

Development and *in vitro*- *in vivo* evaluation of Nitazoxanide sustained release tablets

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Abstract

Objective: The objective of the present investigation was to develop a sustained release (SR) formulation of Nitazoxanide.

Methods: Nitazoxanide Sustained Release tablets were prepared by employing wet granulation method, where hydrophilic polymers were used as drug release retardants.

Results: Based on data obtained from the *in vitro* drug release studies 5%w/w of Methocel K100M was selected as a release retardant. The drug release followed first order kinetics and fickian diffusion.

Conclusion: Present investigation indicates that the developed formulation was able to sustain the drug release.

Keywords: Nitazoxanide, Sustained release, Hydrophilic polymers

1. Introduction

Oral drug delivery has been known for decades as the most widely utilized route of administration among all the routes that have been explored for the systemic delivery of drugs via various pharmaceutical products of different dosage forms. The goal in designing sustained or controlled delivery systems is to reduce the frequency of the dosing or to increase effectiveness of the drug by localization at the site of action, reducing the dose required or provide uniform drug delivery. Controlled release dosage forms provide a better control of plasma drug levels, less dosage frequency, less side effect, increased efficacy and constant delivery.

Nitazoxanide is an anti protozoal agent. The relative bioavailability of the suspension compared to the tablet was 70%. Nitazoxanide is rapidly hydrolyzed to an active metabolite, tizoxanide (desacetyl-nitazoxanide) and excreted in the urine, bile and feces. Approximately two-thirds of the oral dose of nitazoxanide is excreted in the feces and one-third in the urine.

Although conventional tablets of Nitazoxanide available in the market commercially, no study has been done so far for preparing the Nitazoxanide sustained-release tablets. To improve the oral bioavailability and to reduce the dose dependent toxicity there is a need for the development of sustained-release formulations. Hence, in the present study, an attempt has been made to develop the sustained-release matrix tablets of Nitazoxanide using hydrophilic polymers and evaluated for *in vitro* and *in vivo* characteristics.

2. Materials and Methods

Nitazoxanide procured from Chemo Lugano, Hydrophilic polymers like Methocel K100M Prem CR, Methocel K4M Prem CR, PEO WSR 301 and PEO WSR 303 are received from Colorcon, Microcrystalline cellulose (MCC PH 102) obtained from FMC Biopolymer, Plasdone K29/32, from ISP Sales (UK) Ltd., Polyplasdone XL 10 obtained from Ashland, Magnesium Stearate from Ferro Corporation Ltd.

2.1 Preparation of Nitazoxanide sustained release tablets [3-5]:

The Nitazoxanide Sustained release tablets were prepared by employing wet granulation technique. The lubricated blend was evaluated for various physical properties. Then the lubricated blend was compressed using 8 station rotary tableting machine, equipped with 19.0mm × 9.0mm caplet shaped punches at 15 RPM. These compressed tablets were evaluated for various physico-chemical parameters. The composition of the optimized formulation tabulated below:

Table 1: Composition of the Optimized formulation of Nitazoxanide sustained release tablets

Composition	mg/Tablet
Nitazoxanide	1000.00
MCC PH 101	114.60
Polyplasdone XL 10	1.80
Methocel K100M Prem CR	50.00
PVPPK 29/32	30.00
Magnesium Stearate	3.60
Total weight of the tablet	1200.00

2.2 Characterization of the Lubricated Blend [6-7]:**2.2.1 Determination of Bulk Density and Tapped Density**

An accurately weighed quantity of the lubricated blend (W) was carefully poured into the graduated cylinder and volume (V_0) was measured. Then the graduated cylinder was closed with lid and set into the tap density tester (USP). The density apparatus was set for 500 taps and after that the volume (V_f) was measured and continued operation till the two consecutive readings were equal.

The bulk density and the tapped density were calculated using the following formulae.

$$\text{Bulk density} = W/V_0$$

$$\text{Tapped density} = W/V_f$$

Where, W= Weight of the powder, V_0 = Initial volume, V_f = final volume

2.2.2 Compressibility Index (Carr's Index)

Carr's index (CI) is an important measure that can be obtained from the bulk and tapped densities. In theory, the less compressible a material the more flowable it is. CI was calculated by using the following formulae

$$CI = (TD - BD) \times 100 / TD$$

Where, TD is the tapped density and BD is the bulk density.

2.2.3 Hausner's Ratio

It is the ratio of tapped density and bulk density, which was related to inter particle friction and, as such, could be used to predict powder flow properties. Generally a value less than 1.25 indicates good flow properties, which is equivalent to 20% of Carr's index

2.2.4 Angle of Repose

The angle of repose of granules was determined by the funnel-method. The accurately weighed granules were taken in a funnel. The height of the funnel was adjusted in such a manner that the tip of the funnel just touched the apex of the heap of the granules. The granules were allowed to flow through the funnel freely onto the surface. The diameter of the powder cone measured and angle of repose was calculated using the following equation.

$$\tan \theta = h/r$$

Where h and r are the height and radius of the powder cone, θ is the angle of repose.

2.3 Characterization of the Compressed Tablets:**2.3.1 Thickness & Hardness**

Twenty tablets from the representative sample were randomly taken and individual tablet thickness and hardness were measured by using digital Tablet thickness and Hardness tester. Average thickness and hardness were calculated.

2.3.2 Friability Test

From each batch, ten tablets were accurately weighed and placed in the friability test apparatus (Roche friabilator). Apparatus was operated at 25 rpm for 4 minutes and tablets were observed while rotating. The tablets were then taken after 100 rotations, dedusted and reweighed.

% friability was calculated as follows

$$\% \text{ Friability} = (W_1 - W_2) \times 100 / W_1$$

Where W_1 = Initial weight of the 10 tablets, W_2 = Final weight of the 10 tablets after testing.

Friability values less than 1.0% are generally acceptable.

2.3.3 Uniformity of dosage units (by Weight Variation)

To study weight variation individual weights (W_i) of 20 tablets from each formulation were noted. Their average weight (W_A) was calculated. Percent weight variation was calculated as follows.

$$\% \text{ weight variation} = (W_A - W_i) \times 100 / W_A$$

As the total tablet weight was 1200 mg, according to USP, out of twenty tablets $\pm 5\%$ variation can be allowed for not more than two tablets.

2.3.4 Drug Content (Assay) by HPLC

2.3.4.1 Preparation of buffer:

1.6 g of tetra n- butyl ammonium hydrogen sulphate was dissolved in 1000 ml of water and filtered the solution through 0.45μ membrane filter paper.

2.3.4.2 Preparation of mobile phase:

A filtered and degassed mixture of buffer and acetonitrile in the ratio of 45:55.

2.3.4.3 Diluent: Acetonitrile was used as a diluent.

2.3.4.4 Preparation of standard solution:

100 mg of nitazoxanide working standard was accurately weighed and transferred in to a 100 ml volumetric flask. 35 ml of diluent was added and sonicated; made up the volume to 100 ml with diluent and mixed. Transferred 2 ml of this solution to 20 ml volumetric flask and diluted to volume with diluent.

2.3.4.5 Preparation of sample solution:

The tablets were crushed into fine powder and equivalent to 100 mg of nitazoxanide was taken in to 100 ml volumetric flask. 50 ml of diluent was added, allowed for sonication for 15 minutes with intermittent shaking and made up the volume to 100 ml with diluent and mixed well, filtered the solution through 0.45μ nylon filter paper. 2 ml of this solution was transferred to 20 ml volumetric flask and diluted volume with diluent.

2.3.4.6 Chromatographic conditions:

Column: inertsil ODS 3V, 150mmx4.6mmx 5μm or it's equivalent

Flow rate: 1ml/min

Wave length: 240 nm

Injection volume: 10μl

Run time: 10 minutes

The diluent, five replicates of standard solution and two injections of sample solution were separately injected in to the HPLC and measured the peak areas.

Content of Nitazoxanide calculate by using following formula:

$$= \frac{AT}{As} \times \frac{Std.wt}{100} \times \frac{2}{20} \times \frac{100}{sample\ wt} \times \frac{20}{2} \times \frac{P}{100} \times AW = \text{mg/tab}$$

$$\% \text{ of labeled amount} = \frac{\text{Content of Nitazoxanide}}{\text{Label claim in mg}} \times 100$$

Where,

At= peak area of Nitazoxanide in sample solution.

As= average peak area of Nitazoxanide in sample solution.

Std. wt= weight of Nitazoxanide in standard solution.

Sample wt = weight of the sample.

P= potency of Nitazoxanide on as is basis.

AW= average weight of the tablets.

2.3.5 In -Vitro Drug Release (Dissolution) [8]

Reagents, preparation of buffer, mobile phase, diluents, chromatographic conditions and system suitability parameters were adopted same as that of 'assay'.

2.3.5.1 Dissolutions parameters

Dissolution medium: 1000 ml, 0.1 N hydrochloric acid with 10 % hexa decyl trimethyl ammonium bromide.

Apparatus: USP type II (paddle)

RPM: 75

Time: 1st, 2nd, 3rd, 4th, 6th, 8th, 9th and 12th hours

Bath temperature: 25 ±3°C

2.3.5.2 Preparation of dissolution medium:

8.5 mL of hydrochloric acid was transferred in to 1000 mL water and mixed well. 100g of hexadecyl trimethyl ammonium bromide was added and mixed using magnetic stirrer until it dissolved.

2.3.5.3 Procedure:

The diluent, five replicates of standard solution and two injections of sample solution were separately injected in to the HPLC and measured the peak areas'. Amount of Nitazoxanide dissolved at respective time interval (Dn) is calculated by:

$$= \frac{\text{AT} \times \text{WS} \times 5 \times 1000 \times 50 \times \text{P}}{\text{AS} \times 50 \times 50 \times 1 \times 5 \times 100} \times \frac{\text{label claim in mg}}{100} = \text{--- \%}$$

Where,

AT= area of Nitazoxanide in sample solution.

AS= average area of Nitazoxanide in standard solution.

WS=weight of Nitazoxanide working standard taken in mg.

P= purity of Nitazoxanide working standard used (on the basis).

2.3.6 In vivo Studies

2.3.6.1 Experimental design:

Parallel design was selected in which out of six albino male rabbits, three were treated with test formulation (Nitazoxanide sustained release tablets) and another three with reference (Nitazoxanide API).

2.3.6.2 Drug administration sample collection:

Institutional animal ethics committee (1722/RO/ERe/S/13/CPCSEA) approved protocol of the study. Healthy male albino rabbits weighing between 2.0 - 2.5 Kg were used. Rabbits were separated into 2 groups, each consisting of 3 animals. The dose was calculated based on the rabbit weight (i.e. equivalent to human dose of 100 mg per 70Kg). Reference (Nitazoxanide API) and Test formulation (Nitazoxanide sustained release tablets) were given orally via silicone rubber gastric intubation tube to the first group and second group respectively. All the rabbits were housed in individual cages at room temperature, fasted prior to the 12 hours of drug administration and have access to water and food after 4 hours of dosing throughout the study period.

Approximately 2 mL of blood sample was collected at proposed time points such as Pre dose, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 18 and 24 hours through marginal ear vein. All the blood samples were collected into K₂EDTA coated tubes. Samples were centrifuged at 4000 rpm for 5 minutes. The plasma was separated and stored at -70°C until analysis.

2.3.6.3 Sample Preparation

A simple protein precipitation method was used for the extraction of nitazoxanide from the rabbit plasma samples. To an aliquot of 100 µL plasma, 300 µL ACN was added. Inverted and mixed for 15 Sec on a cyclomixer and vortex for 2 min, samples were centrifuged at 3000 rpm for 5 min. The supernatant layer was separated and subject to evaporation under liquid nitrogen. Finally dried samples were reconstituted in the 100 µL of mobile phase and then estimated the content of nitazoxanide using HPLC method.

2.3.6.4 Determination of Nitazoxanide from plasma:

A high performance liquid chromatography (HPLC) method was used for the estimation of Nitazoxanide from rabbit plasma and nifuroxazide used as an internal standard (IS). The 50 mmol/ L⁻¹ KH₂PO₄: acetonitrile: methanol was used as an eluent. Nitazoxanide (tizoxanide) and nifuroxazide were eluted at 22 min and 18 min respectively.

2.3.6.5 Pharmacokinetic analysis:

Pharmacokinetic study was carried out to determine the various parameters such as time to reach maximum concentration (T_{max}), maximum plasma concentration (C_{max}), area under the curve (AUC_{0-inf}). The values of T_{max} and C_{max} were noted from the arrhythmic plot of time versus plasma concentration of Nitazoxanide. The AUC was determined by trapezoidal rule.

3. Results

3.1 Characterization of lubricated blend

The granules for matrix tablets were characterized with respect to angle of repose, bulk density, tapped density and Carr's index (Table 2).

Table 2: Physical Properties of Lubricated Blend

Formulations	Angle of repose (°)	Bulk Density (g/mL)	Tapped Density (g/mL)	Carr's Index (%)	Hausner's ratio
F1	30.01	0.596	0.673	11.4	1.13
F2	31.02	0.590	0.669	11.8	1.13
F3	30.09	0.601	0.692	15.2	1.18
F4	31.02	0.605	0.697	15.5	1.18
F5	30.45	0.602	0.678	11.2	1.13
F6	29.41	0.599	0.684	12.4	1.14
F7	31.42	0.607	0.679	10.6	1.12
F8	29.56	0.605	0.682	11.3	1.13
F9	28.23	0.604	0.680	11.2	1.13
F10	29.81	0.607	0.683	11.1	1.13

3.2 Characterization of the sustained release tablets

3.2.1 Physical Evaluation of tablets

The results of the uniformity of weight, hardness, thickness, friability, and drug content of the tablets are given in Table 3.

Table 3: Physical evaluation of tablets

F. Code	Hardness (kg/cm ²)	Thickness (mm)	Weight (mg)	Friability (%)	Drug content (%)
F1	13.7±1.15	6.77±0.037	1200.8±6.62	0.44	99.25±1.37
F2	13.6±1.0	6.78±0.039	1197.10±7.81	0.47	98.28±0.80
F3	13.7±1.51	6.78±0.04	1202.9±8.26	0.43	99.12±2.47
F4	13.9±1.20	6.80±0.35	1205±10.26	0.41	101.22±0.88
F5	14.1±1.53	6.79±0.41	1200.9±6.99	0.40	100.24±1.25
F6	15.2±3.06	6.80±0.381	1195.7±6.75	0.39	99.53±1.87
F7	16.1±1.53	6.81±0.379	1202.01±7.75	0.38	96.28±1.99
F8	15.1±2.52	6.79±0.32	1205.12±9.51	0.39	97.55±1.14
F9	15.3±1.00	6.78±0.04	1204.5±6.51	0.37	98.34±2.18
F10	15.2±1.00	6.78±0.042	1205.31±7.9	0.38	99.29±0.98

3.2.2 In-vitro drug release studies

Drug release profiles of optimized formulation is described below (Table 4 & Figure 1)

Table 4: Comparative dissolution data of Nitazoxanide SR Tablets 1000 mg – Optimized formulation

Time (Hr)	% Drug release	
	F9	F10
1	21.3 ± 3.5	20.4 ± 3.1
2	34.5 ± 3.3	32.8 ± 3.0
3	45.2 ± 3.0	43.1 ± 2.7
4	60.1 ± 2.6	58.9 ± 2.6
6	70.3 ± 2.1	68.8 ± 2.4
8	81.6 ± 1.9	79.6 ± 1.8
10	91.1 ± 1.5	90.6 ± 1.7
12	98.0 ± 1.0	97.8 ± 0.9

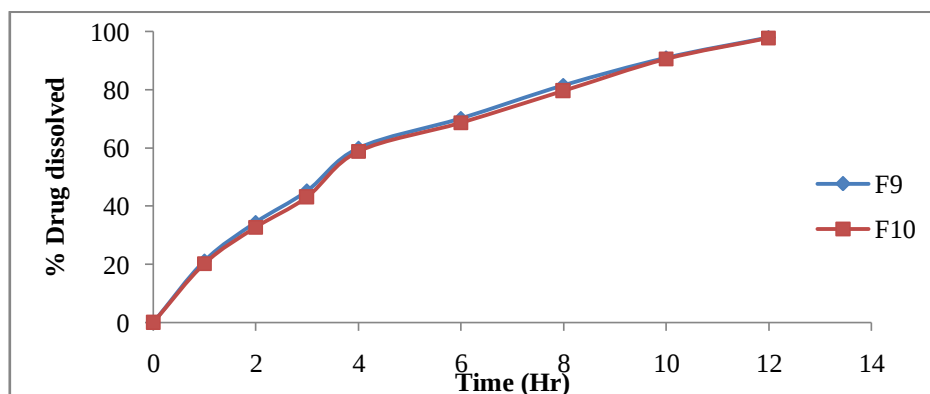


Figure 1: Comparative dissolution profiles of Nitazoxanide SR Tablets 1000 mg – Optimized formulation

3.2.3 In vivo Studies:

The pharmacokinetic parameters of the Nitazoxanide SR tablets were described below.

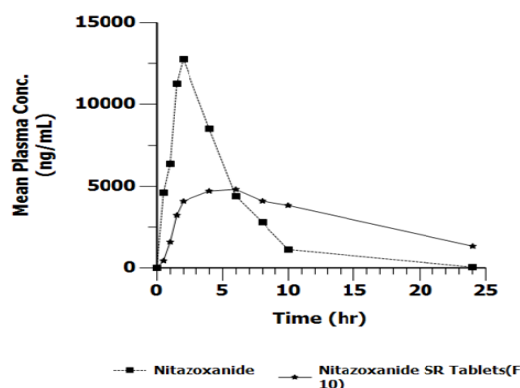


Figure 2: Plasma concentration vs. Time profiles of Nitazoxanide and Nitazoxanide sustained release tablets (F10)

Table 5: Summary of pharmacokinetic parameters for test and reference

S. No.	Pharmacokinetic Parameters	Test formulation (B. No. F10)	Reference
1	C _{max} (ng/mL)	49300	12800
2	T _{max} (h)	4.00	2.00
3	AUC _{0-t} (h*ng/mL)	71800	63600
4	AUC _{0-∞} (h*ng/mL)	91900	63800
5	T _{1/2} (h)	9.95	2.94

4. Discussion

4.1 Characterization of the lubricated blend:

From the obtained results, Angle of repose was less than 35° and Carr's index values were less than 12 for the granules of all the batches indicating good to fair flowability and compressibility. Hausner's ratio was less than 1.25 for all the batches indicating good flow properties. The drug content was more than 90 % for all the granules of different formulations.

4.2 Characterization of the Tablets

All the tablets of different batches complied with the official requirements of uniformity of weight as their weights varied between 1195 and 1204mg.

The hardness of the tablets ranged from 13 to 16 kg/cm² and the friability values were less than 1%. The thickness the tablets ranged from 6.6 to 6.8 mm. All the formulations satisfied the content of the drug as they contained 96 to 102 % of nitazoxanide and good uniformity in drug content was observed. Thus all the physical attributes of the prepared tablets were well within the limits.

From the earlier studies and obtained results it was concluded that the trial F9 (1.5%w/w disintegrant with 5%w/w of polymer) was selected as an optimized formulation. To ensure the reproducibility of the same, F10 was manufactured and satisfactory results were obtained. F10 was evaluated for kinetic modelling, drug release followed the first order kinetics and as the n value is less than 0.45; the mechanism of drug release is found to be Fickian diffusion (Peppas).

From the pharmacokinetic data, the optimized formulation able to sustain the drug release over a period of 12 hours.

From the present investigation it was concluded that once daily sustained release dosage form of Nitazoxanide is successfully developed and which extends the drug release up to 12 hours'.

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