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# Designing of Force Degradative Studies and Development of Validated Stability Indicating UV Spectrophotometric Assay Method for the Estimation of Isosorbide Dinitrate in Tablet Dosage Form

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## ABSTRACT

Isosorbide Dinitrate (ISD) is a moderate to long acting oral organic nitrate which acts as a vasodilator profoundly used in the treatment of angina pectoris, a condition which occurs when the oxygen supply to the myocardium is insufficient for its needs. It is slightly soluble in water and propanol, sparingly soluble in ethanol and freely soluble in methanol. The most economical solvent Distilled water was choosen as a solvent. The drug has absorption maximum (method A) at 304nm, Area under curve (method B) at 300-310 and first order derivative method (method C) at 287nm. Linearity for all three methods was found in the range of 5-25 µg/ml with Correlation coefficient is 0.999, Standard error is 0.001. The drug sample was analyzed by UV spectroscopy using distilled water as solvent and the average content of drug present in the formulation was found to be 99.7%. The % recovery of accuracy studies was found to be 99.1 % for method A, 101.5 % for method B, 100.6 % for method C. The %RSD of precision was found to be 1.11% for intraday, 1.22 % for inter day. The force degradation studies of Isosorbide Dinitrate was done on Stress degradation by hydrolysis under alkaline condition by using 0.1N NaOH was found to be 10.09% for 24hrs, 13.3% for 3days, 14.2% for 5days .Stress degradation by hydrolysis under acidic condition by using 0.1N HCl and degradation was found to be 2.68% for 24hrs, 9.7% for 3days, 11.9% for 5days. Oxidative degradation was done by using hydrogen peroxide (3%v/v) and degradation was found to be 3.22% for 24hrs, 5.06% for 3days, and 8.29% for 5days. The proposed methods can be successfully employed for quality control during manufacture and for assessment of the stability of Isosorbide Dinitrate in bulk samples and its tablet dosage forms.

**Keywords:** Isosorbide Dinitrate, Forced Degradation studies, Tablet dosage forms, UV Spectroscopy, Validation

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# INTRODUCTION

Isosorbide Dinitrate (ISD) is 1,4:3,6-dianhydro-2,5-di-O-nitro-D-glucitol or (3R,3aS,6S,6aS)-6-(nitrooxy)-hexahydrofuro[3,2-b]furan-3-yl nitrate. Its molecular weight 236.1363mg.It is slightly soluble in water and propanol, sparingly soluble in ethanol and freely soluble in methanol. Isosorbide Dinitrate is a moderate to long acting oral organic nitrate which acts as a vasodilator profoundly used in the treatment of angina pectoris, a condition which occurs when the oxygen supply to the myocardium is insufficient for its needs. The vasodilation action of Isosorbide dinitrate is through the relaxing action in blood vessels of nitrates, particularly nitric oxide. This will decrease the oxygen demand of the heart and preventing chest pain.

**Objective of Study**

Literature survey revealed very few analytical methods have been reported for Isosorbide Dinitrate include [HPLC][12] Method available ,to best of our knowledge AUC and UV derivative methods are not available. However, these analytical methods lack stability indicating nature. Also, there was no reported analytical method for the estimation ISD in pharmaceutical dosage forms in presence of their degradation products. In the present investigation, an attempt was made to develop a simple, rapid, precise and accurate stability indicating UV method for estimation of ISD.

# MATERIALS AND METHOD

**Apparatus**

Shimadzu UV -1800 double beam spectrophotometer with 1cm path length supported by shimadzu UV-probe software ,version 2.21 was used for spectral measurements with 10mm matched quartz cells . Shimadzu balance (BL-220H) was used for weighing.

**Chemicals and solvents used**

Hydrochloric acid (AR Grade) , Sodium hydroxide(AR Grade) , Hydrogen peroxide(AR Grade) was purchased from Merck fine chemicals(Mumbai, India), double distilled water was used.

**Drug samples**

Isosorbide Dinitrate (working standard 99.70) was obtained as gift sample from Lara Drugs, Hyderabad, India. A commercial tablet of Isosorbide Dinitrate (Isordil) was purchased from local pharmacy.

**Calibration of proposed methods**

**Preparation of standard stock solution**

Accurately weighed 10mg of ISD working standard in 10mL volumetric flask containing 5ml of water shaken for 5min then remaining volume made up with distilled water. The final

concentration obtained was 1000µg/mL. It was further diluted to get concentration 100µg/ml was prepared with distilled water. From this a series of aliquots were prepared to get concentration ranging from 5-25µg/mL in 10ml vol. flask using distilled water.

**Method A: Zero order spectroscopic method**

The wavelength was selected by preparing a solution of concentration 10µg/mL by diluting the standard solution with distilled water. The solution was scanned in the wave length range of 200-400nm using distilled water as blank. The UV spectrum of Isosorbide Dinitrate showed $\lambda_{max}$ at 305.92 nm. The calibration curve was prepared in concentration range of 5-25µg/mL. Absorbance of each concentration plotted by taking absorbance on y-axis and concentration on x-axis. The regression equation was calculated and the results were shown in figure 2(a) and 2(b) the regression equation was used to estimate the drug content in tablet dosage form.

**Method B: Area under Method**

The AUC method is applicable when abroad peak is obtained. It involves the calculation of integrated value of absorbance with respect to wavelength between two selected wavelengths $\lambda_1$ and $\lambda_2$. The wave length range is selected based upon repeated observations so as to get the linearity between AUC and concentration. The solutions have analytical concentration range of 5-25µg/mL. These solutions were scanned in spectrum mode and AUC spectra was measured between 300nm and 310nm.The regression equation was calculated and the results were shown in figure 3(a) and 3(b).The regression equation was used to estimate the drug content in tablet dosage form.

**Method C: First order derivative spectroscopy**

It involves the conversion of normal spectrum into first derivative spectrum. The derivative spectra have a narrow spectral band width. First, order derivative may swing with greater amplitude than the primary spectra. For example, a spectrum suddenly changes from a positive slope to a negative slope, such as at the peak of a narrow feature. The more distinguishable derivatives are especially useful for separating out peaks of overlapping bands. Thus this derivative method is advantageous for selection of accurate wave length. For the selection of analytical wave length, a solution of concentration 10µg/mL of Isosorbide Dinitrate was prepared and scanned in spectrum mode in wave length range of 200-400nm.The spectrum thus obtained in derivatized in first order. The first order derivative spectrum obtained showed a sharp peak at $\lambda_{max}$ 304nm. The absorbance difference at n=1($dA/d\lambda$) was calculated by software of instrument. The amplitude was measured for all the solutions and plotted against concentration to get calibration curve. The amplitude was linear in the concentration range of 5-25 µg/mL. The regression equation was calculated and results were

shown in figure 4(a) and 4(b). The regression equation was used to estimate the drug content in tablet dosage form

**Figure 1: Structure of isosorbide dinitrate**

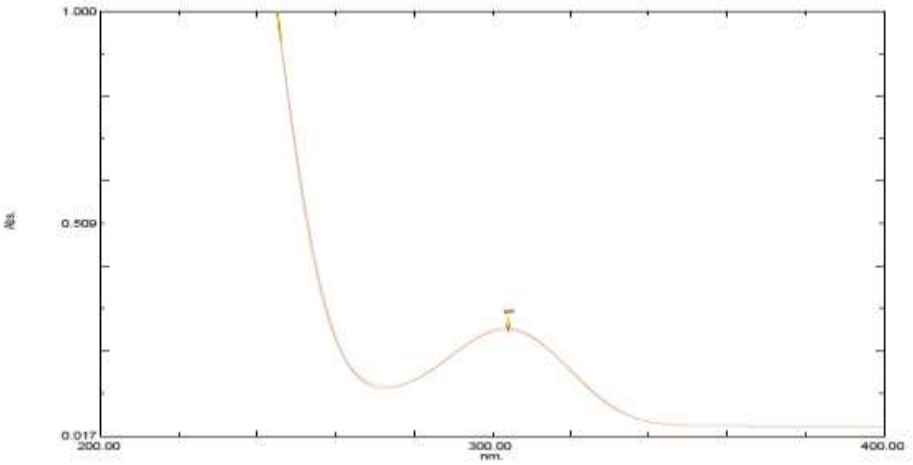


**Figure 2 (a): Zero order spectrum of ISD**

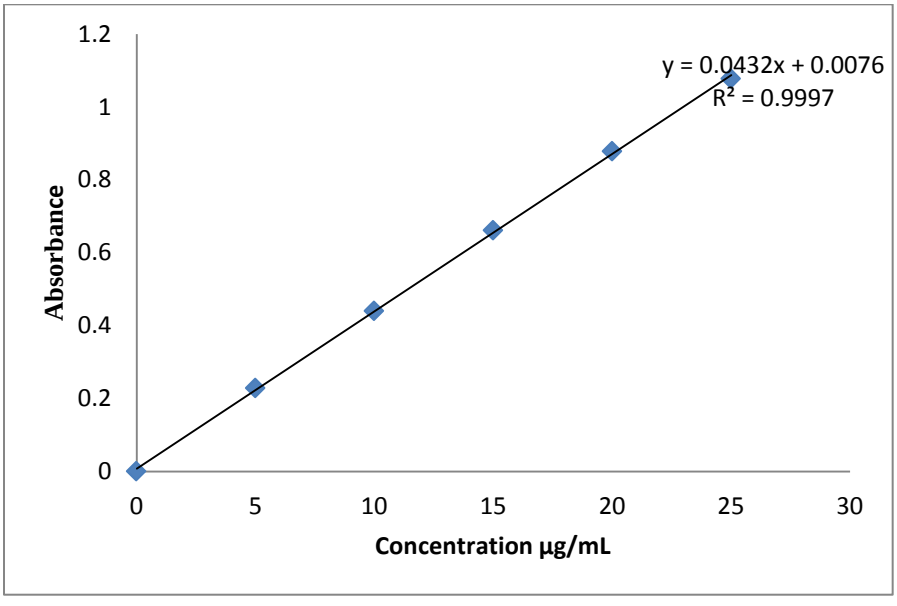


**Figure 2 (b): Calibration curve of ISD by zero order**

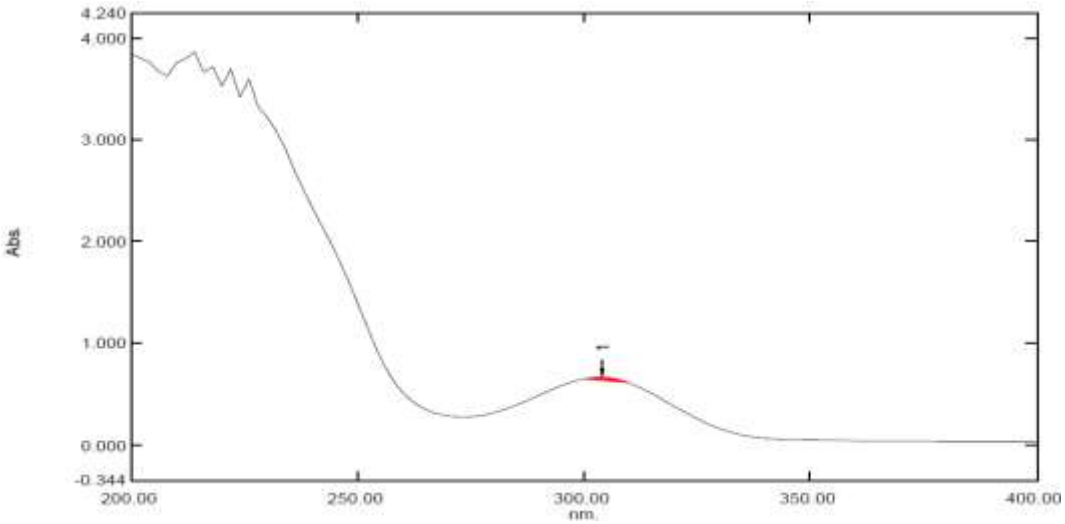


**Figure 3(a): Spectrum of ISD by AUC method**

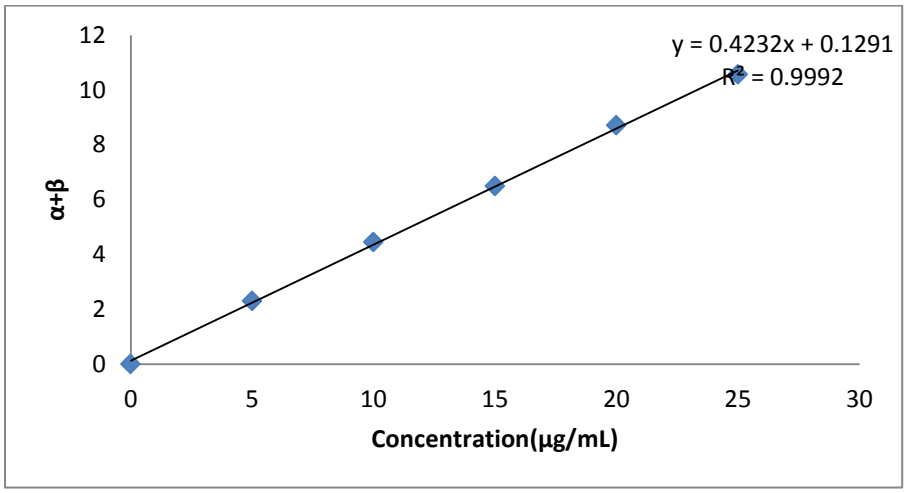


**Figure 3 (b): Calibration curve of ISD by area under curve method**

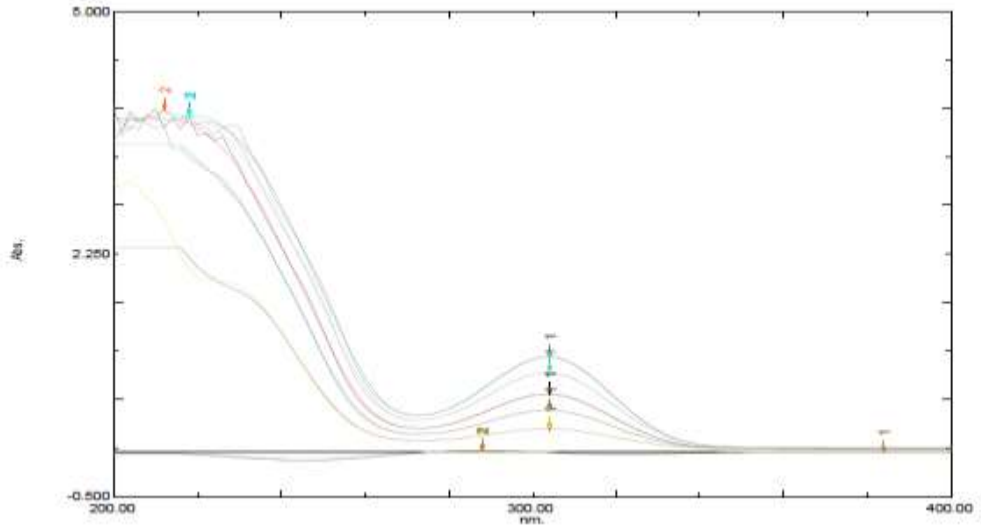


**Figure 4(a): First order derivative spectrum of ISD**

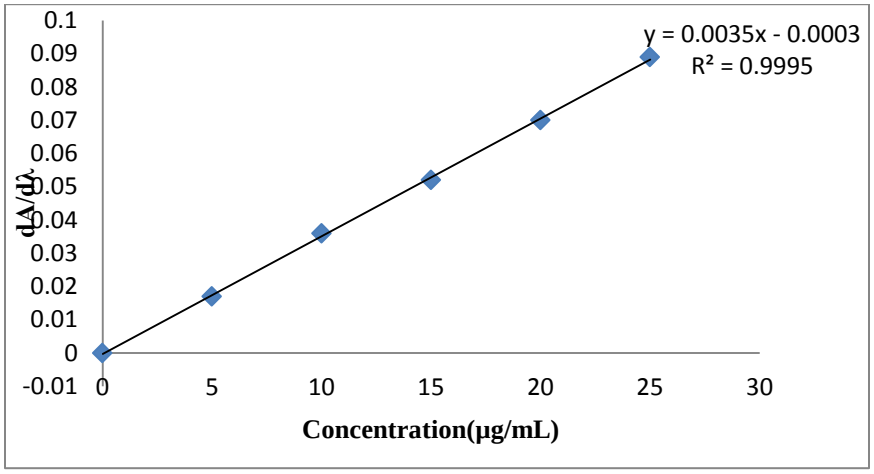


**Figure 4(b): Calibration curve of ISD by first order derivative method**

**Estimation of Isosorbide Dinitrate in Tablets**

**Preparation of sample solution**

For the estimation of Isosorbide Dinitrate in the commercial formulations, 20 tablets (Isordil) each containing 10 mg of Isosorbide Dinitrate were weighed and the average weight was calculated. The tablets were crushed and powdered in glass mortar. For the analysis of drug, quantity of powder equivalent 10 mg of Isosorbide Dinitrate was transferred to 10 mL volumetric flask and dissolved in sufficient quantity of distilled water and volume made up to the mark with distilled water to obtain conc. of 1000 µg/mL of Isosorbide Dinitrate. Then the solution was filtered through whatman filter paper no.41.Further dilutions of the solution were made in distilled water to get required concentration of 10 µg/mL. The concentration of Isosorbide Dinitrate in formulation was determined by above developed methods. The assay procedure was repeated six times (n= 6) for each method.

**Validation of Proposed Methods**

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. The method was validated as per ICH guide lines for different parameters like linearity, accuracy, precision.

**Linearity**

The linearity of the proposed UV spectroscopic methods were evaluated by analyzing different concentrations of standard solutions of Isosorbide Dinitrate and by plotting absorbencies of analyte against concentrations of the analyte. Beer's law was obeyed for all four methods in the concentration range of 5 – 25 µg/mL. A good linear relationship ($R^2$ =0.99) was observed between the concentrations of Isosorbide Dinitrate and the corresponding absorbance. The regression

analysis was made for slope, intercept and correlation coefficient values. The slope, intercept and the correlation coefficient of the drug were shown in the table 2

**Accuracy**

Accuracy is express as degree of closeness of experimental value to the true value. To study the accuracy of the proposed method and to check the interferences from excipients used in the dosage forms, recovery experiments were carried out by standard addition method. This parameter is evaluated by percent recovery studies at concentration levels of 50,100 and 150 % which includes addition of known amounts of Isosorbide Dinitrate working standard to a prequantified sample solution. Each of the dilution was observed six times. The samples were reanalyzed by proposed methods. The amount of Isosorbide Dinitrate was estimated by applying obtained values to regression equation. The percentage recovery of the drug was calculated. The results were shown in the Table 3

**Precision**

Precision is the level of repeatability of results as reported between samples analyzed on the same day (Intra – day) and samples run on three different days (Inter – day). To check the intra – day and inter – day variation of the methods, solutions containing 5, 10 and 15 µg/mL concentrations of Isosorbide Dinitrate were subjected to the proposed spectrophotometric methods of analysis and the recoveries obtained were noted. The precision of the proposed method i.e. the intra and inter – day variations in the absorbance of the drug solutions was calculated in terms of % RSD. Statistical evaluation revealed that relative standard deviation of drugs at different concentration levels for three times was less than 2.0. (Intra – day – 1.09, inter – day – 1.22).The values were shown in table.4

**Degradation Sample Preparation**

**Acid hydrolysis**

Transfer 1ml (1000ug/ml ISD) of above stock solution to10ml volumetric flask and add 1ml of 0.1N HCL and finally make up the volume with distilled water and kept aside for 24hrs at room temperature. From this transfer 0.1ml drug solution into 10ml volumetric flask and neutralize with 1ml of 0.1N NaOH, the final volume made up to with distilled water to get the concentration of 10 µg/mL. The absorbance was measured using above developed methods against blank contain 0.5ml of 0.1N HCl and 0.5ml of 0.1N NaOH in 10ml volumetric flask the final volume made up to the mark with distilled water.

**Base Hydrolysis**

Transfer 1ml (1000ug/ml ISD) of above stock solution to10ml volumetric flask and add 1ml of

0.1N NaOH and finally make up the volume with distilled water and kept aside for 24hrs at room temperature. From this transfer 0.1ml drug solution into 10ml volumetric flask and neutralize with 1ml of 0.1N HCl, the final volume made up to the mark with distilled water to get the concentration of 10 µg/mL. The absorbance was measured using above developed methods against blank contain 0.5ml of 0.1N HCl and 0.5ml of 0.1N NaOH in 10ml volumetric flask the final volume made up to the mark with distilled water.

**Peroxide hydrolysis**

Transfer 1ml (1000ug/ml of ISD) of above stock solution to10ml volumetric flask and add 1ml of 3%v/v of $H_20_2$ and finally make up the volume with distilled water and kept aside for 24 hrs at room temperature from this transfer 0.1ml of above solution in to 10ml volumetric flask and final volume made up to the mark with distilled water to get the concentration of 10µg/mL. The absorbance was measured using above developed methods against blank contain above solution without drug in 10ml volumetric flask the final volume made up to the mark with distilled water.

## RESULTS AND DISCUSSION

The proposed methods for estimation of Isosorbide Dinitrate were found to be simple, precise, accurate and economical for method A, the absorption maxima was found to be at 304 nm, for method B area under the curve in the range of 300 – 310nm was selected for the analysis, for method C, the absorption maxima of first derivative spectra was found to be 287nm.The calibration curve was linear in the concentration range 5-25 µg/mL (table 2). The % assay by the four methods was found to be in the range 98.16 – 101.3% for Isosorbide Dinitrate (table 1). No interference was observed from the pharmaceutical excipients. The recovery studies showed that there methods were accurate and reproducible. The results revealed that any change in the drug concentration could be accurately determined by the proposed method. Accuracy and reproducibility of the proposed methods were further confirmed by percent recovery values, as shown in table.3 Intermediate precision studies for the method % RSD is not more than 2.0 indicate good intermediate precision which were shown in table.4.Hence, the proposed methods were validated in terms of linearity, precision and accuracy. Characteristic parameters and summary of validation parameters for all the three methods were given in table.6. By observing the validation parameters, the methods were found to be simple, accuracy and precise. Hence these methods can be employed for the routine analysis of Isosorbide Dinitrate in tablet formulations.

**Degradation studies**

Results are tabulated in table 5

**Acid hydrolysis**

Upon performance of acid degradation studies 2.68% for 24hrs, 9.7% for 3days, 11.9% for 5days of Isosorbide Dinitrate was degraded.

**Base hydrolysis**

Upon performance of base degradation studies 10.09% for 24hrs, 13.3% for 3days, 14.2% for 5days of was Isosorbide Dinitrate degraded.

**Peroxide hydrolysis**

Upon performance of peroxide degradation studies 3.22% for 24hrs, 5.06% for 3days, 8.29% for 5days of Isosorbide Dinitrate was degraded.

**Table 1: Analysis of Isosorbide Dinitrate (Formulation)**

| Method | Label claim (mg/tablet) | Test concentration (µg/ml) | Amount found (µg/ml) (n= 6) | % assay | % RSD |
|---|---|---|---|---|---|
| A | 10 mg | 10 | 9.94 | 99.5 | 0.94 |
| B | 10 mg | 10 | 10.05 | 98.1 | 0.64 |
| C | 10 mg | 10 | 10.02 | 101.5 | 0.87 |

**Table 2: Optical Characteristics of Proposed Method**

| Parameter | Method A | Method B | Method C |
|---|---|---|---|
| Λmax | 305.92 | 300-310 | 304 |
| Beer's limit (µg/mL) | 5 – 25 | 5 – 25 | 5 – 25 |
| Molar extinction coefficient (L/Mol.cm) | 0.026 | 0.023 | 0.021 |
| Correlation coefficient ($r^2$) | 0.999 | 0.999 | 0.999 |
| Slope (a) | 0.043 | 0.423 | 0.003 |
| Intercept (b) | 0.007 | 0.129 | 0.001 |

**Table 3: Recovery Studies of Isd**

| Conc. taken (µg/mL) | Recovery level (%) | Amount added (µg/mL) | Amount found(µg/mL) (n =6) | | | % Recovery | | |
|---|---|---|---|---|---|---|---|---|
| | | | A | B | C | A | B | C |
| 10 | 50 | 5 | 14.34 | 14.48 | 14.45 | 95.60 | 96.53 | 96.33 |
| 10 | 100 | 10 | 19.96 | 19.77 | 19.84 | 99.3 | 98.85 | 99.20 |
| 10 | 150 | 15 | 24.55 | 24.69 | 24.65 | 99.2 | 98.76 | 98.60 |

**Table 4: Intraday and Inter Day Precision Studies of Isd**

| Concentration taken (µg/mL) | Intra – day precision | | Inter – day precision | |
|---|---|---|---|---|
| | %Recovery±SD; (n=3) | % RSD | %Recovery±SD; (n=3) | % RSD |
| 5 | 98.66±0.173 | 0.93 | 99.92± 0.28 | 0.98 |
| 10 | 97.99±0.753 | 0.86 | 98.70± 0.980 | 0.92 |
| 15 | 98.92 ±1.38 | 1.50 | 99.97 ±1.53 | 1.78 |

**Table 5: Forced Degradation Studies**

| Stress Conditions | Test Concentration taken (µg/mL) | λmax | Normal absorbance | Absorbance of Degradative samples | | |
|---|---|---|---|---|---|---|
| | | | | 24hr | 3Days | 5Days |
| Acidic/0.1N HCl/24hr at room temperature | 10 | 305.92 | 0.434 | 0.426 | 0.398 | 0.388 |
| Basic/0.1N NaOH/24hr at room temperature | 10 | 305.92 | 0.434 | 0.396 | 0.382 | 0.378 |
| Oxidising 3%v/v/24hr at room temperature | 10 | 305.92 | 0.434 | 0.420 | 0.412 | 0.398 |

**Table 6: Summary of Validation Parameters of Isosorbide Dinitrate**

| Parameter | Method A | Method B | Method C |
|---|---|---|---|
| $\lambda_{max}$ | 304 nm | 300 – 310 nm | 287 nm |
| Beer's limit (µg/mL) | 5-25 | 5-25 | 5-25 |
| Linearity indicated by correlation coefficient | 0.999 | 0.999 | 0.999 |
| LOD(µg/mL) | 0.064 | | |
| LOQ(µg/ mL) | 0.211 | | |
| Precision indicated by % RSD | 0.93 | 0.98 | 1.68 |
| Accuracy indicated by % recovery | 99.2 | 101.3 | 100.5 |

## CONCLUSION

A simple, rapid, accurate and precise stability-indicating UV method has been developed and validated for the routine quantitative analysis of Isosorbide Dinitrate in API and its dosage forms. The results of stress testing undertaken according to the ICH guidelines reveal that the method is specific and stability- indicating. The proposed method has the ability to separate these drugs from their degradation products in tablet dosage forms and hence can be applied to the analysis of routine quality control samples and samples obtained from stability studies.

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