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Formulation development and *in vivo* evaluation of mouth dissolving films containing Palonosetron HCl

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Abstract

Fast dissolving drug delivery systems have gained patient acceptability and popularity in the recent times. The purpose of this work was to develop mouth dissolving oral films of Palonosetron HCl is an antiemetic drug especially used in the prevention and treatment of chemotherapy-induced nausea and vomiting . An attempt was made to prepare oral dissolving films by solvent casting method. 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 F13 was10sec and drug release was found to be very fast i.e. 99.52% of within 10 min when compared with innovator product i.e 80.5%. 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 Palonosetron 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. Therefore, the oral fast dissolving film is considered to be potentially useful for the treatment of emesis disease where quick onset of action is desired, also improved patient compliance.

Keywords: Palonosetron, emesis, mouth dissolving oral films, disintegration time, HPMC, Pharmacokinetics.

Introduction

Mouth dissolving films offers an elegant route for systemic drug delivery. The improved systemic bioavailability results from bypassing first pass effect and permeability due to a well supplied vascular and lymphatic drainage.Also, large surface area of absorption, easy ingestion and swallowing, pain avoidance make the oral mucosa a very attractive and selective site for systemic drug delivery [1,2] . There is a growing demand for novel dosage forms to cater the needs of pediatric and geriatric population. in order to assist or satisfy these patients, several fast disintegrating drug delivery systems have been developed and marketed. However such fast disintegrating solid preparations suffer from certain major drawbacks including fear of choking/swallowing, fragility and friability and requirement of specialized and expensive packaging [3]. Fast dissolving drug-delivery systems were first developed in the late 1970s as an alternative to conventional dosage forms for pediatric and geriatric patients who experience difficulties in swallowing traditional oral solid-dosage form [4]. The fast dissolving drug delivery system are specially designed for the drugs which have extensive first pass metabolism and have low dose, for the enhancement of bioavailability [5].

These systems consist of the solid dosage forms that disintegrate and dissolve quickly in the oral cavity without the administration of water. Research and development in the oral drug delivery segment has led to transition of dosage forms from simple conventional tablets or capsules to modified release tablets or capsules to oral disintegrating tablet (ODT) to wafer to the recent development of oral fast dissolving films (OFDFs). Amongst the plethora of avenues explored for the rapid drug releasing products, oral strip technology is gaining much attention [6].

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 [7].

Palonosetron HCl hydrochloride is a white to off-white crystalline powder. It is freely soluble in water, soluble in propylene glycol, and slightly soluble in ethanol and 2- propanol. It prevents acute and delayed nausea and vomiting associated with initial and repeat courses of moderately emetogenic cancer chemotherapy. Palonosetron HCl hydrochloride is being administered intravenously, as a single dose, 30 minutes before chemotherapy, or administered as a single oral capsule one hour before chemotherapy [8]. The present study is aim to formulate and characterize the fast dissolving oral films of Palonosetron HCl hydrochloride by solvent casting method for rapid onset of action in the management of migraine attack and also to improve the bioavailability of the drug.

Materials and methods

Materials

Palonosetron HCl Hydrochloride was generous gift sample from MSN labs, Hyderabad, India. Hydroxy Propyl Methyl Cellulose (HPMC E3, E6 & E15) was gifted by Nectar life sciences, Hyderabad, Maltodextrin DE6 and Aspertame was gifted by MSN Labs, Xanthan gum was obtained from Matrix Labs, Hyderabad. Propylene glycol, Vanillin, Citric acid are laboratory grade, all other chemicals used were of analytical grade.

Methods

Determination of dose of Palonosetron HCl hydrochloride

Amount of drug required per film = 0.5 mg of Palonosetron HCl
Therefore, 4 films require 2mg of drug
Area of the petridish (πr2) ==3.5*4.5*4.5=64cm2
6 films of 4 cm2 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 cm2*6=24 cm2
Amount of drug in area considered= 3mg
Area not considered= Total area of petridish-Area considered
= 64-24= 40 cm2
4 cm2 film contains 0.5 mg of drug therefore 40 cm2 contains 5mg of drug
Amount of drug in area not considered= 5mg
Therefore,
Total drug dose = (Amount of drug in area considered) + (Amount of drug in area not considered) = 5mg + 3mg = 8mg
Therefore, an approximate amount of 8mg drug was considered per petridish9

Preparation of Palonosetron HCl hydrochloride films

It was aimed to prepare to Fast Dissolving Oral Films of Palonosetron HCl Hydrochloride whose dose was 0.5mg per 4cm2 film. 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 foe 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. Palonosetron HCl 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°C in a hot air oven for 6 hours. The petridish 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 petridish. Small films of 4cm2 were cut from one big film and packed primarily in aluminium foil and secondarily in 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. The composition of Palonosetron HCl hydrochloride fast dissolving oral films with different HPMC grades are shown in Table 1, 2 &3

Table: 1 Formulation trials using HPMC E3

CODE & INDREDIANTS	F1	F2	F3	F4	F5	F6
PALONOSETRON HCL (mg)	8	8	8	8	8	8
HPMC E3 (mg)	200	220	240	260	280	300
MALTODEXTRIN	160	150	140	140	120	110
PROPLYLENE GLYCOL	80	80	90	90	100	100
XANTHAN GUM (mg)	10	10	8	8	6	6
TWEEN 80	5	5	5	5	5	5
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

Table: 2 Formulation trials using HPMC E6

CODE & INDREDIANTS	F7	F8	F9	F10	F11	F12	F13
PALONOSETRON HCL (mg)	8	8	8	8	8	8	8
HPMC E6 (mg)	200	210	220	230	240	250	260
MALTODEXTRIN	180	180	160	160	150	150	130
PROPLYLENE GLYCOL	80	80	90	90	100	100	110
XANTHAN GUM (mg)	10	10	8	8	8	6	6
TWEEN 80	5	5	5	5	5	5	5
ASPARTAME (mg)	20	20	20	20	20	20	20
CITRIC ACID (mg)	10	10	10	10	10	10	10
WATER(ml)	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
AMARANTH	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

Table: 3 Formulation trials using HPMC E15

CODE & INDREDIANTS	F14	F15	F16	F17	F18	F19	F20
PALONOSETRON HCL (mg)	8	8	8	8	8	8	8
HPMC E15(mg)	200	210	220	230	240	250	260
MALTODEXTRIN	180	180	160	160	150	150	130
PROPLYLENE GLYCOL	80	80	90	90	100	100	110
XANTHAN GUM (mg)	10	10	8	8	8	6	6
TWEEN 80	5	5	5	5	5	5	5
ASPARTAME (mg)	20	20	20	20	20	20	20
CITRIC ACID (mg)	10	10	10	10	10	10	10
WATER(ml)	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
AMARANTH	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

Evaluation of Palonosetron HCl 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 done10.

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.

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.
%Drug content
Three films (4 cm2 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 227 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.

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

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 below[14]:

$$\text{Tensile strength} = \frac{\text{Load at failure}}{\text{Strip thickness X Stripwidth}}$$

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 Almotriptan malate 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 227 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 Colorimetry (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. The DSC thermograms were recorded for pure drug, HPMC E15, Maltodextrin, Drug and HPMC mixture and optimized formulation. 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 0C / 75 % RH for the best formulations for 6 months. The patches were characterized for the drug content and other parameters 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 250C, RH 45% and 12 h 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 Palonosetron HCl Hydrochloride 0.5 mg) and Innovator (Palozen soft gelatine capsule 0.5mg). 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 & 24.00h after doing. 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 C18 column (ODS-UG-5, 250 ×4.6 mm, 5μ) and the mobile phase consisting of phosphate buffer (0.025M sodium dihydrogen phosphate pH adjusted to 6.9 with triethylamine) and Acetonitrile (65:35) at a flow rate of 1 ml/min with UV detection at 240 nm. The retention time of Palonosetron HCl hydrochloride was 4.722 min. internal standard zolmitripton was used. The retention time was 2.290min 17

Pharmacokinetic Analysis

The pharmacokinetic parameters, peak plasma concentrations (Cmax) and time to reach peak concentration (tmax) were directly obtained from concentration time data. In the present study, AUC0-t refers to the AUC from 0 to 24 hrs, which was determined by linear trapezoidal rule and AUC0-α refers to the AUC from time at zero hours to infinity.

The AUC0-α was calculated using the formula AUC0-t + [Clast/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 (Vd), total clearance (CIT) 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 Palonosetron HCl oral films

It was aimed to prepare fast dissolving oral films of Palonosetron HCl with the dose of 0.5mg per 4 cm^2 film. Total 20 formulations were prepared each using three different polymers, HPMC E3, HPMC E6 and HPMC E15.

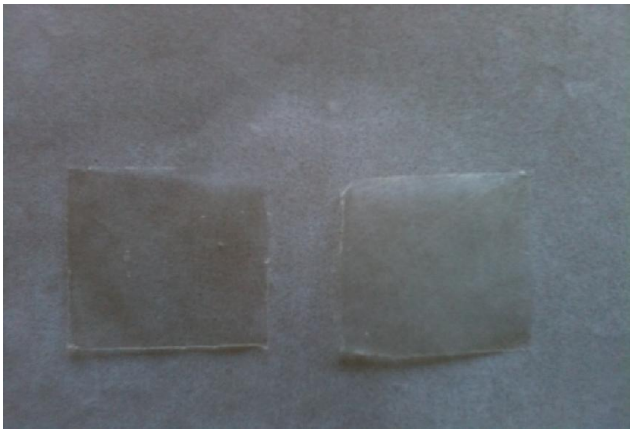
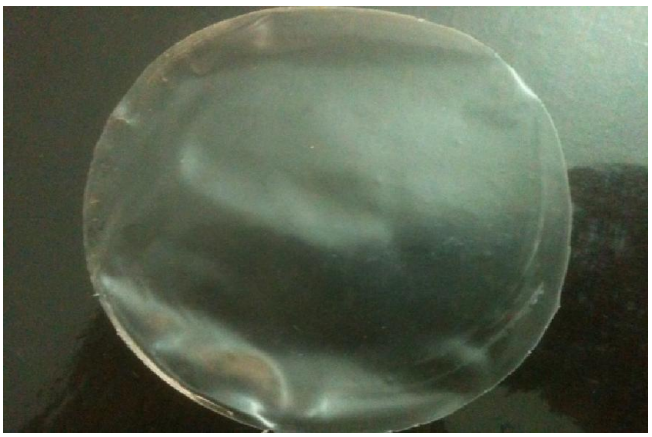


Figure 1: Palonosetron HCl Hydrochloride Films

Physical Characterization of films

Physical characterization of FDOFs was carried out by visual



inspection and the following results were observed.

The films were evenly colored and no migration of colour was observed. The increased thickness of film is attributed to the increase in the amount of HPMC. F1, F2, F3, F4, F5, F6, F8, F9, F10 and F14 were found to brittle in nature due to insufficient amount of plasticizer added to the formulation. F5 and F6 are difficult to peel whereas others separated easily.

F3, F4, F7, F12, F20 were found to be thick. F13& F19 were found to be excellent in film forming property, non-tacky, thin, flexible and easy to peel. Other formulations were found to be good film forming property. The films obtained from all the formulations had smooth surface on either side. Formulations prepared using HPMC E3 was not evaluated for physical parameters and other tests as they fail to satisfy the preliminary characteristics of films due to their poor film forming ability (Table 4).

Evaluation of fast dissolving oral films of Palonosetron HCl Hydrochloride

Thickness & Weight variation

Thickness of all mouth dissolving films was measured with Digital Vernier Caliper (Mitutoyo) and found to be in the range of 74±2 to 90±1 µm (Table 5). The optimized film has thickness of 82±1. A result of thickness measurement showed that as the concentration of polymer increases, thickness of mouth dissolving film also increases. The weight variation of the formulations was in the range of 22.53 to 32.43 mm, 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+4 to 115+2 (Table 5). The optimized film (F13) has folding endurance value of 115+2, 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 6.5 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. 8 to 98.22. % (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 shown in Table 5. The disintegration time of optimized formulation (F13) was found to be 10 sec, which was very less and desirable for quick onset of action (Table 5 & Figure 2 & 3).

Table 4: Preliminary Characterisation of FDOF'S

Code and properties	Film property	Track property	Ease of handling
F1	Poor	Non-tacky	Thick & brittle
F2	Poor	Non-tacky	Thick & brittle
F3	Average	Non-tacky	Slightly thick & brittle
F4	Average	Non-tacky	Slightly thick & brittle
F5	Average	Non-tacky	Thin, brittle, difficult to peel
F6	Average	Tacky	Thin, brittle, difficult to peel
F7	Good	Tacky	Thick, easy to peel
F8	Poor	Non-tacky	Brittle
F9	Poor	Non-tacky	Brittle, slightly Opaque
F10	Average	Non-tacky	Brittle, Opaque
F11	Average	Non-tacky	Opaque, easy to peel
F12	Average	Tacky	Thick, easy to peel
F13	Excellent	Non-Tacky	Soft, Thin, easy to peel
F14	Poor	Non-tacky	Brittle
F15	Good	Non-tacky	Opaque, easy to peel
F16	Good	Non-tacky	easy to peel
F17	Good	Non-tacky	Slightly Opaque
F18	Good	Tacky	Soft, easy to peel
F19	Excellent	Non-tacky	Thin, easy to peel
F20	Good	Non-tacky	Soft, Thick, easy to peel

Table 5: Physical evaluation of fast dissolving oral films of Palonosetron HCl Hydrochloride

Code	Thickness (µm)	Weight Variation (mg)	Folding Endurance (count)	Surface pH	% Drug Content	*In vitro* disintegration time (sec)
F6	74±2	27.1±0.12	56±1	6.55±0.03	95.8±0.23	25±2
F7	76±1	29.1±0.17	62±1	6.68±0.04	95.58±1.2	26±2
F8	81±3	32.43±0.95	54±2	6.54±0.11	92.44±0.6	22±2
F9	80±2	28.56±0.20	54±4	6.82±0.01	92.3±0.1	27±2
F10	83±2	28.36±0.20	62±1	6.76±0.01	83.4±0.6	25±2
F11	88±1	27.1±0.12	67±3	6.90±0.02	95.5±0.2	23±2
F12	86±2	28.14±0.21	66±4	6.62±0.1	93.68±0.15	15±2
F13	82±1	26.66±3.21	115±2	6.75±0.05	98.22±0.22	10±2
F14	84±0	32.53±0.95	83±4	6.77±0.05	95.32±0.3	18±2
F15	90±1	27.1±0.12	101±5	6.93±0.02	92.4±0.6	13±2
F16	85±0	24.3±3.05	96±2	6.77±0.02	86.7±2.2	25±2
F17	82±2	22.53±0.95	110±3	6.67±0.01	90.5±1.8	17±2
F18	85±1	28.56±0.20	83±4	6.77±0.05	97.32±0.3	15±2
F19	82±1	27.1±0.12	108±2	6.81±0.01	96.7±0.1	13±2
F20	81±2	24.26±0.26	99±1	6.78±0.01	97.6±1.6	14±2

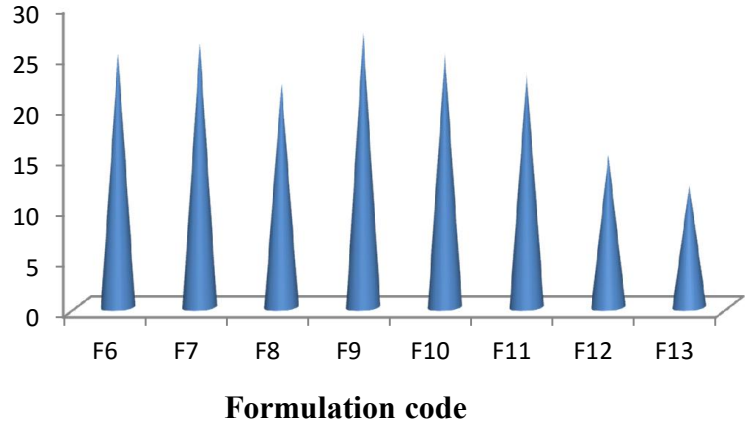


Figure 2: *In vitro* Disintegration Time of HPMC E6 formulations

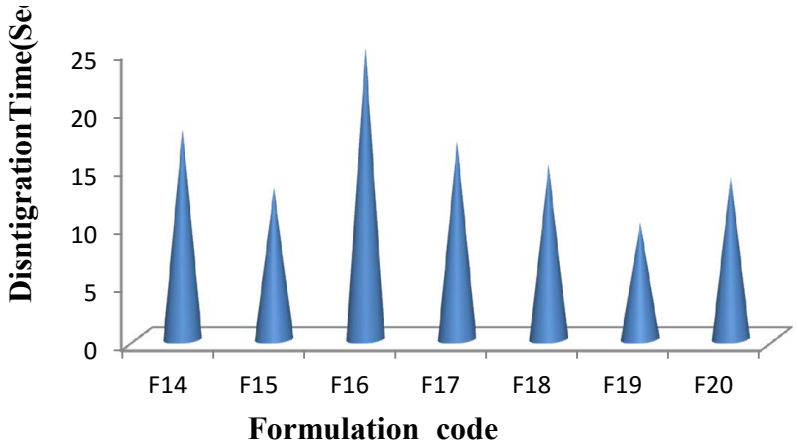


Figure 3: *In vitro* Disintegration Time of HPMC E15 formulations

Tensile strength and Percent Elongation

The tensile testing gives an indication of the strength and elasticity of the film, reflected by the parameters, tensile strength and elongation at break. Tensile strength and percent elongation of all prepared formulation is shown in Table 6, found to be within the limits. Results revealed that optimized formulation (F13) showed better tensile strength (10.4 g/cm2) and moderate % elongation (9.7).

Table 6: Tensile Strength and Percentage Elongation

Formulation Code	Tensile Strength (g/cm^2)	(%) Elongation
F13	10.4	9.7
F15	9.8	9.2
F19	9.4	8.8
F20	9.7	8.3

In vitro dissolution studies

In-vitro drug dissolution study of formulation batches F6 to F13

The cumulative % drug release for the formulations F7 to F20 are tabulated in Table 7, 8 & Figure 4 and 5. The optimized formulation (F13) shows highest percent of drug release 99.52 by the end of 10min. The cumulative % drug release studies of optimized formulation F13 was compared with the marketed PALOGEN (0.5mg) and the drug release of F13 and innovator was found to be 99.52 and 80.12 respectively after 10min.

Table 7: Cumulative Percentage Drug Release for HPMC E6

Time (min)	Formulation Code						
	F7	F8	F9	F10	F11	F12	F13
0	0	0	0	0	0	0	0
1	37.45±1.5	40.04±1.4	32.2±3.01	35.7±1.11	42.5±2.045	45.2±2.55	48±1.52
2	52.11±1.12	56.48±2.55	50.2±1.25	54.3±2.21	58.3±2.21	60.1±3.01	69.6±1.82
4	67.47±1.22	72.2±30	66.85±2.74	64.5±2.09	74.2±2.66	76.2±2.54	90±1.05
6	78.2±2.03	80.1±20	76.2±2.13	74.5±1.88	94.1±59	93.2±1.33	96.4±1.22
8	82.3±2.1	89.6±26	86.2±5.1	83.2±2.88	98.1±2.69	96.2±2.31	99.4±2.69
10	95.05±1.86	94.8±1.88	92±1.75	93±2.11	92.1±2.12	93±3.22	99.52±1.2

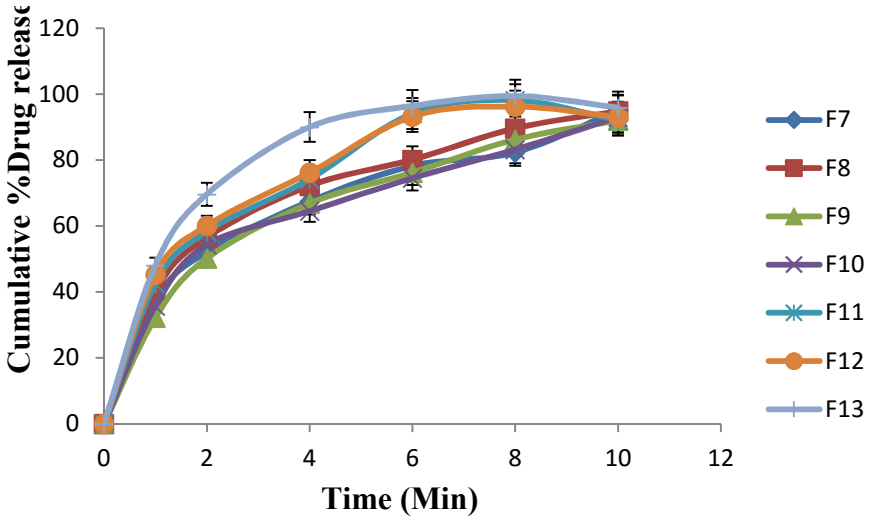
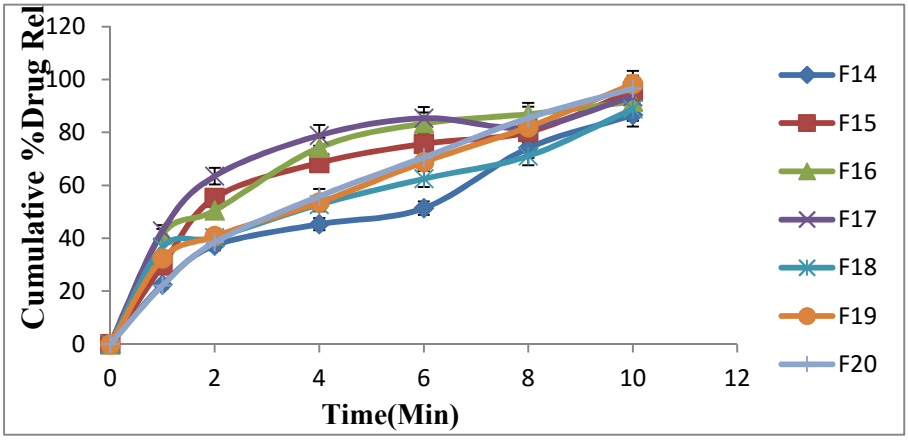


Figure 4: Cumulative Percentage Drug Release for HPMC E6

Table 8: Cumulative Percentage Drug Release for HPMC E15

Time (min)	Formulation Code						
	F14	F15	F16	F17	F18	F19	F20
0	0	0	0	0	0	0	0
1	22.45±1.5	29.7±1.4	41.5±3.01	42.8±1.11	36.6±2.045	32.35±2.55	22.12±1.21
2	37.25±1.12	55.3±2.55	50.5±1.25	63.5±1.68	40.23±2.21	40.81±1.62	38.52±1.4
4	45.31±2.74	68.5±2.72	74.3±2.09	78.9±1.25	52.77±2.66	53.55±2.27	55.87±1.7
6	51.36±2.68	75.7±3.05	83.4±3.55	85±2.36	62.47±1.88	68.8±3.21	70.55±1.5
8	74.11±2.03	80.2±2.65	86.9±2.88	81.9±2.55	71.1±0.96	82.04±1.8	85.5±2.04
10	86.54±1.86	95.6±1.88	91.7±1.75	93.5±2.71	88.9±1.48	96.35±1.18	96.6±3.11

Figure 5: Cumulative Percentage Drug Release for HPMC E15



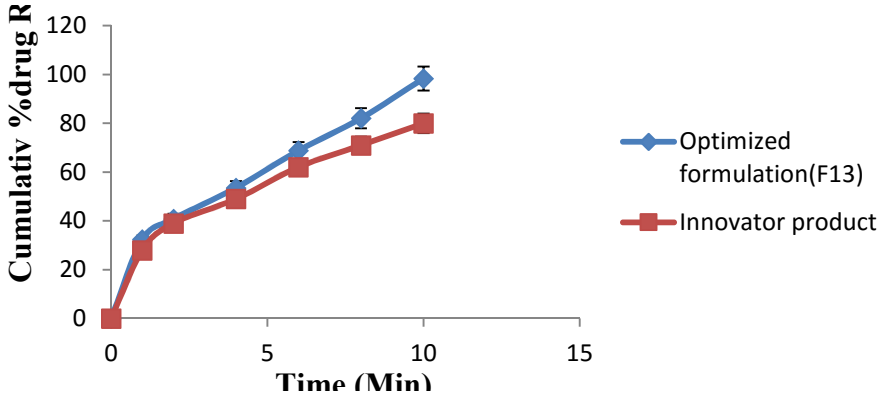


Figure 6: Comparison of Cumulative drug release of F13 with innovator product PALOZEN-0.5 mg soft gelatin capsules.

Drug excipient interactions studies by FTIR

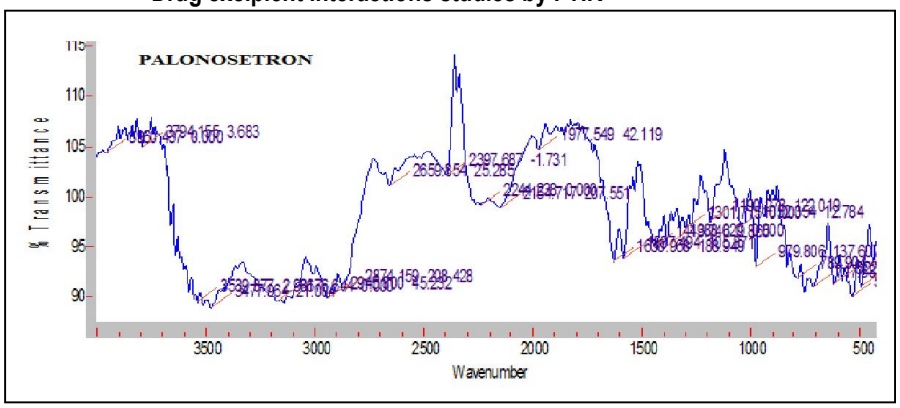


Figure 7: FTIR Spectroscopy of Palonosetron HCl Hydrochloride Pure Drug

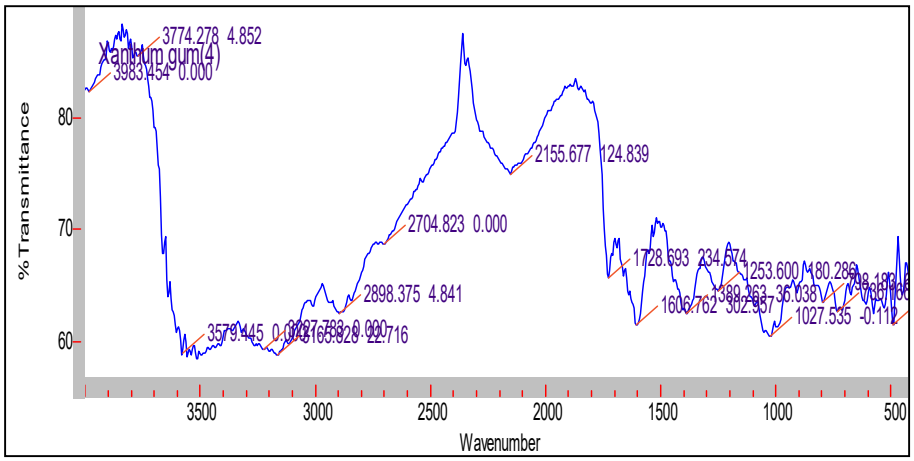


Figure 8: FTIR Spectroscopy of Palonosetron HCl Hydrochloride + Maltodextrin+ Xanthan gum + HPMC E6

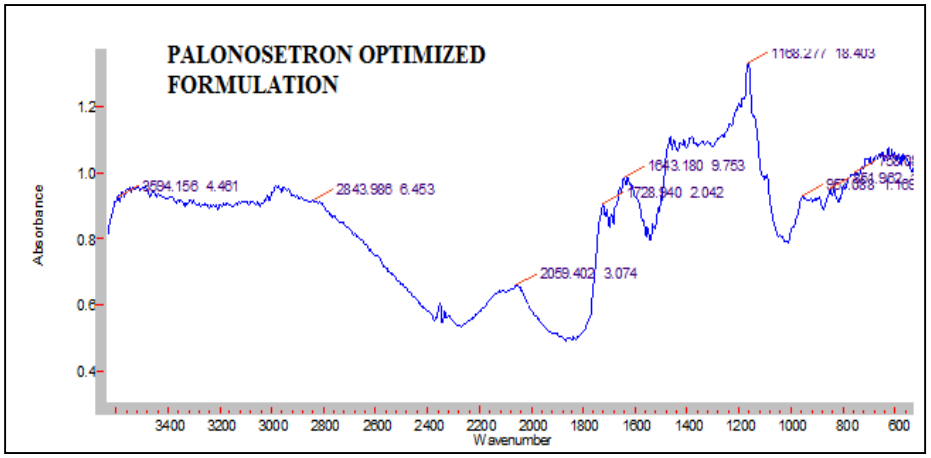


Figure 9: FTIR Spectroscopy of Palonosetron HCl Hydrochloride optimized formulation F13

Interpretation of FTIR data

The FTIR spectra of pure Palonosetron HCl Hydrochloride displayed band at 2874 cm-1 due to C-H stretch, at 1710 cm-1 due to C=O stretching, at 1659 cm-1 due to heterocyclic C=C stretching. The spectra also showed bands at 1188 cm-1 due to C-N stretching. The FTIR spectrum of film containing Palonosetron HCl Hydrochloride exhibited characteristic bands consistent with the molecular structure of Palonosetron HCl Hydrochloride such as bands at 2843 cm-1due to C-H stretch, at 1728 cm-1 due to C=O stretching, at 1643 cm-1 due to heterocyclic C=C stretching, at 1188 cm-1 due to C-N stretching. Thus, the presence of characteristic absorption bands of Palonosetron HCl Hydrochloride and the film containing Palonosetron HCl Hydrochloride suggest that there is no interaction takes place between the drug and excipients used in the formulation.

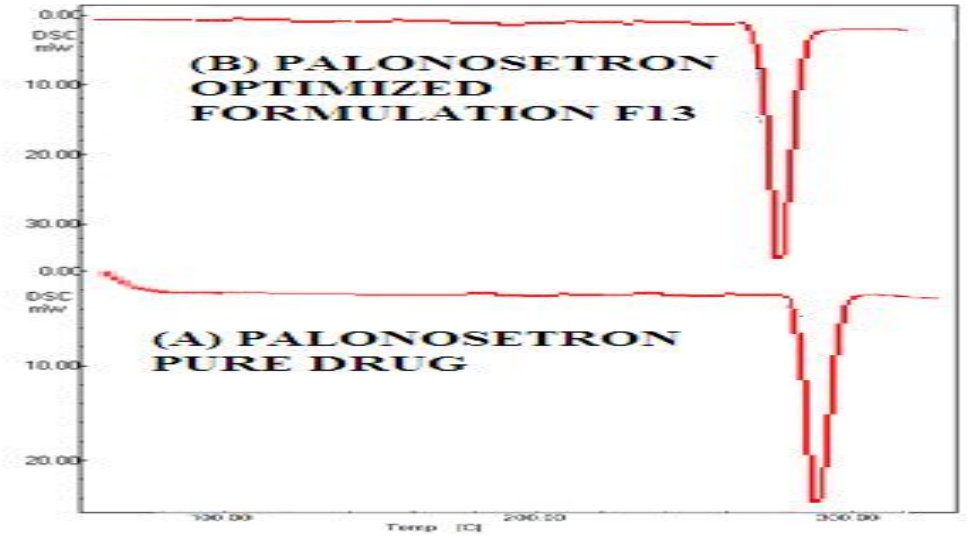


Figure 10: DSC thermogram of Palonosetron HCl pure drug (A) and optimized formulatin F13 (B)

Interpretation of DSC data

DSC Thermo gram revealed that there is no considerable change observed in melting endotherm of pure drug (290) and drug in optimized formulation (F13) (286). It indicates that there is no interaction takes place between drug and other excipients used in the formulation.

Stability Studies

Optimized formulation (F13) was selected for stability studies on the basis of fast 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 9.

Table 9: Physico-chemical characteristics of optimized formulation (F19) stored at 40 ±2ºC /75 ±5%RH

Retest Time For Optimized formulation	Disintegrating Time (sec)	% Drug Content	In-vitro drug release profile (%)	Transparency
0 days	10± 2	98.22	99.52	Transparent
30 days	11± 1	97.45	98.14	Transparent
60 days	11± 9	97.06	97.68	Transparent
90 days	12± 2	96.54	96.23	Transparent
180 days	12± 6	96.54	96.23	Transparent

Pharmacokinetic study

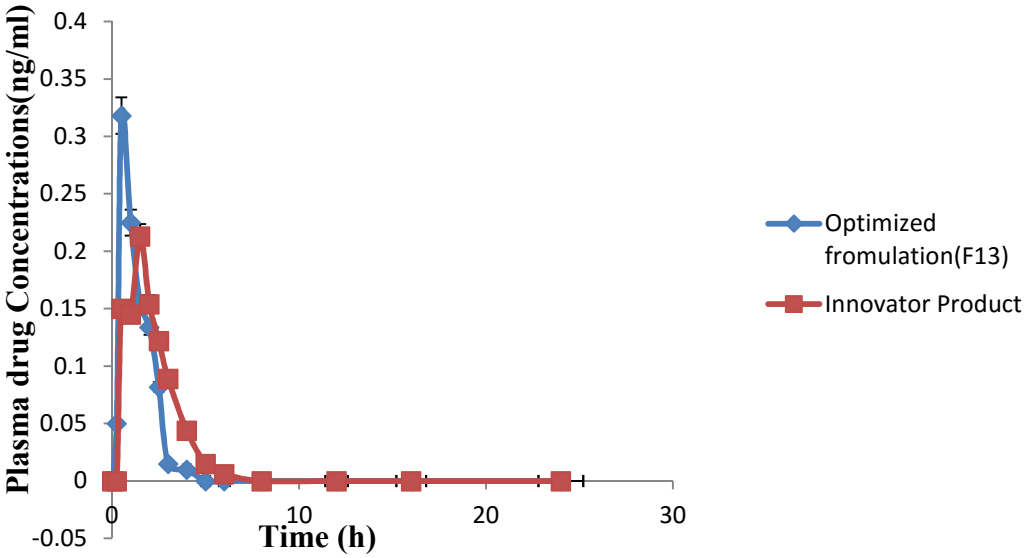


Figure 11: Plasma concentrations at different time intervals of Palonosetron HCl Hydrochloride Optimized formulation (F13) and Innovator

Table 10: Comparison of pharmacokinetic parameters of Palonosetron HCl Hydrochloride between the optimized formulation film and innovator (Palozen soft gelatine capsule 0.5mg) in Rabbits (mean ± SD, n = 6).

Parameters	Optimized formulation Film	Innovator
C_{max}(ng/ml)	0.318±0.1	0.213±0.1
AUC_{0-t}(ng hr/ml)	1.06±0.44	0.95±0.26
$AUC_{0-\infty}$(ng hr/ml)	1.14±0.14	1.04±0.12
T_{max}(h)	0.50±0.5	1.5±0.1
$t_{1/2}$(h)	1.253 ± 0.519	2.664 ± 0.01
K_{el} (hr^{-1})	1.336 ± 0.11	1.196

Pharmacokinetic parameters comparison for Palonosetron HCl Hydrochloride optimized film and innovator product

The mean Palonosetron HCl Hydrochloride plasma concentrations - time profiles for the prepared Palonosetron HCl Hydrochloride film and innovator are shown in figure 11. The bioavailability parameters for the both test film and reference standard are summarized in Table 10. Mean time to reach peak drug concentration (Tmax) was 0.50±0.5h and 1.5±0.1h for the optimized and commercial formulations, respectively, while mean maximum drug concentration (Cmax) was 0.318±0.1ng/ml and 0.213±0.1ng/ml, respectively. The statistical comparison of AUC0-∞ and AUC0–t indicated no significant difference between the two treatments, and there was a significant difference for the Cmax and Tmax was observed in this study. As the prepared films were exhibited immediate release and capsules were shown prolonged release, as prepared formulation shown immediate release so it was shown significantly increased maximum concentration when compared with marketed product.

Summary and conclusion

Fast dissolving Oral film of Palonosetron HCl was formulated by using solvent casting method with different concentrations of HPMC-E3, E6 and E15. 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. Dissolution studies were performed for FDOFs excluding batches that showed poor film forming property. Among the prepared formulations F13 showed minimum disintegration time 10 sec. Formulation F13 was shown fast release of the drug 99.52% within 10min 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 F13 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 (PALOZEN) and optimized formulation was compared and found to be the drug released was 72% within 7min from the marketed product, whereas from optimized formulation (F13) the drug release was 96.4% within 7min. Therefore it can be a good alternative to conventional Palonosetron HCl capsules.

The in vitro dissolution profiles of marketed product (PALOGEN 0.5mb) and optimized formulation (F13) was compared and found to be 80.12% within 10min from the marketed product, whereas from optimized formulation (F13) the drug release was 99.52% respectively within 10min. Therefore it can be a good alternative to conventional Palonosetron for immediate action.

In vivo study exhibited both optimized formulation and innovator shown comparable drug -plasma level- time profiles in vivo evaluation of the films confirmed their potential as an innovative dosage form to achieve immediate action and improvement in the bioavailability of the drug. Therefore, the oral dissolving film is considered to be potentially useful for the treatment of emesis where quick on set of action, improved patient compliance and comfort is expected. FDOFs are suitable dosage forms in disease conditions like chemotherapy induced nausea and vomiting (CINV) as these dosage forms are patient compliant as well as show rapid onset of action as they are quick dissolving dosage forms. It can be especially useful for geriatric, bedridden, and non-cooperative patients due to its ease of administration.

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