



Preformulation Study of Dolasetron: An Insight for Formulation and Development of Nasal Formulation

Mansi Rathod, Dharmik Mehta, Pragna Shelat, Punit Parejiya

Department of Pharmaceutics, K. B. Institute of Pharmaceutical Education and Research, Kadi Sarva Vishwavidyalaya, Gandhinagar, Gujarat, India

ABSTRACT

The objective of preformulation study is to develop the elegant, stable, effective and safe dosage form by establishing kinetic profile, compatibility with other formulation excipients and to establish physico-chemical parameter of new drug substance. This could provide important information for formulation design or support the need for molecular modification. So, in the present study preformulation studies were performed on Dolasetron (DS) to assess its suitability for nasal formulation. DS is a specific and selective serotonin subtypes 3 (5-HT) receptor antagonists, used to treat chemotherapy induced nausea and vomiting. The authenticity of DS was established by DSC and FITR spectra. An UV spectrophotometric method and HPLC method were employed for determination of DS in bulk and blood plasma respectively. Saturation solubility, micromeritical properties, melting point, pH, hygroscopicity and stability profile were studied. The UV method was linear in the range of 5-50 µg/ml. The low % CV values of intra-day and inter-day variations revealed that the proposed method is robust. The retention time of DS in HPLC method was found to be 2.8 min. The method was proven robust by obtaining very high regression coefficient value (0.999). The results of the physicochemical study of drug revealed suitability of DS for nasal route. Moreover, the drug was found stable in both solid as well as liquid state at different conditions.

Keywords: Preformulation, dolasetron, nasal formulation, bioavailability, stability

*Corresponding Author Email: mansirathod18@yahoo.in

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INTRODUCTION

Preformulation is a group of studies that focus on the physicochemical properties of a drug candidate that could affect the drug performance and the development of dosage form (1). Also, it could provide important information for formulation design or support the need for molecular modification. Every drug has intrinsic chemical and physical properties which have been considered before development of pharmaceutical formulation. This property provides the framework for drugs combination with pharmaceutical ingredients in the fabrication of dosage form (2).

The objective of preformulation study is to develop the elegant, stable, effective and safe dosage form by establishing kinetic profile, compatibility with other formulation excipients and establish physic-chemical parameter of new drug substance. The classic preformulation study requires drug characterization in solid as well as liquid phase. Preformulation can help in cost cutting for effective therapeutic development of the product.

The therapeutic efficacy of drug product intended to be administered by the oral route mainly depends on its absorption by the gastrointestinal tract. However, for a drug substance to be absorbed, it is needed to be solubilized. Majority of new chemical entities as well as classical molecules are found to be poorly water soluble in nature. To deliver such drugs in a better way, the issue of poor aqueous solubility needs to be addressed by formulation scientist.

Dolasetron (DS) mesylate, chemically (2 α ,6 α ,8 α ,9 $\alpha\beta$)-octahydro-3-oxo-2,6-methano-2H-quinolizin-8-yl-1H-indole-3-carboxylate mono methane sulfonate monohydrate, is an highly specific and selective serotonin subtype 3 (5-HT) receptor antagonist, used to treat chemotherapy induced nausea and vomiting (3). 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. Dolasetron mesylate is official in USP30-NF25 with HPLC method for its estimation.

In present study, dolasetron, a well known anti-nauseant and anti-emetic drug has been taken as a candidate drug. This is due to poor solubility and extensive first pass metabolism in the gut wall and liver. *In vivo*, the drug is rapidly converted into its major active metabolite, hydrodolasetron, which seems to be largely responsible for the drug's pharmacological activity. The antiemetic activity of the drug is brought out through the inhibition of 5-HT₃ receptors present both centrally (medullary chemoreceptor zone) and peripherally (GI tract).

Nasal drug delivery system conventionally used for local delivery of drugs for the treatment of nasal allergies and infections. In recent years research established that the nasal route is safe and

acceptable alternative to oral and parenteral administration of drugs. Nasal route provides advantages like rapid absorption and onset of action, elicitation of local immune response, avoidance of hepatic metabolism, self medication, ease of applicability and needle free drug application (4). Certain polar drug molecules with poor oral absorption are suitable for nasal administration. Though, nasal route possesses certain disadvantages like improper technique of administration leads to mechanical loss of the drug, certain absorption enhancers may lead to histological damages, risk of local side effects and limited volume of formulation delivery into nostrils, can be an alternative to oral route.

The relevant aspects of physicochemical, formulation and physiological factors of nasal cavity that must be considered during the process of discovery and development of new drugs for nasal delivery drugs as well as in their incorporation into appropriate nasal pharmaceutical formulations.

MATERIALS AND METHOD

Dolasetron mesylate was provided as a gift sample by M/s Emcure Pharmaceuticals, Pune, India. Drug was used as such, without any further purification. Methanol and acetonitrile were purchased from M/s Finar Limited, Ahmedabad, India. Phosphate buffer pH 6.8 and double distilled water were prepared in laboratory.

Preformulation study is the mandatory step in formulation and development of pharmaceutical products for best selection of appropriate dosage form and choice of excipients. In the drug preformulation studies, DS was tested for following parameters:

Analytical preformulation

Quantification of drug in bulk

Quantification of DS in bulk was done by UV spectroscopic method which is mentioned below (Birajdar et al., 2015):

Apparatus

An UV spectrophotometric analysis was performed on a double beam ultraviolet spectrophotometer (Shimadzu-1800, Japan), with a 1.00 cm quartz cells. The instrument settings were optimized to produce a spectrum with about 80% full-scale deflection and acceptable noise level. Each spectrum was recorded in triplicate. For each replicate measurement the cell was refilled with fresh solution.

Preparation of phosphate buffer pH 6.8

Dissolve 28.80 gm of di-sodium hydrogen phosphate and 11.45gm of potassium dihydrogen phosphate in sufficient water to produce 1000 ml. Ultraviolet absorption in the range 200 to 400 nm of 10µg/ml solution in phosphate buffer (pH 6.8) was measured by UV spectrophotometer.

Preparation of DS standard solution

A stock solution containing 1000 µg/ml DS was prepared by dissolving 25 mg DS in 5 ml of methanol in a 25 ml of volumetric flask and volume was made upto 25 ml with the methanol. The same has been repeated for phosphate buffer pH 6.8. From these stock solutions, suitable aliquots were taken and diluted using appropriate solvent to get dilutions of 5-50 µg/ml. The determinations were conducted in triplicate and studied for three days to check intra and inter day variations.

Preparation of calibration curve

Calibration curve was constructed at concentrations range 5-50 µg/ml. Absorbance of each solution was measured at the wavelength of 285 nm. Calibration curve was constructed for DS by plotting absorbance versus concentration at 285 nm wavelength. The determination was conducted in triplicate.

Quantification of drug in plasma

HPLC method was employed for determination of DS in human plasma is as described below (5):

Instruments, reagents and chromatographic conditions

| | |
|----------------|--|
| Instrument | HPLC shimadzu |
| Column | C ₈ |
| Mobile phase | ACN: Water: Ammonium formate (450:440:110) |
| Flow rate | 1 ml |
| Injection vol. | 20 µl |
| PDA | 285 |
| Rt | 2.8 mins |
| Run time | 12 mins |

Preparation of stock solution

The stock solution of DS was prepared by dissolving 20 mg into 100 ml ACN separately. Aliquots were subsequently diluted with ACN to yield stock solutions 0.1 g/l. All prepared stock solutions were stored at 4° C and also checked for intra and inter day variations.

Extraction procedure

Plasma sample (1 ml), working internal standard (10 µl) and NaOH solution (0.1 ml) were added to a test tube. The samples were mixed and then extracted with 5 ml ethyl acetate using vortex (Remi Instruments, Mumbai, India) for 3 min and centrifuged (Remi Centrifuge, Mumbai, India) at 25000 rpm for 5 min. The top organic layer (4 ml) was transferred to another tube and evaporated to dryness at 50° C under a gentle steam of nitrogen. The residue was reconstituted in 0.1 ml of mobile phase, centrifuged at 10000 rpm at 4° C for 10 min and then upper aliquot of this (20 µl) was injected in HPLC for analysis.

Physicochemical and micromeritical preformulation

Saturation solubility study

DS was taken in excess amount in clean and dry volumetric flask and dispersed in 50 ml purified water. The dispersion was shaken well and volume was adjusted to 100 ml followed by 10 min of shaking using flask shaker-orbital shaker (CIS-24 Remi, India). The solution was kept aside for 15 min and 5 ml aliquot was withdrawn from supernatant and analyzed using standardized analytical method. The procedure was repeated for different solvent (phosphate buffer pH 6.8, methanol and ethanol).

Melting point

Melting point was determined by capillary fusion method in melting point apparatus. A capillary was sealed at one end filled with a small amount of DS and the capillary was kept inverted i.e. sealed end downwards into the melting point apparatus.

pH of 10% DS solution

The pH of 10% DS solution (10 mg/100 ml) was determined using digital pH meter.

Hygroscopicity

Different samples of 25 mg of DS was placed in glass petri dishes and exposed to different humidity conditions in desiccators (previously calibrated) for a specific time period. The amount of moisture absorbed was determined by gravimetric analysis.

Aqueous state stability study

DS was accurately weighed and made into dispersion in distilled water. The solution was exposed to various conditions for specific time and checked for DS content periodically. The stability of drug in presence of light, oxygen, moisture, pH and ionic strength were also tested.

Differential Scanning Calorimetry (DSC)

DSC analysis was performed using Netzsch DSC 204, Tokyo, Japan. The Samples were heated in an open aluminium pans at a rate of 100 per min⁻¹ in a 30 to 300° C temperature range under a nitrogen flow of 40 ml/min.

Fourier Transform Infrared (FTIR) Spectroscopy (6)

FTIR spectra were obtained by Shimadzu FTIR Model 8400-S spectrometer. The spectra was recorded as a dispersion of the sample in potassium bromide in IR disk (2 mg sample in 200 mg KBr) with the scanning range of 400 to 4000 cm^{-1} and the resolution was 1 cm^{-1} .

Micromeritical properties

Particle size and particle size distribution was determined by calibrated microscope. Bulk density and angle of repose were determined by standard procedure using standard density apparatus and fixed funnel method respectively. The Carr's index (%) and the Hausner's ratio were calculated using following equations:

$$\text{Carr's index (\%)} = \frac{\text{TBD} - \text{LBD}}{\text{TBD}} \times 100$$

$$\text{Hausner's ratio} = \frac{\text{TBD}}{\text{LBD}}$$

RESULT AND DISCUSSION

Analytical methods

The development of spectrophotometry methods for the determination of drugs has been increased considerably in recent years because of their importance in pharmaceutical analysis. Based on the experimental data the standard calibration curves were plotted. The regression analysis showed very good correlation ($r^2=0.996$ in methanol and 0.998 in phoshphate buffer). The method was linear in the range of 5-50 $\mu\text{g/ml}$. These solutions obeyed Beer-Lambert's law in concentration range of 5-50 $\mu\text{g/ml}$ in methanol and phosphate buffer. The overlaid spectrum of DS is shown in Figure 1.

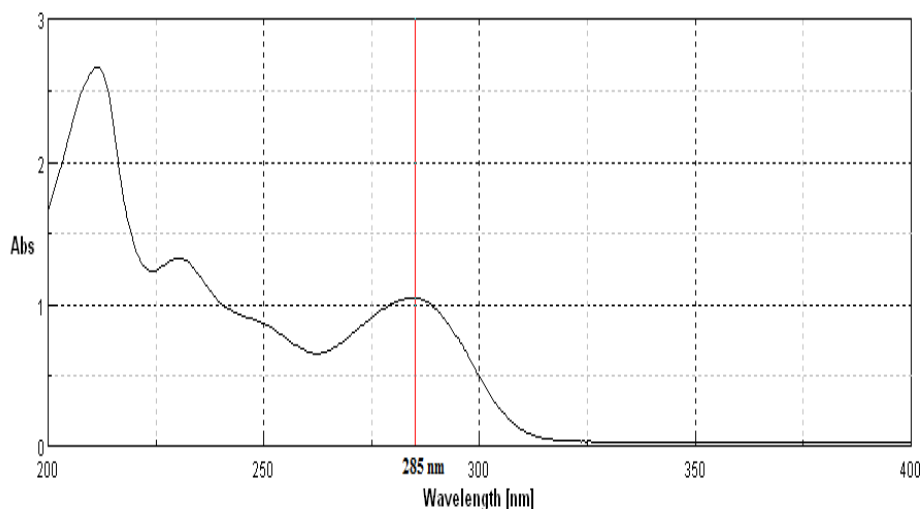


Figure 1: The UV spectra of DS

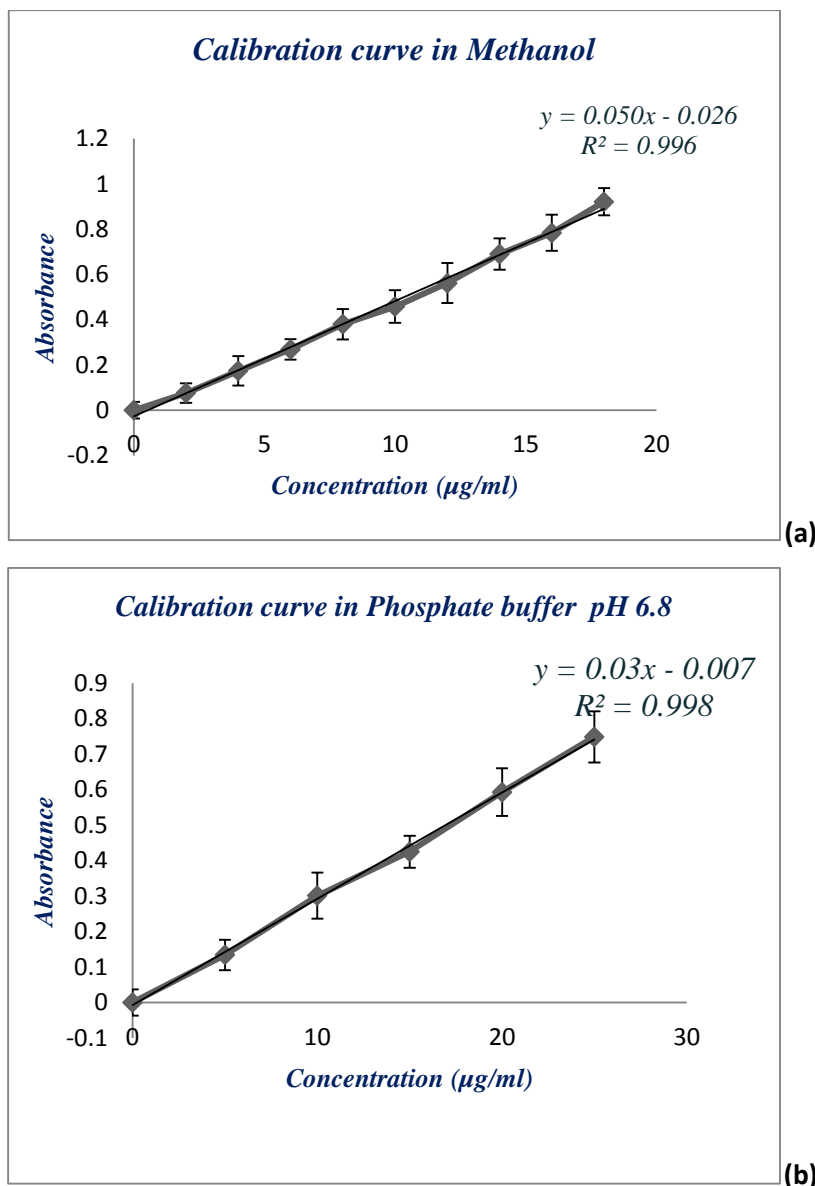


Figure 2: (a) calibration curve in methanol (b) calibration curve in phosphate buffer pH 6.8

Figure 2(a) shows calibration curve in methanol and (b) shows in phosphate buffer. The accuracy of analytical method is the closeness of test results obtained from that method to the true value. Accuracy is the measure of exactness of analytical method. Accuracy of the assay method was evaluated in triplicate at three concentration levels (5, 15, 25 $\mu\text{g/ml}$) and showed minor variation in concentration data. Result of intra-day and inter-day precision is expressed in % RSD. UV spectra of three different concentrations (5, 15, 25 $\mu\text{g/ml}$) were taken on the same day and the values of the relative standard deviation were calculated to determine intra-day precision. Percent RSD for Intraday assay precision was found to be 0.0503. Inter-day assay precision was found to be 0.0907. The low % CV values of intra-day and inter-day variations revealed that the proposed method is robust. According to the equation, the LOD was found to be 0.24 and 0.21

for methanol and phosphate buffer respectively. LOQ was found to be 0.82 and 0.85 µg/ml for methanol and phosphate buffer respectively. This data shows that this method is sensitive for the determination of DS.

The reliability of analytical findings is a matter of great importance in forensic and clinical toxicology, as it is of course a prerequisite for correct interpretation of pharmacokinetics and toxicological findings. HPLC analysis of biological samples, such as plasma, requires sample preparation or clean-up prior to injecting into the HPLC system. The mobile phase in developed HPLC method was optimized and selected by taking different proportions of ACN and ammonium formate which resulted acceptable asymmetry and theoretical plates. The HPLC system was equilibrated with the initial mobile phase composition, followed by 10 injections of the same standard. These 10 consecutive injections were used to evaluate the system suitability on each day of method validation. The retention time of DS was found to be 2.8 min. The method was proven robust by obtaining very high regression coefficient value (0.999). The sensitivity and selectivity of method was proven by value of LOD and LOQ which are 3.171728 and 9.611298 respectively. Figure 3 shows linearity curve of DS by HPLC method.

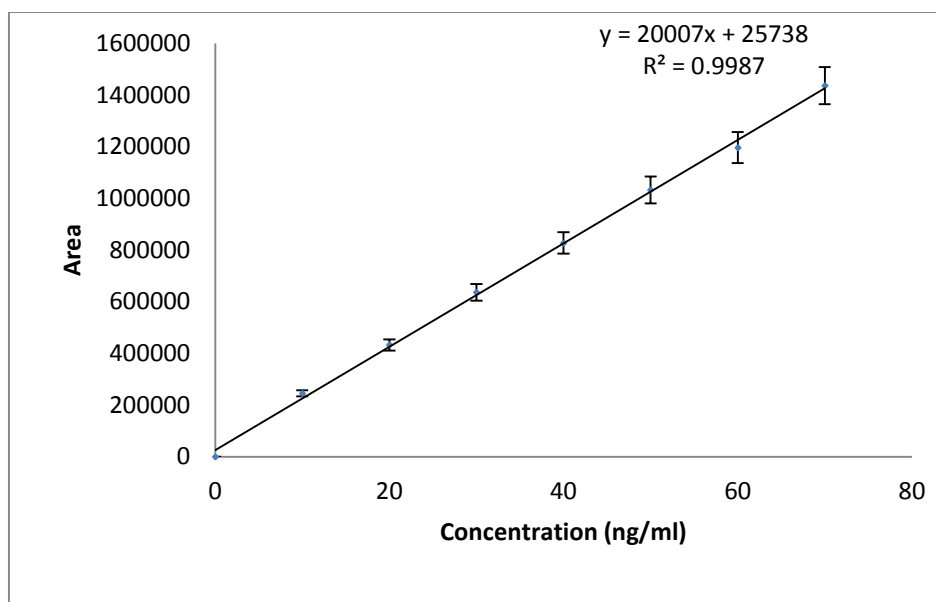


Figure 3: Linearity curve of DS by HPLC method

Physicochemical and micromeritical preformulation

The results of physicochemical and micromeritical studies are depicted in Table 1. Various pharmacokinetics parameters were collected from literature. Acceptable Organoleptic properties, solubility profile and physicochemical properties of DS establish its nasal administration profile. DSC thermogram of DS is shown in Figure 4.

Table 1: Physiochemical, derived and pharmacokinetics data for DS

| Sr. no. | Parameter | Observation |
|---------|--|-----------------------------|
| 1. | Organoleptic properties | |
| 1.1 | Colour | White fine powder |
| 1.2 | Odour | Odourless |
| 1.3 | Taste | Unpleasant, slightly bitter |
| 2. | Solubility | |
| 2.1 | Aqueous solubility | 0.291mg/100 ml |
| 2.2 | pKa | 12.18 |
| 2.3 | Partition co efficient (Octanol-water, 25 °C) | 308±40mg/L |
| 3. | Physicochemical property | |
| 3.1 | Melting point (°C) | 236-239 |
| 3.2 | pH | 6.5 |
| 3.3 | Molecular weight | 324.38 |
| 3.4 | Purity (%) | 99.98 |
| 4. | Derived property | |
| 4.1 | Bulk density | 0.495±0.031 |
| 4.2 | Tapped density | 0.573±0.043 |
| 4.3 | Carr's index | 17.24 |
| 4.4 | Hausner's ratio | 1.22 |
| 4.5 | Angle of repose | 23.22°±1.44 |
| 5. | Pharmacokinetic parameters (Reported) | |
| 5.1 | Half life (h) | 8.1 |
| 5.2 | Protein binding | ~ 92% |
| 5.3 | Cmax (µg/L) | 55.00 |
| 5.4 | Tmax (h) | 0.7-0.8 |
| 5.5 | Bioavailability (%) | 54% |

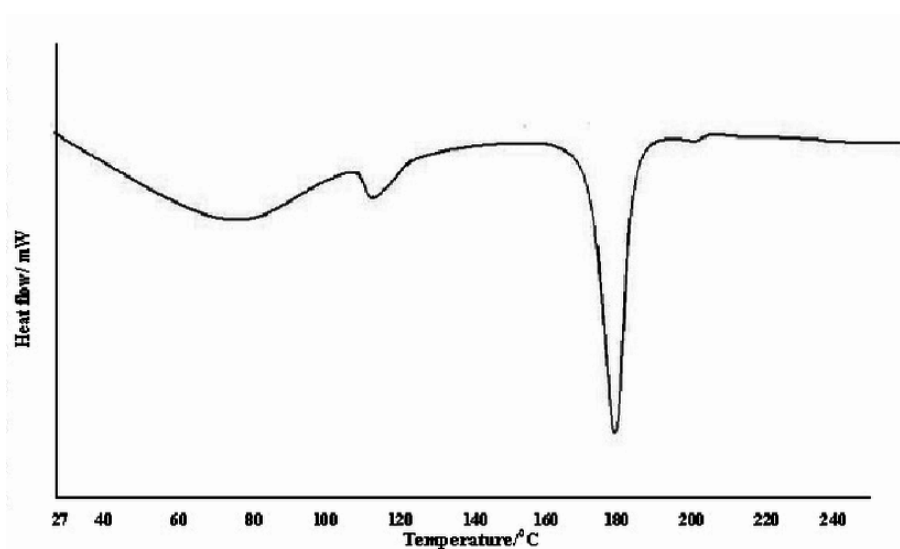


Figure 4: DSC thermogram of dolasetron

The characteristic absorption peaks of DS in FT-IR spectra as shown in Figure 5 proves stable and pure drug profile. Further, stability of DS has been also assessed at various temperatures, moisture, light, oxidation and pH condition. The results obtained from stability study under preformulation exhibited stable characteristics of drug at different storage conditions which are shown in Table 2.

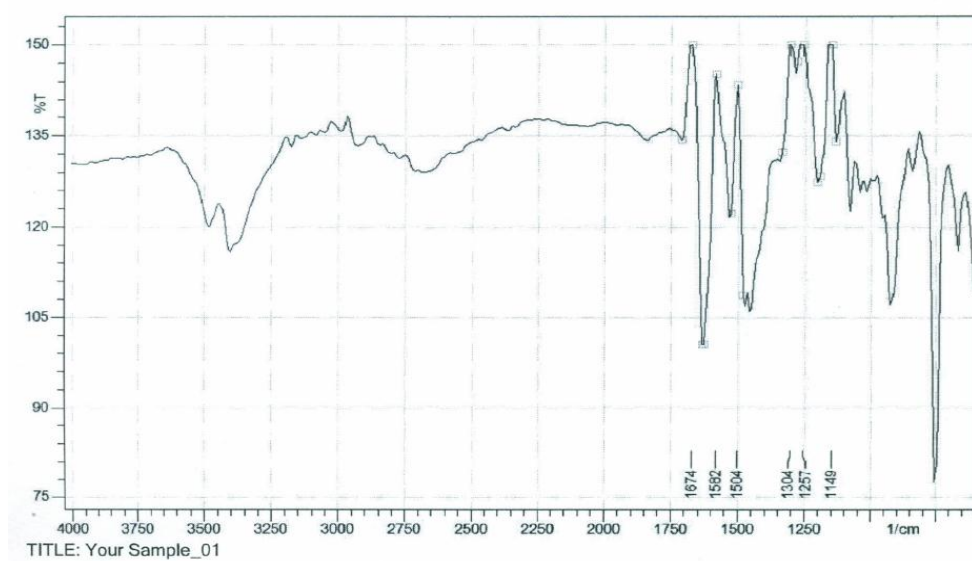


Figure 5: FT-IR spectra of dolasetron

Table 2: Drug stability under preformulation study at different conditions

| No. | Influencing factor | Test sample | Packing material | Storage condition | Storage time(weeks) | Physical degradation | Drug content |
|-----|------------------------|---|--|-------------------|---------------------|----------------------|--------------|
| 1 | Moisture | Pure drug | Open container | 25°C/75 % RH | 0 | No | 97.78±0.32 |
| | | | | | 1 | No | 98.79±0.29 |
| 2 | Temperature | Pure drug | 50 ml glass container with twist-off closure | 70°C | 0 | No | 99±0.74 |
| | | | | | 2 | No | 100.43±0.82 |
| | | | | | 4 | No | 98.45±0.45 |
| | | | | | | | |
| 3 | Temperature + Moisture | Pure drug substance with absorbed water at 25°C/75 % RH | 50 ml glass container with twist-off closure | 70°C | 0 | No | 99.24±0.34 |
| | | | | | 2 | No | 100.23±0.11 |
| | | | | | 4 | No | 99.31±0.58 |
| 4 | Oxidation | 1%aqueous solution in 0.35 H ₂ O ₂ solution | 25 mL glass flask with glass stopper | 50°C | 0 | No | 99.12±0.63 |
| | | | | | 1 | No | 100.66±0.31 |
| | | | | | 3 | No | 98.78±0.38 |
| 5 | Light | Pure drug substance | Open petridish | Xenon lamp | 24 hrs | No | 99.45±0.33 |
| | | | | | 48 hrs | No | 101.34±0.68 |
| | | 1%aqueous | Brown glass | Xenon | 24 hrs | No | 97.87±0.35 |

| | | | | | | | |
|---|----------|----------------|---------------|--------|----|------------|-------------|
| | solution | flask | lamp | 48 hrs | No | 98.94±0.23 | |
| | | Open | Xenon | 24 hrs | No | 99.45±0.89 | |
| | | petridish | lamp | 48 hrs | No | 98.87±0.54 | |
| | | Brown glass | Xenon | 24 hrs | No | 99.34±0.92 | |
| | | flask | lamp | 48 hrs | No | 98.56±0.78 | |
| 6 | pH | 1%aqueous | 25 ml glass | 60°C | 0 | No | 98.34±0.32 |
| | | solution pH | flask with | | 1 | No | 98.24±0.73 |
| | | 1, 2, 3, 4, 5, | glass stopper | | 3 | No | 100.89±0.81 |
| | | 6, 7, and 8 | | | | | |

CONCLUSION

From the results of the different preformulation studies, it can be concluded that DS is suitable for nasal formulation. The pH of DS was found to be 6.5 proving no irritancy on nasal mucosa. Stability study under preformulation studies revealed stable characteristics of drug in both solid as well as aqueous state confirming final stability of formulation. So, in a nut shell it can be concluded that, due to high first pass metabolism of DS, the later can be administered via nasal route to achieve improved bioavailability. This study also suggests that DS can be delivered in nasal spray.

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