several years. About 80% or greater of all SVPs commercially available are prepared by aseptic processing. LVPs usually involve intravenous infusion, dialysis, or irrigation fluids containing electrolytes, sugar, amino acids, blood, blood products, and fatty lipid emulsions. SVP formulations are simple formulations compared with other pharmaceutical dosage forms, composed of active ingredients, solvent system (preferably aqueous), minimal number of excipients, in the appropriate container and closure packaging system. Formulation scientists have severe restrictions in number and choice of added substances because of safety considerations[1].

*** Corresponding Author**
E.mail: nagraj.ys@gmail.com

On the other hand, if the fish farmers can produce an Ofloxacin is member of fluoroquinolone class of antimicrobial drugs. It is active against a wide range of Gram +ve and Gram –ve organisms. Ofloxacin is preferred in the treatment of adults with acute exacerbations of chronic bronchitis and community-acquired pneumonia caused by susceptible organisms. It is the most potent fluoroquinolone against *S. pneumoniae* and demonstrates excellent *in vitro* activity versus penicillin, macrolide, cephalosporin, and quinolone-resistant strains. Ofloxacin retains good activity against Gram-negative organisms and is active against atypical pathogens although it has shown to be effective in treatment of RTIs.

The aim of the present study is to formulate and evaluate the parenteral dosage form containing Ofloxacin. The objectives of the study are, to study the solubility behavior of the drug in different solvents, to develop an analytical method for assay of Ofloxacin, to design and formulate a stable parenteral formulation of Ofloxacin, to evaluate prepared parenteral formulations of Ofloxacin.

Material and Methods[2-5]

Preformulation Studies

Solubility studies of Ofloxacin in different solvents: Excess of drug was added to different solvents in 10 ml stoppered volumetric flasks. Then Drug was made to dissolve in the solvent by placing the volumetric flask in the shaker bath at 25° C for 6 hours. The volumetric

flasks were then placed at room temperature for 24 hours. The solutions were filtered and appropriate dilutions were made to measure absorbances at 300nm using UV visible spectrophotometer, and water as blank. The data are given in Table 2.

Effect of Temperature on Stability of Drug: 1% Ofloxacin solution in PEG-200 is filled into vials. The vials were sealed and placed at refrigeration, room-temperature, 50°C, 75°C and 95°C for 1 week and observed for colour change and crystal growth. The samples placed at refrigeration and room temperature served as controls. The data are given in Table 3.

Light Stability of Drug: 1% of Ofloxacin solution in 20% PEG-200 is filled in to 20ml glass vials (amber and clear).Also samples of drug substance are placed in an open Petridish to expose a large surface. Drug and dilutions placed in a light-resistant amber coloured glass vials, foil wrapped and in a cardboard box as controls. This is carried out for 4 weeks with weekly examinations for visible colour change or precipitation in solution in clear vials, the compound can be considered as potentially light sensitive and should be handled accordingly. The data are given in Table 4.

Effect of Oxygen on Drug: 1% of Ofloxacin in PEG-200 is filled into vials and placed at 30°C and 40°C. One group is purged and another group is sealed with air. Solutions are observed for colour change and drug content. The data are given in Table 5 and 6.

Formulation development

Attempts were made to develop a stable parenteral formulation using cosolvent/s along with other excipients. The dose selected for formulation was 250 mg of Ofloxacin in 1ml solvent. The prepared formulations contain the following ingredients along with their concentrations are given in Table 1.

Table 1: Concentration of different ingredients used in various trial formulations

Ingredients	Formulation (%) in grams					
	C1	C2	C3	C4	C5	C6
Ofloxacin	25	25	25	25	25	25
PEG-200	45	-	-	-	45	-
Resorcinol	4	-	-	4	4	-
Glycerin	-	-	5	-	5	5
Benzyl alcohol	1.5	1.5	1.5	1.5	1.5	1.5
Methyl Paraben	0.198	0.198	0.198	0.198	0.198	0.198
Propyl Paraben	0.022	0.022	0.022	0.022	0.022	0.022
Sodium metabisulphite	0.1	0.1	0.1	0.1	0.1	0.1
Water for Injection	q s	q s	q s	q s	q s	q s

Thus prepared formulations were assayed for drug content respectively and 10ml of these were placed at 5°C, room temperature (RT), 37°C, 40°C and 45°C for six weeks and observed for crystal growth, clarity, pH change, and drug content.

Post formulation evaluations

Assay of Formulations

Reference Solution Preparation

100ml of stock reference solutions for each formulation was prepared. The composition of the reference stock solution was similar to that of the respective formulations excluding the drug and also they were diluted similarly as the formulations were diluted using water. This resulting solution is used as reference solution (blank) in comparison with the prepared formulations to measure the % drug content by measuring the absorbencies using Shimadzu UV-Visible spectrophotometer. The amount of Ofloxacin was determined from standard calibration curve. The data are given in Table 7.

Sterilization Studies: The injection samples were taken in glass syringe, the membrane filter holder was attached to the syringe. A prefilter of 1.5 micrometers was placed in this holder, after which filters of 0.22, 0.45, 1.2 and 1.5 micrometers were placed successively and tested whether the injection sample could pass through these membrane or not. The data are given in Table 8 and 13.

Stability Studies

For any pharmaceutical dosage form stability of the prepared formulation is a very basic and important factor, from point of view of safety of the patient being treated with and to get a safe and maximum therapeutic response of the drug.

The provision of rapid means of quality control, which ensures that no unexpected changes in the stored product are occurred like: Crystal growth, pH changes, Clarity and % Drug content. The data are given in Table 9 to12.

Crystal Growth

10 ml of the each prepared formulations C1, C5 were placed at refrigeration, room temperature, 37°C, 40°C and 45°C respectively for six weeks and observed for crystal growth. The data are given in Table 15.

pH Changes

10ml of the each prepared formulations C1, C5 were kept at different temperatures/conditions such as refrigeration, room temperature, 37°C, 40°C, 45°C and

under light. At regular time intervals the samples were examined for pH changes for six weeks using a digital pH meter. The data are given in Table 14.

Clarity

10ml of the formulations were placed at refrigeration, room temperature, 37°C, 40°C and 45°C for six weeks and observed for colour change or turbidity. The data are given in Table 16.

% Drug Content

The drug content of the formulations C1, C5 were determined by following the same procedures as mentioned in assay. The estimates were done at intervals of one week upto six weeks. The data are given in Table 17 and 18.

Results and Discussion

FT-IR spectrum of pure Ofloxacin

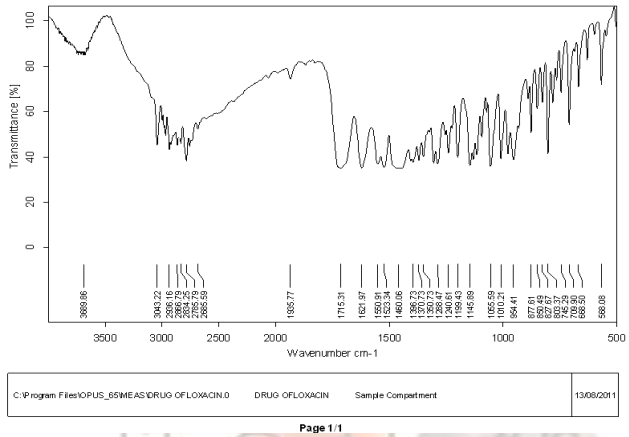

Table 2: Solubility profile of Ofloxacin in different solvents

Solvents	Absorbance* at 270 nm	Conc. mg/ml	Standard deviation
DM Water	0.736	92.1439	± 0.108
0.1NaoH	0.292	36.5571	± 0.003
Ascorbic acid	0.279	34.9295	± 0.026
Nicotinamide	0.058	7.2613	± 0.009
20% PEG 400	0.295	36.9327	± 0.004
Resorcinol	0.322	40.3129	± 0.006
Tween 80	0.296	37.0579	± 0.008
Span 80	0.264	33.0516	± 0.006
Urea	0.385	48.20031	± 0.007
20% Glycerine	0.317	39.6870	± 0.003
Polysarbate 80	0.744	93.1455	± 0.003

Stability evaluation

Various stress tests are performed on solid and solution samples to establish the effect of heat, light and oxygen on the drug substance stability.

Heat stability

Table 3: Heat stability profile of Ofloxacin

Temperature (°C)	Duration (weeks)			
	1	2	3	4
Refrigeration	-	-	-	-
Room temperature	-	-	-	-
40	-	-	-	-
50	+	+	+	+
75	+	+	+	+

+ Colour change, - No colour change

Light stability

Table 4: Light stability study of Ofloxacin

Withdrawal week	Observations	
	Clear	Amber
1	-	-
2	-	-
3	+	-
4	+	-

- Clear, + Turbidity

Effect of oxygen

Table 5: Test for colour change after a week

Temperature (°C)	Air sealed vials	Perged vials
25	+	-
30	+	+

+ colour change, - no colour change

Estimation of drug content

Table 6: Drug content in freshly prepared drug solution

Absorbance at 270 nm	Concentration in µg/ml	Concentration in mg/ml
0.794	9940.53	9.9405

Formulation development

A stable parenteral formulation of water soluble drug Ofloxacin was formulated after performing trials with various solvents. Thus prepared formulations were subjected for various tests and results are discussed in the following section.

Table 7: % Drug content of various formulation trials containing Ofloxacin

Formulation	Absorbance* at 286nm	Drug content (mg/ml)	% Drug content
C1	0.202	252.890	101.156
C2	0.209	261.658	104.663

C3	0.200	250.390	100.156
C4	0.205	256.651	102.660
C5	0.204	255.398	102.159
C6	0.200	250.390	100.156

* Each value is an average of three determinations

Table 8: Filter pore size and filterability of the formulations of Ofloxacin

Formulation	Filter pore size(µm)	Observation
C1	0.22	+
	0.45	+
	1.2	+
	1.5	+
C2	0.22	+
	0.45	+
	1.2	+
	1.5	+
C3	0.22	+
	0.45	+
	1.2	+
	1.5	+
C4	0.22	+
	0.45	+
	1.2	+
	1.5	+
C5	0.22	+
	0.45	+
	1.2	+
	1.5	+
C6	0.22	+
	0.45	+
	1.2	+
	1.5	+

+ Injection passes through. - Injection does not pass through

All the formulations were found to be easily passing through all the pore size filters and hence 0.22 µm pore size filter was selected to filter all the prepared formulations separately.

None of the formulations showed turbidity or signs of microbial growth (except the positive control) at the end of incubation period, indicating all the formulations were sterile and thus all the formulations are subjected to further evaluations.

Post Formulation Studies

Effect of different temperature on crystal growth

Table 9: Effect of different temperature on crystal growth

Formulation	RT	40°C	Light
C1	-	-	-
C2	-	-	-
C3	+	+	+
C4	-	-	-
C5	-	+	+
C6	-	-	-

+ Crystal growth, - No crystal growth

In the formulations C1, C5, C4 and C6 no crystals were developed after two weeks. So C1, C5, C4 and C6 are stable at temperatures studied.

Effect of different temperature on clarity

Table 10: Effect of different temperature on clarity

Formulation	RT	40°C	Light
C1	-	-	-
C2	-	-	-
C3	+	+	-
C4	-	-	-
C5	-	+	-
C6	-	-	-

+ Turbid, - Clear

C1, C2, C4 and C6 are clear after two weeks. So C1, C2, C4 and C5 are stable at temperatures studied.

Effect of different temperature on colour change

Table 11: Effect of different temperature on colour change

Formulation	5°C	RT	40°C
C1	-	-	-
C2	-	-	-
C3	+	+	+
C4	-	-	-
C5	-	+	+
C6	-	-	+

+colour change, - no colour change.

C1, C5 and C5 show no colour change up to 40^0C after two weeks. So C1, C5 and C5 are stable at temperatures studied.

Scale up studies

Assay of the formulations

Table 12: Drug content of C1, C5

Formulation	Absorbance* at 291.5nm	Drug content (mg/ml)	% Drug content
C1	0.202	252.89	101.156
C5	0.209	261.658	104.663

* Each value is an average of three determinations

Sterilization studies and sterility testing

Filtration

The results of filterability show that both the formulations of Ofloxacin passes through all the four membrane filters. Hence they can be sterilized by filteration.

Table 13: Filter pore size and filterability of the formulations of Ofloxacin

Formulation	Filter pore size (µm)	Observation
C1	0.22	+
	0.45	+
	1.2	+
	1.5	+
C5	0.22	+
	0.45	+
	1.2	+
	1.5	+

+ Injection passes through. - Injection does not pass through.

The results of filterability show that both the formulations of Ofloxacin passes through all the four membrane filters. Hence they can be sterilized by filteration.

Accelerated stability studies

pH Changes

Table 14: pH changes of formulation C1, C5 at different temperatures/conditions on ageing

Formulation	Withdrawal Week	37°C	40°C	Light
C1	0	4.18	4.18	4.18
	1	4.25	4.25	4.28
	2	4.25	4.27	4.30
	3	4.29	4.29	4.30
	4	4.32	4.33	4.35
	5	4.24	4.32	4.34
	6	4.20	4.27	4.29
C5	0	4.06	4.06	4.06
	1	4.13	4.12	4.15
	2	4.19	4.17	4.19
	3	4.25	4.24	4.27
	4	4.25	4.21	4.24
	5	4.23	4.21	4.20
	6	4.15	4.19	4.17

Crystal growth

Table 15: Crystal growth of formulation C1, C5 at different temperatures/conditions on ageing

Formulation	Withdrawal Week	37°C	40°C	45°C
C1	0	-	-	-
	1	-	-	-
	2	-	-	-
	3	-	-	-
	4	-	-	-
	5	-	-	-
	6	-	-	-
C5	0	-	-	-
	1	-	-	-
	2	-	-	-
	3	-	-	-
	4	-	-	-
	5	-	-	-
	6	-	-	-

+crystal growth, - no crystal growth

No crystal growth was observed in the formulations at different temperatures/conditions.

Clarity studies

Table 16: Clarity of formulation C1, C5 at different temperatures/conditions on ageing

Formulation	Withdrawal Week	37°C	40°C	45°C
C1	0	-	-	-
	1	-	-	-
	2	-	-	-
	3	-	-	-
	4	-	-	-
	5	-	-	-
	6	-	-	-
C5	0	-	-	-
	1	-	-	-
	2	-	-	-
	3	-	-	-
	4	-	-	-
	5	-	-	-
	6	-	-	-

+ turbid, - clear

All the formulations were clear at different temperatures/ conditions.

Drug content

Table 17: Percent drug content of formulation C1 at different temperatures/conditions on ageing

Sample withdrawal (week)	% Drug Content		
	37°C	40°C	Light
0	101.156	101.156	101.156
1	101.015	100.973	101.000
2	100.873	100.761	100.728
3	100.798	100.537	100.569
4	100.569	100.365	100.296
5	100.470	100.107	100.100
6	100.017	99.897	99.901

Table 18: Percent drug content of formulation C5 at different temperatures/conditions on ageing

Sample withdrawal (week)	% Drug Content		
	37°C	40°C	Light
0	104.066	104.066	104.066
1	103.857	103.810	103.829

2	103.628	103.698	103.725
3	103.501	103.427	103.500
4	103.389	103.251	103.317
5	103.109	103.006	103.108
6	102.961	102.894	102.996

Conclusion

The concept of parenteral formulations containing Ofloxacin offers a suitable, practical approach to achieve desired stable parenteral preparation with solubility of drug in suitable solvent composition. In present work, parenteral formulation of Ofloxacin was prepared successfully by using different concentrations and combinations of PEG-200 in formulation design. These formulations were expected to be stable for sufficiently long time. The conclusions arrived from the above results indicated that the parenteral formulation containing Ofloxacin developed was found to be complying satisfactorily with all the evaluation tests performed and was stable for sufficiently longer duration of time.

References

1. Lachmann Leon, Patrick Deluca, Michael J. Akers,Kinetic Principles and Stability Testing., chapter 26 in the Theory and Practice of Industrial Pharmacy, Bombay: Varghese Publishing House, 1987, page-902.
2. Gopal Krishna, Hodnick WF, Lang W, Lin X, Karra S, Mao J, Almassian B. Pharmaceutical Development and Manufacturing of a Parenteral Formulation of a Novel Antitumor Agent. AAPS PharmSciTech.2001; 2 (3):14.
3. Nahar M, Jain NK. Formulation and evaluation of saquinavir injection. Indian J Pharm Sci 2006; 68:608-14
4. Anupama B. Formulation and evaluation of rofecoxib injection. M.Pharm dissertation: Rajiv Gandhi University of Health Sciences; 2007.
5. Kenneth EA, Leon L, Herbert AL. Pharmaceutical dosage forms: Parenteral medications. Vol 1. Marcel Dekker Inc: New York and Basel; 1989. p. 89-137.
6. Kenneth EA, Leon L, Herbert AL. Pharmaceutical dosage forms: Parenteral medications. Vol 1. Marcel Dekker Inc: New York and Basel; 1989. p.529.
7. Nahar M, Jain NK. Formulation and evaluation of saquinavir injection. Indian J Pharm Sci 2006; 68:608-14
8. Jusko WJ, Gretch M, Gassett R. J Amer Med Assoc 1973; 225:176.
9. Korttila K, Sothman A, Andersson P. Acta Pharmacol Toxicol 1976; 139:104.
10. Yalkowsky SH, Valvani SC. Int Clin Pharm 1977; 11:417.
11. Swamy PV, Sushma P, Chirag G, Prasad K, Younus AM, Raju SA. Parenteral formulation of zopiclone. Int J Pharm Sci 2008; 70(1):96-9.
12. Subramanyam CVS. Text book of physical pharmaceutics. 2nd ed: Vallabha prakashan ; 200.p. 65-75
13. Subrmanyam CVS, Thimmasetty J. Laboratory manual of physical pharmaceutics. 1st ed. Vallabha prakashan: Delhi; 2002. p. 116-25.
14. Paranjothy KLK, Praveen KA. Stability testing of pharmaceuticals 1979; 17(4): 114-19.