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Design, Optimization and In Vivo Evaluation of Granisetron HCl Mouth Dissolving Films

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ABSTRACT

Present work aimed at preparing quick onset of action of Granisetron HCl which is beneficial in emesis, aiding in the enhancement of bioavailability and is very convenient for administration without the problem of swallowing and using water. The films were prepared by using different grades of HPMC E3, E6 and E15**,** maltodextrin DE6 and other polymers by solvent casting method. They were evaluated for physical characteristics such as thickness, uniformity of weight, folding endurance, drug content, surface pH, percentage elongation and tensile strength and results were found to be satisfactory. The formulations were subjected to disintegration and in-vitro drug release test. The in vitro disintegration time of the optimized formulation F9 was 9 sec and drug release was found to be very fast i.e. 97.8% of within 8 min when compared with innovator product i.e 70.8%. *In vivo* studies confirmed that their potential as an innovative dosage form to improve the bioavailability and considered to be potentially useful for the treatment of emesis where quick onset of action is desirable. DSC and FTIR data revealed that no interactions takes place between the drug and polymers used in the optimized formulation. From the above results, it can be a good alternative to conventional Granisetron Hydrochloride tablets in the treatment of emesis. *In vitro* and *in vivo* evaluation of the films confirmed their potential as an innovative dosage form to improve delivery and quick onset of action of Granisetron Hydrochloride.

Keywords: Granisetron Hydrochloride, fast dissolving oral films, emesis, HPMC, bioavailability studies.

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INTRODUCTION

The oral route is one of the most preferred routes of drug administration as it is more convenient, cost effective, and ease of administration lead to high level of patient compliance. The oral route is problematic because of the swallowing difficulty for pediatric and geriatric patients who have fear of choking. Recent developments in the technology have presented viable dosage alternatives from oral route for pediatrics, geriatric, bedridden, nauseous or noncompliant patients. The buccal cavity is an attractive route of administration for systemic drug delivery. Oral mucosa has a rich vascularisation and offers higher permeability to many drugs. It has been well known that after buccal and sublingual administration drug solutes are rapidly absorbed in to the reticulated vein and are then drained into the systemic circulation[1,2]. The oral fast dissolving dosage forms, also known as fast melt, fast disintegrating dosage forms, are relatively novel dosage technology that involves rapid disintegration or dissolution of the dosage forms, into a solution or suspension in the mouth without need of water[3,4,5,6,7]. The dosage form begins to disintegrate immediately after coming into contact with saliva, the complete disintegration normally occurring within 30 to 50 seconds[8]. The solution containing active ingredients is swallowed, and the active ingredients are then absorbed through gastrointestinal epithelium to reach the target and to produce the desired effect[9].

In some cases such as motion sickness, sudden episode of allergic attack or coughing, fear of choking and an unavailability of water, the swallowing of tablet or capsules may become difficult. To overcome these difficulties, several fast dissolving drug delivery systems have been developed[10]. It was developed on the basis of technology of the transdermal patch. The delivery system consists of a very thin oral strip, which is simply placed on the patient's tongue or any oral mucosal tissue, instantly wet by saliva the film rapidly hydrates and adheres onto the site of application. It then rapidly disintegrates and dissolves to release the medication for oromucosal and intra gastric absorption. Technology Catalysts forecasts the market for drug products in oral thin film formulations was valued of $500 million in 2007 and could reach $2 billion in 2012. Based on upward global growth trends of the past decade, the fast dissolving dosage market could produce revenues of $13 billion by 2015.

Vomiting, also known as emesis, throwing up, among other terms, is the involuntary, forceful expulsion of the contents of one's stomach through the mouth and sometimes the nose. Vomiting can be caused by a wide variety of conditions, it may present as a specific response to ailments like gastritis or poisoning, or as a non-specific sequela of disorders ranging from brain tumors and

elevated intracranial pressure to overexposure to ionizing radiation. The feeling that one is about to vomit is called nausea, which often proceeds, but does not always lead to, vomiting[11]. Granisetron hydrochloride is chemically endo-1-methyl-N-(9-methyl-9- azabicyclo non-3-yl)-H-indazole-3-carboxamide hydrochloride, a selective 5-HT3 receptor antagonist, which may have beneficial therapeutic effects in the treatment of vomiting and nausea resulting from cancer therapy[12]. It has an improved side effect and tolerability profile, a lower risk of drug interactions and a longer duration of action than other 5-HT3 receptor antagonists. It is also an effective and well-tolerated agent in the management of chemotherapy-induced, radiotherapy-induced and post-operative nausea and vomiting in adults and children[13]. Its main effect is to reduce the activity of the vagus nerve, which is a nerve that activates the vomiting centre in the medulla oblongata. Granisetron hydrochloride undergoes extensive hepatic first pass metabolism with a bioavailability of 60%. The terminal elimination half-life is 3 to14 hours after oral administration. Granisetron hydrochloride is about 65% bound to plasma proteins[14].

MATERIALS AND METHOD

Materials:

Granisetron Hydrochloride was generous gift sample from Aurobindo Pharma Ltd, Hyderabad, India. Hydroxypropylmethylcellulose (HPMC E3, E6 & E15) was gifted by Nectar life sciences, Hyderabad, Maltodextrin DE6 and Aspartame was obtained from MSN Labs, Hyderabad. Xanthan gum was obtained from Matrix Labs, Hyderabad, Propylene glycol and Glycerine are from Laboratory Grade. Vanillin and Citric acid are from SD FINE CHEM. LTD, Mumbai. All other chemicals used were of analytical grade.

Methods:

Determination of dose of Granisetron HCl:

Amount of drug required per film =1.12 mg of Granisetron HCl

Therefore, 4 films require 4.48 mg of drug

Area of the petridish (πr^2) ==3.14*4.5*4.5=63.5cm^2

6 films of 4 cm^2 each i.e. (2cm*2cm) can be obtained freely per petridish.

Area not required is the one remaining after cutting the films from the centre of petridish. This is obtained as

Area considered= Sum of the areas of number of films taken= 4 cm^2*6=24 cm^2

Amount of drug in area considered= 6.72mg

Area not considered= Total area of petridish-Area considered

$= 63.5\text{-}24 = 39.5\ cm^2$

4 cm^2 film contains 1.12mg of drug therefore 39.5 cm^2 contains 11.2 mg of drug

Amount of drug in area not considered=11.2mg

Therefore,

Total drug dose = (Amount of drug in area considered) + (Amount of drug in Area not considered) = 6.72mg + 11.2 mg =17.92.

Therefore, an approximate amount of 17.92mg drug was considered per Petridish[15]

Preparation of Granisetron hydrochloride films

It was aimed to prepare to fast dissolving oral films of Granisetron Hydrochloride whose dose was 1.12mg per 4cm^2 films. The procedure was carried out on a digital magnetic stirrer using a medium sized magnetic bead.

Film forming polymers hypromellose and maltodextrin were weighed accurately, added to a small amount of water in a small beaker, covered with an aluminium foil and soaked for 24 hours to ensure complete hydration. Xanthan gum was added the next day in small amounts and the solution was stirred on a magnetic stirrer at 75rpm for first half an hour and later 50rpm for 1.5 hours. Then, propylene glycol was added and stirring continued for 30min at 50rpm.

Granisetron Hydrochloride drug, Aspartame, citric acid, vanillin and amaranth were dissolved in sufficient quantity of water and added to the polymer mixture. This film forming solution then stirred well to obtain a homogenous solution. Dry and clean petridish was selected and the solution was poured into it. Drying was carried out at 45 ºC in a hot air oven for 6 hours. The Petri dish was then removed and left aside to cool down to room temperature. The film was then peeled carefully using a surgical scalpel by making a small incision in the film on one side of the Petri dish. Small films of 4cm^2 were cut from one big film and packed primarily in a aluminium foil and secondarily in a self-sealing polythene to ensure least moisture penetration. The formulation was carried out using three different polymers, hypromellose E3, hypromellose E6 and hypromellose E15 and the resulting films were evaluated[16].

The composition of Granisetron Hydrochloride fast dissolving oral films with different HPMC grades are shown in Table 1, 2 & 3.

Table 1: Formulation trails using HPMC E3

Formulation code & ingredients	F1	F2	F3	F4
Granisetron hydrochloride (mg)	17.92	17.92	17.92	17.92
HPMC E3 (mg)	225	250	275	300
Maltodextrin (mg)	130	120	110	100
Propylene glycol	50	60	70	80

xanthan gum (mg)	10	10	8	8
Aspartame (mg)	20	20	20	20
Citric acid (mg)	10	10	10	10
water(ml)	Q.S	Q.S	Q.S	Q.S
vanilla	Q.S	Q.S	Q.S	Q.S
Amaranth	Q.S	Q.S	Q.S	Q.S

Table 2: Formulation trails using HPMC E6

formulation code& ingredients	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**
Granisetron Hydrochloride(mg)	17.92	17.92	17.92	17.92	17.92	17.92	17.92	17.92
HPMCE6(mg)	200	210	220	230	240	250	260	270
Maltodextrin(mg)	180	180	170	170	160	160	150	150
Xanthangum(mg)	10	10	8	8	8	6	6	6
Glycerine (mg)	70	70	80	80	90	90	100	100
Aspartame (mg)	20	20	20	20	20	20	20	20
Citric acid (mg)	10	10	10	10	10	10	10	10
Water (ml)	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S
Vanilla	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S
Amaranth	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

Table 3: Formulation trails using HPMC E15

Formulation Code & Ingredients	**F13**	**F14**	**F15**	**F16**	**F17**	**F18**
Granisetron Hydrochloride(mg)	17.92	17.92	17.92	17.92	17.92	17.92
HPMCE15(mg)	170	180	190	200	210	220
Maltodextrin(mg)	190	180	170	170	160	150
Xanthangum(mg)	10	8	7	6	5	5
Glycerine (mg)	90	100	100	110	110	100
Aspartame(mg)	20	20	20	20	20	20
Citric acid(mg)	10	10	10	10	10	10
water (ml)	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S
vanilla	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S
Amaranth	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

Evaluation of Granisetron Hydrochloride fast dissolving oral films:

Physical characterization of FDOFs:

Physical characterization of FDOFs can be carried out by visual inspection for characteristics such as colour, thickness, brittleness, peeling ability, transparency, surface smoothness, tack property and film forming capacity.

Peeling ability is measured as the easy or difficulty in separating the film from the release liner.

Transparency is checked by placing the film against an illuminated background and viewing carefully to find any opacity.

Film forming capacity is the ability of the film forming polymer to form an efficient film, thin enough and also with sufficient drug loading ability. Film forming capacity may be rated as poor, average, good and excellent based on the overall examination.

The prepared films were subjected for in vitro evaluation tests like Thickness, Folding Endurance, Surface pH, Morphological properties, %Drug content and content uniformity, Tensile strength, Percent elongation, In vitro Disintegration time, In vitro Dissolution studies and in vivo studies on rabbits.

Surface pH

The film to be tested was placed in a Petri dish and was moistened with 0.5 ml of distilled water and kept for 30s. The pH was noted after bringing the electrode of the pH meter in contact with the surface of the formulation and allowing equilibration for 1 min. The average of three determinations for each formulation was done[17].

Weight variation and thickness

For evaluation of film weight and thickness films were taken and weighed individually on a digital balance. The film thickness was measured using Digital Vernier caliper (Mitutoyo) at six different places and the average value was calculated[18]**.**

Folding endurance

The folding endurance is expressed as the number of folds (Number of times the film is folded at the same place) required to break the specimen or to develop visible cracks. This also gives an indication of brittleness of the film. A strip of 2.5 cm × 2.5 cm was subjected to folding endurance by folding the patch at the same place repeatedly several times until a visible crack was observed, and the values were reported[19].

% Drug content

Three films (4 cm^2 of each) were transferred in to separate graduated flasks containing 100 ml of phosphate buffer pH 6.8 and continuously stirred for 2 hrs. The solutions were filtered, suitably diluted and analyzed at 308 nm and the drug content was calculated.

Percent Elongation

This mechanical property was evaluated using the Instron universal testing instrument (Model F. 4026, Instron Ltd., Japan) with a 5 kg load cell. The percentage increase in the length of a film (L_2), when it is pulled under standard conditions of stress just before the point of break is known as percent elongation. The initial length of a film is L_1, the increase in length is (L_2-L_1). It is measured in terms of percentage. Percent elongation and tensile strength was carried for only 4 best formulations.

$$\text{Percent elongation} = (L_2 - L_1)/ L_1 \text{ X Cross sectional area X } 100$$

Tensile strength

Tensile strength is the maximum stress applied to a point at which the strip specimen breaks. Film strip of dimension 5 × 2 cm^2 and free from air bubbles or physical imperfections was held between two clamps positioned at a distance of 3 cm apart. A cardboard was attached on the surface of the clamp via a double sided tape to prevent the film from being cut by the grooves of the clamp. During measurement, the strips were pulled at the bottom clamp by adding weights in pan till the film breaks. The force was measured when the films broke. It is calculated by the applied load at rupture divided by the cross-sectional area of the strip as given in the equation

Tensile strength = Load at Failure/ Strip thickness X Strip Width

In vitro disintegration studies

Disintegration test was performed to ensure the disintegration of the film in phosphate buffer pH 6.8. One film from each formulation was introduced into one tube of disintegration apparatus IP. A disc was added into the tube. The assembly was suspended in a beaker containing phosphate buffer pH 6.8 and the apparatus was operated until the film disintegrated.

In vitro dissolution studies

The phosphate buffer pH 6.8 was taken as the dissolution medium to determine the drug release. The dissolution profile of quick release films of Granisetron was carried out in USP basket type apparatus containing 300 ml of the phosphate buffer pH 6.8. The film was placed in the basket, maintained at 37 ± 0.5°C and the agitation speed was 50 rpm. Aliquots (5 ml) of the dissolution medium were withdrawn at 1, 2, 4, 6, 8, 10 and 12 minutes time intervals and the same amount was replaced with the fresh medium. Samples were analyzed spectrophotometrically at 308 nm and the cumulative percentage of drug release was calculated.

Drug Excipient Compatibility Studies

The drug excipient compatibility studies were carried out by Fourier Transmission Infrared Spectroscopy (FTIR) method and Differential Scanning Calorimetry (DSC) method.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra for pure drug, physical mixture and optimized formulations were recorded using a Fourier transform Infrared spectrophotometer. The analysis was carried out in Shimadzu-IR affinity 1 spectrophotometer. The IR spectrum of the samples was prepared using KBr disks by means of hydraulic pellet press at pressure of seven to ten tons.

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) studies were carried out using DSC 60, having TA60 software, Shimadzu, Japan. Accurately weighed samples were placed on aluminium plate, sealed

with aluminium lids and heated at a constant rate of 5ºC /min, over a temperature range of 0 to 250ºC.

Stability studies

The stability study of the optimized fast-dissolving films was carried out under different conditions according to ICH guidelines. The film was packed in the aluminium foil and stored in a stability chamber for stability studies. Accelerated Stability studies were carried out at 40 ^{0}C/ 75 % RH for the best formulations for 6 months. The patches were characterized for disintegration time, %drug content, Transparency and in vitro drug release studies during the stability study period**.**

Pharmacokinetic Study

Animal Preparation

Twelve New Zealand white rabbits of either sex rabbits were (weighing 2-3 kg) selected for this study, all the animals were healthy during the period of the experiment. Animals were maintained at room temperature 25^{0}C, RH 45% and 12h alternate light and dark cycle with 100 % fresh air exchange in animal rooms, uninterrupted power and water supply and rabbits were fed with standard diet and water ad libitum. The protocol of animal study was approved by the institutional animal ethics committee.

***In vivo* Study design**

The rabbits were fasted overnight before administration of the formulations (ODF contain Granisetron Hydrochloride 1mg) and Innovator (KYTRIL ODT 1mg). The rabbits were randomly divided into two groups each group contains six animals. The group A rabbits were anaesthetized with intravenous injection of pentobarbital in a dose of 25mg/kg then positioned on table with the lower jaw supported in a horizontal position and the ODF was carefully placed on the rabbit tongue. The innovator was administered orally to group B with equivalent to animal body weight.

Blood samples for pharmacokinetic analysis were obtained at different time intervals 0, 0.25, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 8.00, 12.00, 16.00, 20.00 & 24.00h after dosing. Blood samples were collected in heparinised tubes and were centrifuged for 10min at 3,000 rpm at room temperature.

Preparation of Plasma Samples for HPLC Analysis

Rabbit plasma (0.5 ml) samples were prepared for chromatography by precipitating proteins with 2.5 ml of ice-cold absolute ethanol for each 0.5 ml of plasma. After centrifugation the ethanol was transferred into a clean tube. The precipitate was re suspended with 1 ml of acetonitrile by vortexing for 1 min. After centrifugation (5000 – 6000 rpm for 10 min), the acetonitrile was added to the ethanol and the organic mixture was taken to near dryness by a steam of nitrogen at room

temperature. Samples were reconstituted in 200 µ1 of 70 % of acetonitrile and 30% water was injected for HPLC analysis.

For HPLC an Inertsil ODS 3V, 250x4.6 mm, column with 5 µm particle size and the mobile phase consisting of phosphate buffer pH 6.0 and acetonitrile in the ratio of 70:30, v/v. The mobile phase was pumped at a flow rate of 1.0 mL/min and detection was done by UV detector at 263 nm. Internal standard Dexamethasone was used. The retention time was 4.746 min and 1.988 respectively Granisetron Hydrochloride and Dexamethasone. (20)

Pharmacokinetic Analysis

The pharmacokinetic parameters, peak plasma concentrations (C_{max}) and time to reach peak concentration (t_{max}) were directly obtained from concentration time data. In the present study, AUC_{0-t} refers to the AUC from 0 to 24 hrs, which was determined by linear trapezoidal rule and $AUC_{0-\alpha}$ refers to the AUC from time at zero hours to infinity.

The $AUC_{0-\alpha}$ was calculated using the formula $AUC_{0-t} + [C_{last}/K]$ where C_{last} is the concentration in µg/ml at the last time point and K is the elimination rate constant.

Various pharmacokinetic parameters like area under the curve [AUC], elimination half life (t½). Volume of distribution (V_d), total clearance (Cl_T) and mean residence time for each subject using a non compartmental pharmacokinetic programme. The pharmacokinetic parameters were performed by a non compartmental analysis using Win Nonlin 3.3® pharmacokinetic software (Pharsight Mountain View, CA USA). All values are expressed as the mean ±SD. Statistical analysis was performed with Graph Pad InStat software (version 3.00, Graph Pad Software, San Diego, CA, USA) using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test. Difference with $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Preparation of Granisetron hydrochloride oral films

It was aimed to prepare fast dissolving oral films of Granisetron hydrochloride with the dose of 6.25mg per 4 cm^2 film. Total 25 formulations were prepared using three different polymers, HPMC E3, HPMC E6 and HPMC E15 (Figure 1).

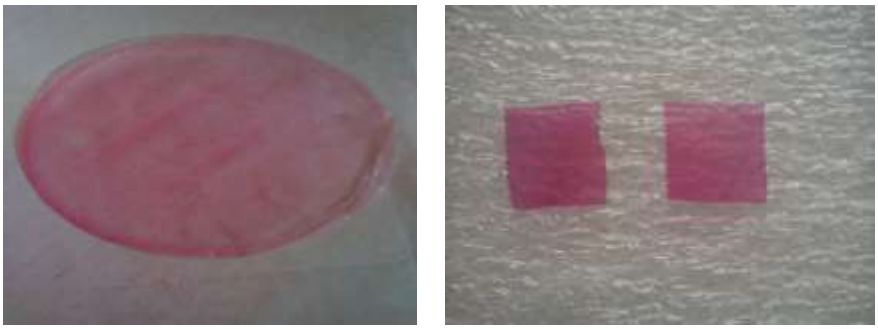

Figure 1: Granisetron Hydrochloride films

Physical characterization of films:

The films were evenly coloured and no migration of colour was observed. F1 to F3, F5 to F10, and F13 to F15 films with more HPMC were found to be thick. F1 to F3, F5 to F8, F13 were found to be brittle in nature and difficult to peel whereas others separated easily. Increased opacity and decreased transparency is due to increase in the amount of maltodextrin proportionately to HPMC. Slightly opaque and opaque formulations were found to be F6 to F8, F13 and F14.The films obtained from all the formulations had smooth surface on either side.F4, F8, F10, F14 were found to have more tackiness which indicates that the physical handling of these films is difficult. Also, soft films as in F14 and F18 are difficult to handle. Because of poor film forming property of formulations F1-F4 did not precede further (Table 4).

Table 4: Physical characteristics of formulations F1-F18

Code and properties	Film property	Tack property	Ease of handling
F1	Poor	Non-tacky	Thick & brittle
F2	Poor	Non-tacky	Slightly thick & brittle
F3	Poor	Non-tacky	Slightly thick & brittle
F4	Poor	Non-tacky	Thin, brittle, difficult to peel
F5	Average	Non-tacky	Brittle
F6	Average	Non-tacky	Brittle, opaque
F7	Average	Non-tacky	Opaque, easy to peel
F8	Average	Tacky	Thick, easy to peel
F9	Excellent	Non-tacky	Thin, easy to peel
F10	Good	Tacky	Thick, easy to peel
F11	Excellent	Non-tacky	Thin, easy to peel
F12	Good	Non-tacky	Thin, easy to peel
F13	Poor	Non-tacky	Brittle
F14	Good	Non-tacky	Easy to peel
F15	Good	Non-tacky	Opaque, easy to peel
F16	Excellent	Non-tacky	Thin, easy to peel
F17	Excellent	Non-tacky	Thin, easy to peel
F18	Good	Non-tacky	Soft, thick, easy to peel

Evaluation of fast dissolving oral films of Granisetron hydrochloride:

Thickness & Weight variation

Thickness of all mouth dissolving films was measured with Digital Vernier caliper (Mitutoyo). All the mouth dissolving formulations of different polymers are show thickness value in the range of 74±2 to 88±1mm (Table 5). A result of thickness measurement showed that as the concentration of polymer increases, thickness of mouth dissolving film also increases. A result showed that as the concentration of polymer increases weight of film also increases. The weight variation of the formulations was in the range of 57.14±0.6 to 86.0±0.3mm, which was acceptable.

Folding endurance

Folding endurance gives an indication of brittleness of the film. It was shown that as the concentration of polymer and plasticizer increases, folding Endurance of mouth dissolving film increases. The folding endurance value of the prepared films ranged from 54±2 to 106±4 (Table 5). The optimized film (F9) has folding endurance value of 106±4, which was desirable.

Surface pH

Surface pH of all mouth dissolving films prepared by using different polymers was found to be in the range of 6.7 to 6.9 pH **(Table 5)**, which was close to the neutral pH, which indicated that films may have less potential to irritate the sublingual mucosa, and hence, more acceptable by the patients.

%Drug content

All the fast dissolving oral films were found to contain an almost uniform quantity of the drug, as per content uniformity studies indicating reproducibility of the technique. Drug content in the films was evaluated and the values were found to be between 95.78±1.4 to 100.5±0.2. % **(Table 5)** for three different cuts from each film. As per the USP requirements, the films found to meet the criteria for content uniformity. No significant difference in the drug content among the films indicated good content uniformity.

In vitro disintegration studies

The disintegrating time of all the formulations was ranges from 10±2± to 38±2 sec. *In vitro* disintegrating time for mouth dissolving film using HPMC E6 was ranges from 14±2 to 38±2 sec, the results were depicted in table and the disintegrating time for the films made by the polymer HPMC E15 was ranges from09±2 to 25±2. The disintegration time of optimized formulation (F9) was found to be 9 sec, which was very less and desirable for quick onset of action.

Table 5: Physical evaluation of fast dissolving oral films of Granisetron hydrochloride

Formulation code	Thickness (µm)	Weight variation (mg)	Folding Endurance (Count)	Surface pH	% Drug content	In vitro Disintegration Time (sec)
F5	74±2	57.14±0.6	58±2	6.55±0.03	98.8±0.2	25±2
F6	75±1	52.22±0.1	54±2	6.74±0.01	92.44±0.46	24±2
F7	80±3	58.4±0.1	62±1	6.54±0.011	89.65±0.14	30±2
F8	82±2	60.2±0.1	64±4	6.6±0.02	95.5±0.2	22±2
F9	84±2	65.55±0.3	106±4	6.97±0.11	99.8±0.1	09±2
F10	88±1	62.55±0.1	70±1	6.88±0.01	98.7±0.8	18±2
F11	86±2	70.5±0.3	95±4	6.93±0.02	86.7±2.2	14±2
F12	80±1	71.5±0.5	86±3	6.89±0.010	97.32±0.3	20±2
F13	87±0	73.5±0.4	93±1	6.92±0.01	98.7±0.1	18±2
F14	91±1	79.2±0.3	96±2	6.95±0.01	98.7±1.6	23±2
F15	88±0	82.1±0.4	86±5	6.67±0.01	92.4±0.6	18±2
F16	85±2	80.2±0.3	90±2	6.93±0.02	96.7±2.2	15±2
F17	87±1	84.3±0.1	96±1	6.94±0.01	98.6±0.45	14±2
F18	88±1	86.0±0.3	98±1	6.89±0.03	97.7±0.55	18±2

Tensile Strength and Percent Elongation

Tensile strength test was performed for selected formulations that are more similar to the optimized formulation.

Table 6: Tensile Strength and Percent Elongation

Formulation code	Tensile Strength (g/cm2)	Percent Elongation
F9	11.6	9.8
F11	10.8	8.2
F16	9.7	8.1
F17	10.7	9.3

(Mean ±SD n=3)

***In vitro* dissolution studies**

Table7: Cumulative Percent Drug Release for HPMC E6 (mean ± SD n =3)

Formulation code Time(Min)	F5	F6	F7	F8
0	0	0	0	0
1	12.3±1.2	17.8±2.4	23.3±1.1	25.5±2.4
2	22.9±1.0	28.3±1.2	38.9±3.4	40.8±1.0
4	40.2±2.2	42.3±1.0	55.6±1.0	60.6±1.7
6	56.9±1.3	58.7±2.4	67.7±2.3	82.3±1.5
8	73.8±1.2	72.5±1.0	85.2±2.2	90.1±1.1
10	80.2±2.2	82.3±1.6	90.2±2.2	90.1±1.0

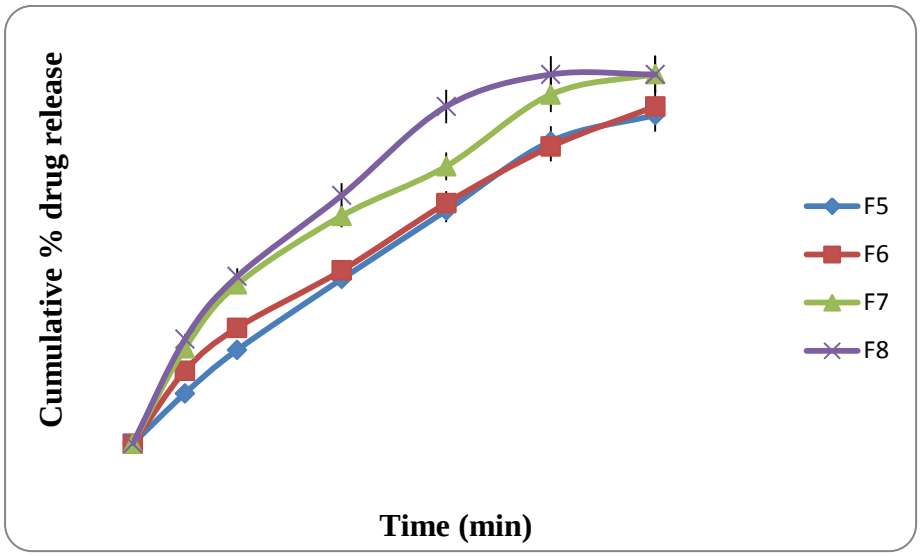

Figure 2: Cumulative Percent Drug Release for HPMC E6, F5-F8

Table 8: Cumulative % Drug Release for HPMC E6 (mean± SD n=3)

Formulation code Time(Min)	F9	F10	F11	F12
0	0	0	0	0
1	27.3±1.6	24.35±1.24	30.1±1.2	42.5±2.3
2	43.9±2.3	35.8±1.62	42.5±1.23	63.5±1
4	65.05±2.5	53.55±2.27	63.6±2.35	78.9±0.22
6	88.5±1.7	73.4±3.2	81.1±1.22	85±1.22
8	97.8±1.34	95.8±2.5	94.5±2.4	94.1±0.34
10	98.6±2.55	95.6±2.0	96.6±2.33	95.2±2.4

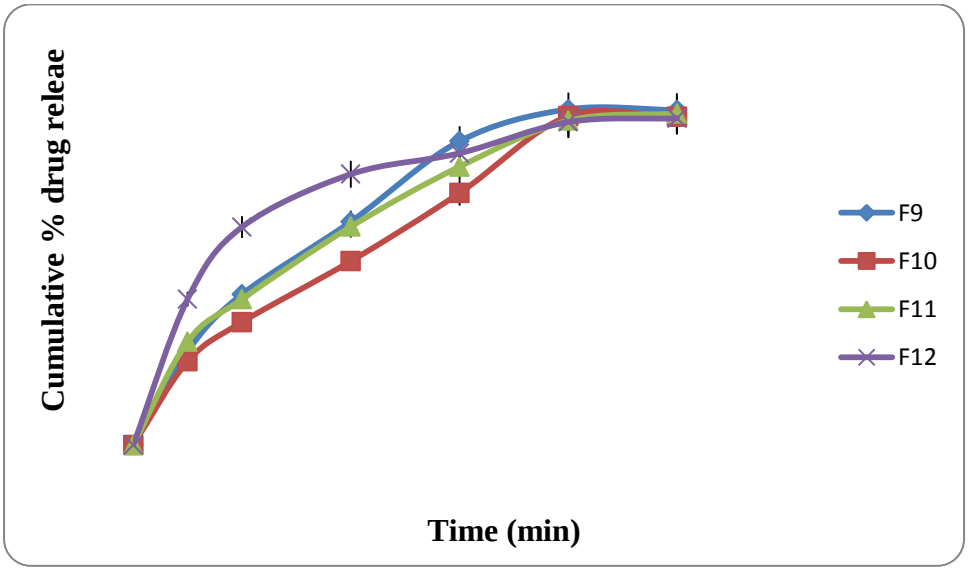

Figure 3: Cumulative % Drug Release for HPMC E6, F9-F12 (mean± SD n=3)

Table 9: Cumulative % Drug Release for HPMC E15 (mean± SD n=3)

Formulation Code Time(Min)	F13	F14	F15	F16	F17	F18
0	0	0	0	0	0	0
1	34.45±0.3	36.6±3.01	40.04±2.8	42.2±0.98	40.04±2.33	39.0±3.45
2	47.4±1.33	38.0±1.25	56.48±1.25	52.9±3.4	56.48±1.4	57.5±0.5
4	58.25±1.7	40.5±1.2	66.85±2.21	67.7±3.5	66.85±0.45	68.6±2.45
6	66.47±2.99	59.6±1.88	75.65±0.96	84.5±0.5	85.2±1.45	83.0±1.2
8	75.77±3.67	77.8±2.3	82.6±1.48	95.3±2.3	97.2±1.44	96.3±1.5
10	89.3±1.2	90.3±2.67	93.2±1.88	95.0±1.99	97.2±1.44	96.3±1.5

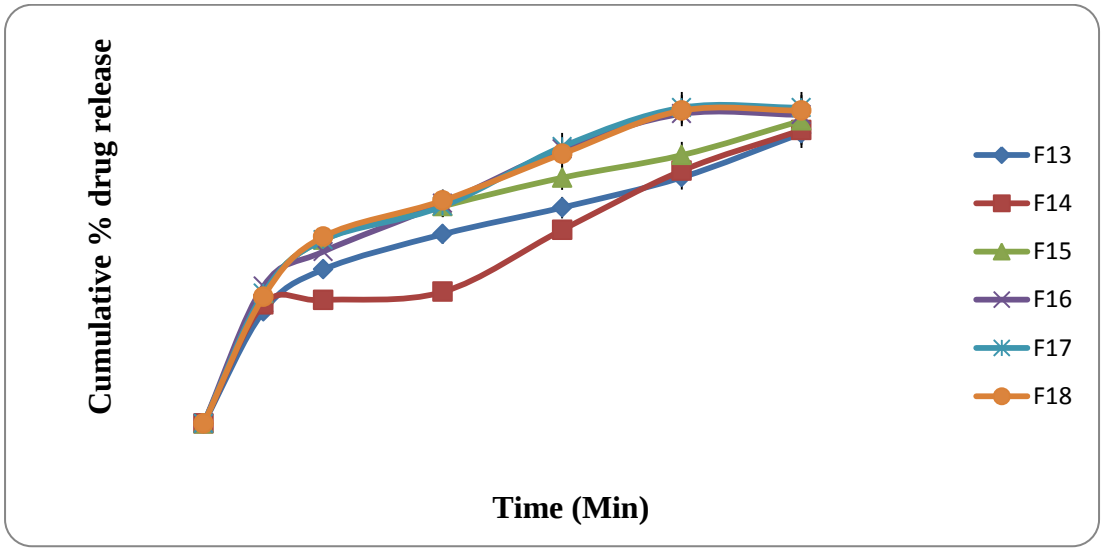

Figure 4: Cumulative Percent Drug Release for HPMC E15, F13-F18

Comparison of optimized formulation with innovator product

From the above studies F9 was found optimum for the formulation of Granisetron Hydrochloride FDOFs. Thus cumulative drug release studies of F9 formulation was compared with the marketed product

Table 10: Comparison of Cumulative Drug Release of F9 with Innovator Product

Time (min) and formulation code	1	2	4	6	8	10
F9	27.3±1.6	43.9±2.3	65.05±2.5	88.5±1.7	97.8±1.34	98.6±1.5
Kytril 1mg ODT	20.8±0.5	35.5±1.2	48.0.5±1.0	61.0±0.5	70.8±1.4	78.5±2.22

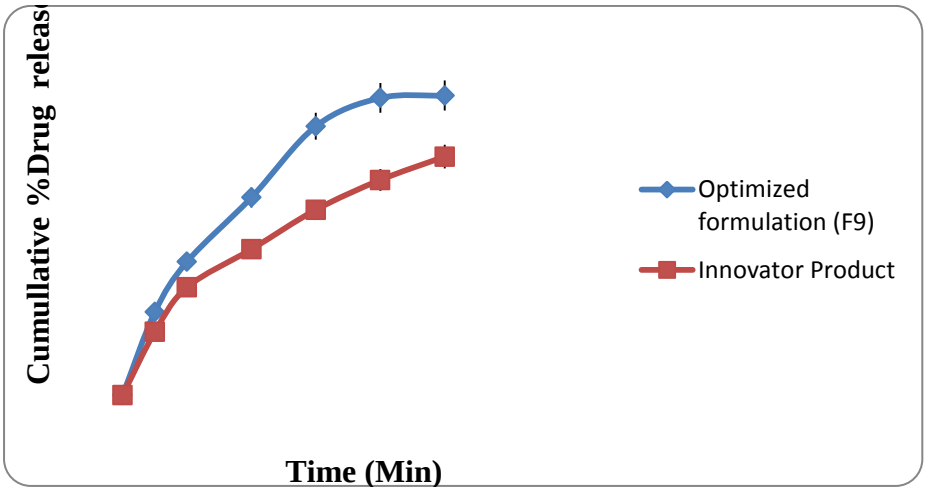

Figure 5: Comparison of Cumulative drug release of F9 with Innovator product KYTRIL 1mg ODT Tablet.

Drug excipient interactions studies by FTIR Spectroscopy

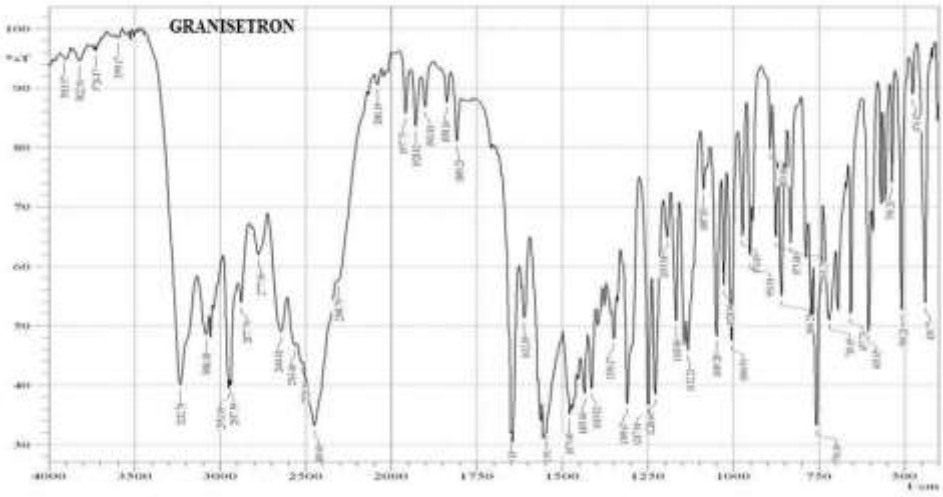

Figure 6: FTIR Spectroscopy of Granisetron Hydrochloride Pure Drug

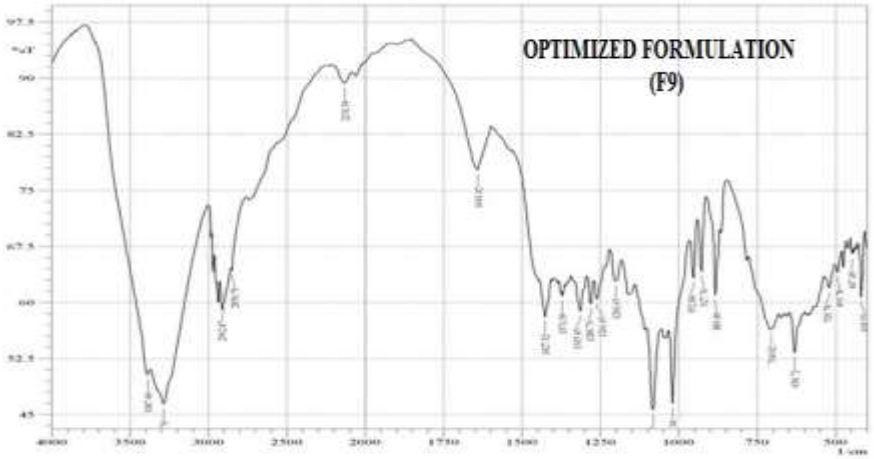

Figure 7: FTIR Spectroscopy of Granisetron Hydrochloride Formulation F9

Interpretation of FTIR data

The FTIR Spectra of pure Granisetron Hydrochloride displayed band at 3400 cm^{-1} due to C-H stretch, at 1673 cm^{-1} due to C=O stretching at 1550 due to hetero cyclic C=C stretching . The spectra also showed bands at 1253 due to C-N stretching at 1612 due to N-H stretching. The FTIR spectrum of film containing Granisetron Hydrochloride such as bands at 3392 cm^{-1} due to C-H stretch , at 1677 cm^{-1} due to C=O stretching at 1560 due to hetero cyclic C=C stretching , at 1260 due to C-N stretching , at 1617 due to N-H stretching. Thus, the presence of characteristic absorption bands of Granisetron Hydrochloride and the film containing Granisetron Hydrochloride suggest that there is no interaction takes place between the drug and excipients used in the formulation.

Drug excipient compatibility studies by DSC

Figure 8: DSC thermogram of Granisetron HCl pure drug (A) and optimized formulatin F9 (B)

Interpretation of DSC Data

DSC thermogram revealed that there is no considerable change observed in melting endotherm of Granisetron pure drug (219) and drug in optimized formulation (220) (Figure 8). It indicates that there is no interaction takes place between drug and other excipients used in the formulation.

Stability Studies

Optimized formulation F9 was selected for stability studies on the basis of high cumulative % drug release. Stability studies were conducted for 6 months according to ICH guidelines. From these results it was concluded that, optimized formulation is stable and retained their original properties with minor differences which depicted in the table 11.

Table 11: Physico-chemical characteristics of optimized formulation stored at 40 ±2ºC /75 ±5%RH

Retest Time For Optimized formulation	Disintegrating Time (sec)	Percent Drug Content	In-vitro drug release profile (%)	Transparency
0 days	09± 2	99.8±0.1	98.6	Transparent
30 days	10± 8	99.06±0.14	97.5	Transparent
60 days	11± 6	98.06±0.12	96.8	Transparent
90 days	11± 2	97.54±0.26	96.1	Transparent
180 days	12± 1	97.14±0.26	95.9	Transparent

Pharmacokinetic study:

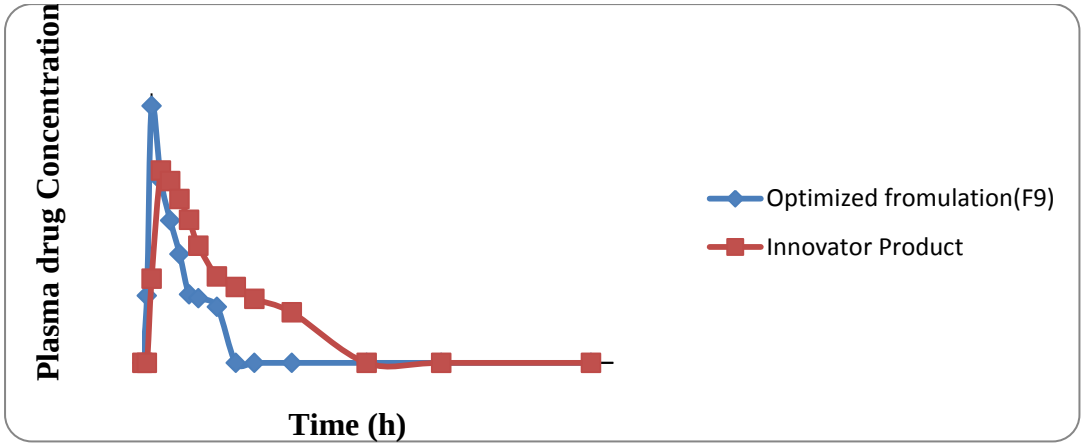

Figure 9: Plasma concentrations at different time intervals

12: Comparison of pharmacokinetic parameters of Granisetron Hydrochloride between the film and innovator (KYTRIL ODT 1mg) in Rabbits (mean ± SD, n = 6).

Parameters	Optimized formulation F9	Innovator product (KYTRIL ODT 1mg)
C_{max} (ng/ml)	0.498±0.4	0.343±0.1
AUC_{0-t} (ng h/ml)	1.688±0.74	1.547±0.16
$AUC_{0-\infty}$ (ng h/ml)	1.946±0.14	1.911±0.12
T_{max} (h)	0.50±0.5	1.0±0.1
$t_{1/2}$ (h)	1.453 ± 0.519	2.364 ± 0.01
K_{el} (h-1)	1.036 ± 0.18	1.496 ± 0.93

Pharmacokinetic parameters comparison for Granisetron Hydrochloride optimized film and innovator (KYTRIL ODT 1mg):

The mean Granisetron Hydrochloride plasma concentrations - time profiles for the prepared Granisetron Hydrochloride film and innovator are shown in **figure 9.** The bioavailability parameters for the both test film and reference standard are summarized in **Table 12.** Mean time to reach peak drug concentration (T_{max}) was 0.50±0.5h and 1.0±0.1h for the optimized and commercial formulations, respectively, while mean maximum drug concentration (C_{max}) was 0.498±0.4ng/ml and 0.343±0.1ng/ml, respectively. C_{max} was significantly increased when

compared with marketed product. The statistical comparison of t_{max}, $AUC_{0-\infty}$ and AUC_{0-t} indicated no significant difference between the two treatments,

SUMMARY AND CONCLUSION

Fast dissolving Oral film of Granisetron Hydrochloride was formulated by using solvent casting method with different concentrations of HPMC-E3, E6 and E15. Film forming property of various grades of HPMC was investigated based on preliminary characteristics of various batches of FDOFs. Formulations with HPMC E3 were not evaluated for other physical parameters due to their poor film forming ability, tack property and ease of handling or peeling. The bitter taste of the drug was masked by Aspartame and Vanilla flavour.

Formulations with HPMC E6 and E15 were evaluated for their physical characteristics, thickness, folding endurance, tensile strength, disintegration time, drug content uniformity and drug release characteristics.

Dissolution studies were performed for FDOFs excluding batches that showed poor film forming property.

Among the prepared formulations F9 showed minimum disintegration time 9 sec. Formulation F9 released 97.8% of drug within 8 min when compared to the other formulations. Based on the physicochemical properties like tensile strength, folding endurance, thickness, disintegration results and dissolution studies, it was concluded that F9 finalized as optimized formulation.

DSC and FTIR data revealed that no interactions takes place between the drug and polymers used in the optimized formulation.

The in vitro dissolution profiles of marketed product (KYTRIL) and optimized formulation was compared and found to be the drug released was 70.8% and 97.8% respectively within 8min. Therefore it can be a good alternative to conventional Granisetron Hydrochloride tablets.

In vivo evaluation of the films confirmed their potential as an innovative dosage form to improve delivery and quick onset of action of Granisetron Hydrochloride, which is essential in the management of emesis. FDOFs are suitable dosage forms in disease conditions like nausea and vomiting as these dosage forms are patient compliant as well as show rapid onset of action as they are quick dissolving dosage forms.

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