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Research Article

Preparation and Evaluation of Sparfloxacin Parenteral Dosage Form

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ABSTRACT

Sparfloxacin 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, 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 sparfloxacin. Sparfloxacin is a water insoluble drug. The effects of various co solvents in the solubility of sparfloxacin have been evaluated. Sparfloxacin was tried with co solvents such as PEG-400, Propylene glycol, Glycerin, Ethanol, Tween 80. The drug was made into injection formulation for administration with infusions. PEG-400 was selected as co solvent and formulation have been formulated in different combinations along with ethyl alcohol. Various batches of sparfloxacin 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 testing in order to predict approximate shelf-life of the product.

Keywords: Sparfloxacin, fluoroquinolone, infusions, 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 SVIs commercially available are prepared by aseptic prosing. LVIs usually involve intravenous infusion, dialysis, or irrigation fluids containing electrolytes, sugar, amino acids, blood, blood products, and fatty lipid emulsions. SVI 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.

However, because many important therapeutic agents are poorly soluble or totally insoluble in water, oily solvents and water-miscible co-solvents are used to produce ready-to-use solutions. The solubility enhancing agents must be selected with great care as it must not be irritating, toxic or sensitizing and it must not exert any adverse effect on the ingredients of the formulation. Solubilizers are used to enhance and maintain the aqueous solubility of poorly water-soluble drugs examples of solubilizing agents that are miscible with water that are used as cosolvents, include dioxolanes, PEG 400 and 600, propylene glycol, glycerin and ethyl alcohol. Among these propylene glycol is most widely use, as it's safe and it also believed to inhibit crystal growth in most of the parenterals, which is a major concern in stability of parenterals. SVIs must be sterile and free from pyrogens and foreign particulate matter. These are major characteristics to distinguish sterile dosage forms from any other pharmaceutical product. Filtration is a process of physical removal of organisms by passing through proof filter, which is used for the sterilization of thermo labile solutions.

Drugs with sufficient solution stability will still require certain formulation, packaging or storage conditions to maintain stability during shelf of drugs in SVIs as they are generally unstable.
Sparfloxacin is member of fluoroquinolone class of antimicrobial drugs. It is active against a wide range of Gram $_{+}$ve and Gram -ve organisms, with chemical name 5-amino-1-cyclopropyl-7-[(3S,5R)-3,5-dimethylpiperazin-1-yl]-6,8-difluoro-4-oxoquinoline-3-carboxylic acid and molecular formula $C_{19}H_{22}F_2N_4O_3$. It is used in treatment of urinary tract infection.

MATERIALS AND METHODS

List of ingredients used is given in Table 1.

Preformulation Studies[2,3,4,5,6]

Solubility studies of Sparfloxacin in different solvents (saturation solubility method)

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 286nm using UV visible spectrophotometer, and water as blank. The data are given in Table 3.

Effect of Temperature on Stability of Drug

1% Sparfloxacin solution in 0.0.1N NaOH 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 4.

Light Stability of Drug

1% of Sparfloxacin solution in 0.0.1N NaOH is filled in to 20ml glass vials (amber and clear).
Also samples of drug substance are placed in an open perti dish 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 5.

Effect of Oxygen on Drug

1% of Sparfloxacin in 0.0.1N NaOH 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 6 to 11.

FORMULATION DEVELOPMENT

Attempts were made to develop a stable parenteral formulation using cosolvent/s along with other excipients. The dose selected for formulation was 400 mg of Sparfloxacin in 2ml solvent. The prepared formulations contain the following ingredients along with their concentrations are given in Table 2.

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 Sparfloxacin was determined from standard calibration curve. The data are given in Table 12.

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 13.

STERILITY TESTING

Direct Transfer Method

Aliquots of the samples are transferred aseptically into fluid thioglycolate medium and soybean casein digest medium. The inoculated thioglycolate medium is incubated at 32°C and soybean casein digest samples at 22°C for 7 days. Likewise negative and positive controls are prepared. The data are given in Table 14.

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.

Crystal Growth

10 ml of the each prepared formulations F5 and F6 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

10 ml of the each prepared formulations F5 and F6 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 16.

Clarity

10 ml 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 17.

% Drug Content

The drug content of the formulations F5 and F6 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 18 to 21.

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Compatibility of rubber closures with F5 and F6

The formulation was filled in 10 ml glass vials and stoppered with west gray butyl rubber stoppers. Vials were also stoppered with west gray Teflon-coated stoppers and served as controls. The vials were placed upright and inverted in the stability chambers maintained at 40°C with 75% RH, 25°C with 60% RH, and refrigeration. The vials were inverted to obtain maximum exposure of the rubber closure to the formulation for four weeks and the contents of the vials were periodically analyzed for drug content. The data are given in Table 22.

Effect of silastic tubing on F5 and F6

The effect of silastic tubing on formulation was tested by immersing the tubing in the formulations F5 and F6 for 24 hours at room temperature. The tubing was also immersed in the formulation vehicle for the same period of time and this served as a control. Then all solutions were analyzed by UV spectrophotometric method to determine any loss of drug via adsorption. The data are given in Table 25.

Dilution study[7,8,9,10]

Precipitation of drug often occurs upon injecting a formulation into body fluids. The amount of precipitation can be correlated with the rate at which the drug is injected. Method for determination of such effect is dilution study.

The serial dilutions of formulations were prepared in ratio of 20:50 to 20:500 and stored at room temperature and examined visually for the appearance of crystals and turbidity upto 24 hours. The data are given in Table 26 to 29.

Shelf Life Determination[11,12,13,14]

The injections stored at 37 ºC, 40ºC, 45ºC and light were subjected to the shelf life determination studies

Prediction of Shelf Life

The log percentage drug retained (undecomposed) values of the four formulations F5 and F6 stored at 37 ºC, 40 ºC and 45 ºC are given in Table 30 and are graphically represented in Figure 3 to 6. The results obtained of the above graphs using Arrhenius plot shelf life of the all four formulations were predicted.

RESULTS AND DISCUSSION

Table 3: Solubility profile of Sparfloxacin in different solvents

Sl. No	Solvents	Dilution	Absorbances* at 291.5nm	Concentration mg/ml	Standard deviation
1	DM Water	10	0.7796	0.1055	± 0.108
2	0.1N NaOH	10000	0.283667	38.41986	± 0.021
3	0.1N HCl	50	0.134667	0.091196	± 0.003
4	5% PEG	50	0.21	0.142212	± 0.026
5	10% PEG	50	0.97933	0.663205	± 0.009
6	5% Propylene glycol	50	0.42	0.284424	± 0.006
7	10% Propylene glycol	100	0.11933	0.161625	± 0.008
8	20% Propylene glycol	100	0.162	0.219413	± 0.006
9	10% Glycerine	100	0.12133	0.164334	± 0.007
10	20% Glycerine	100	0.13433	0.181941	± 0.003
11	Ethanol	100	0.7623	1.03246	± 0.010
12	Tween 80	100	0.4186	0.56704	± 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.

1. Heat stability

Table 4: Heat stability profile of sparfloxacin

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

+ Colour change, - No colour change

2. Light stability

Table 5: Light stability study of sparfloxacin

Withdrawal week	Observation
1	-
2	-
3	-
4	-

- Clear, + Turbidity

3. Effect of oxygen

Table 6: Test for colour change after a week

Temperature(°C)	Air sealed vials	Perged vials
30	+	-
40	+	+

+ colour change, - no colour change

4. Estimation of drug content

Table 7: Drug content in freshly prepared drug solution

Absorbance at 291.5nm	Concentration in µg/ml	Concentration in mg/ml
0.703	9844.21	9.8442

Table 8: Drug content in perged vials at 30°C

Absorbance at 291.5nm	Concentration in µg/ml	Concentration in mg/ml
0.701	9816.20	9.8162

Table 9: Drug content in air sealed vials at 30°C

Absorbance at 291.5nm	Concentration in µg/ml	Concentration in mg/ml
0.612	8569.92	8.5699

None of the above solutions were found to be sensitive to oxygen.

FORMULATION DEVELOPMENT

A stable parenteral formulation of slightly water soluble drug Sparfloxacin 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 10: Percentage Drug content of various formulation trials containing Sparfloxacin

Formulation	Absorbance* at 286nm	Drug content (mg/ml)	% Drug content	Standard deviation
F1	0.8343	204.6746	102.3373	± 0.0015
F2	0.8273	202.3574	101.4787	± 0.0020
F3	0.8430	206.8007	103.4003	± 0.0026
F4	0.8345	204.7589	102.1564	± 0.0010
F5	0.8337	204.511	102.2555	± 0.0023

* Each value is an average of three determinations

Table 11: Filter pore size and filterability of the formulations of Sparfloxacin

Formulation	Filter pore size(µm)	Observation
F1	0.22	+
	0.45	+
	1.2	+
	1.5	+
F2	0.22	+
	0.45	+
	1.2	+
	1.5	+
F3	0.22	+
	0.45	+
	1.2	+
	1.5	+
F4	0.22	+
	0.45	+
	1.2	+
	1.5	+
F5	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 12: Effect of different temperature on crystal growth

Formulation	RT	40°C	50°C
F1	+	+	+
F2	+	-	-
F3	-	-	-
F4	+	+	-
F5	-	-	-
F6	-	-	-

+ Crystal growth, - No crystal growth

In the formulations F3, F5 and F6 no crystals were developed after two weeks. So F3, F5 and F6 are stable at temperatures studied.

Effect of different temperature on clarity

Table 13: Effect of different temperature on clarity

Formulation	RT	40°C	50°C
F1	+	+	+
F2	+	+	+
F3	-	-	-
F4	-	+	+
F5	-	-	-
F6	-	-	-

+ Turbid, - Clear

F3, F5 and F6 are clear after two weeks. So F3, F5 and F6 are stable at temperatures studied.

Effect of different temperature on colour change

Table 14: Effect of different temperature on colour change

Formulation	5°C	RT	40°C	50°C
F1	-	-	+	+
F2	-	-	+	+
F3	-	-	+	+
F4	-	-	+	+
F5	-	-	-	+
F6	-	-	-	+

+colour change, - no colour change.

F5 and F6 shows no colour change up to 40°C but at 50°C colour change was observed. So F5 and F6 are stable at temperatures studied.

SCALE UP STUDIES

ASSAY OF THE FORMULATIONS

Table 15: Drug content of F5 and F6

Formulation	Absorbance* at 291.5nm	Drug content (mg/ml)	% Drug content	Standard deviation
F5	0.7616	20.632	103.160	±0.0006
F6	0.7586	20.550	102.754	±0.0006

* Each value is an average of three determinations

STERILIZATION STUDIES AND STERILITY TESTING

FILTRATION

Table 16: Filter pore size and filterability of the formulations of sparfloxacin

Sl. No	Formulation	Filter pore size (µm)	Observation
1	F5	0.22	+
		0.45	+
		1.2	+
		1.5	+
2	F6	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 sparfloxacin passes through all the four membrane filters. Hence they can be sterilized by filteration.

DIRECT TRANSFER METHOD

Table 17: The growth of bacteria in soya bean casein digest medium and fluid thioglycollate medium after seven days

Formulation	Soyabean-casein digest medium (SCDM)	Fluid thioglycollate medium (FTM)
F5	-	-
F6	-	-

- Clear; + Turbid

Both the formulations pass the sterility test.

ACCELERATED STABILITY STUDIES
pH CHANGES

Table 18: pH changes of formulation F5 and F6 at different temperatures/conditions on ageing

Formulation	Withdrawal Week	37°C	40°C	45°C	Light
F5	0	10.13	10.13	10.13	10.13
	1	10.22	10.16	10.12	10.19
	2	10.21	10.16	10.15	10.23
	3	10.18	10.12	10.21	10.25
	4	10.17	10.17	10.25	10.26
	5	10.23	10.24	10.28	10.28
	6	10.26	10.21	10.39	10.34
F6	0	10.17	10.17	10.17	10.17
	1	10.20	10.21	10.31	10.17
	2	10.20	10.26	10.32	10.19
	3	10.21	10.26	10.42	10.22
	4	10.26	10.35	10.43	10.23
	5	10.28	10.39	10.51	10.28
	6	10.32	10.38	10.55	10.32

CRYSTAL GROWTH

Table 19: Crystal growth of formulation F5 and F6 at different temperatures/conditions on ageing

Formulation	Withdrawal Week	37°C	40°C	45°C	Light
F5	0	-	-	-	-
	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
	4	-	-	-	-
	5	-	-	-	-
	6	-	-	-	-
F6	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 20: Clarity of formulation F5 and F6 at different temperatures/conditions on ageing

Formulation	Withdrawal Week	37°C	40°C	45°C	Light
F5	0	-	-	-	-
	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
	4	-	-	-	-
	5	-	-	-	-
	6	-	-	-	-
F6	0	-	-	-	-
	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
	4	-	-	-	-
	5	-	-	-	-
	6	-	-	-	-

+ turbid, - clear

All the formulations were clear at different temperatures/ conditions.

DRUG CONTENT

Table 21: Percent drug content of formulation F5 at different temperatures/conditions on ageing

Sample withdrawal (week)	% Drug Content			
	37°C	40°C	45°C	Light
0	103.1603	103.1603	103.1603	103.1603
1	102.9345	102.8894	102.9345	103.1151
2	102.8894	102.7991	102.5282	103.2054
3	102.8445	102.3476	102.3476	103.0248
4	102.7088	101.7607	101.9413	102.9345
5	101.6253	101.535	99.5936	102.5734
6	101.3544	101.3093	99.1873	102.3928

Table 22: Percent drug content of formulation F6 at different temperatures/conditions on ageing

Sample withdrawal (week)	% Drug Content			
	37°C	40°C	45°C	Light
0	102.754	102.754	102.754	102.754
1	102.212	102.212	100.451	102.979
2	101.219	101.670	99.909	102.618
3	100.812	100.722	99.774	102.121
4	100.541	100.722	99.638	101.128
5	100.180	100.406	99.187	101.038
6	99.819	99.729	98.614	100.451

COMPATIBILITY OF RUBBER CLOSURES WITH F5 AND F6

Table 23: Compatibility of F5 with rubber closures

Withdrawal week	% Drug content		
	5°C	25°C	40°C
0	103.1603	103.1603	103.1603
1	103.1603	103.1603	103.0248
2	103.0248	103.07	102.7991
3	103.0248	102.754	102.3476
4	102.9345	102.6185	101.7156

Table 24: Compatibility of F6 with rubber closures

Withdrawal week	% Drug content		
	5°C	25°C	40°C
0	102.754	102.754	102.754
1	102.6185	102.6637	102.0316
2	102.5282	102.167	101.5801
3	101.9413	101.7607	100.8578
4	101.8962	101.3995	100.7223

EFFECT OF SILASTIC TUBING ON F5 AND F6

Silastic tubing is generally used to transfer the product from one container to another and for vial filling operations. Drug adsorption into these tubing materials may result in substantial loss of potency. The recovery of sparfloxacin from formulations that had been in contact with silastic tubing for 24 hours was 100%, indicating no loss of drug via absorption into the tubing. The nature of solvents may affect the ability of the tubing to dispense the solution uniformly during the filling operation.

DILUTION STUDY

Table 25: Dilution study of F5

Dilution (v/v)	Time (hours)									
	Normal saline					5% w/v Dextrose solution				
	0	2	4	8	24	0	2	4	8	24
20:50	-	-	-	-	-	-	-	-	-	-
20:100	-	-	-	-	-	-	-	-	-	-
20:200	-	-	-	-	-	-	-	-	-	-
20:300	-	-	-	-	-	-	-	-	-	-
20:400	-	-	-	-	-	-	-	-	-	-
20:500	-	-	-	-	-	-	-	-	-	-

+ Crystals, - Clear

Table 26: Dilution study of F6

Dilution (v/v)	Time (hours)									
	Normal saline					5% w/v Dextrose solution				
	0	2	4	8	24	0	2	4	8	24
20:50	-	-	-	-	-	-	-	-	-	-
20:100	-	-	-	-	-	-	-	-	-	-
20:200	-	-	-	-	-	-	-	-	-	-
20:300	-	-	-	-	-	-	-	-	-	-
20:400	-	-	-	-	-	-	-	-	-	-
20:500	-	-	-	-	-	-	-	-	-	-

+ Crystals, - Clear

PREDICTION OF SHELF LIFE

The log percentage drug retained (undecomposed) values of the formulations F5 and F6 are shown below.

Table 27: Percentage drug retained (log values) of the formulations F5 and F6 stored at different temperatures on ageing

Formulations	Temperature conditions	Withdrawal week v/s log % drug content						
		0	1	2	3	4	5	6
F5	37°C	2.0000	1.9990	1.9988	1.9987	1.9981	1.9935	1.9923
	40°C	2.0000	1.9988	1.9985	1.9966	1.9941	1.9931	1.9921
	45°C	2.0000	1.9990	1.9973	1.9966	1.9948	1.9847	1.9829
	Light	2.0000	1.9998	1.9998	1.9994	1.9990	1.9975	1.9967

F6	**37°C**	2.0000	1.9977	1.9935	1.9917	1.9906	1.9890	1.9874
	40°C	2.0000	1.9977	1.9954	1.9913	1.9913	1.9900	1.9870
	45°C	2.0000	1.9902	1.9878	1.9872	1.9866	1.9847	1.9821
	Light	2.0000	2.0000	1.9994	1.9973	1.9931	1.9927	1.9902

From the log percentage retained (undecomposed) potencies of the drug different intervals of time, loss lines are plotted for each temperature under study for the three formulations. From these loss lines the time required to have 90% potency of the drug at room temperature (25°C) is calculated.

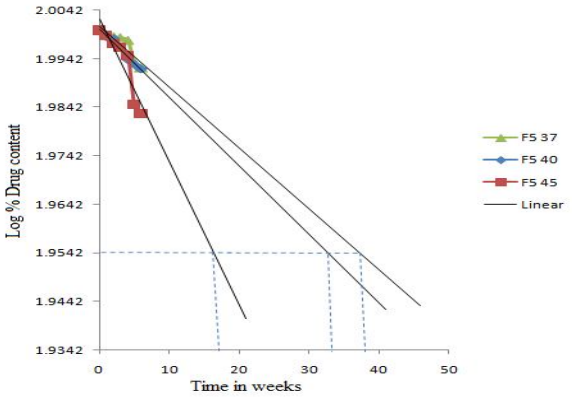

Fig. 1: Loss line of formulation F5

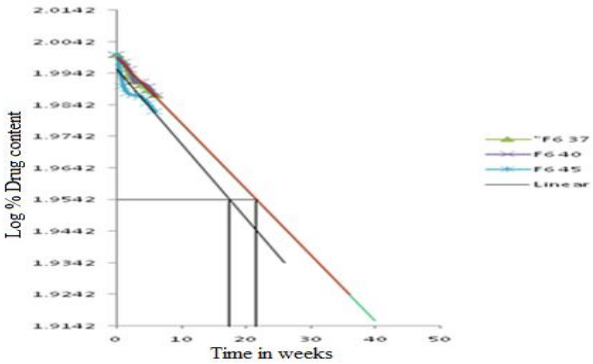

Fig. 2: Loss line of formulation F6

The t_{90}% values of formulation F5 and F6 at different temperatures are obtained from the figures 5.5 and 5.6. These values are shown in the table 5.27 and 5.28.

These t_{90} values are then converted to log t_{90}. The log t_{90} values are plotted against their corresponding reciprocal absolute temperature ($1/T * 10^{-3}$) for each formulation. The line thus obtained is then extrapolated and the time required to have 90% potency of the drug at room temperature (25°C) is calculated.

Table 28: t_{90} values of formulations F5 and F6 at different temperatures

Formulation	**Time required to have 90% potency in weeks**		
	37°C	**40°C**	**45°C**
F5	38.25	33.42	17.05
F6	24.28	24.28	19.72

Table 29: Log t_{90} values of formulations F5 and F6 at different temperatures

Formulation	Time required to have 90% potency in weeks		
	3.22	3.19	3.14
F5	1.5826	1.524	1.2317
F6	1.3852	1.3852	1.2949

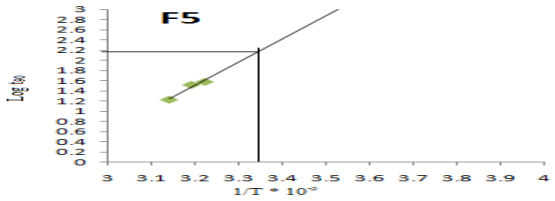

Fig. 3: t_{90} values (at room temperature) of formulations F5

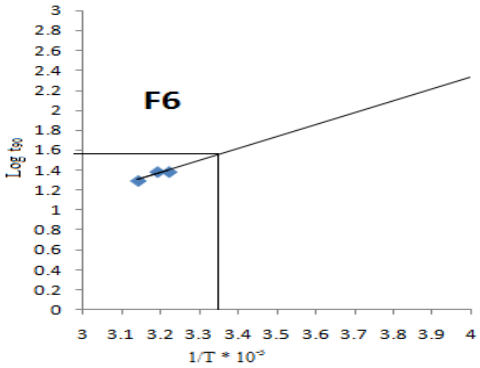

Fig. 4: t_{90} values (at room temperature) of formulations F6

Table 30: Shelf life values of Sparfloxacin injection formulations F5 and F6

Formulation	Intercept values	Shelf life in weeks	Shelf life in years
F5	2.19	154.88	3.22
F6	1.58	38.01	0.79

From the results it is clear that formulation F5 is more stable than the formulation F6.

CONCLUSION

The conclusion arrived from this research work indicated that the parenteral formulation containing Sparfloxacin developed was found to be complying satisfactorily with all the evaluation tests performed and was stable for sufficiently longer duration of time. Further studies are needed to evaluate these formulations for its performance *in vivo* and bioequivalence to establish its potency and efficacy.

Table 1: List of ingredients used

Ingredients	Source
Sparfloxacin	Elegant Drugs Pvt. Ltd. Chalamatti, Hubli, Karnataka.
NaOH	S.d. fine Chem. Limited. Mumbai
Propylene Glycol	S.d. fine Chem. Limited. Mumbai
Polyethyleneglycol-400	S.d. fine Chem. Limited. Mumbai
Glycerin	S.d. fine Chem. Limited. Mumbai
Benzyl alcohol	S.d. fine Chem. Limited. Mumbai
Methyl paraben	S.d. fine Chem. Limited. Mumbai
Propyl paraben	S.d. fine Chem. Limited. Mumbai
Sodium metabisulphite	S.d. fine Chem. Limited. Mumbai
Hydrochloric acid	S.d. fine Chem. Limited. Mumbai

Table 2: Concentration of different ingredients used in various trial formulations

Ingredients	Formulation (%)					
	F1	F2	F3	F4	F5	F6
Sparfloxacin	2	2	2	2	2	2
NaOH	0.4	0.3	0.4	0.3	0.35	0.2
PEG-400	10	10	-	15	-	10
Ethyl alcohol	-	10	10	-	20	20
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
10% HCl (to adjust the pH)	q s	q s	q s	q s	q s	q s

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