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Design and Evaluation of Medicated Antiemetic Lozenges

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ABSTRACT

For the treatment of emesis in patients, metoclopramide hydrochloride was developed as oral retentive lozenges. Although there are existing dosage forms such as syrups and pills on the market, there is still a need for novel dosage forms that function effectively and locally. As a result, the goal of this study is to create, produce, and test Metoclopramide hydrochloride lozenges utilizing a mix of polymers such as sodium carboxy methyl cellulose, hydroxy propyl methyl cellulose, and hydroxy methyl cellulose at various doses. The formulation MT9, which contains 2% Hydroxy propyl methyl cellulose, was judged to be the most promising of the ten. *In vitro* drug release was 86.65 percent in 30 minutes with this formulation. All of the formulations were tested for hardness, content homogeneity, friability, weight fluctuation, and moisture content, among other things. The produced formulations have a hardness of 10-12 Kg/cm^2 and are devoid of grit. IR Spectral analysis was used to check all of the formulations for medication excipient interactions. Patients suffering from emesis may find the lozenges to be an appealing alternative formulation. There were no drug-excipient interactions, according to IR spectroscopy investigations. The created metoclopramide hydrochloride lozenges could stay in the mouth for extended periods, indicating that lozenges of metoclopramide hydrochloride might be used to treat emesis.

INTRODUCTION:

Vomiting is the reflex action of ejecting stomach contents via the mouth and occasionally through the nose, whereas nausea is the sensation of being about to vomit. Emesis and nausea can be caused by some factors, including medication consumption, stomach irritants, chemotherapy, radiation, and gastrointestinal infections. [1] The emetic centre (Chemoreceptor Trigger Zone) in the medulla oblongata is stimulated, resulting in vomiting. Reduced gastric tone is associated with nausea. [2] Metoclopramide inhibits 5-HT3 receptors in the peripheral nervous system. Unlike other D2-receptor antagonists, high dose metoclopramide blocks 5-HT3 receptors and has been shown to reduce cisplatin-induced emesis [3].The GI tract is the body's main source of serotonin, and it's been believed that chemotherapy causes serotonin to be released from the GI tract's enterochromaffin cells, which subsequently drives emesis via the vagus, greater splanchnic nerve, and the brain's area postrema. Urinary excretion of 5-hydroxy indole acetic acid, the primary metabolite of serotonin, increases after cisplatin treatment, and this rise corresponds to the frequency of bouts of emesis [4].

Lozenges are flavoured medicated dosage forms that are delivered and kept in the mouth or throat and typically include one or more medicaments in a sweetened foundation. Lozenges are utilised for juvenile and geriatric patients who are unable to swallow solid oral dose forms, as well as drugs that are designed to release slowly over time to maintain a steady amount of drug in the oral cavity [5, 6]. Lozenges are a common and unique medication delivery technique, as well as a more inventive dosage form and oral confectionery goods. It is most likely beneficial for administering medicine either locally or continuously through the mouth.The reasons for this preference include ease of administration for elderly and pediatric patients, as well as widespread patient acceptability. Development of novel drug delivery methods for current drugs that improve effectiveness, minimize first-pass hepatic metabolism, eliminate the requirement for water consumption, and boost bioavailability while lowering dosage frequency [7, 8].

MATERIALS AND METHODS:

Metoclopramide hydrochloride was supplied as a gift sample by Leads Pharmaceuticals, Hyderabad, A.P. Liquid glucose, HPMC, HEC and Sucrose were purchased from Rakesh chemicals Pvt. Ltd., Mumbai and Himedia labs, Mumbai respectively. Sodium lauryl sulphate and Citric acid were purchased from SD Fine chemicals, Mumbai.

UV Spectroscopy:

Preparation of Standard Calibration Curve of Metoclopramide Hydrochloride in Distilled water: 100 mg of metoclopramide hydrochloride was dissolved in 100 ml of distilled water by slight shaking (1000 mcg/ml). 1 ml of this solution was taken and made up to 50mlwith distilled water, which gives 20 mcg/ ml concentration (stock solution). From the stock solution, concentrations of 2, 4, 6, 8 and 10 µg/ml in distilled water were prepared. The absorbance of diluted solutions was measured at 271nm and a standard plot was drawn using the data obtained. The correlation coefficient was calculated.

Preparation of Standard Calibration Curve of Metoclopramide Hydrochloride inpH 6.8 Phosphate Buffer:100 mg of Metaclopramide hydrochloride was dissolved in 100 ml of pH 6.8phosphate buffer by slight shaking (1000 mcg/ml). 1 ml of this solution was taken and made up to 50 ml with pH 6.8 phosphate buffer, which gives 20 mcg/ ml concentration (stock solution).From the stock solution, concentrations of 2, 4, 6, 8 and 10 µg/ml in pH 6.8 phosphate buffer were prepared. The absorbance of diluted solutions was measured at271 nm and a standard plot was drawn using the data obtained. The correlation coefficient was calculated, the absorbance data of the above concentrations [9, 10].

Methods Employed in the Present Investigation:

Phase-I Studies: Preparation of Lozenges with & without Added Hydrocolloids [11, 12]

Lozenges were prepared following heating and congealing method. The various steps involved in the preparation of lozenges are:

- Step-1: The desired quantity of sugar was dissolved in water by heating and stirring in a copper kettle until sugar was completely dissolved. Corn syrup was added when the cooking temperature reaches 110ºC. Cooking was then continued to145 - 156ºC till the syrupy base becomes thick.

- Step-2: The finished cooked syrup (154.4ºC) was then placed in vacuum chamber which was maintained at 274 mm Hg for about 30 minutes to remove the traces of water molecules and to give plasticity to the base prepared.

- Step-3: The mixing was done manually. During the mixing cycle, the temperature ofcandy base (154ºC) was brought to 90ºC to form a solidified mass. A hydrogenated vegetable oil-based lubricant was spread onto the table surface to alleviate this condition. At this stage the

Drug, mucoadhesive polymers, citric acid, other excipients such as Colour and flavoring agents were added manually and mixed thoroughly.

- Step-4: Then the pour the mixture into mold of desired shape and size and then allow cooling it at room temperature.

- Step-5: Then wrapping the lozenges with polyethylene wraps and store.

Table No. 1: Working formulae to prepare tablet lozenges

Parameters	**Formulation**									
	MT0	**MT1**	**MT2**	**MT3**	**MT4**	**MT5**	**MT6**	**MT7**	**MT8**	**MT9**
Sugar (gms)	68	68	68	68	68	68	68	68	68	68
Liquid glucose (gms)	28.6	28.6	28.6	28.6	28.6	28.6	28.6	28.6	28.6	28.6
Drug (gms)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Hydroxy Ethyl Cellulose (gms)	-	1	1.5	2	-	-	-	-	-	-
Sodium CMC (gms)	-	-	-	-	1	1.5	2	-	-	-
Hydroxy Propyl Methyl Cellulose (gms)	-	-	-	-	-	-	-	1	1.5	2
Citric Acid (gms)	1	1	1	1	1	1	1	1	1	1
Flavoring Agent (gms)	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.
Coloring Agent (mg)	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.
Total Weight (gms)	100	100	100	100	100	100	100	100	100	100

* Each tablet lozenges contains 10 mg of Drug.

* Each tablet lozenges contains weight of 3 gms

Figure No. 1: Models of prepared Metoclopramide hydrochloride lozenges

Phase-II Studies: Characterization of prepared medicated lozenges.

A) Weight variation test: In the present work Lozenges were formulated to weigh3 grams containing a 10 mg of drug. The USP weight variation test was run by weighing 20 lozenges individually, calculating the average weight and comparing the individual tablet weights to the average weight. Standard Limits: 85 <80mg-10%, 80-250-7.5%, >250mg-5%. [13]

B) Hardness Test: Tablets require a certain amount of strength or hardness and resistance to friability to withstand mechanical shocks of handling in manufacture, packaging and shipping. The lozenge was compressed between the holding anvil and the piston connected to a direct force reading gauge of a Monsanto hardness tester. Five lozenges were chosen at random by this test from all formulations and the mean values were recorded. [14]

C) Drug Content Estimation: Ten lozenges are taken and weighed, average weight of each lozenges is calculated. All the lozenges were powdered in a glass motor and equivalent of 10 mg is taken and placed in 100 ml volumetric flask. Drug is extracted with Methanol and makes a suitable dilution of 24mcg/ml and measure the absorbance at lambda max 261 nm against blank.

D) Thickness: To produce lozenges of uniform thickness during production and between productions for the same formulation, can be exercised to employ the same volume of fill and the same pressure. Tablet lozenges were measured with the help of "Screw gauge" pre-calibrated. The thickness was measured, by observing which was prethickness at three different lozenges. [15]

E) Diameter: The diameter of individual lozenges was measure which permits accurate measurements and provides information on the variation between lozenges.

F) Moisture content determination: Add about 20ml of anhydrous methanol to the titration vessel and titrate to the amperometric end point with Karl fischer reagent. Quickly add 2g of Lozenge samples, stir for 1minute and again titrate to the amperometric end point with the Karl Fischer reagent. The difference between the two titrations gives the volume (v) of Karl Fischer reagent by the sample. The minimum water equivalent is3.5mg of water/ml of Karl Fischer reagent. Hence percent of the water w/w in the given sample may be calculated by the following expression.

$$water\ \%\left(\frac{w}{w}\right) = \frac{\mathbf{v}\ X\ 3.5}{wt.of\ Sample\ (mg)} X100$$

Phase- III: Stability studies: All the Prepared formulations MT0, MT1, MT2, MT3, MT4, MT5 and MT6, MT7, MT8, MT9, were subjected to stability studies at different temperatures i.e.,30±2°C & 65±5% RH and 40±2°C & 75±5% RH for the period of six months. Drug content estimation was carried during intervals of 15 days. After the stability studies, there was no such considerable change in hardness of tablet and no change in weight, thickness and drug content.[16]

Phase-IV Drug excipient interaction studies: The selected formulations will be subjected for IR analytical techniques.

Phase- V: *In- vitro* drug dissolution studies: All the formulations prepared to subjected for in- vitro drug release studies at the salivary pH conditions (6.4) using reported modified USP dissolution method. USP XXIII Dissolution test apparatus was used by taking 100 ml of pH 6.4buffer in 250 ml beaker lozenge was placed in it, rotating paddle at a speed of 50 rpm and temperature 37±1ºC was maintained. 5 ml aliquots were withdrawn at 5, 10, 15, 20, 25 and 30 minutes intervals, after each withdrawal of a sample an equal volume of dissolution medium was added to the dissolution vessel. The filtered samples were diluted and analyzed spectrophotometrically at 271.0 nm.[17, 18]

RESULTS AND OBSERVATION

UV Spectroscopy:

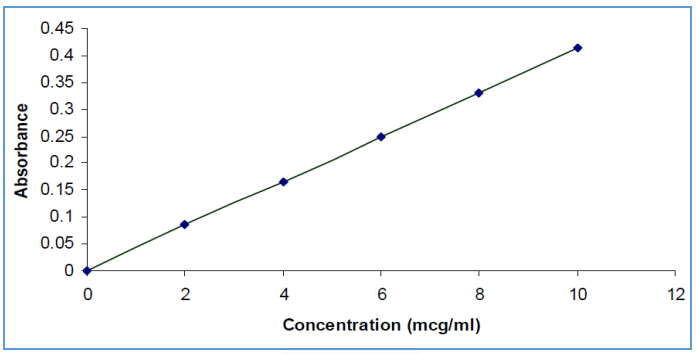

Figure No. 2: Standard Graph of Metoclopramide hydrochloride in Distilled water

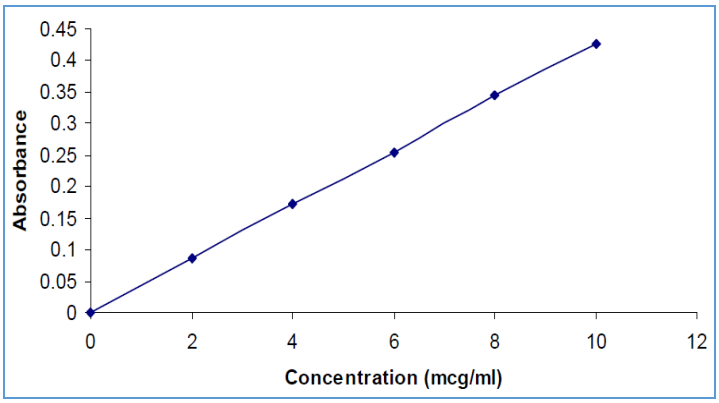

Figure No. 3: Standard calibration data of Meatoclopramide HCl in pH 6.8

Physiochemical Characterization of prepared lozenges:

Table No. 2: Physico-chemical parameters of prepared Metoclopramide HCl lozenges

Parameters	Formulation									
	MT0	**MT1**	**MT2**	**MT3**	**MT4**	**MT5**	**MT6**	**MT7**	**MT8**	**MT9**
Hardness test (Kg/cm2)	11.05±0.19	11.18±0.38	11.09±0.26	11.01±0.47	11.14±0.22	11.05±0.19	11.09±0.11	10.9±0.6	11.03±0.05	11.13±0.06
Weight Variation (gm)	1.97±0.01	1.92±0.01	1.90±0.02	1.94±0.02	2.02±0.03	2.01±0.02	1.98±0.02	1.95±0.01	1.93±0.05	2.05±0.02
Thickness (mm)	3.24	3.26	3.32	3.45	3.27	3.28	3.31	3.22	3.41	3.31
Drug Content (%)	99.04± 0.86	99.140± 3.35	100.14± 3.39	102.62± 0.45	101.24±1.66	99.045± 0.53	100.44± 0.57	102.52±0.5 9	99.68±1.04	101.29± 2.37
Diameter (mm)	20.11±0.04	20.07±0.37	20.03±0.39	20.04±0.53	20.27±0.50	20.23±0.59	20.13±.60	20.09±0.01	20.17±0.02	20.18±0.05
Moisture Content (%)	1.95±0.19	1.93±0.16	1.92±0.04	1.85±0.13	1.86±0.12	1.89±0.03	1.78±0.06	1.94±0.005	1.92±0.01	1.87±0.009

* Each reading is a mean of three replicates.

* Each lozenge contains 10 mg of Metoclopramide hydrochloride

* Each lozenge weight of 3 gms

Stability Study:

Table No. 3: Stability studies of prepared Metoclopramide hydrochloride lozenges (MT0 with MT7 & MT8 MT9)

Time in days	Physical changes	Hardness Kgs/cm²	MT0 % drug content	MT7 % drug content	MT8% drug content	MT9% drug content
1	No change	10-11.5	97.56±0.64	97.17±1032	98.28±0.53	97.56±1.17
15	No change	10-11.5	98.33±0.03	97.64±0.49	98.46±0.45	98.71±0.86
30	No change	10-11.5	98.39±0.96	98.86±0.43	97.25±0.22	97.42±0.09
45	No change	10-11.5	97.70±0.47	97.60±0.57	97.65±0.56	98.60±0.25
60	No change	10-11.5	98.02±0.97	97.49±0.55	97.32±0.74	98.46±0.21
75	No change	10-11.5	97.83±0.54	98.25±0.07	97.84±0.28	98.52±0.99
90	No change	10-11.5	97.17±1.02	96.24±0.05	97.41±0.49	97.23±0.56
105	No change	10-11.5	97.17±0.49	98.21±0.09	96.87±0.57	97.56±0.23
120	No change	10-11.5	97.64±0.43	97.12±0.53	97.64±0.41	96.78±0.34
135	No change	10-11.5	98.86±0.57	99.87±0.43	97.89±0.96	97.18±0.87
150	No change	10-11.5	97.60±0.55	98.96±0.08	98.47±0.47	98.65±0.91
165	No change	10-11.5	97.49±0.07	97.82±0.05	97.55±0.12	97.85±0.32
180	No change	10-11.5	98.25±0.36	96.24±0.31	98.32±0.36	96.08±0.45

* Each reading is a mean of three replicates.

* Each lozenge contains 10 mg of Metoclopramide hydrochloride

* Each lozenge weight of 3 gms

FTIR Spectra of drug and excipients for compatibility Study:

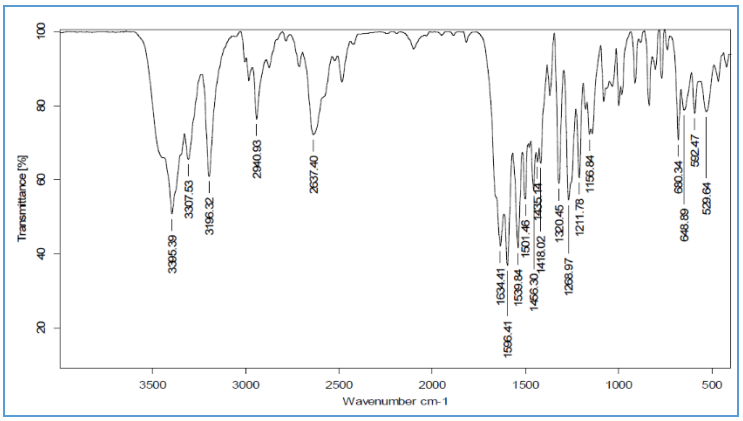

Figure No. 4: FT-IR spectrum of Metoclopramide hydrochloride

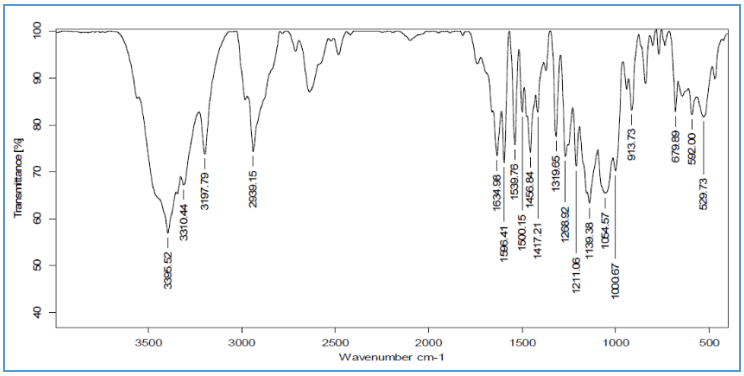

Figure No. 5: FT- IR spectrum of Metoclopramide hydrochloride and HEC

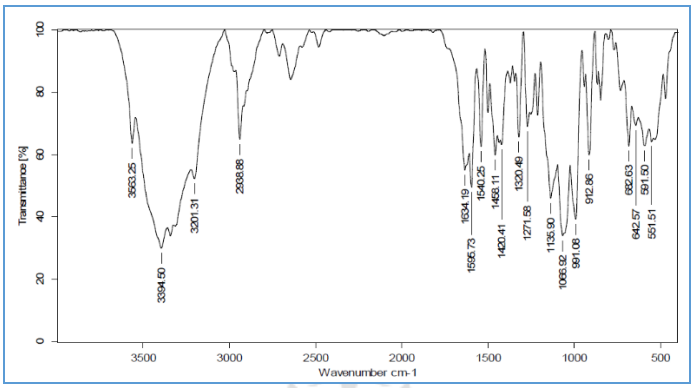

Figure No. 6: FT-IR spectrum of Metoclopramide hydrochloride and Na CMC

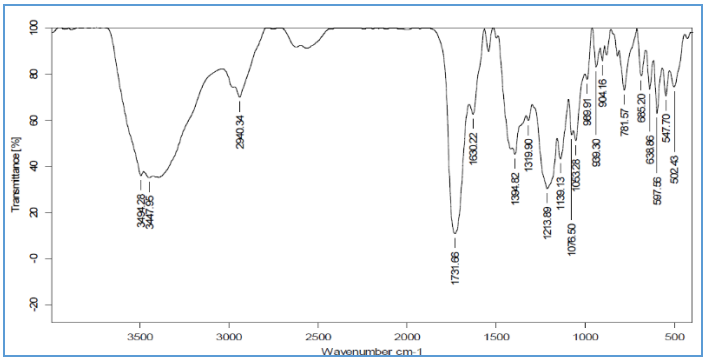

Figure No. 7: FT-IR spectrum of Metoclopramide hydrochloride and HPMC

***In-vitro* Drug Release Study:**

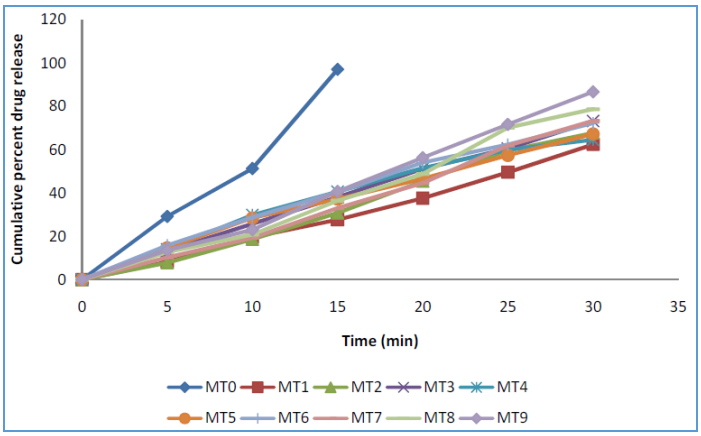

Figure 8: Comparative studies of Metoclopramide hydrochloride lozenges with and without hydrocolloids (MT0, MT1, MT2, MT3, MT4, MT5, MT6, MT7, MT8, and MT9)

* Each lozenge contains 10 mg of Metoclopramide hydrochloride

* Each lozenge weight of 3 gms

DISCUSSION

Metoclopramide hydrochloride Lozenges: Metoclopramide hydrochloride was the drug of choice for the treatment of emesis. In the present work three different mucoadhesive polymers were used for the preparation of Nine Metoclopramide hydrochloride lozenges formulations MT1(HEC1%), MT2 (HEC 1.5%), MT3 (HEC 2%), MT4 (Na CMC 1%), MT5 (Na CMC1.5%), MT6 (Na CMC 2%), MT7 (HPMC 1%), MT8 (HPMC 1.5%), MT9 (HPMC 2%). The formulation MT0 was without added hydrocolloid and evaluated for the utility in the treatment.

UV Spectroscopy: Absorption maxima for Metoclopramide hydrochloride when observed under UV–visible spectrophotometer were found to be at 271 nm. This was observed from the peak in Fig. 1 and 2.

Phase-I Studies: The prepared lozenges were chocolate flavoured spherical in shape, 3 gms in weight with 20 mm diameter and chocolate brown in colour.

Phase-II Studies: Physico-chemical properties of prepared tablet lozenges:

Hardness Studies: As shown in table no.2 the hardness of the lozenges prepared were 11.05 without hydrocolloids, 11.18 with HEC 1%, 11.09 with HEC 1.5%, 11.01 with HEC 2%, 11.19 with Na CMC 1%, 11.05 with Na CMC1.5%, 11.09 with Na CMC 2%, 10.90 with HPMC 1%,11.03 with HPMC 1.5%,11.03 with HPMC 2%, in Kg/cm² respectively.

Weight Variation: The weight variation of the lozenges was not more than 5%.

Drug Content: The drug content of the lozenge was within 95 to 105%.

Thickness: As shown in Table No.2 the hardness of the lozenges prepared were3.24 without hydrocolloids, 3.26 with HEC 1%, 3.32 with HEC 1.5%, 3.41 with HEC 2%, 3.17 with Na CMC 1%, 3.28 with Na CMC 1.5%, 3.31 with Na CMC2%, 3.22 with HPMC 1%, 3.41 with HPMC 1.5%, and 3.31 with HPMC 2% in mm respectively.

Diameter: As shown in Table No.2 the diameter of the lozenges prepared were20.11 without hydrocolloids, 20.07 with HEC 1%, 20.03 with HEC 1.5%, 20.04with HEC 2%, 20.27 with Na CMC 1%, 20.23 with Na CMC 1.5%, 20.13 with Na CMC 2%, 20.27 with HPMC 1%, 20.23 with HPMC 1.5%, and 20.13 with HPMC 2% in mm respectively.

Moisture content: The moisture content of the lozenges was within 2%.

Phase-III Studies:

Stability studies: The stability studies showed that there was no considerable change in hardness, weight, thickness and drug content. The data are shown in Table No. 17, 18 & 19.

Phase- IV Studies:

Infra- red spectrophotometric analysis for drug- excipient interactions: Drug excipient interactions were ruled out in the promising formulations Metoclopramide hydrochloride by IR spectroscopic studies using KBr pellet method.

Phase-V: *In- vitro* Drug Dissolution Studies.

- Without added hydrocolloid (MT0): The drug dissolution studies indicated that in 15 minutes time 97.02% of the drug was dissolved.

- With added Hydroxy Ethyl Cellulose 1% (MT1): The drug dissolution studies indicated that in 30 minutes time 62.41% of the drug was dissolved.

- With added Ethyl Cellulose 1.5% (MT2): The drug dissolution studies indicated that in 30 minutes time 67.68% of the drug was dissolved.

- With added Hydroxy Ethyl Cellulose 2% (MT3): The drug dissolution studies indicated that in 30 minutes time 73.20% of the drug was dissolved.

- With added Sodium Carboxy Methyl Cellulose 1% (MT4): The drug dissolution studies indicated that in 30 minutes time 64.52% of the drug was dissolved.

- With added Sodium Carboxy Methyl Cellulose 1.5% (MT5): The drug dissolution studies indicated that in 30 minutes time 67.19% of the drug was dissolved.

- With added Sodium Carboxy Methyl Cellulose 2% (MT6): The drug dissolution studies indicated that in 30 minutes time 72.21% of the drug was dissolved.

- With added Hydroxy Propyl Methyl Cellulose 1% (MT7): The drug dissolution studies indicated that in 30 minutes time 73.27% of the drug was dissolved.

- With added Hydroxy Propyl Methyl Cellulose 1.5% (MT8): The drug dissolution studies indicated that in 30 minutes time 78.68% of the drug was dissolved.

- With added Hydroxy Propyl Methyl Cellulose 2% (MT9): The drug dissolution studies indicated that in 30 minutes 86.65% of the drug was dissolved.

CONCLUSION:

Sucrose-based medicated hard-boiled lozenges of Metoclopramide hydrochloride will be an alternative dosage forms for emesis patients. These will have additional advantages of patient compliance, convenience and comforts for efficient treatment including low dose, immediate onset of action, reduced dosage regimen and economy.

REFERENCES:

1. Tripathi KD. Essentials of Medical Pharmacology. 5thEd. New Delhi: Jaypee Brothers Medical Publishers (p) Ltd; 2003. p. 7-10, 599-609.
2. Brunton LL, Parkar KL, editors. Goodman & Gillman's Manual of Pharmacology & therapeutics. NY: The McGraw-Hill; 2008. p. 644-50.
3. Gralla RJ, Itri LM, Pisko SE et al. Antiemetic efficacy of high-dose Metoclopramide: Randomized trials with placebo and Prochlorperazine in patients with chemotherapy-induced nausea and vomiting. N Eng J Med 1981; 305: 905–909.
4. Cubeddu LX, Hoffmann IS. Participation of serotonin on early and delayed emesis induced by initial and subsequent cycles of cisplatinum-based chemotherapy: effects of antiemetics. J Clin Pharmacol 1993; 33: 691–697.

5. Bangale, G. S., G. V. Shinde, D. G. Umalkar, and K. S. Rajesh. "Natural mucoadhesive material based buccal tablets of nitrendipine-formulation and in-vitro evaluation." J Pharm Res (2011) 4: 33-38.
6. Galey, William R., H. K. Lonsdale, and Sergio Nacht. "The in vitro permeability of skin and buccal mucosa to selected drugs and tritiated water." Journal of Investigative Dermatology 67, (1976) 6: 713-717.
7. Guideline, ICH Harmonised Tripartite. "Stability testing of new drug substances and products." Q1A (R2), current step 4 (2003): 1-24.
8. Lakshmi, B. Moulika, K. Brahma, G. Swathi, S. Sravani, P. Indira Rao, and P. Shailaja. "Formulation and evaluation of domperidone candy lozenges." World J Pharm PharmSci (2017) 6: 12.
9. Rawe RC, Sheskey PJ, Owen SC. Handbook of Pharmaceutical Excipients, 5th Edition. London Pharmaceutical Press: 2006; 346,120, 330, 111, 611, 624.
10. Rawe RC, Sheskey PJ, Owen SC. Handbook of Pharmaceutical Excipients, 5th Edition. London Pharmaceutical Press: 2005; 120-23.
11. Pattanayak D and Das S: Formulation development and optimization of medicated lozenges for pediatric use. Int J Pharm Sci Res 2012; 3(1): 138-140.
12. Shojaei HA: Development of medicated lozenges. Journal of Pharmaceutical Science1998; 1(1): 15-30.
13. Rawe RC, Sheskey PJ, Owen SC. Handbook of Pharmaceutical Excipients, 5th Edition. London Pharmaceutical Press: 2006; 299-300.
14. Herbert A, Lieberman, Lachman L. Pharmaceutical Dosage forms-tablets series. Medicated Lozenges. 2nd edition Marcel Dekker. 1981; 1: 459-487
15. Swarbric, Boyller, Encyclopedia of Pharmaceutical Technology. 2nd edition Marcel Dekker. 2002; 3: 2775.
16. ICH Guidelines, Stability testing of active pharmaceutical ingredients and finished pharmaceutical products, WHO Technical Report Series, No. 953, Annex. 2009; 2: 87-130.
17. Brahmankar DM and Jaiswal Sunil B. Biopharmaceutics and Pharmacokinetics- A Treatise. In-vitro drug dissolution testing models, Vallabh Prakashan, Delhi.1995; 290-293.
18. Mehra Gilhotra R and Mishra Dina N. Preparation, characterization and Invitro Dissolution studies of surface cross-linked ocular Films of Gatifloxacin. Ethiop Pharm. Journal. 2008; 28: 1-8.