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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ISOSORBIDE DINITRATE AND HYDRALAZINE HCL IN COMBINED PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

*A simple, sensitive ,linear, precise and accurate PR-HPLC method for simultaneous estimation of isosorbide dinitrate and hydralazine hydrochloride, in tablet formulation was developed, validated and forced degradation studies were conducted. The chromatographic separation of the two drugs was achieved on INERTSIL ODS C_{18}(150 x 4.6 *5µ) column in an isocratic mode. The mobile phase consisting of 0.1M sodium dihydrogen phosphate:methanol in the ratio of 80:20 v/v and was delivered at a flowrate of 1ml/min and effluents were monitored at 270nm. The retention time of was found to be 1.679 and 3.430 min, respectively. Calibration curves were linear with acorrelation coefficient of 0.994 for HYD, and 0.997 for ISDN over the concentration range of 160-480 µg/ml for ISDN, and 300-900 µg/ml for Hydralazine HCl and precise with (%RSD <2). The method was validated as per the ICH guidelines and drugs were found to be stable under forced degradation conditions and can be employed for routine Q.C.analysis.*

Key words: *Isosorbide dinitrate, Hydralazine HCl, RP – HPLC, Simultaneous estimation, Method validation and ICH guidelines*

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INTRODUCTION:

Analytical chemistry is often described as the area of chemistry responsible for characterizing the composition of matter, both qualitatively (what is present) and quantitatively (how much is present).Analytical chemistry is not a separate branch of chemistry, but simply the application of chemical knowledge.

Pharmaceutical Analysis is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter. It is mainly involved in the qualitative identification or detection of compounds and quantitative measurements of the substances present in bulk and pharmaceutical preparation.
High-performance liquid chromatography (HPLC) is a form of liquid chromatography to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by injecting a plug of the sample mixture into the column. The different components in the mixture pass through the column at different rates due to differences in their partition behavior between the mobile liquid phase and the stationary phase

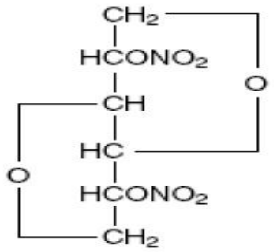


Figure 1:Structure of ISDN

Nitrates are vasodilators (dilators of blood vessels). Blood returning from the body in the veins must be pumped by the heart through the lungs and into the body's arteries against the high pressure in the arteries. In order to accomplish this work, the heart's muscle must produce and use energy ("fuel"), and this requires oxygen. Angina pectoris (angina) or "heart pain" is due to an inadequate flow of blood (and oxygen) to the muscle of the heart. Nitrates, including isosorbide dinitrate, correct the imbalance between the flow of blood and oxygen to the heart and the work that the heart must do by dilating (expanding) the arteries and veins in the body. Dilation of the veins reduces the amount of blood that returns to the heart that must be pumped. Dilation of the arteries lowers the pressure in the arteries against which the heart must pump. As a consequence of both effects, the heart works less and requires less blood and oxygen.

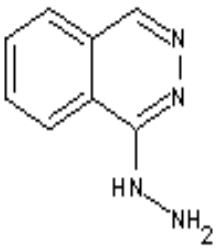


Figure2:Structure of Hydralazine.HCl

(Apresoline) hydralazine hydrochloride USP, is an antihypertensive, available as 10-, 25-, 50-, and 100-mg tablets for oral administration. Its chemical name is 1-hydrazinophthalazine monohydrochloride Hydralazine hydrochloride, USP is a white to off-white, odorless crystalline powder. It is soluble in water, slightly soluble in alcohol, and very slightly soluble in ether. It melts at about 275°C, with decomposition, and has a molecular weight of 196.84.

Directly relaxes vascular smooth muscle to cause peripheral vasodilation, decreasing arterial BP and peripheral vascular resistance. Hydralazine binds to and activates gated potassium channels on vascular smooth muscle. The result is an efflux of potassium and a subsequent hyperpolarization of the cell. This prevents calcium-mediated activation and constriction of the smooth muscle, resulting in vasodilation.

MATERIALS AND METHODOLOGY

Chemicals and reagents: Isosorbide dinitrate and hydralazine hcl standard drugs, sodium dihydrogen phosphate were obtained from lara drugs, kukatpally, hyderabad. Methanol and water used were HPLC grade (RANKEM). Commercially available tablets ISOLAZINE are obtained from local market.

Instrument:

Waters HPLC e2695 series consisting pump, Auto sampler, photodiode array detector, Thermostat column compartment connected with Waters (alliance) Empower-2 software.

Chromatograhic Conditions:

The mobile phase consisting of 0.1M sodium dihydrogen phosphate and methanol (HPLC grade) in the ratio of 80:20v/v was pumped into the column at a flow rate of 1.0 mL/min. It was an isocratic elution. The column used was INERTSIL ODS C_{18}, 4.6x150 m, 5µ at 25°C. The detection was monitored at 270 nm using PDA detector and the run time was 10min.

Mobile Phase Preparation:

Mix 800 ml of sodium dihydrogen phosphate and 200 ml of methanol in the ratio 80 : 20 %v/v.

Standard Stock Solution Preparation:

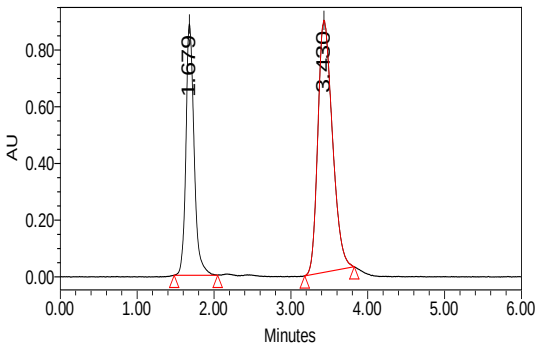
Weigh and transfer 80 mg of isosorbide dinitrate & 150 mg of hydralazine hcl working standard into 50 mL volumetric flask, add 10 mL of diluent and sonicated to dissolve and dilute to volume with diluent.

Standard Preparation:

Transfer 5 mL of standard stock solution into 25 mL volumetric flask and dilute to volume with diluent.

Sample Preparation:

Accurately weighed 20 tablets and calculated average weight of those tablets and crushed. Transfer the tablet powder of weight about 808.4 mg of sample into 50 ml of volumetric flask add water and sonicate for 30 mins and make up the volume with water and filtered through the 0.45 µm Millipore filter paper Transfer above solution 5 ml into 25 ml volumetric flask and make up the volume with mobile phase.



ISDN-Hydralazine HCl Optimized chromatogram

Optimized Method

Retention Time	USP Resolution	USP Tailing	USP Plate Cou
1.679	---	1.26	3080
3.430	6.50	1.28	4583

Observation:

In the above method, both ISDN and Hydralazine HCl are separated well with good resolution, good symmetrical factor. The theoretical plates observed for both the peaks are also within the range and the same are eluted within a run time of 10min. This method is suitable for Validation.

RESULTS AND DISCUSSION

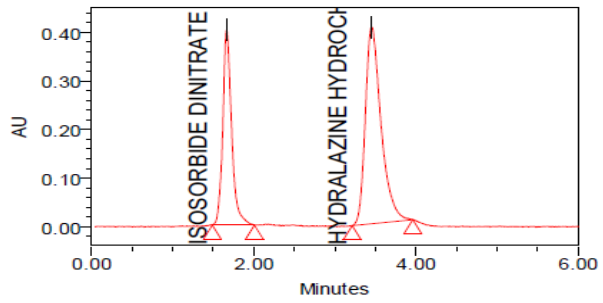
PRECISION

The relative standard deviation (%RSD) of the six assay preparations of ISDN-Hydralazine HCl was calculated and it was found to be 0.03% and 0.19% respectively

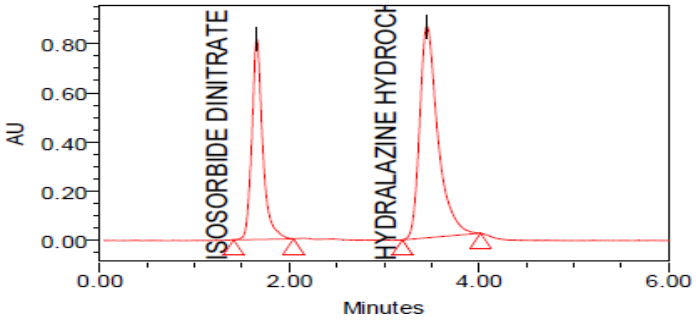
	HYDRALAZINE HCL				ISDN	
	SampleName	**Inj**	**RT**	**Area**	**RT**	**Area**
1	precision1	1	3.466	11885939	1.669	6486791
2	precision2	1	3.459	11856019	1.662	6481691
3	precision3	1	3.461	11876618	1.663	6483231
4	precision4	1	3.476	11839783	1.666	6483189
5	precision5	1	3.465	11842824	1.664	6485118
6	precision6	1	3.453	11891941	1.661	6484307
Mean			3.463333	11865520.6	1.66416666667	6484054.5
Std.dev			0.007763	22387.232	0.002926886	1771.8123
% RSD			0.22%	0.19%	0.18%	0.03%

ACCURACY

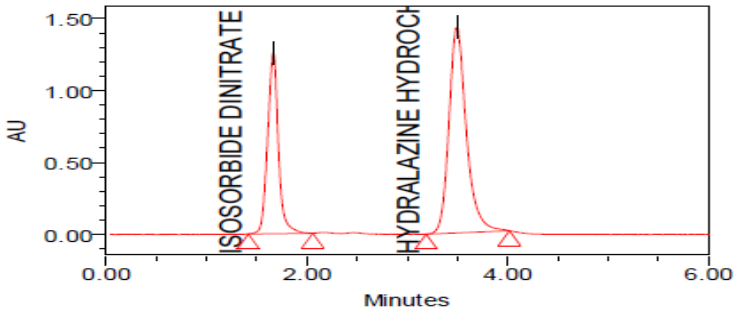
To study the accuracy of the method, recovery studies were carried out. To the formulation equivalent to 80 mg of ISDN and 150 mg of Hydralazine HCl at the levels of 50%, 100% and 150% was added pure ISDN and Hydralazine Hcl and made up to the mark with Mobile phase and filtered through Whatmann filter paper and chromatograms were recorded. The concentration of drug present in resulting solution was determined using developed procedure and percentage recovery and percentage RSD were calculated.



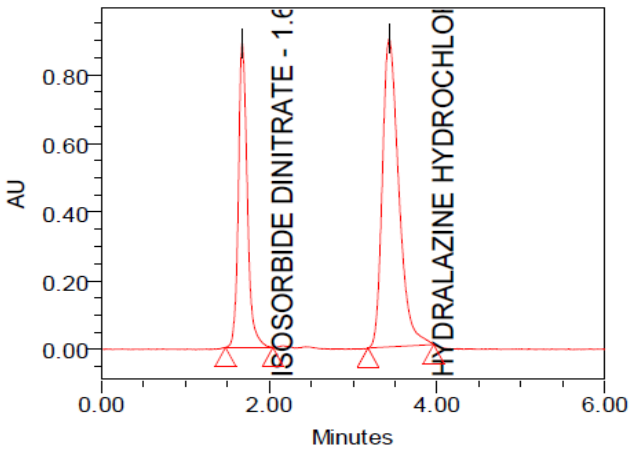
Accuracy 50% -1 Chromatogram Using Sample Drug Sample in 100 µg/Ml Drug



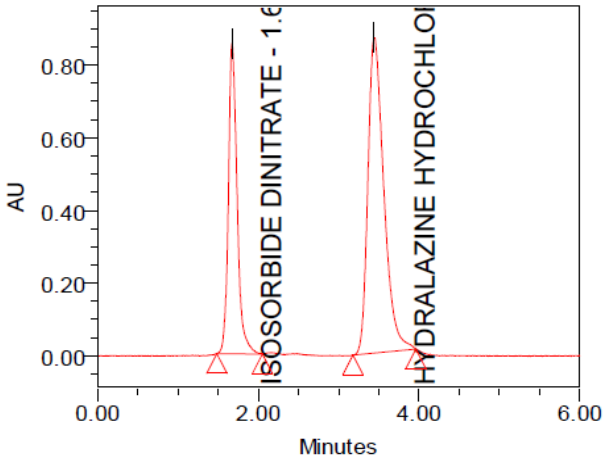
Accuracy100% -1 Chromatogram Using in 50 µg/mL



Accuracy -150% -1 Chromatogram Using Sample Drug in 150 µg/mL



Standard chromatogram -1



Standard chromatogram-2

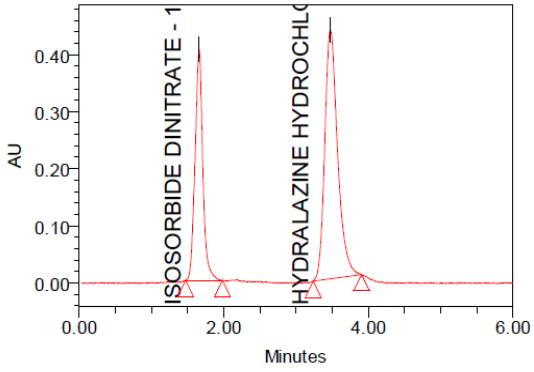
ASSAY:

Assay values for precision

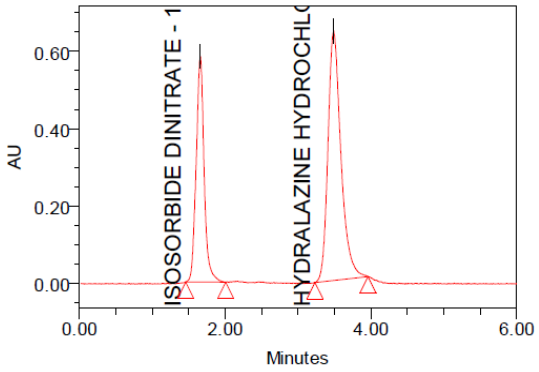
S.No	Sample Weight	Sample Area-1	Sample Area-2	% Assay	% Assay
1	808.64	6486791	11885939	99	100
2	808.64	6481691	11856019	99	99
3	808.64	6483231	11876618	99	100
4	808.64	6483189	11839783	99	99
5	808.64	6485118	11842824	99	99
6	808.64	6484307	11891941	99	100
Avarage Assay:				99	100
STD				0.03	0.19
%RSD				0.03	0.19

LINEARITY

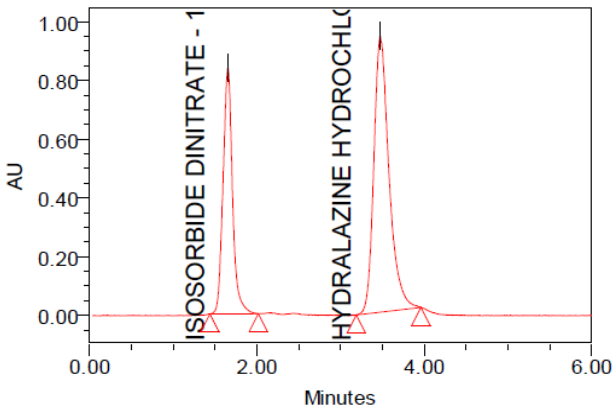
Aliquots of standard ISDN and Hydralazine Hcl stock solution (0.2 ml to 0.8 ml) (1ml=1000µg/mL) were taken in different 10 ml volumetric flasks and diluted up to the mark with the diluents such that the final concentrations of ISDN and Hydralazine Hcl are in the range of 300-900 µg/mL. Each of these drug solutions (10µL) was injected three times in to the column, and the peak area and retention time were recorded. Evaluation was performed with PDA detector at 210 nm and a calibration curve graph were obtained by plotting peak area versus concentration.



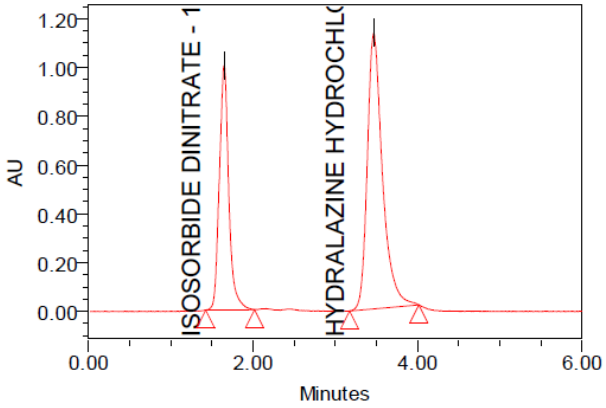
Linearity -50% Chromatogram



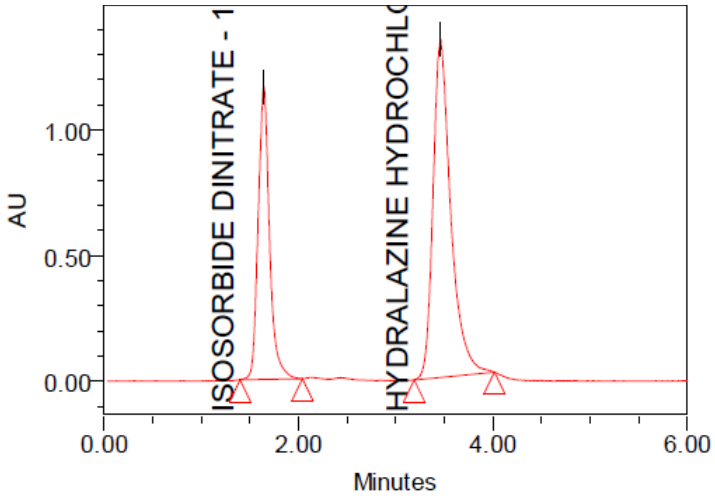
Linearity -75% Chromatogram



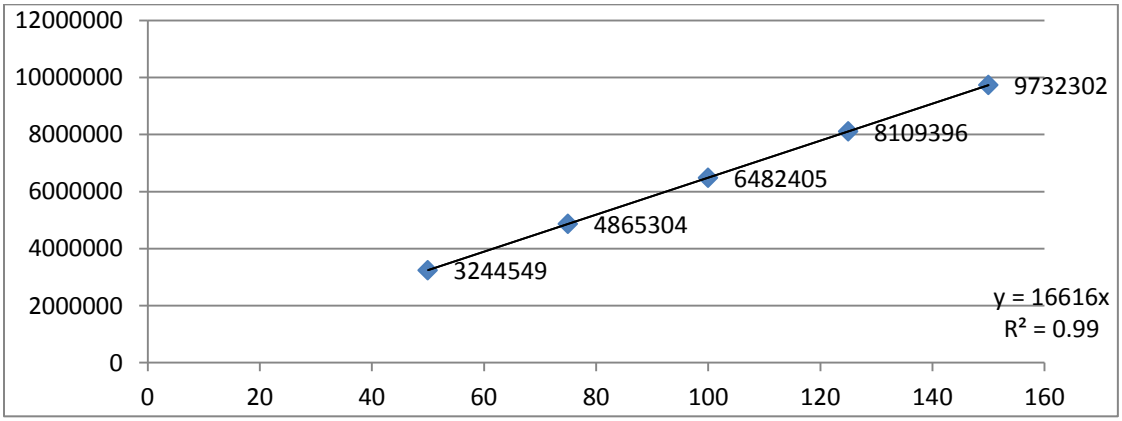
Linearity -100% Chromatogram



Linearity -125% Chromatogram

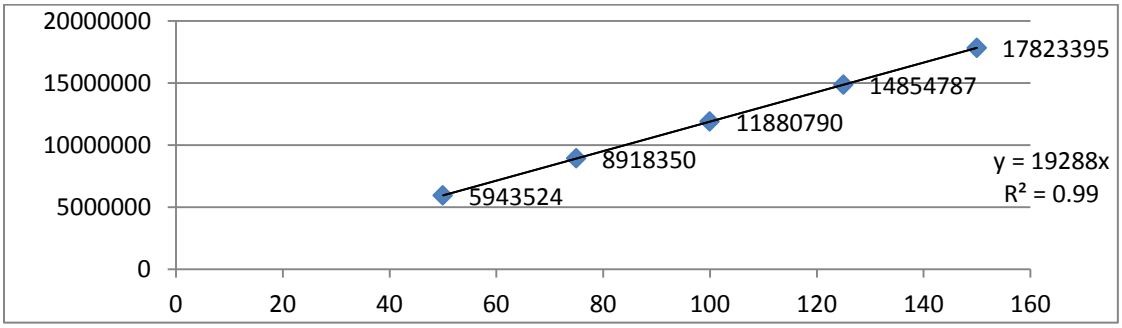


Linearity -150% Chromatogram



Conc in ug /ml

STD Calibration Curve – ISDN



Conc in ug /ml

STD Calibration Curve – Hydralazine HCl

LINEARITY VALUES

HYDRALAZINE HCL					ISDN	
	SampleName	**Inj**	**RT**	**Area**	**RT**	**Area**
1	linearity 50%	1	1.660	3244549	1.660	3244549
2	linearity 75%	1	1.659	4865304	1.659	4865304
3	linearity 100%	1	1.654	6482405	1.654	6482405
4	linearity 125%	1	1.648	8109396	1.648	8109396
5	linearity 150%	1	1.643	9732302	1.643	9732302
Mean			3.4736	11884169.4	1.6528	6486791.2
Std.Dev			0.010310189135	4695378.62355	0.00725947656515	2564544.85006
% RSD			0.30%	39.51%	0.44%	39.53%

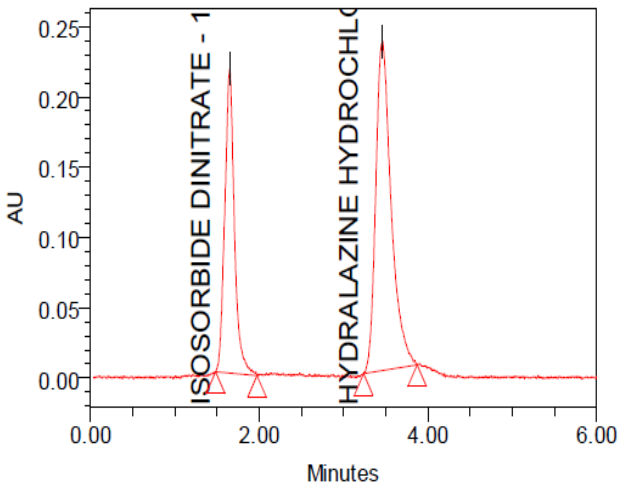
LIMIT OF DETECTION (LOD)

From the linearity data calculate the limit of detection and quantitation, using the following formula. LOD= $\frac{3.3}{\sigma\sigma}$ = standard deviation of the response
SS = slope of the calibration curve of the analyte.

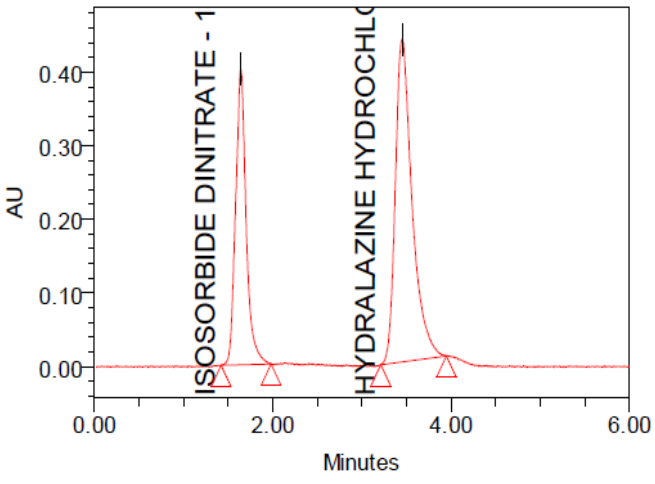
The limit of detection (LOD) and limit of quantification (LOQ) for ISDN was found to be 2.697 µg/mL & 8.989 µg/mL.

.LIMIT OF QUANTITATION (LOQ):

LOQ = $\frac{10\ \sigma}{}$ σ = standard deviation of the response SS = slope of the calibration curve of the analyte.
The limit of detection (LOD) and the limit of quantification (LOQ) for Hydralazine Hcl was found to be 2.4259 µg/mL & 8.0863 µg/mL



Chromatograms illustrating LOD of 0.5% Working Standards



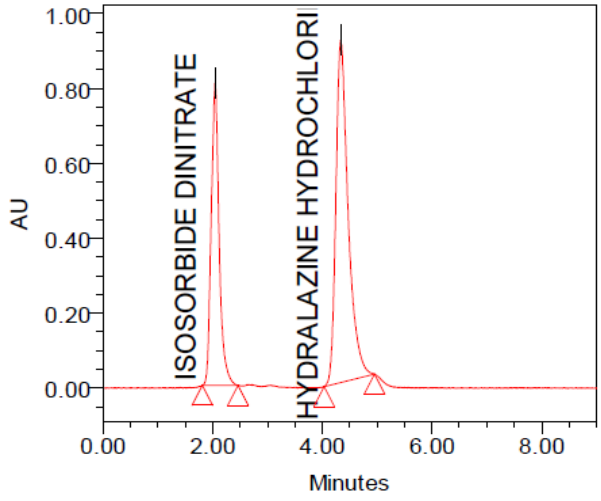
Chromatograms illustrating LOQ of 0.5% Working Standards

LOD AND LOQ VALUES

HYDRALAZINE HYDR0CHLORIDE					ISOSORBIDE DINITRATE				
CONC%	**Area**	**ug/ml**	**LOD**	**LOQ**	**CONC %**	**Area**	**ug/ml**	**LOD**	**LOQ**
50	5943524	300	S/N	742	50	3244549	160	S/N	356
75	8918350	450	2.4259	8.0863	75	4865304	240.00	2.697	8.989
100	11880790	600			100	6482405	320.00		

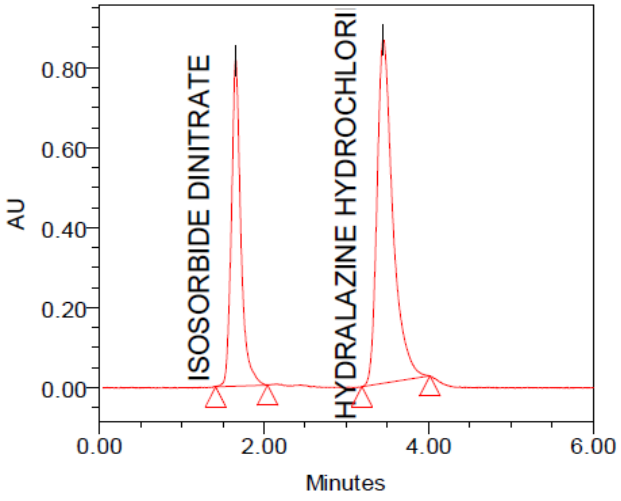
ROBUSTNESS

In order to prove that the method is robust, flow rate of the mobile phase (±0.2ml/min) and the column temperature (±5°c) are varied. The results showed that they have passed the system suitability parameters.



Chromatograms for robustness studies

Flow change (0.8 ml/min)



Chromatograms for robustness studies

Flow change (1.00 ml/min)

ROBUSTNESS RESULTS

DEGRADATION PROFILE:

Acid: Tranfer 814.50 mg weight of sample into a 50 ml of volumetric flask and add 10 ml of 0.1n HCl and sonicate 30 min and add 10 ml of 0.1n NaOH make up with mobile phase. Transfer above solution 5

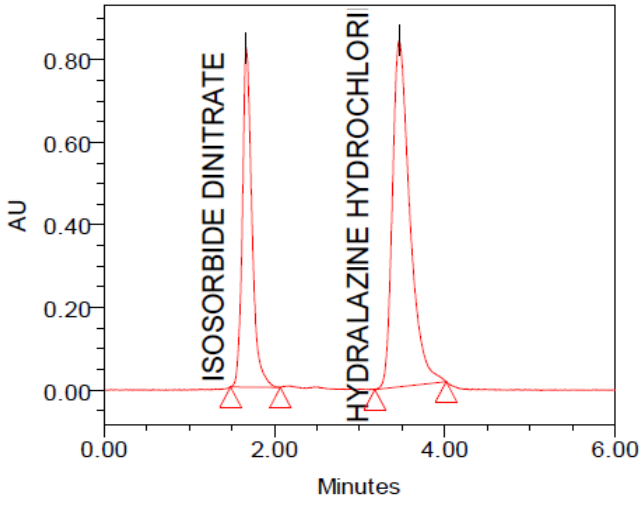
ml into 25 ml volumetric flask dilute to volume with mobile phase.

BASE: Transfer 814.50 mg weight of sample into a 50 ml volumetric flask and add 10 ml of 0.1N NaOH and sonicate 30 min and add 10 ml of HCl make up volume with mobile phase. Tranfer above solution 5ml into 25 ml volumetric flask dilute to volume with mobile phase.

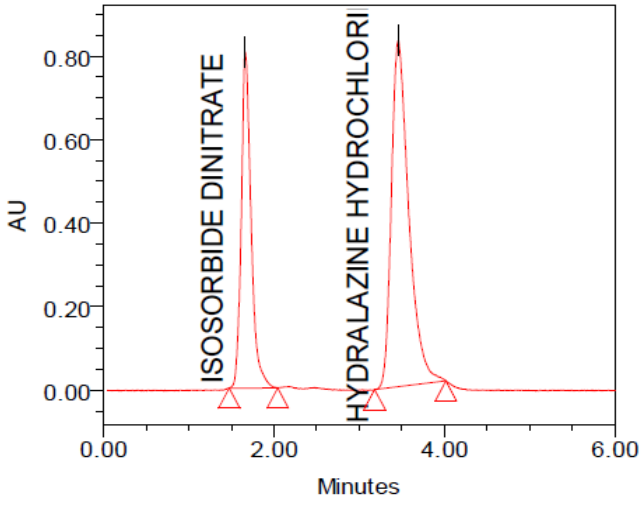
PEROXIDE: Transfer 814.50 mg weight of sample into a 50 ml of volumetric flask and add 10ml peroxide and sonic make up volume with mobile phase. Transfer above solution 5ml into 25 ml volumetric flask dilute to volume with mobile phase.

HEAT: Before sample weighing exposes the sample at 10535°C. Transfer the 814.50 mg weight of sample into a 50 ml volumetric flask and add 15ml of mobile phase and sonicate 30 min and make up with mobile phase. Transfer above 5ml into 25ml volumetric flask dilute to volume with mobile phase.

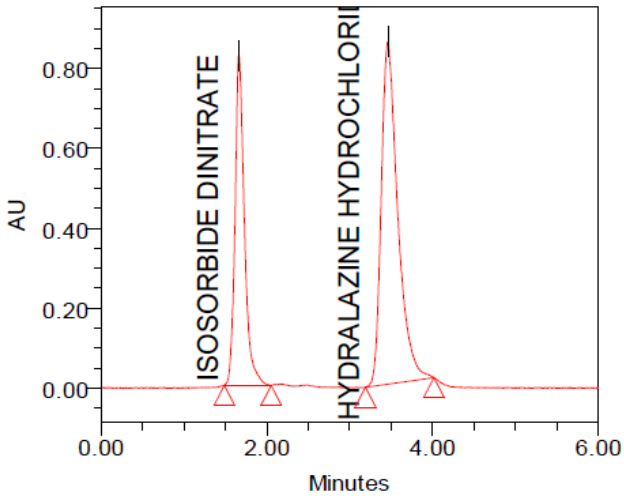
LIGHT: Before weighing sample expose the sample in light for 24 hrs. Transfer the 814.50 mg of sample into a 50 ml volumetric flask and add 15 ml of mobile phase and sonicate 30 min and make up with mobile phase. Transfer above solution 5 ml into 25 ml volumetric flask dilute to volume with mobile phase.



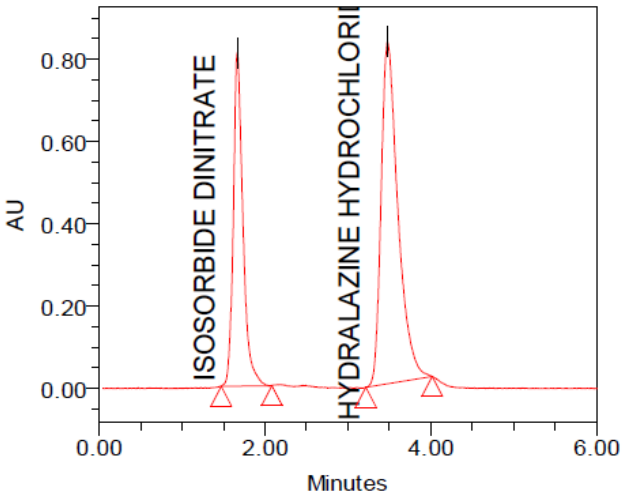
Chromatograms for Degradation – ACID



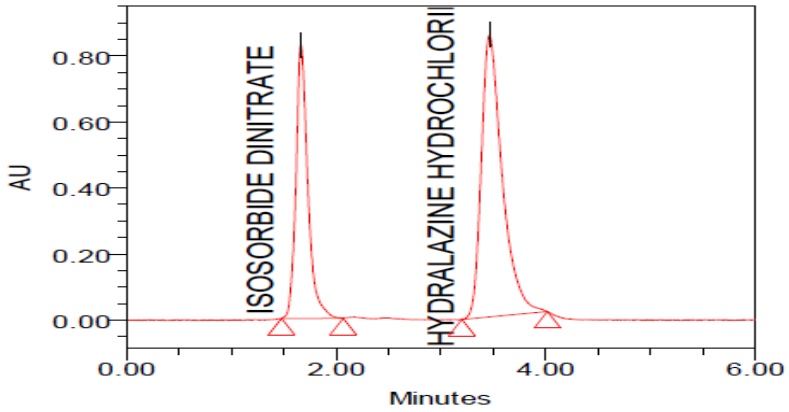
Chromatograms for Degradation - Base



Chromatograms for Degradation -Peroxide



Chromatograms for Degradation - Heat



Chromatograms for Degradation - Light

CONCLUSION:

There are no reports on the stability indicating RP- HPLC determination of ISDN and Hydralazine Hcl in tablets in the literature prior to commencement of this work. The proposed method is simple, rapid, accurate, precise and specific. In Reverse Phase HPLC method it was found that the retention time for ISDNwas 1.679 min and the retention time for Hydralazine Hcl was 3.430 min. A mixture of sodium dihydrogen phosphate and methanol 80:20 v/v was found to be most suitable to obtain a peak well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (ISDN r=0.99 & Hydralazine Hcl r=0.99) was observed. The assay of ISDN was found to be 99% & the assay of Hydralazine Hcl was found to be 100%. From the recovery studies it was found that about 100% of drug was recovered which indicates high accuracy of the method. It is suitable for the routine analysis of in pharmaceutical dosage form. The limit of detection (LOD) and limit of quantification (LOQ) for ISDN was found to be 2.697 µg/mL & 8.989 µg/mL. The limit of detection (LOD) and the limit of quantification (LOQ) for Hydralazine Hcl was found to be 2.4259 µg/mL & 8.0863 µg/mL.

The above proposed method obviates the need for any preliminary treatment and is simple, sensitive and reliable and can be used for the routine determination of ISDN and Hydralazine Hcl in bulk sample and in tablets.

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