International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.7, No.1, pp 01-09, 2014-2015

A Newer RP - HPLC Method for the Estimation of Isosorbide Dinitrate in Tablet Formulation

M.Madhu[1], V.Sreeram[2*], A.V.D.Nagendrakumar[3], T.V.Reddy[4]

[1]Department of chemistry, P.B. Siddhartha College of Arts & Science, Vijayawada, Krishna (Dt) -520010.A.P.INDIA
[2]Department of chemistry, A.G.& S.G. Siddhartha College of Arts & Science, Vuyyuru, Krishna (Dt) -521165.A.P. INDIA.
[3]Department of Chemistry, GITAM University, Visakhapatnam-530 045, Andhra Pradesh, India
[4]Department of H & S, Malla Reddy college of Engineering, Secunderabad., India

Abstract: A simple, specific, accurate and precise reverse phase high performance liquid chromatographic method is developed and validated for the estimation of Isosorbide Dinitrate in tablet dosage form. The expected separation and peak shapes were obtained on µ Bondapak C18 10 µm (3.9 x 300 mm) column. To have an ideal separation of the drug under isocratic conditions, mixtures of solvents like methanol, Ammonium Sulphate with or without different buffers indifferent combinations were tested as mobile phases on a µ Bondapak C18 10 µm (3.9 x 300 mm). A mixture of Methanol:0.1M Ammonium Sulphate in the ratio of 50:50 v/v was proved to be the most suitable of all the combinations since the chromatographic peak obtained was better defined and resolved and almost free from tailing. The flow rate was 1.0ml/min and effluents were monitored at 210 nm. The retention time for Isosorbide Dinitrate was 6.3 min. The method was validated for accurate, precise, simple, sensitive and rapid and can be applied successfully for the estimation of Isosorbide Dinitrate in bulk and in pharmaceutical formulations without interference and with good sensitivity. The average percentage recovery varies from 100.0 to 1007. The % RSD for intermediate precision is 0.5 %.The intermediate precision and repeatability complies as they differ by 2.9 %. The proposed method was successfully applied for the quantitative determination of Isosorbide Dinitrate in tablet formulation. The method was validated as per the ICH guidelines and can be employed for routine quality control analysis.
Key Words: Isosorbide Dinitrate, HPLC, Linearity, Validation, 210 nm.

1. Introduction:

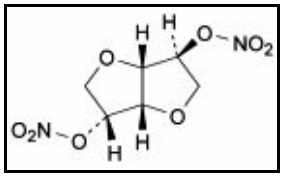

Fig:1 Isosorbide Dinitrate

The Molecular formulae for Isosorbide Dinitrate is found to be $C_6H_8N_2O_8$. Diluted Isosorbide Dinitrate is a dry mixture of Isosorbide Dinitrate and Lactose monohydrate Undiluted Isosorbide Dinitrate is a fine, white, crystalline powder, very slightly soluble in water, very soluble in acetone, sparingly soluble in alcohol. The solubility of the diluted product depends on the diluent and its concentration. It is used for heart related

chest pain, in addition to other medications for congestive heart failure, and for esophageal spasms.[1] It is on the World Health Organization's List of Essential Medicines, a list of the most important medication needed in a basic health system.[2] Monali Pambhar et.al.,[3] developed a method which was carried out on an Hypersil C18 (250 x 4.6 mm, 5 µm) column, with a mobile phase consisting of Acetonitrile: Phosphate buffer pH 3.6 (30:70 v/v) at a flow rate of 0.8 ml/min. The retention time of Hydralazine Hydrochloride and Isosorbide Dinitrate were 3.59, 6.56 min. respectively. S.Hasan Amrohi et.al., [4]described a simple, precise, accurate and stability-indicating RP-HPLC method 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. 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. K. Neelima et.al.,[5] proposed that the chromatographic separation of the three drugs was achieved on Zodiac C18 (250 mm × 4.6 mm) 5µ column in an isocratic mode. The mobile phase consisting of 0.01M 0.01 M Ammonium acetate : Acetonitrile : Methanol in the ratio of 50:30:20 v/v and pH adjusted to 3 using ortho phosphoric acid was delivered at a flow rate of 1ml/ min and effluents were monitored at 270 nm. The retention time of Hydralazine, Isosorbide Dinitrate was found to be 2.337 and 3.413 min, respectively. Calibration curves were linear with a correlation coefficient of 0.994 for HYD , and 0.997 for ISD over the concentration range of 45-105µg/ml for HYD, and 24-56 µg/ml for ISD and precise with (%RSD<2). The method was validated as per the ICH guidelines and can be employed for routine quality control analysis. Carolyn S. Olsen et.al.,[6] described that the nitrate ester dosage forms were dissolved in methanol, filtered, and injected directly into the liquid chromatography. A variable-wavelength UV detector, operated at 220 nm, and a reverse-phase C_{18} microporous silica column were employed. The mobile phase was methanol-water (40:60). Rajan K Verma et.al.,[7] developed a reversed phase HPLC method using C_{18} column was developed for the quantitative determination of isosorbide mononitrate (IMN) in the bulk material and extended release dosage forms. The method was specific to IMN and able to resolve the drug peak from the pharmacopoeial impurities and formulation excipients. The method was accurate, precise, and linear within the desired range. In addition to analysis of assay and dissolution samples, the method was successfully used for analysis of drug–excipient compatibility samples of IMN used for development of extended release formulations in our laboratory and their subsequent stability studies. The method was also used for analysis of IMN in commercially available raw material.

2. Experimental

2.1 Instrumentation:

Peak HPLC containing LC 20AT pump and variable wavelength programmable UV-Visible detector and Rheodyne injector was employed for investigation. The chromatographic analysis was performed on a µ Bondapak C18 10 µm (3.9 x 300 mm). Degassing of the mobile phase was done using a Loba ultrasonic bath sonicator. A Denwar Analytical balance was used for weighing the materials.

2.2 Chemicals and Solvents :

The reference sample of Isordil 5 mg Tablets was obtained from Cipla, Mumbai. The Formulation Isosorbide Dinitrate was procured from the local market. Methanol, Acetonitrile used was of HPLC grade and purchased from Merck Specialities Private Limited, Mumbai, India.

2.3 The mobile phase :

A mixture of Methanol:0.1M Ammonium sulphate in the ratio of 50:50v/v was prepared and used as mobile phase.

2.4 The Standard solution of the drug :

Accurately weigh 80 mg Isosorbide Dinitrate 25 % standard into a 200 ml volumetric flask. Add about 60 ml of mobile phase and sonicate for 15 minutes. Cool and make up to volume with mobile phase. Filter through a 0.45 µm filter, discarding the first 10 mls of the filtrate.

2.5 Sample (tablet) solution:

Weigh 20 tablets and grind to a fine powder. Accurately weigh 72.0 mg of finely powdered tablets into a 100 ml volumetric flask. Add about 50 ml of mobile phase and sonicate for 15 minutes. Cool and make up to volume with mobile phase. Filter through a 0.45 µm filter, discarding the first 10 mls of the filtrate.

3. Method Development

A systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wave length and choice of stationary and mobile phases. The following studies were conducted for this purpose.

3.1 Detection wavelength:

The spectrum of 10ppm solution of the Isosorbide Dinitrate in methanol was recorded separately on UV spectrophotometer. The peak of maximum absorbance wavelength was observed. The spectra of Isosorbide Dinitrate were showed maximum absorbance at 210nm.

3.2 Choice of stationary phase and Mobile Phase:

Finally the expected separation and peak shapes were obtained on µ Bondapak C18 10 µm (3.9 x 300 mm) column. A mixture of Methanol:0.1M Ammonium sulphate in the ratio of 50:50 v/v was proved to be the most suitable of all the combinations since the chromatographic peak obtained was better defined and resolved and almost free from tailing.

3.3 Flow rate:

Flow rates of the mobile phase were changed from 0.5 – 1.5 mL/min for optimum separation. It was found from the experiments that 1.0 mL/min flow rate was ideal for the successful elution of the analyte.

4. Validation of Proposed Mehtod:

The proposed method was validated as per ICH guidelines. The parameters studied for validation were specificity, linearity, accuracy, system suitability and Rage.

4.1 Linearity

The linearity of an assay method is its ability to elicit test results, which are directly proportional to the concentrations of drug actives in samples in a given range. Proof of linearity justifies the use of single- point calibrations. The correlation coefficient of the regression line for Isosorbide Dinitrate should be greater than or equal to 0.990. The Y-intercept of the line should not be significantly different from zero, i.e. the assessment value (z) falls within the specified limits only when +2>z>-2. Five solutions containing 50, 75, 100, 125 and 150 % of Isosorbide Dinitrate relative to the working concentration of 40mg/ tablet were prepared. 20.1, 30.0, 39.9, 50.2, and 60.1mg portions of Isosorbide Dinitrate were weighed into separate 50 ml volumetric flask. Mobile phase was added to each and ultrasonicated for 15 minutes. Samples were filtered through a 0.45µm filter before use. The correlation coefficient for Isosorbide Dinitrate is 0.9996. The plot is a straight line, and the assessment value (z) falls within the specified limit at 0.07. The method is therefore linear within the specified range. The Calibration Curve is shown in the Fig: 2. and the results are tabulated in the Table: 1.

Table:1 Linearity Resutls

Sample Number	Concentration	Response 1	Response 2	Average Response
50%	0.240000	1458882.0000	1454525.0000	1456703.5000
75%	0.320000	1831252.0000	1835992.0000	1833622.0000
100%	0.400000	2306120.0000	2305433.0000	2305776.5000
125%	0.500000	2930001.0000	2928423.0000	2929212.0000
150%	0.600000	3396251.0000	3381761.0000	3989006.0000

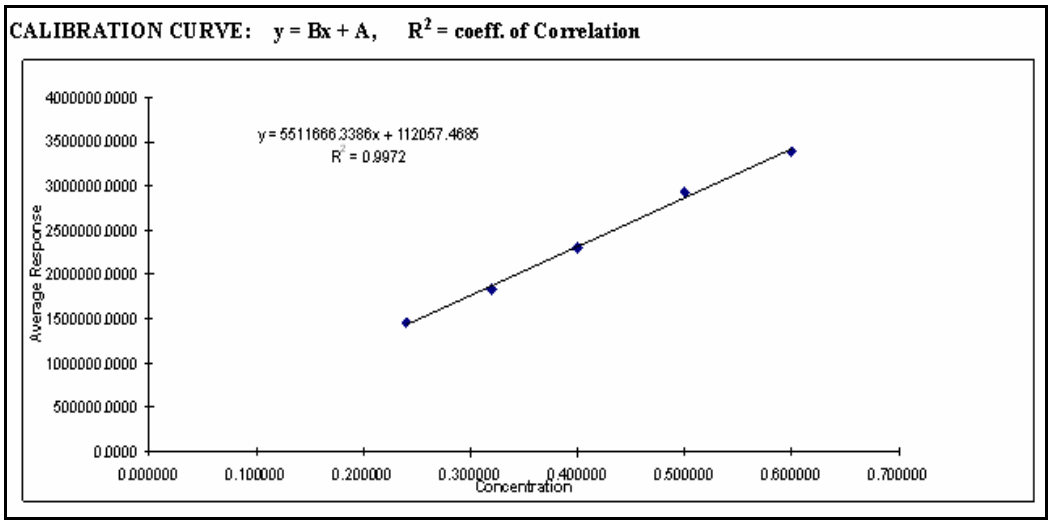

Fig: 2: Linearity Curve

4.2 Specificity

Specificity of an analytical procedure is its ability to assess unequivocally the analyte in the presence of components that may be expected to be present. The placebo solution must contain no components, which co-elute with Isosorbide Dinitrate peak. The peak purity results from the photo diode-array analysis must show that Isosorbide Dinitrate peak is pure, i.e. the purity angle (PA) must be less than the threshold angle (TH). The solutions listed below were injected using the conditions specified in the method of analysis. No components are seen to co-elute with the Isosorbide Dinitrate peak, and the purity angle is less than the threshold for both the drug active and product. Isosorbide Dinitrate is stable under UV light exposure. Isosorbide Dinitrate can therefore be considered spectrally pure. The results are shown in the Fig:3 to Fig:11.

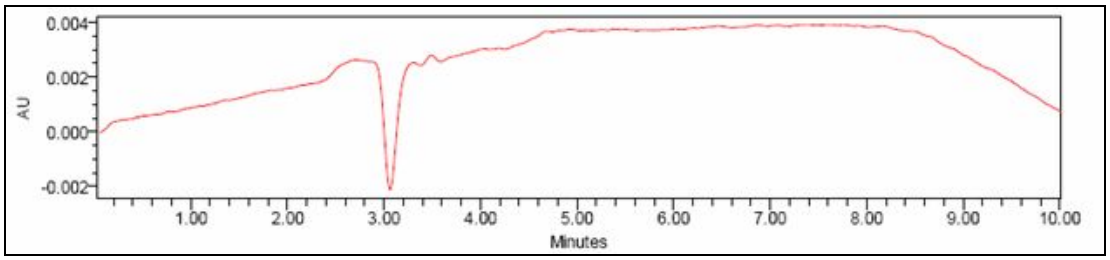

Fig: 3. Chromatogram – 1: No significant peak detected

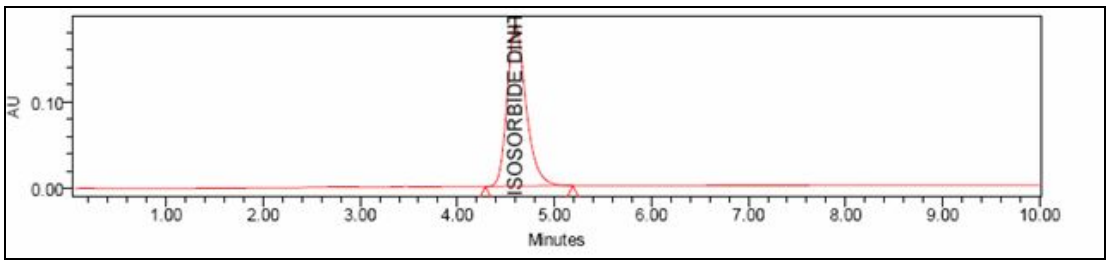

Fig: 4. Chromatogram – 2: Peak due to Isosorbide Dinitrate

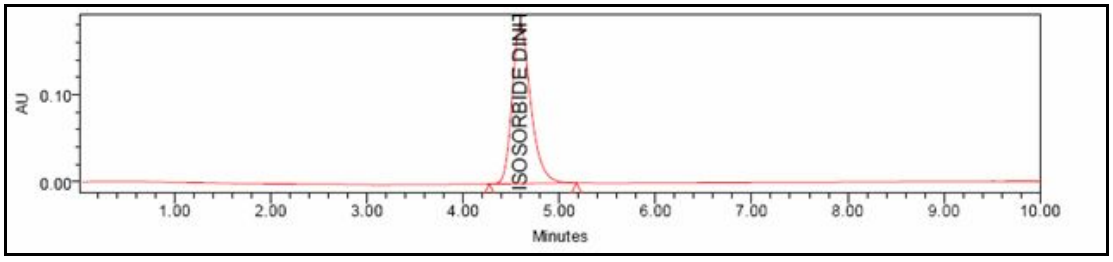

Fig: 5. Chromatogram - 3 Product - Peak due to Isosorbide Dinitrate

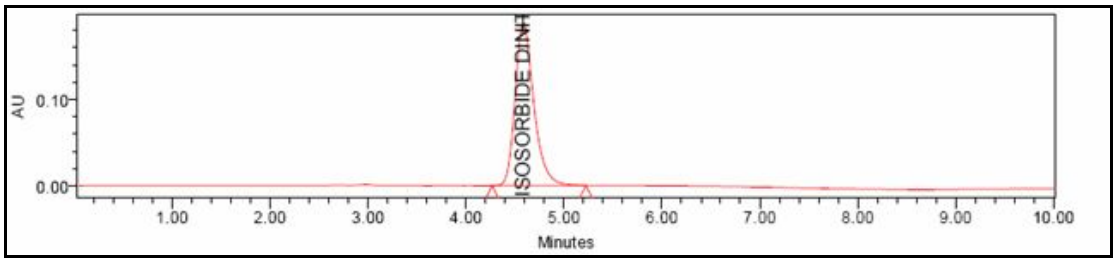

Fig: 6. Chromatogram – 4; Active – UV stress: Peak due to Isosorbide Dinitrate

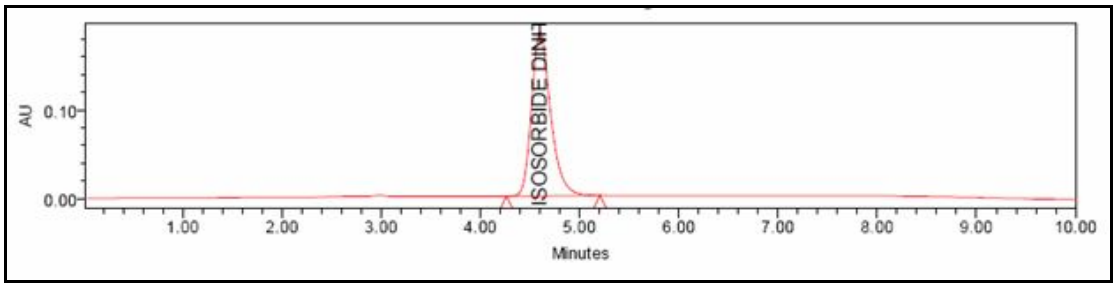

Fig: 7. Chromatogram – 5: Product – UV stress: Peak due to Isosorbide Dinitrate

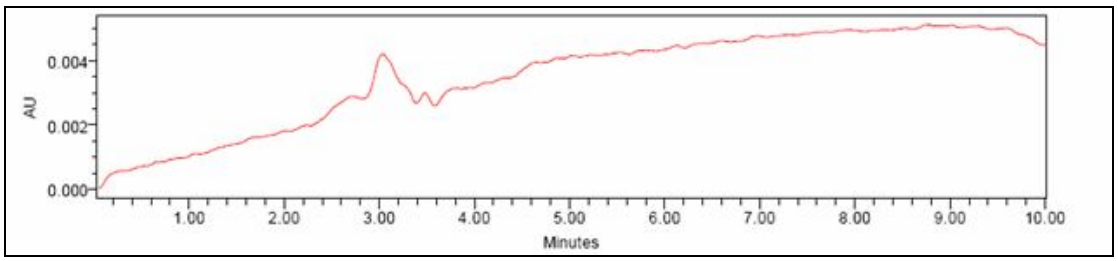

Fig: 8. Chromatogram – 6: Placebo – No significant peak detected

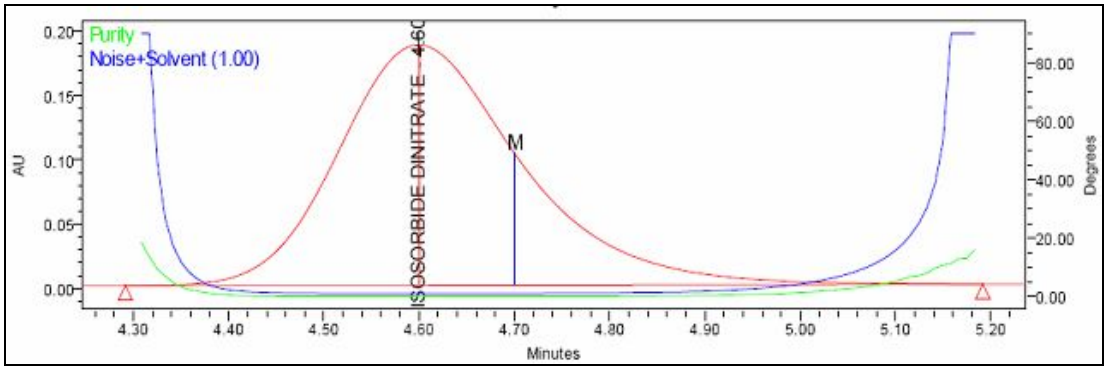

Fig: 9. Chromatogram – 6 Purity angle < Threshold (0.047 < 1.065)

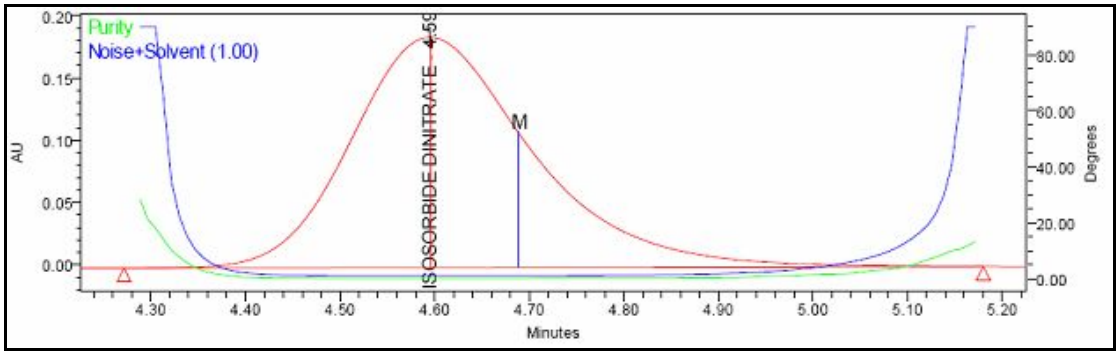

Fig: 10. Chromatogram – 6 Purity angle < Threshold (0.038 < 1.053)

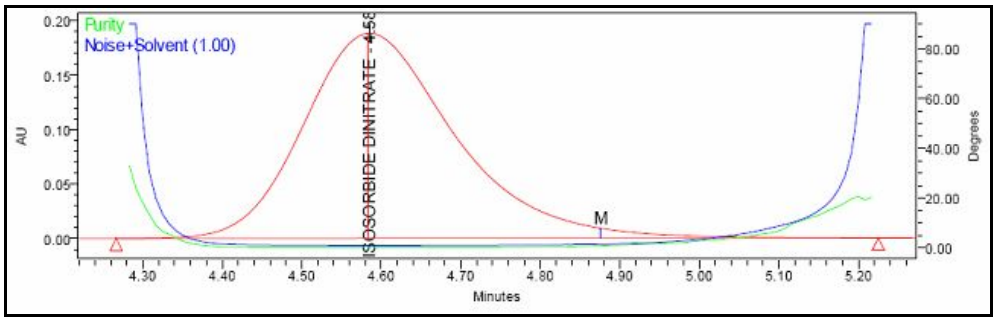

Fig: 11. Chromatogram – 6 Purity angle < Threshold (0.071 < 1.043)

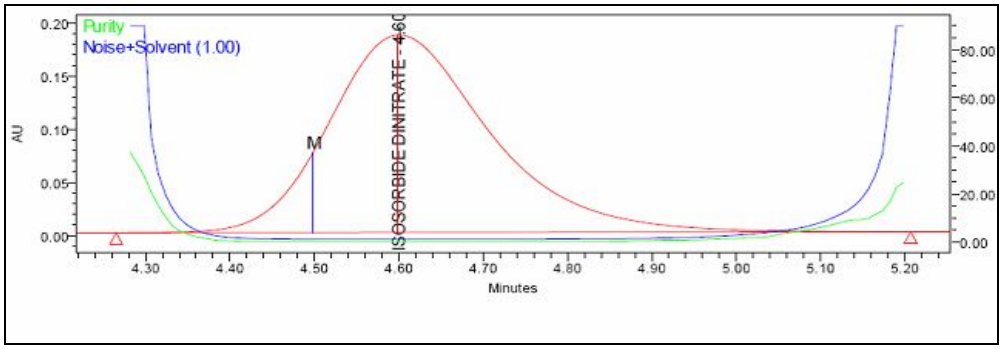

Fig: 12. Chromatogram – 6 Purity angle < Threshold (0.041 < 1.036)

4.3 System Sutability

System suitability is a measure of the performance and chromatographic quality of the total analytical system – i.e. instrument and procedure. The requirements for system suitability for this method are: The % RSD of the peak responses due to the Isosorbide Dinitrate for the six replicate injections must be less than or equal to 2.0 %.Tailing less than 2 and USP plate count not less than 2000. Six replicate injections of working standard solution were injected according to the method of analysis. The percentage relative standard deviation (% RSD) for the peak responses was determined. The %RSD is 0.15% for Isosorbide Dinitrate, and therefore complies with the specified requirements The method complies with the requirements specified by the system suitability. The Results are tabulated in the Table:2.

Table: 2 System Suitability Results

Sample Number	Response
1	4727412
2	4745586
3	4734929
4	4731488
5	4729596
Mean	4733802
%RSD	0.15

4.4 Accuracy

The accuracy of an analytical method expresses the closeness of test results obtained by that method to the true value. The percentage recovery of the active compounds, for each solution prepared, must be within 98.0 – 102.0 % of the actual amount. Bromazepam Samples were weighed to contain known concentrations of Isosorbide Dinitrate to result in concentrations representing respectively 50, 75, 100, 125 and 150 % of Isosorbide Dinitrate relative to the working concentration. The above samples were injected in duplicate according to the method of analysis. From the accuracy results above, the percentage recovery values for the Isosorbide Dinitrate satisfy the acceptance criteria for accuracy across the range of 50 % - 150 %. The results are shown in the Table:3.

Table: 3 Accuracy Results

Sample %	Theoretical Active	Actual Active	% Recovery	Average % Recovery
50	4.99	4.99	100.0	100.0
		4.99	100.0	
75	7.49	7.57	101.1	100.7
		7.51	100.3	
100	9.98	9.96	99.8	100.7
		10.13	101.5	
125	12.48	12.49	100.1	100.1
		12.48	100.0	
150	14.97	15.05	100.5	100.4
		15.02	100.3	

4.5 Method Precision

The precision of an analytical procedure expresses the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogenous sample.

4.6 Repeatability

The % RSD due to the Isosorbide Dinitrate concentration for the six samples must be less than or equal to 2.0 %. Six separate sample preparations of batch 254423 were analysed according to the method of analysis. The % RSD due to the Isosorbide Dinitrate concentration for the assay meets the requirements for repeatability at 0.9 %. The Uniformity of the content of Isosorbide Dinitrate in ten units must be between 85 – 115 % and % RSD must be less than or equal to 6.0 %. The results are shown in the Table:4. And Table:5.

Table: 4 Repeatability Results

Sample number	Results (mg / tab)
	Isosorbide Dinitrate
1	5.0
2	4.9
3	5.0
4	5.0
5	5.0
6	5.0
Mean	5.0
% RSD	0.9

Table: 5 % RSD Results

Sample number	**Results (%)**
	Isosorbide Dinitrate
1	100.8
2	104.5
3	102.8
4	100.6
5	99.6
6	99.3
7	97.5
8	100.0
9	100.2
10	100.6
Mean	100.6
% RSD	1.9

4.7 Intermediate Precision

Intermediate Precision of an analytical procedure expresses intra-laboratory variations of the repeatability test performed: by a different analyst, on a different day, and using different reagents, mobile phases and solvents. The % RSD due to the Isosorbide Dinitrate concentration for the six samples must be less than or equal to 2.0 %. The mean results obtained in the repeatability and the intermediate precision must not differ by more than 3.0 %. Six separate sample preparations of batch 254423 were assayed according to the method of analysis. The % RSD for intermediate precision is 0.5 %. The intermediate precision and repeatability comply as they differ by 2.9 %. Results are tabulated in Table:6.

Table: 6 Repeatability Results

Sample	Results (mg /tab)
	Isosorbide Dinitrate
1	4.9
2	4.9
3	4.8
4	4.9
5	4.8
6	4.8
Mean	4.8
% RSD	0.5

Table: 7 Precision Results

Sample	Mean Results (mg/tab)
	Isosorbide Dinitrate
Repeatability	5.0
Intermediate Precision	4.8
Mean	4.9
% RSD	2.9

4.8 Range

Range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. Based on the accuracy results, the range for the assay of Isordil 5 mg Tablets is 2.5 – 7.5 mg/tab of the Isosorbide Dinitrate, which represents 50 % to 150 % of the working concentration.

5. Declaration on the Validity Of The Method

The method for the assay of the of Isordil 5 mg Tablets complies with the requirements for linearity, specificity, system suitability, method precision and accuracy across the range of 50 % to 150 % of the working concentration. The method is therefore acceptable as valid and stability indicating.

6. References:

1. "Isosorbide Dinitrate/Mononitrate". The American Society of Health-System Pharmacists. Retrieved Apr 21, 2014.
2. "WHO Model List of EssentialMedicines". *World Health Organization*. October 2013. Retrieved 22 April 2014.
3. Monali Pambhar, Bijal Kotecha, Gayatri Zala, Ketan Dadhania, " Analytical Method Development And Validation For Simultaneous Estimation Of Isosorbide Dinitrate And Hydralazine Hydrochloride In Their Combined Pharmaceutical Dosage Form By Rp-Hplc Method", Inventi Rapid: Pharm Analysis & Quality Assurance , 2014 , Article Id- " Inventi:Ppaqa/1405/14.

4. S.Hasan Amrohi[*1], Mahesh Nasare[1], Afra Nazneen[1], Prakash V Diwan[1]" Stability Indicating RP-HPLC Method for the Estimation of Isosorbide 5-Mononitrate in Bulk Drug and its Pharmaceutical Dosage Form**"** Am. J. PharmTech Res. 2013; 3(1): 420 – +429.
5. K. Neelima*[1]and Y. Rajendra Prasad [2]; " Analytical Method Development And Validation For Simultaneous Estimation Of Hydralazine, Isosorbide Dinitrate In Bulk and Tablet Formulation By RP-HPLC" ; IJPSR; 2014 , 5(4): 1290 – 1294.
6. Carolyn S. Olsen[*] and Henry S. Scroggins; " High-performance liquid chromatographic determination of the nitrate esters isosorbide dinitrate, pentaerythritol tetranitrate, and erythrityl tetranitrate in various tablet forms" ; Journal of Pharmaceutical Sciences; 1984; 73(9): 1303–1304.
7. Rajan K Verma, Sanjay Garg; "A validated high performance liquid chromatographic method for analysis of isosorbide mononitrate in bulk material and extended release formulations"; Journal of Pharmaceutical and Biomedical Analysis; 2002; 30(3), :583–591.

*****