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Stability Indicating RP-HPLC Method for the Estimation of Isosorbide 5-Mononitrate in Bulk Drug and its Pharmaceutical Dosage Form

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ABSTRACT

A simple, precise, accurate and stability-indicating reverse phase high performance liquid chromatography (RP-HPLC) method is developed for estimation of Isosorbide 5-Mononitrate in bulk drug and tablet dosage form. The method employed, with reverse phase phenomenex® Luna 5µ C18 (2) 100A (250 × 4.60 mm) column in an isocratic mode, with mobile phase of methanol: water: acetonitrile in the ratio 55:28:17 (%v/v/v). The flow rate was 1.0 ml/min and effluent was monitored at 217 nm. Retention time was found to be 4.391±0.015 min. The method was validated in terms of linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) etc. in accordance with ICH guidelines. Linear regression analysis data for the calibration plot showed that there was good linear relationship between response and concentration in the range of 1- 9 µg/ml respectively. The LOD and LOQ values for were found to be 2.5 and 10 ng/ml respectively. No chromatographic interference from tablet excipients and degradants were found. The proposed method was successfully used for estimation of Isosorbide 5-Mononitrate in tablet dosage form.

Keywords: Isosorbide 5-mononitrate, RP-HPLC, Validation, Stability-indicating method.

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INTRODUCTION

Isosorbide 5-mononitrate[1] (ISMN) chemically 8-nitrooxy-2, 6-dioxabicyclo [3.3.0] is used to decrease the severity and frequency of anginal episodes which is caused by coronary artery disease and to reduce the need for sublingual nitroglycerin. By stimulating cyclic GMP, ISMN relaxes vascular smooth muscles. It also decreases afterload (arterial resistance) and preload (left ventricular pressure). Chemical structure of ISMN was shown in Figure.1

Literature survey revealed that there are few methods reported for estimation of ISMN by using GC-MS[2], HPLC method in bulk drug and extended release formulations[3], gel permeation chromatography[4], supercritical fluid chromatography[5] and RP-HPLC method for simultaneous determination of aspirin and ISMN in formulation[6]. However none of the reported methods have described a stability indicating RP-HPLC method development for determination of ISMN. Hence the present work aims to develop a simple, precise, sensitive, economic and validated stability indicating RP-HPLC method for the determination of ISMN in bulk drug and its tablet dosage form.

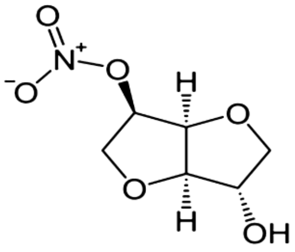

Figure 1 Structure of ISMN

MATERIALS AND METHODS

HPLC grade acetonitrile and methanol were obtained from Merck specialties private limited (Mumbai, India), HPLC grade water and analytical grade chemicals were obtained from Qualigen Fine chemicals, (Mumbai, India). Sodium hydroxide pellets, Hydrochloric acid, Hydrogen peroxide were obtained from SD Fine chemicals, Mumbai. Pure drug sample of ISMN was obtained from RA Chem Pharma Limited (Hyderabad, India). Isonorm (20 mg) tablets are purchased from the local pharmacy.

Various equipments like HPLC system(SHIMADZU-SPD 20A), analytical weighing balance, sonicator, Injector (Rheodyne, 20µl), mobile phase reservoir, pH meter, vaccum filter pump, Millipore filtration kit, water bath, sample filtration assembly and glassware’s were used throughout the experiment. The analysis was carried on a reversed-phase high performance liquid chromatographic system with isocratic elution mode using UV detector.

Optimization of chromatographic conditions:

The chromatographic conditions were optimized by different means (using different mobile phase, different flow rate and different detection wavelength).

Standard and sample preparation:

The standard and sample stock solutions were separately prepared by dissolving standard and sample in a solvent mixture of methanol, water and acetonitrile in the ratio 55:28:17 (%v/v/v) and diluting with the same solvent (after optimization of all conditions).

Stability Indicating Studies[8, 9]

Stress degradation studies like acid hydrolysis, basic hydrolysis, photolytic degradation, dry heat degradation, wet heat degradation and oxidative degradation were carried out.

RESULTS AND DISCUSSIONS

Selected wavelength and mobile phase

Mobile phase for preparation of various samples were finalized after studying the solubility of ISMN pure drug in different solvents (water, methanol, acetonitrile, dichloromethane, 0.1N NaOH, 0.1NHCl). The optimum mobile phase consisted of methanol, water and acetonitrile in the ratio 55:28:17(%v/v/v), selected as it was found to give best resolution and peak at a retention time of 4.391±0.015 min.

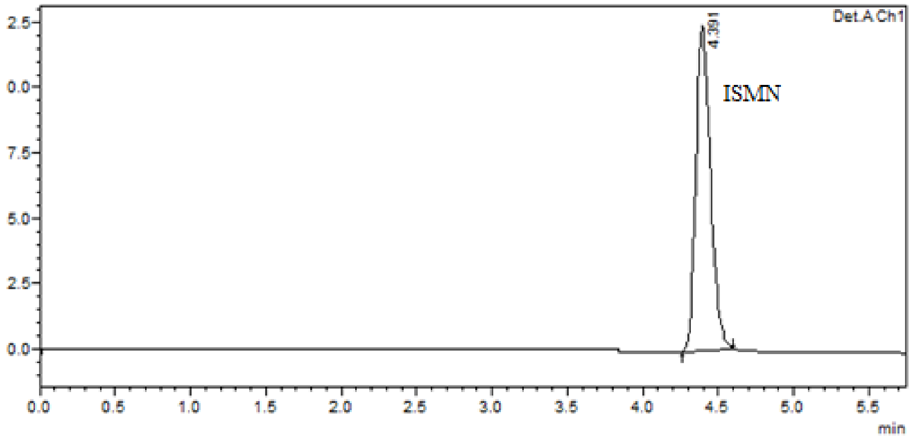

Figure. 2 Optimized chromatogram of ISMN Using Mobile Phase Composition of Methanol: Water: Acetonitrile (55:28:17 %v/v/v)

Detection wavelength was selected after scanning the standard solution of drug over 200 to 800nm. From the U.V spectrum of ISMN it is evident that most of the HPLC work can be accomplished in the wavelength of 217 nm conveniently. Further, a flow rate of 1.0 ml/min and an injection volume of 20 µl were found to be the best analysis. HPLC spectrum of ISMN (10 µg/ml) in optimized conditions is shown in Figure 2.

Validation

The analytical method was validated with respect to parameters such as linearity, precision, specificity and accuracy, limit of detection (LOD), limit of quantitation (LOQ) and robustness in compliance with ICH guidelines. [7]

Linearity and Range:

The linearity of an analytical procedure is the ability to obtain test results that are directly proportional to the concentration of an analyte in the sample. The calibration curve showed good linearity in the range of 1 – 9 µg/ml, for ISMN (API) with correlation coefficient (r^2) of 0.999. A typical calibration curve has the regression equation of $y = 9180.x – 112.0$ for ISMN. Results are given in Table 1.

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

The LOD and LOQ of ISMN were calculated by mathematical equation.

LOD= 3.3×standard deviation ÷ slope and LOQ=10×standard deviation ÷ slope. The LOD of was found to be 2.5 ng/ml and the LOQ of ISMN was found to be 10 ng/ml. Results are given in Table 1.

Table 1. Summary of validation parameters for the proposed method

Parameter	ISMN
Linearity	1 – 9 µg/ml
Intercept (c)	112.0
Slope (m)	9180
Correlation coefficient	0.999
LOD	2.5 ng/ml
LOQ	10 ng/ml

Precision:

The Precision of the method was studied in terms of intraday and interday precision of sample injections (5µg/ml). Intraday precision was investigated by injecting six replicate samples of each of the sample on the same day. The % RSD was found to be 0.534. Interday precision was assessed by analysis of the 6 solutions on three consecutive days. The % RSD obtained was found to be 0.925. Low % RSD values indicate that the method is precise. The results are given in table 2.

Table 2. Precision studies

Precision	ISMN (5 µg/ml)
Interday precision (n=3), % RSD*	0.925
Intraday precision (n=6), % RSD*	0.534

Robustness:

Small deliberate changes in chromatographic conditions such as change in temperature (± 2^0C), flow rate (± 0.1ml/min) and wavelength of detection (± 2nm) were studied to determine the robustness of the method. The results were in favor of (% RSD < 2%) the developed RP-HPLC method for the analysis of ISMN. The results are given in table 3.

Table 3. Robustness studies

Change in Parameters	% RSD
Flow rate 0.9 ml/min	0.54
Flow rate 1.1 ml/min	0.87
Temperature (25°C)	1.88
Temperature (21°C)	0.33
Wavelength of detection (215 nm)	0.79
Wavelength of detection (220 nm)	0.09

Accuracy:

To study the accuracy of method, recovery studies were carried out by spiking of standard drug solution to preanalyzed sample at three different levels i.e., at 50, 100, and 150%. The resultant solutions were then reanalyzed by the proposed method. At each level of the amount, six determinations were performed. From the data obtained, the method was found to be accurate. The % recovery and %RSD were calculated and presented in Table 4.

Table 4. Accuracy (Recovery studies)

Drug	Amount added(µg/ml)	Total Amount Found(µg/ml)	% Recovery	% RSD
ISMN	6	5.89	98.16	0.1221
	8	8.12	100.01	0.9632
	10	10.03	100.3	1.2405

Assay of Tablet (Isonorm-20) dosage form:

The proposed RP-HPLC method was applied to the estimation of ISMN in Isonorm - 20 mg and drug content in each sample were calculated by comparison with the appropriate standard solution of the drug. No interference due to excipients was detected in the chromatograms produced. The results are given in table 5.

Table 5. Assay of ISMN in Tablet (Isonorm 20) dosage form:

Drug	Labeled Amount (mg/Tablet)	Total Amount Found (mg/ Tablet)	% Label Claim	% RSD
ISMN	20	19.54	97.70	0.60

STABILITY INDICATING STUDIES

According to Singh and Bakshi [10] the, stress testing suggests a target degradation of 20-80 % for establishing stability indicating nature of the method. RP-HPLC study of samples obtained on stress testing of ISMN under different conditions using mixture of methanol, water and acetonitrile in the ratio 55:28:17(%v/v/v) as a mobile solvent system suggested the following degradation behavior.

1. Acid hydrolysis:

An accurately weighed pure drug (10 mg) was transferred to a clean and dry round bottom flask (RBF). 30 ml of 0.1 N HCl was added to it. It was refluxed in a water bath at 60^0C for 4 hours. Drug became soluble after reflux which was insoluble initially. Allowed to cool at room temperature. The sample was then neutralized using 2N NaOH solution and final volume of the sample was made up to 100ml with water to prepare 100ppm solution. It was injected into the RP-HPLC system against a blank of methanol: water: Acetonitrile 55:28:17(%v/v/v) after optimizing the mobile phase composition, chromatogram was recorded and shown in Figure. 3.

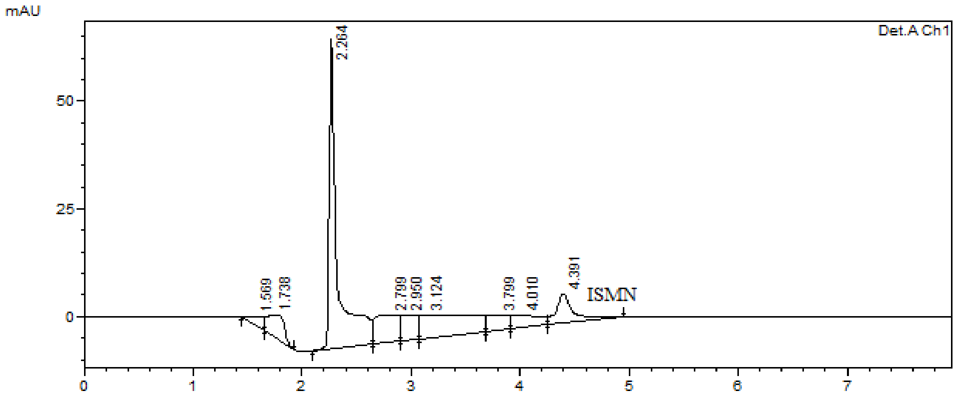

Figure. 3 Chromatogram showing the degraded products in Acidic degradation

2. Basic hydrolysis:

An accurately weighed 10 mg of pure drug was transferred to a clean and dry RBF. 30 ml of 0.1N NaOH was added to it. It was refluxed in a water bath at 60^0C for 4 hours. Drug became soluble after reflux which was insoluble initially. It was allowed to cool at room temperature. The sample was then neutralized using 2N HCl solution and final volume of the sample was made up to 100ml with water to prepare 100ppm solution. It was injected into the RP-HPLC system against a blank of methanol: water: Acetonitrile 55:28:17(%v/v/v) after optimizing the mobile phase composition, chromatogram was recorded and shown in Figure. 4.

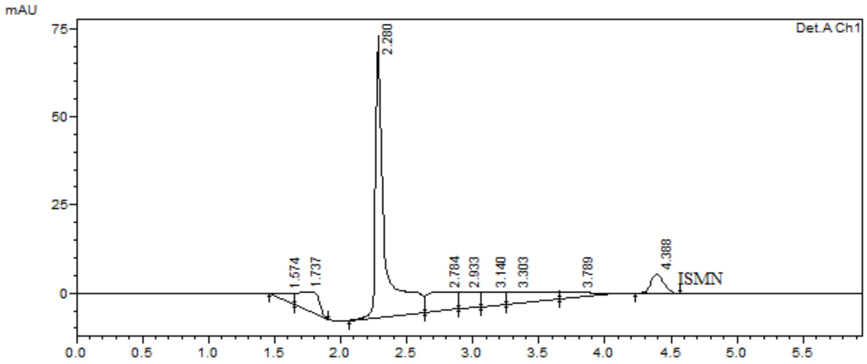

Figure. 4 Chromatogram showing the degraded products in Basic degradation

3. Dry heat degradation:

Approximately weighed 10 mg of pure drug was taken in a clean and dry petridis and covered with an aluminum foil to protect it from light. The petridis was kept in an incubator at 60^{0}C for 72 hours without interruption. An accurately weighed 1 mg of a dry heated drug was transferred to a clean and dry 10 ml volumetric flask. It was dissolved using mobile phase and final volume was made up to 10 ml. The above sample (100 ppm concentration) was injected into the RP-HPLC system against a blank of methanol: water: Acetonitrile 55:28:17(%v/v/v) after optimizing the mobile phase composition, chromatogram was recorded and shown in Figure. 5.

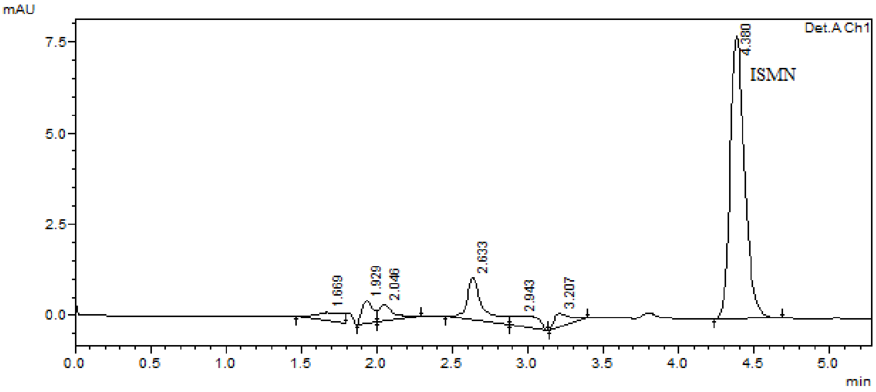

Figure. 5 Chromatogram showing the degraded products in Dry heat degradation

4. Wet heat degradation:

Accurately weighed 10 mg of a pure drug was transferred to a clean and dry RBF. 30 ml of HPLC grade water was added to it. Then, it was refluxed in a water bath at 60^{0}C for 6 hours uninterruptedly. After the completion of reflux, the drug became soluble and the mixture of drug and water was allowed to cool at room temperature. Final volume was made up to 100 ml with HPLC grade water to prepare 100 ppm solution. It was injected into the RP-HPLC system against a blank of methanol: water: Acetonitrile 55:28:17(%v/v/v) after optimizing the mobile phase composition, chromatogram was recorded and shown in Figure. 6.

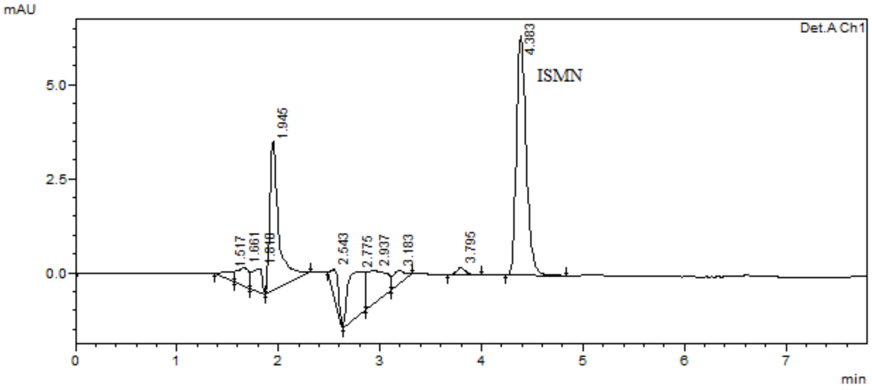

Figure 6 Chromatogram showing the degraded products in Wet heat degradation

5. Photolytic degradation:

Approximately 10 mg of a pure drug was taken in a clean and dry petridis. It was kept in a UV chamber at 254 nm wavelength for 24 hours without interruption. Accurately weighed 1 mg of the UV exposed drug was transferred to a clean and dry 10 ml volumetric flask. The UV exposed drug was dissolved using mobile phase. The above sample (100 ppm concentration) was injected into the RP-HPLC system against a blank of methanol: water: Acetonitrile 55:28:17(%v/v/v) after optimizing the mobile phase composition, chromatogram was recorded and shown in Figure. 7.

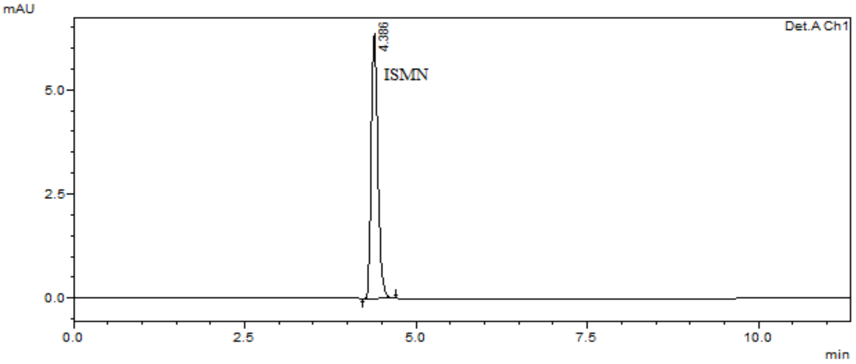

Figure. 7 Chromatogram showing the degraded products in Photolytic degradation

6. Oxidation with (3%) H_2O_2:

Accurately weighed 10 mg of a pure drug was taken in a clean and dry 100 ml volumetric flask. 30 ml of 3% H_2O_2 and a little methanol was added to it to make it soluble and then kept as such in dark for 24 hours. Final volume was made up to 100 ml using water to prepare 100 ppm solution. The above sample was injected into the RP-HPLC system. The chromatogram was recorded and shown in Figure. 8.

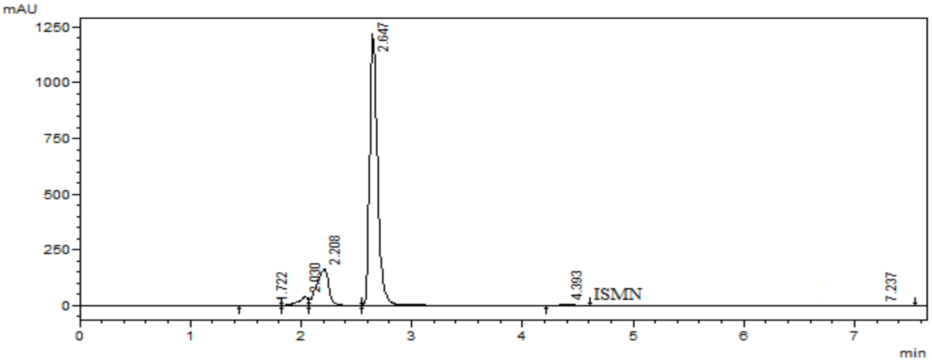

Figure. 8 Chromatogram showing the degraded products in H_2O_2

In all degradation studies, except photolytic degradation there was a significant formation of degradation products when compared to that of a standard. This indicates that, the drug may be degraded to low molecular weight non-chromophoric compounds.

CONCLUSION

A simple, sensitive and selective stability indicting RP-HPLC method has been developed and validated for the analysis of ISMN in bulk dug and tablet dosage form. Based on peak purity results, obtained from the analysis of force degradation samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of ISMN indicated that the developed method is specific for the estimation of ISMN in presence of degradation products. Further the proposed RP-HPLC method has excellent precision, sensitivity and reproducibility. Even though no attempt has been made to identify the degraded products, proposed method can be used as stability indicating method for assay of ISMN in commercial formulations.

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