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Asheesh Singh
Alpa Laboratories Ltd; Pithampur; Dhar (MP) – 454775

Vinayaditya Singh
Ipca Laboratories Limited Pithampur, Dhar (MP)-454775

Jagrati Singh
Daksh college of pharmacy, Chhatarpur (MP)

Naveen Sharma
Rajiv Gandhi College of pharmacy, (MP)-462026

Correspondence

Asheesh Singh
Alpa Laboratories Ltd; Pithampur; Dhar (MP) – 454775

E mail: asheesh_parihar@yahoo.com

Formulation and Evaluation of Trovafloxacin: The Next Generation of Fluoroquinolones as Sustained Release Tablet

Asheesh Singh,Vinayaditya Singh, Jagrati Singh, Naveen Sharma

ABSTRACT

Trovafloxacin is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class and is used to treat severe or life-threatening bacterial infections or bacterial infections that have failed to respond to other antibiotic classes. It is sold under various brand names, such as Trovan and Tavanic, the most common. Trovafloxacin is considered to be same as Ofloxacin by the U.S. Food and Drug Administration (FDA), with the exception of the potency shown in vitro against mycobacteria. In vitro, it is, in general, twice as potent as ofloxacin, whereas d-ofloxacin is less active against mycobacteria. The advantage of administering a single dose of a drug that is released over an extended period of time instead of numerous doses is now a day's area of interest for formulation scientists in Pharmaceutical industry. There are several advantages of sustained release drug delivery over conventional dosage forms like improved patient compliance due to less frequent drug administration, maximum utilization of the drug, increased safety margin of potent drug, reduction of fluctuation in steady-state drug levels, reduction in healthcare costs through improved therapy and shorter treatment period. Wide varieties of polymers like Hydroxy Propyl Methyl Cellulose (HPMC), Carboxy Methyl Cellulose (CMC), Ethyl Cellulose (EC), Cellulose Acetate Phthalate, HPMC K100M, Xanthan gum, Carrageenan gum, Karaya gum, HPMC K15, Carbopol 971P and Carbopol 974P etc. are available for retarding the release rate of drugs hence sustains the action of drugs.

Keywords: Trovafloxacin, sustained release tablet, tablet, drug delivery system

1. INTRODUCTION

A tablet is a pharmaceutical dosage form. It comprises a mixture of active substances and excipients, usually in powder form, pressed or compacted from a powder into a solid dose. The excipients can include diluents, binders or granulating agents, glidants (flow aids) and lubricants to ensure efficient tableting; disintegrants to promote tablet break-up in the digestive tract; sweeteners or flavours to enhance taste; and pigments to make the tablets visually attractive. A polymer coating is often applied to make the tablet smoother and easier to swallow, to control the release rate of the active ingredient, to make it more resistant to the environment (extending its shelf life), or to enhance the tablet's appearance[1]. Now a day's conventional dosage forms of drugs are rapidly being replaced by the new and the novel drug delivery systems. Amongst, these the controlled release/sustained release dosage forms have become extremely popular in modern therapeutics. Matrix system is the release system which prolongs and controls the release of the drug, which is dissolved or dispersed. A matrix is defined as a well-mixed composite of one or more drugs with gelling agent i.e. hydrophilic polymers. Introduction of matrix tablet as sustained release (SR) has given a new breakthrough for novel drug delivery system in the field of Pharmaceutical technology [2].

Sustained release constitutes any dosage form that provides medication over an extended time or denotes that the system is able to provide some actual therapeutic control whether this is of a temporal nature, spatial nature or both. Sustained release system generally do not attain zero order type release and usually try to mimic zero order release by providing drug in a slow first order. Repeat action tablet are an alternative method of sustained release in which multiple doses of drug are an alternative method of sustained release, in which, multiple doses are contained within a dosage form and each dose is released at a periodic interval. Delayed release system, in contrast, may not be sustaining, since often the function of these dosage forms is to maintain the drug in the dosage for some time before release, for example. Enteric coated tablet[3]. A sustained release dosage form will provide a therapeutic concentration of the drug in the blood that is maintained throughout the dosing interval with a reduction in a peak concentration ratio[4-5]. Numerous drug delivery techniques have been developed to sustain the release of drugs, including triple-layered tablets (Geomatrix® technology) and osmotic pumps with laser drilled holes (OROS® technology). These technologies are intricate and relatively expensive to manufacture. Thus, there remains an interest in developing novel formulations that allow for sustained release of drugs using readily available, inexpensive excipients[6]. Sustained release, sustained action, prolonged action controlled release, extended released, depot release are the terms used to identify drug delivery systems that are designed to achieve a prolonged therapeutic effect by continuously releasing medication over an extended period of time after administration of single dose of drug. In some SR formulations, the drug dissolves into the matrix, and the matrix physically swells to form a gel, allowing the drug to exit through the gel's outer surface. There are certain considerations for the formation of sustained-release formulation,

- If the active compound has a long half-life (over 6 hours), it is sustained on its own.
- If the pharmacological activity of the active compound is not related to its blood levels, time releasing has no purpose.
- If the absorption of the active compound involves an active transport, the development of a time-release product may be Problematic.

Finally, if the active compound has a short half-life, it would require a large amount to maintain a prolonged effective dose. In this case, a broad therapeutic window is necessary to avoid toxicity; otherwise, the risk is unwarranted and another mode of administration would be recommended [7-10].

2. MATERIALS AND METHODS

Trovafloxacin was supplied from Ranbaxy Lab., Devas, INDIA. Citric Acid and Sodium Bicarbonate was a kind gift from Rankem lab. Mumbai, INDIA. HPMC and EC was a kind gift from Sulab lab. Baroda. Ethanol and methanol was purchased from Sigma Lab, New Delhi, INDIA. All other Excipients used in our work were of analytical grade.

Preformulation studies were the first step in the rational development of dosage form of a drug substance. The objective of Preformulation studies is to develop a portfolio of information about the drug substance, which is useful to develop formulation. Preformulation can be defined as investigation of physical and chemical properties of drug substance alone and when combined with excipients. Preformulation investigations was designed to identify those physicochemical properties and excipients that may influence the formulation design, method of manufacture, and Pharmacokinetic-biopharmaceutical properties of the resulting product**.** A fixed amount of drug was taken, and then distilled water was added and observes the solubility visually. Solubility study should be performed for Trovafloxacin to determine in which solvent it is soluble, for that various solvents like ethanol, methanol, 0.1N NaOH, phosphate Buffer pH 7.4 was used, for determining the solubility the drug should be dissolved in individual solvent in 1:10 ratio (Drug: Solvent) and visually observed for its solubility. The Melting point was determined by the capillary method using Digital Melting point apparatus. The capillary tube was fused and filled by pressing the open end gently into pure drug sample and packed by tapping the bottom of the capillary on a hard surface so that the drug packed down into the bottom of the tube. When the drug was packed into the bottom of the tube, the tube was placed into the slot of the apparatus, the apparatus was started and the temperature was noted at which the drug melt.

2.1 Analytical Estimation by UV

2.1.1 Determination of Wavelength of Maximum Absorbance (λmax)

10 mg of drug was weighed accurately and transferred to 100 ml of volumetric flask. Then Methanol was added to dissolve the drug completely. The volume was made up to 10 ml with Methanol. The prepared sample was 100 µg / ml. 10 ml of above solution was than transferred to another 100 ml volumetric flask and diluted it upto the mark with Methanol. This sample was 10 µg / ml.

2.1.2 Preparation of stock solution

Trovafloxacin (10 mg) was dissolved in 1mL Methanol and volume was made upto 10 mL volumetric flask using

Methanol. Five microliters of stock solution (1 mg/mL) was further diluted with Methanol to 10 mL. This solution (100µg/mL) was further diluted to Methanol to obtain solutions of 2 to 10 µg/mL. Absorption of each solution was measured at 294.8 nm using Systronics UV-2203 UV/Vis double beam spectrophotometer and Methanol as a reference standard. Trovafloxacin (10 mg) was dissolved in 1mL Sodium hydroxide (0.1N NaoH) and volume was made upto 10 mL volumetric flask using Sodium hydroxide (0.1N NaoH). Five microliters of stock solution (1 mg/mL) was further diluted with Sodium hydroxide (0.1N NaoH) to 10 mL. This solution (100 µg/mL) was further diluted to Sodium hydroxide (0.1N NaoH) to obtain solutions of 2 to 10 µg/mL. Absorption of each solution was measured at 290 nm using Systronics UV-2203 UV/Vis double beam spectrophotometer and Sodium hydroxide (0.1N NaoH) as a reference standard.

2.1.3 Preparation of dilutions from stock solution

From this stock solution 2, 4, 6, 8, 10 ml was pipette out in 100 ml calibrated volumetric flask and dilutions of 2, 4, 6, 8, 10 µg/ml was obtained. The absorbance of these solutions was taken on double beam U.V. spectrophotometer using λmax at 294.8 nm. The absorbance values were plotted against concentration (µg/ml) to obtain the standard calibration curve. Same procedure was fallowed for 0.1 N NaoH and Phosphate buffer pH 6.8.

2.1.4 Preparation of Trovafloxacin Sustained Release tablet

Tablets were prepared by conventional wet granulation method. The various excipients used were listed in table. Ingredients except glidants and lubricants were thoroughly mixed and passed through sieve no. 16. Granulation was done with a solution of calculated quantity of PVP K30 in sufficient isopropyl alcohol. The wet mass was passed through sieve no. 60 and dried at 50°C for 2hrs. The dried granules were lubricated with magnesium stearate and talc and compressed into tablets using single station tablet punch machine. Various formulation of Trovafloxacin Effervescent sustained release tablets shown in table 1.

2.2 Evaluation of tablets

Thickness and diameter of tablets were determined using Vernier caliper. Five tablets from each batch were used, and an average value was calculated. For each formulation, the hardness of five tablets was determined using the Monsanto Hardness tester (Cadmach). The friability of a sample of 10 tablets was measured using a Friability tester (Electro Lab). Ten tablets were weighed, rotated at 25 rpm for 4 minutes. Tablets were reweighed after Removal of fines (dedusted) and the percentage of weight loss was calculated. Twenty tablets were randomly selected from each batch individually weighed, the average Weight and standard deviation of 20 tablets was calculated. On immersion of tablets of different formulations in 0.1N HCl solution at 37±5°C, the tablets floated, and remained; the results of the total floating time (TFT) were shown in Table. Twenty tablets were taken and amount of drug present in each tablet was determined. The tablets were crushed in a mortar and the powder equivalent to 100mg of drug was transferred to 100ml standard flask. The powder was dissolved in 5ml of Methanol and made up to volume with phosphate buffer pH 6.8. The sample was mixed thoroughly and filtered through a 0.45µ membrane filter. The filtered solution was diluted suitably and analyzed for drug content by UV spectrophotometer at a λmax of 273 nm using phosphate buffer pH 6.8 as blank.

2.2.1 In vitro Disintegration test

The disintegration time was measured using disintegration test apparatus. One tablet was placed in each tube of the basket. The basket with the bottom surface made of a stainless steel screen (mesh no. 10) was immersed in water bath at 37 ± 2°C. The time required for complete disintegration of the tablet in each tube was determined using a stop watch. To be complied with the Pharmacopoeial standards, dispersible tablets must disintegrate within 20 min 20 sec. when examined by the disintegration test for tablets.

2.2.2 Dissolution rate studies

In vitro drug release of the sample was carried out using USP- type II dissolution apparatus (Paddle type). The dissolution medium, 900ml 0.1N HCl was placed into the dissolution flask maintaining the temperature of 37±0.5°C and rpm of 50. One Trovafloxacin tablet was placed in each basket of dissolution apparatus. The apparatus was allowed to run for 10 hours. Sample measuring 10ml were withdrawn after every 1 hour up to 10 hours using 10ml pipette. The fresh dissolution medium (37°C) was replaced every time with the same quantity of the sample.

2.2.3 Stability studies

Stability studies were aimed at determining the result of aging and storage under various conditions on the formulated extended release tablets. It was carried out to evaluate the stability of Trovafloxacin in formulated tablets after storing at different temperatures for 30 days. The Prepared tablets were kept at four different temperatures 4°C, 25°C, 40°C, 50°C for 30 days. At different intervals was evaluated for all physical parameter.

Table 1: Various formulation of Trovafloxacin Sustained release tablets

Excipients	Wet granulation mg/tablet							
	F1	F2	F3	F4	F5	F6	F7	F8
Trovafloxacin	250	250	250	250	250	250	250	250
HPMC	50	50	50	50	-	-	-	-
Ethyl cellulose	-	-	-	-	50	50	50	50
Lactose	25	30	20	25	25	30	20	25
Citric acid	25	20	30	20	25	20	30	20
Sodium bicarbonate	20	25	15	20	20	25	15	20
Talc	20	15	25	25	20	15	25	25
PEG	10	10	10	10	10	10	10	10

Table No.2: Solubility profile of Trovafloxacin in ratio (1:10)

S. No	Solvent	Ratio	Solubility
1.	Trovafloxacin: Ethanol	1:10	Sparingly soluble
2.	Trovafloxacin: Methanol	1:10	Freely soluble
3.	Trovafloxacin:Ethyle Acetate	1:10	Slightly soluble
4.	Trovafloxacin: Sodium Hydroxide(0.1N NaOH)	1:10	Freely soluble
5.	Trovafloxacin: Phosphate Buffer (pH)	1:10	Freely soluble
6.	Trovafloxacin: Glacial Acetic Acid	1:10	Freely soluble

Table 3: Partition coefficient of Trovafloxacin

S. No	Solvent	Wave length	Absorbance
1.	Ethyl Acetate	290.0	2.494
2.	Distilled water	290.0	2.998

Table 4: Result of study of physical parameters of Trovafloxacin and formulation (F1-F8)

Material	Angle of repose(Degree)	Bulk density(gm/ml)	Tapped density(gm/ml)	Compressibility index	Hausner ration
Trovafloxacin	38.04	0.593±0.008	0.790±0.008	30.30±0.78	0.725±0.01
F1	35.31	0.582±0.002	0.732±0.007	27.33±0.73	0.721±0.01
F2	36.15	0.581±0.008	0.730±0.006	28.33±0.72	0.723±0.01
F3	34.82	0.576±0.002	0.728±0.005	27.30±0.68	0.720±0.01
F4	37.69	0.570±0.007	0.729±0.003	29.30±0.65	0.726±0.03
F5	37.30	0.580±0.003	0.735±0.004	30.30±0.61	0.730±0.04
F6	39.28	0.585±0.003	0.732±0.006	32.80±0.64	0.728±0.06
F7	36.46	0.582±0.004	0.742±0.003	36.24±0.70	0.720±0.03
F8	38.04	0.582±0.006	0.740±0.008	38.23±0.61	0.718±0.01

Table No. 5: Results of Post Compression Properties of Trovafloxacin Sustained Release Tablets

Formulation code	Thickness (mm)	Hardness (kg/cm2)	Weight variation (mg)	Friability (%)	Drug content (%)	Total floating duration (h)
F1	3.53±0.05	4.8	328.19± 2.94	0.58 ± 0.10	98.33± 0.92	8
F2	3.94± 0.10	4.4	332.18 ± 3.77	0.51 ± 0.08	97.20 ± 0.34	10
F3	3.96± 0.05	4.5	335.33 ± 1.50	0.38 ± 0.12	99.60 ± 1.39	>12
F4	3.95± 0.05	4.7	336.30 ± 3.30	0.16 ± 0.04	98.14 ± 1.69	>12
F5	3.93± 0.10	5.2	327.13 ± 2.83	0.31 ± 0.07	99.21 ± 1.07	>12
F6	4.03± 0.06	5.3	332.16 ± 2.33	0.27 ± 0.05	99.50± 1.81	>12
F7	4.05± 0.05	4.8	338.18 ± 3.11	0.29 ± 0.08	99.34 ± 0.37	>12
F8	3.98± 0.05	4.5	327.04 ± 2.56	0.34 ± 0.12	98.31± 0.91	>12

3. RESULT AND DISCUSSION

3.1 Melting point

The sample of Trovafloxacin was identified and characterized as per the Pharmacopoeial requirements. The sample drug (Trovafloxacin) was found to be showing a melting point of 218-220°C. The above obtained results were in accordance with pharmacopeial requirement.

3.2 Solubility

Solubility studies of Trovafloxacin is shown in Table 2.

3.3 Partition coefficient

These Partition coefficient as the Absorbance of organic layer (2.494) in divided as the Absorbance of organic layer (2.998) as the value 0.83. Partition coefficient of Trovafloxacin is shown in Table 3.

3.4 Standard Calibration Curve of Methanol

This different concentration as the 2,4,6,8 & 10 Absorption as 0.177, 0.236, 0.344, 0.451 & 0.553. The correlation coefficient as 0.991 The calibration curve for Trovafloxacin in Methanol is in the concentration range of 2-10 µg /ml as shown in following figure 1. The Wavelength of maximum absorbance (λmax) in Methanol was found to be 294.8 nm and the graph is shown in figure 1.

3.5 Standard Calibration Curve 0.1N NaOH

These different concentrations as the 2,4,6,8 & 10 Absorption as 0.192, 0.253, 0.322, 0.451 & 0.524. The correlation coefficient as 0.98. The calibration curve for Trovafloxacin in 0.1N NaoH is in the concentration range of 2-10 µg /ml as shown in following figure. The Wavelength of maximum absorbance (λmax) in 0.1N NaoH was found to be 290.0 nm. and the graph is shown in figure 2.

Physical parameter for the formulations prepared by sustained release technique is shown in Table No.4: Bulk density was found to be between 0.570±0.007 to 0.593±0.008 g/ml and tapped density between 0.728±0.005 to 0.790±0.008g/ml, Compressibility index between 27.30±0.68 to 38.23±0.61%, Hausner ration between 0.720±0.01to 0.730±0.04 and angle of repose was found to be between 34.82 to 39.28^0, indicating fair to good flow properties.

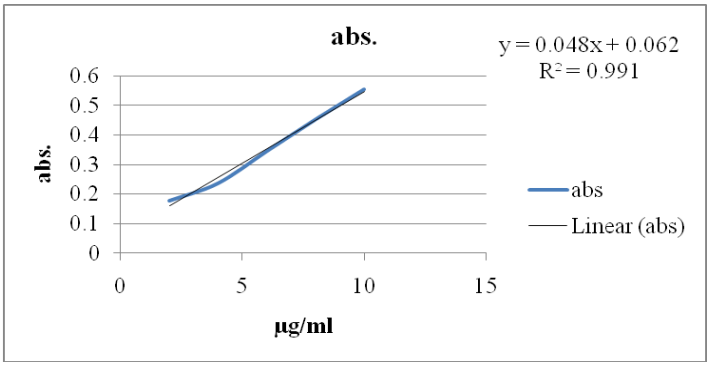

Figure.1: Calibration curve of Trovafloxacin in Methanol

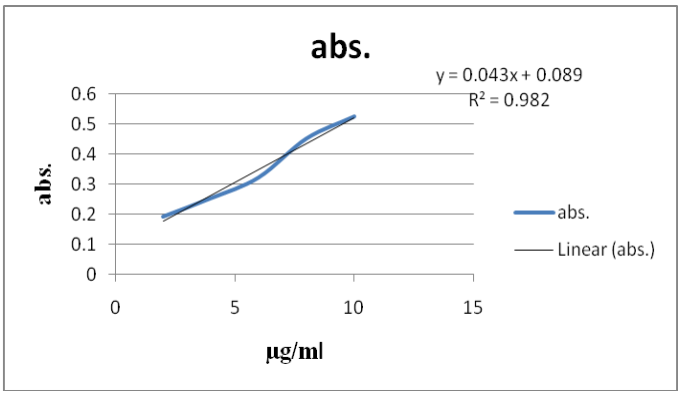

Figure.2: Calibration curve of Trovafloxacin in 0.1N NaOH

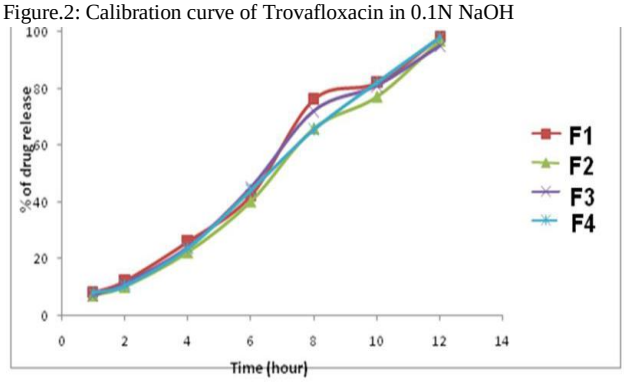

Figure 3: % of Drug release of Trovafloxacin floating tablet using HPMC

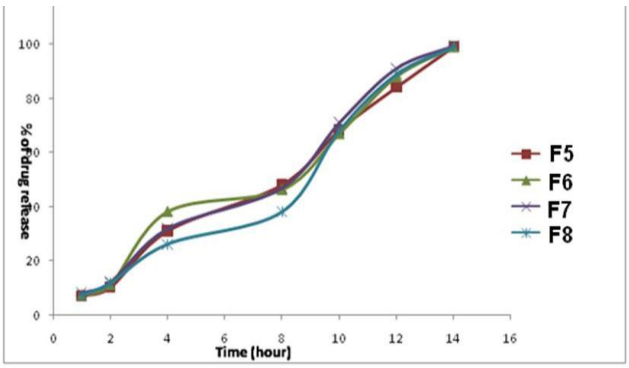

Figure 4: % of Drug release of Trovafloxacin using Ethyl cellulose

Table 6: In vitro drug release study of floating tablet

Time (hr)	% of Drug Release							
	F1	F2	F3	F4	F5	F6	F7	F8
1	08.23	07.14	07.24	08.23	07.23	07.45	08.32	07.26
2	12.32	10.23	11.45	10.45	10.45	11.23	12.23	11.87
4	26.23	22.42	24.23	23.76	31.23	38.23	32.13	26.28
8	42.45	40.32	45.23	44.23	48.23	46.32	47.14	38.21
10	76.34	66.11	72.21	65.71	68.34	67.02	71.13	68.24
12	82.23	77.33	81.11	82.34	84.23	88.13	91.23	89.12
14	98.32	97.13	95.13	98.35	99.12	99.13	99.56	99.25

Table 7: Change in In-Vitro Drug Release Profile of Optimized Formulation during Stability Study

Product code	Temperature	In-vitro % Drug release profile			
		At 0 week	At 2 weeks	At 3weeks	At 4 weeks
F7	4°C	97.93	97.7	97.04	97.43
	25°C	99.48	98.63	98.34	98.14
	40°C	98.65	98.67	98.34	98.23
	50°C	99.36	98.69	98.47	98.03

3.6 In Vitro Drug Release

The in vitro drug release from tablets containing citric acid and heat treated sodium bicarbonate in ration of 8:10, was above 95% and drug release from tablets s containing citric acid and sodium bicarbonate in ration of 8:10 and was above 65% within 10 minutes. The drug release profiles of all prepared tablets are shown in table 6.

The change in In-vitro drug release of optimized formulations during stability study as selection of product code as F7 choose the higher % drug release. The different temperature of optimized formulation as 4°C, 25°C, 40°C, 50°C. After zero weeks of formulation as 97.93, 99.48, 98.65, 99.36, After two weeks of formulation as 97.7, 98.63, 98.67, 98.69. These are after three weeks of formulation as 97.04, 98.34, 98.34, and 98.47 and after four weeks of formulation as 97.43, 98.14, 98.23, and 98.03.

4. CONCLUSION

Compared to investigate a new molecule, it is better to do the research and development of already existing molecules by solving the problem of confrontation due to their awkward use particularly in case of drugs like antibiotics. The advantages of sustain released dosage form is that they can often be taken less frequently than conventional formulation of same drug, helpful in increasing the efficiency of drug and they keep steadier levels of drug in the blood stream. All this sustain release formulation comes with reasonable cost. Difference between sustained and controlled release is that controlled release is perfectly zero order release that is drug release with time irrespective of concentration. On the other hand, sustained release implies slow release of drug over a time period. Sustain released formulation may or may not be controlled release. It can be easily conclude that development of sustained release dosage form which will prolong the drug release leading to minimize the peak and valley effect in plasma and provide patients compliance.

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