Metronidazole retention enema experiment and study

1. Introduction

1. 1. Rectal dosage forms

The most common and usually most convenient route for administering conventional pharmaceutical dosage forms is orally, where the drug dissolves in the gastric and/or intestinal fluids and is absorbed to reach the site of action. Dissolution and absorption of the drug from the gastro-intestinal environment depends upon many factors e. g. the physico-chemical properties of the drug, enzymes, metabolism, pH of the fluids etc. and these are considered as major drawback when localized drug delivery in the colon is required. Colonic drug delivery system offers advantages over oral dosage forms in improving the efficacy and achieving high concentrations with minimal side-effects1.

1. 1. 1. Advantages and limitations of rectal dosage forms

Advantages:

- Safe and painless form of administration.
- Drugs liable to degradation in the gastrointestinal tract can be administration.
- First pass elimination (drugs liable to degrade before reaching the site of action) of high clearance drugs is partially owing to bypassing the liver.
- Even larger doses can be administered.
- Drugs can be administered rectally in the long term care of geriatric and terminally ill patients.

- Administration of rectal suppositories or capsules is a simple procedure that can be under taken even by unskilled healthcare personnel and patients.

Limitations:

- Patient's acceptability and compliance is poor, especially for long term therapy.
- Suppositories can leak.
- Drug absorption from suppositories is slow in comparison to oral or intravenous administration.

1. 2. Different rectal drug formulations

Many formulations are developed for rectal use and these include suppositories (in the form of solid dose suspensions and emulsions), irrigations, gelatine capsules (used for non-steroidal anti-inflammatory drugs, encapsulated in a soft gelatine capsules as a reverse micellar solution for rectal application) 2, and enemas.

Enemas are aqueous solutions are suspensions intended for instillation into the rectal region for evacuation of bowel and to treat microbial infections. Enemas are of two types – macro enemas (100ml or more) and micro enemas (<100ml). Macro enemas are evacuation enemas and micro enemas are retention enemas, may be used for antimicrobial action3.

1. 3. Anatomy of Rectum and lower colon

The terminal 15- 19cm portion of the large intestine is rectum; it has a circumference of 15-35cm. The rectal pH is around 7-8. The absorptive area of rectum is less when compared to

that of small bowel because the rectal surface area is 200-400cm2 compared to that 200m2 of small bowel, due to very much shorter surface area per unit length 4. Generally medications for rectal delivery are better absorbed as weakly alkaline solutions. For rapid absorption of the medication aqueous solutions are preferable rather than suppositories or suspensions. Rectal absorption takes place by active absorption and for maximum retention without any rectal urgency to vacate bowels small volume of the fluid is recommended5. The diagram with a section of colon is shown below in fig-1.

1. 3. 1. Physiological considerations of colon

1. 3. 1a. Intestinal colonic micro flora

The human colonic atmosphere supports over 400 distinct species of bacterium with a population of 1011 to 1012 CFU/ml with mostly Eubacterium, Bacteriodes etc6. The enzymes produced by these bacterium has wide spectrum of action, that being hydrolytic and reductive in nature, these enzymes are actively involved in many processes, such as steroidal transformation, protein and carbohydrate fermentation, and destruction of mutagenic metabolism. Nitroreductase, azoreductases, and N-oxide and sulfoxide reductases are the most extensive reductive enzymes produced by the intestinal flora7.

1. 3. 1b. Colonic motility

Under the normal physiological conditions the colonic motility is described as irregular alternation of inactive, non-propagative, segmental contractions and infrequent propagative contractions that can be further classified into

high amplitude contractions (> 100 mmHg) and low amplitude contractions (<50 mmHg). Propagative contractions frequently originate in caecum and ascending colon and travel aborally over long segments of colonic walls and further diminish in distal colon. The low amplitude propagative contractions occurrence is frequent than high amplitude propagative contractions, which are observed early in the morning and postprandial8, 9. More frequently these high amplitude propagative contractions are observed in diarrheal-predominant irritable bowel syndrome, less frequently in idiopathic constipated patients10.

1. 3. 1c. Ascending colonic volume

The ascending colonic volume was found to be 170±40 ml as per the studies conducted on healthy subjects using single photon emission computed tomography11.

- Metronidazole

Metronidazole is a nitro-imidazole bactericidal agent primarily used against obligate anaerobic bacteria including Bacteroides, Clostridium spp., and certain protozoal parasites like Trichomonas vaginalis, Entamoeba histolytica, Giardia lamblia, Blastocysistis hominis, Balantidium coli and also some of the facultative anaerobes Gardnerella vaginalis and Helicobacter pylori16.

Gram negative anaerobes like bacteroides and fusobacterium species and the gram positive anaerobes like Peptosteptococci and clostridium species typically test sensitive to metronidazole. It is particularly used against

Helicobacter pylori associated to duodenal ulcers and gastritis. Metronidazole is also used against anaerobic bowel flora for the prophylaxis and for the treatment of Crohn’s disease where patients might develop complications of infections in bowel12. Metronidazole supports the overgrowth of aerobic microbial flora of the large intestine by reducing the number of anaerobic micro-organisms with acceptable profile of adverse effects13.

1. 4. 1. Anti microbial action of Metronidazole

Metronidazole as anti microbial agent was first introduced in 1959 for the treatment of Trichomonas vaginalis infections, and used subsequently for invasive giardiasis and amebiasis. Metronidazole is highly effective and show rapid onset of action against anaerobic infections. Antimicrobial action of Metronidazole is mainly due to the toxic intermediates which are produced during the reduction of the compound14. Interaction of these intermediates with deoxyribonucleic acid in protozoan inhibits nucleic acid synthesis and there by exerts antimicrobial effects15. Mechanism of action of Metronidazole is shown in the fig-2.

1. 4. 2. Physico-chemical properties

Chemically metronidazole is 2-methyl-5-nitroimidazole-1-ethanol or 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole. Its formula is C4H9N3O and its chemical formula is in the fig-3.

Metronidazole is white to pale yellow coloured, odourless, crystalline powder. It is sparingly soluble in water, alcohol and slightly soluble in ether16. It is generally stable in aqueous solutions of pH 2. 0-7. 017.

1. 4. 3. Metabolism of Metronidazole

Metronidazole is metabolised in the liver into two metabolites. These metabolites include 2-hydroxy-methyl-5-nitroimdazole-1-acetic acid (HM) and 2-methyl-1-2-nitroimidazole-1-acetic acid (MAA). Hydroxy metronidazole is pharmacologically active with antimicrobial action and its potency is 30% to that of metronidazole and the acetic acid metabolite of metronidazole is pharmacologically inactive but its activity is noticed in patients with renal dysfunction, small amount of metronidazole is oxidised to acetamide18. The metabolization pathway and chemical formulas can be seen in the figure 419.

Study objective

Metronidazole is commercially available in the form Tablets, Suppositories, Gels, and suspensions20. But metronidazole in the form of enema is not available commercially. Olumide F et al. 1976, described metronidazole in the form of enema (2gm. of metronidazole in 200ml of normal saline) for management of severe intestinal amoebiasis21. No extensive pharmaceutical data about the formulation and stability of the metronidazole enema is available at this moment. Hence, the development of a metronidazole enema was undertaken.

The present objective of the study is to formulate Metronidazole retention enema in a suitable aqueous media using carbopol and tragacanth as suspending and viscosifying agents. Chemical stability of the formulated enema is analysed with an analytical method: high performance liquid

chromatography (HPLC) and viscosity of the formulation is checked by Brook field viscometer.

Aim

To formulate a metronidazole retention enema and analyze physico-chemically.

2. 0. Materials and Method

2. 1. Chemicals

Metronidazole (98. 9% pure) used is a gift sample from Thrope Laboratories (India) Ltd, Mumbai. 2-methyl-5-nitro-imidazole is used as a standard impurity. Cardopol (35% w/v),

tragacanth, methyl paraben, propyl paraben, tris buffer were purchased from Aldrich. The methanol and acetonitrile used were of high performance liquid chromatography (HPLC) grade along with distilled water; other materials namely potassium hydrogen phthalate (KHP), sodium hydroxide (NaOH), sodium chloride (NaCl), potassium di-hydrogen phosphate (KH2PO4), acetic acid (CH3C OOH), sodium acetate (CH3COONa), sodium citrate (Na3C6H5O7) and 0. 1M hydrochloric acid (HCl) were of analytical quality.

2. 2. Instrumentation

- High Performance Liquid Chromatography apparatus and conditions

The liquid chromatography used was a Varian Prostar HPLC system (Model 410), equipped with an auto sampler serial mode system with a 20µl loop. Detection is accomplished with a UV-Visible detector. Integration and the system parameters were controlled by Galaxy software running on a PC.

- Viscometer

The viscometer used was Brook field viscometer (Model LVDV – II + PRO); displays temperature (C or F), viscosity, % scales, shear rate, shear stress, speed, % torque, and spindle. Technical specifications include 0. 01 to 200/rpm, viscosity range of 1 – 6Million cP, with 4 supplied spindles.

2. 3. Preparation of buffer solutions22, 23

2. 3. 1. Preparation of Phthalate buffer

Phthalate buffer was prepared by dissolving 20. 42g of potassium hydrogen phthalate in 1000ml of water (0. 1M). The pH was adjusted with 0. 1M hydrochloric acid, 0. 1M sodium hydroxide. Preparation of different pH ranges of phthalate buffer is shown in the table-1:

2. 3. 4. Preparation of tris buffer

Tris buffer was prepared by dissolving 12. 11g of tris (hydroxymethyl aminomethane) in 1000ml of water (0. 1M). The pH of the buffer was adjusted with 0. 1M hydrochloric acid. Preparation of different ph ranges of tris buffer is shown in the table-4.

2. 4. Pre-formulation studies

2. 4. 1. Pre-formulation solubility studies

The main problem associated with developing any of the solution formulation of a compound is its aqueous solubility. Metronidazole is poorly aqueous soluble drug24. For enhancing the poorly aqueous solubility of drug there are several alternatives and these include pH manipulation, co-solvency, surfactants, chelating agents and emulsion formation25.

In the present experiment the solubility of metronidazole, 2-methyl-5-nitro-imidazole in water, buffered solutions of phosphate, acetate and tris in various pH ranges were determined by adding a weighed amount of drug to the solvent by stirring with a glass rod at 20±2oC. Excess amount of solvent was added until the drug completely dissolved in that solvent.

2. 5. Stability Indicating HPLC studies

The stability studies are particularly demonstrated to analyse the quality, concentration and purity of the pharmaceutical dosage form. For demonstrating stability of pharmaceutical dosage forms HPLC is prominently used. Szepesi et al. 26 described some special stability-indicating requirements for HPLC and these include:

2. 5. 1. Stability- indicating assay

The peaks of the drug substance and its decomposition product should not elute at same time; any decrease in the active drug concentration should be detected by the method.

Stability-indicating purity:

The resolutions between the active component peak and the adjacent peak should be higher to identify any decomposition of the active component similar in its structure formed during different storage conditions.

2. 5. 3. The main impurity peak(s) should be separated from degraded product peak(s) of different chemical structure, so that evaluation of purity and assay are carried out together.

2. 5. 4. The peaks of the degraded products or secondary degraded products formed by the decomposition of by-product can also be separated from other peaks.

2. 6. Stability-indicating purity:

Pre-formulation stability studies were conducted to determine the stability of metronidazole in water and various buffered pH ranges using reverse phase high performance liquid chromatography (HPLC).

All the solutions were stored at room temperature 20±2oC for approximately 20 days in glass beakers and then analysed for stability.

Results and Discussion

Solubility study of Metronidazole

The aqueous solubility and pH solubility profiles for Metronidazole and 2-methyl-5-nitro-imidazole are shown in Table-7, 8, 9. Overall solubility of Metronidazole and 2-methyl-5-nitro-imidazole is determined at all pH values. Both Metronidazole and 2-methyl-5-nitro-imidazole exhibited high solubility at a pH range â‰¥4. For example, at room temperature the aqueous solubility of Metronidazole was 100mg/50ml, respectively. Metronidazole, being a weak base, appears to dissolve maximally at a pH â‰¥ 4. 0.

High performance liquid chromatography method development

The new method developed in this present study was very closely related to that of British Pharmacopoeia. British pharmacopoeia specifies reverse phase chromatography carried out using stainless steel column (20cmÃ-4. 6 mm) packed with octadecylsilyl silica gel of particle size 10µm (spherisorb ODS), using a mixture of 30 volumes of methanol and 70 volumes of 0. 01M

potassium di-hydrogen orthophosphate as the mobile phase with a flow rate of 1ml per minute and a detection wavelength of 315nm.

To establish a new stability indicating chromatographic nature of the HPLC method, we have changed the column to 5µm C18 (150 Ã- 4. 60 mm i. d., Phenomenex) stainless steel column, packed with Sphereclone octadecylsilane (ODS) and an (eluent) mobile phase to carry out the stability analysis in the solution at ambient room temperature with a flow rate of 1. 0ml/min and at a detection wave length of 325nm.

Each chromatographic run required about 10 minutes and the elution time obtained for metronidazole and the standard impurity were different for different mobile phases.

For methanol: KH2PO4 in the volume of 30: 70v/v retention times of drug and the impurity was1. 89min and 2. 00min, for methanol: KH2PO4 in the volume of 80: 20v/v retention times were very narrow, like wise all the mobile phase in different proportion showed a little retention time gap between drug and impurity. When acetonitrile and sodium citrate was used in the volumes of 10: 90 v/v retention time gap of 2min was achieved. Elution of Metronidazole and 2-methyl-5-nitro-imidazole was achieved with a retention time of 3. 19Min and 4. 99Min respectively.

Stability indicating solubility studies

Metronidazole was observed to be relatively stable in water and buffer pH conditions. The results obtained in our stability indicating solubility study showed an agreement with solubility and studies conducted by Yunqi et al., 200527.

Test for clarity and sedimentation

Sedimentation was observed when the water (400C) solubilized metronidazole is stored for 20 days, this might be due to the super saturation of the solution during solubilization of the drug at high temperature. Rest of the solutions were free from particles and sedimentation when observed against a black and white back ground.

Solubility studies of metronidazole and its standard impurity (2-methyl-5-nitro-imidazole), indicates solubility of metronidazole was more in aqueous solvents including various buffered pH systems. These solubility profiles help to understand the chemical nature (polarity) of drug and the impurity.

Solubility of metronidazole was observed to be high in acetate buffer (100mg/30ml) than any other buffer solutions. Taking the solubility profiles and colonic pH (5. 5-7) into consideration, acetate buffer of pH 6 was used to formulate enema

4. Formulation of enema

Our aim to formulate retention enemas is based on the studies conducted by Nyman-Pantelidis et al., 1994. There study proved low viscous enemas superiority over high viscous enemas in retention and colonic spread28.

4. 1. Preparation of metronidazole- tragacanth enema

Metronidazole-tragacanth enema was prepared by simple titration technique using motor and pestle. Metronidazole retention enema prepared was an aqueous formulation with a viscosity of 6. 00 cPas, containing metronidazole in a buffered pH. The formulation includes tragacanth as suspending and viscocifying agent. Methyl paraben as a preservative and NaOH (0. 1M) was

used to maintain the pH of the formulation. Metrinidazole was dissolved in buffered solution and then added to tragacanth gum, while triturating methyl paraben was added and pH of the final formulation is maintained at pH-6 using NaOH. The composition of the enema prepared in the study contains 1g of Metronidazole as shown in table 9.

Preparation of metronidazole- carbopol enema

For the treatment of anaerobic infection of colon we have formulated Metronidazole as enema using carbopol, a high molecular weight polymer of acrylic acid cross linked to allyl sucrose. Carbopol increases the viscosity of the enema and so help in retaining in the colon for maximum efficacy of metronidazole29.

Metronidazole was dissolved in buffered solution 1. 0g/300ml. This solution was added under constant stirring to carbopol. Methyl paraben was dissolved in water and under constant stirring this mixture was added to the suspension. The pH of the final formulation is maintained at 6 by the addition of NaOH. The composition of the enema formulated is shown below in the table-10.

Storage of enemas for stability and viscosity studies

The formulated enemas were filled in 100 ml glass beakers and stored for 2 days at refrigerated conditions 40C, room temperature 200C and at accelerated conditions 400C and analysed for stability and viscosity.

Stability studies of the formulated enemas

Both the enemas were found to be stable for 3 days at various temperatures (2-100C, 25±20C, 40±20C). The chromatograms show no peak of degraded metronidazole. Chromatograms of both the enemas is shown in the fig-9, fig-10.

Viscosities of the formulated enemas

The viscosity measurements for both rectal enemas were performed by Brook's field viscometer, using spindle 62 revolving at 22 rpm. Both the rectal enemas were prepared with a viscosity of 6. 0 cP using tragacanth and carbopol. The viscosities of both rectal enemas were then analysed after storage for 2 days at 2-100C, 20±20C, and 40±20C respectively and there results are shown in table-10.

There was a little variation of viscosities in the formulated enemas after their storage at various temperatures, and this variation is seen especially in enemas stored at accelerated temperature (40±20C) for 3 day. Because viscosity is inversely related to temperature, as temperature increases viscosity decreases.

Test for clarity and sedimentation

Both the formulated enemas were clear without any particles when observed against a black and white background. When the enemas are further analysed, phase separation was observed in the enema formulated using tragacanth and stored at 2-100C, 20±20C, and 40±20C.

Conclusion

The solubility studies results indicate that metronidazole showed a good solubility at various pH levels (â‰¥4. 0). Maximum solubility of metronidazole was shown in acetate buffer of pH 6 (100mg/30ml). The stability studies indicate that metronidazole was stable at all the pH ranges without any degradation.

The Metronidazole enema formulated using tragacanth and carbopol was chemically stable with no degradation when stored for a period of 3 days at 2±100C, 20±20C, 40±20C respectively. And there is no remarkable effect on the viscosities and pH of the enemas when stored at these temperatures.

Future work

In the present study stability studies were conducted for only 3 days, the results would be more appropriate if the stability studies of the formulated enema were carried for some more days.

The new methodology developed for demonstrating High Performance Liquid Chromatography indicating stability studies using acetinitrile and sodium citrate in the volumes of 10: 90v/v should be validated.