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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION OF ISOSORBIDE DINITRATE AND HYDRALAZINE HCL IN TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

A simple, precise, rapid, specific and accurate reverse phase high performance liquid chromatography method was developed for simultaneous estimation of Isosorbide Dinitrite and Hydralazine HCl in pharmaceutical dosage form. Chromatographic separation was performed on Agilant Zorbax (C_{18}) (4.6mm x 250mm, 5µm) column, with mobile phase comprising of mixture of buffer (pH6.5, adjusted with potassium dihydrogen phosphate), acetonitrile in the ratio of 70:30 v/v, at the flow rate 0.8 ml/min. The detection was carried out at 274 nm. The retention times of Isosorbide Dinitrite and Hydralazine HCl were found to be 3.6 and 2.7 mins respectively with a run time of 6 mins, theoretical levels for Isosorbide Dinitrite and Hydralazine HCl were 8055 and 7525 respectively, with a resolution of 6.57. As per ICH guidelines the method was validated for linearity, accuracy, precision, limit of detection and limit of quantitation, robustness and ruggedness. Linearity of Isosorbide Dinitrite was found in the range of 50-150 µg/mL and that for Hydralazine HCl was found to be 50-150 µg/mL. The correlation coefficient for Isosorbide Dinitrite and Hydralazine HCl were 1 and 0.999 respectively. The LOD values for Isosorbide Dinitrite and Hydralazine HCl were 2.95 and 2.88 µg/mL respectively. The LOQ values for Isosorbide Dinitrite and Hydralazine HCl were and 9.8 and 9.6 µg/mL respectively. This demonstrates that the developed method is simple, precise, rapid, selective, accurate and reproducible for simultaneous estimation of Isosorbide Dinitrite and Hydralazine HCl tablet dosage form.

Keywords: Isosorbide Dinitrate, Hydralazine HCl, RP-HPLC, Validation.

INTRODUCTION

Isosorbide Dinitrate Fig. (A1) is described chemically as 1,4:3,6-dianhydro-d-glucitol-2,5-dinitrate and it is a white to off-white crystalline powder with the empirical formula $C_6H_8N_2O_8$ and a molecular weight of 236.14 [1-4]. It is freely soluble in organic solvents such as alcohol, chloroform and ether, but is only sparingly soluble in water [5].

Hydralazine Hydrochloride Fig. (A2) is described chemically as 1-hydrazinophthalazine mono hydrochloride and it is a white to off-white crystalline powder with the empirical formula $C_8H_8N_4 \cdot HCl$ and a molecular weight of 196.64. It is soluble in water, slightly soluble in alcohol, and very slightly soluble in ether [6].

MATERIALSANDMETHODS

Equipment

Chromatographic separation was performed on HPLC system–Waters e2695 model, PDA Detector, equipped with a solvent delivery pump, sample injector and column thermostats. Empower system softwarewasappliedfordatacollecting andprocessing.

Chemicals and reagents

Acetonitrile (HPLC grade) was used. Buffer used was pH-6.5 (pH adjusted with potassium dihydrogen phosphate). (Reference standards Isosorbide Dinitrate and Hydralazine HCl were obtained from Rainbow Labs.

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Isolazine Tablets of Isosorbide Dinitrate (20mg) and Hydralazine HCl (37.5mg) manufactured by Lupin pharmaceuticals Ltd., were procured from local market.

Preparation of standard solutions

Accurately weighed and transferred 80 mg of Isosorbide Dinitrate and 150 mg of Hydralazine HCl working standard into a 50mL clean dry volumetric flask and added about 30mL of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. (Stock solution). From this, 5 ml of the solution was pipetted into another 25ml volumetric flask and diluted up to the mark with diluent.

Preparation of sample solution

Accurately weighed and transferred tablet powder equivalent to 20 mg of Isosorbide Dinitrate and 37.5 mg of Hydralazine HCl in to a 50mL clean dry volumetric flask and added about 30mL of diluent. It was sonicated to dissolve completely and made volume up to themark with the same diluent. (Stock solution)From this, 5 mL of the solution was pipetted into another 25ml volumetric flask and diluted up to the mark with diluent.

Preparation of buffer

Take 1000mL of HPLC grade water. The pH was adjusted to 6.5 with potassium dihydrogen phosphate [6].

Optimized Chromatographic Conditions

Diluent **:** Potassium Dihydrogen Phosphate : Acetonitrile.
Mobile phase: Buffer p^H 6.5: KH2P04
: Acetonitrile (70:30v/v)
Flow rate : 0.8mL/min
Column : Agilant Zorbax (C_{18}) (4.6mm x 250mm, 5µm)
Detector wavelength : 274nm
Injection volume : 10µL

METHODVALIDATION

Linearity

Solutions were prepared under the concentrations of Isosorbide Dinitrate, which corresponding to 50, 75, 100, 125 and 150% respectively, concentrations of Hydralazine HCl which corresponding to 50, 75, 100, 125 and 150 % respectively of the test solution concentration. Each solution was injected, linearity was evaluated by linear-regression analysis [1].

Accuracy

Accuracy was determined by the recovery studies at three different concentrations (corresponding to50, 100 and 150% of the test solution concentration) by addition of known amounts of standard to pre-analysed sample preparation. For each concentration, three sets were prepared and injected [2].

Precision

Precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements [3].

Robustness

The robustness was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions. Thefactors chosen forhis study were the flow rate (±0.1ml/min), temperature. [4].

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ was calculated from linear curve using formulae
LOD=3.3*σ /slope, LOQ=10*σ /slope
(Where σ=the standard deviation of the response
And S= Slope of calibration curve) [7]

Specificity

Specificity was checked for the interference of impurities in the analysis of blank solution and injecting sample solution under optimized chromatographic conditions to demonstrate separation of both Isosorbide Dinitrate and Hydralazine HCl from impurities [8-9].

Table1.Analysis data of formulation (Isolazine)

Injection	Label claim(mg)	Assay (%)
Isosorbide Dinitrate	20mg	99.8%
Hydralazine HCl	37.5mg	99.07

Table 2.Resultof Linearity

S. No	Isosorbide Dinitrate		Hydralazine HCl	
	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area
1	50	3006497	50	2073071
2	75	4514426	75	3117605
3	100	6014830	100	4155452
4	125	7524785	125	5191111
5	150	9024624	150	6233973

Table 3. System suitability studies

Parameters	Hydralazine HCl	Isosorbide Dinitrate	Acceptance criteria
Theoretical plates	7525	8055	Not less than 2000
Tailing factor	1.26	1.18	Not more than 2
Resolution	-	6.57	Not less than 2

Table 4. Recovery studies for Isosorbide Dinitrate and Hydralazine HCl

DRUG	Spiked level%	Amount taken (µg/ml)	Amount found (µg/ml)	Percent recovery n=3	Mean recovery
Isosorbide Dinitrate	50	159.403	159.41	100	99.99
	100	319.040	318.78	99.92	
	150	478.443	478.68	100.05	
Hydralazine HCl	50	297.082	296.52	99.81	99.99
	100	594.600	593.54	100.12	
	150	891.682	892.17	100.05	

n- Number of replicate injections

Table 5.LODand LOQ for Isosorbide Dinitrate and Hydralazine HCl

DRUG	LOD	LOQ
Isosorbide Dinitrate	2.9538	9.8462
Hydralazine HCl	2.880	9.600

Table 6. Results of Precision

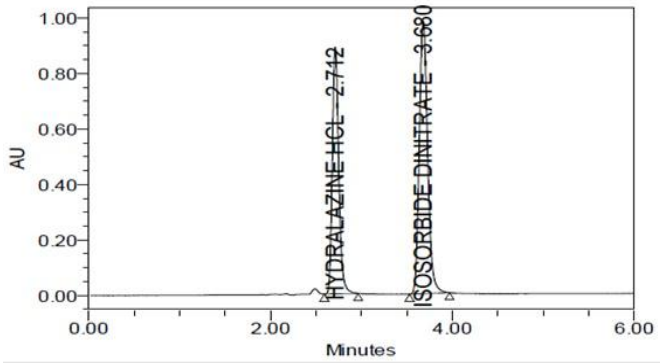
S.No	Sample Weight	Hydralazine HCl	Isosorbide Dinitrate	% Assay	% Assay
1	816.80	4152654	6015301	99.03	99.69
2	816.80	4151358	6014887	99.00	99.68
3	816.80	4157111	6022331	99.14	99.80
4	816.80	4155766	6026107	99.10	99.86
5	816.80	4147812	6012400	98.91	99.64
6	816.80	4163440	6016738	99.29	99.71
Average Assay:				99.08	99.73
STD				0.13	0.09
%RSD				0.13	0.09

Table 7. Results of Robustness study

S. no	Parameter	Condition	Theoretical levels		Tailing factor		Retention time	
			Isosorbide Dinitrate	Hydralazine HCl	Isosorbide Dinitrate	Hydralazine HCl	Isosorbide Dinitrate	Hydralazine HCl
1	Flow rate	0.7min/ml	9751	8885	1.16	1.26	4.63	3.35
		0.9min/ml	7500	6584	1.17	1.28	3.09	2.23
2	Temperature	24^0C	8064	7531	1.16	1.25	3.69	2.71
		26^0C	8099	7583	1.17	1.25	3.70	2.69

Fig 1. The chemical structure of Isosorbide Dinitrate(A1) And Hydralazine HCl (A2)

Fig 2. Chromatogram of Isosorbide Dinitrate and Hydralazine HCl standard



Fi 3. Chromatogram of Isosorbide Dinitrate and Hydralazine HCl sample

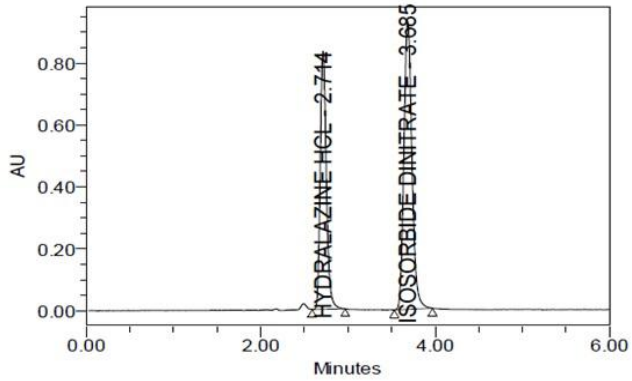


Fig 4.Calibrationcurvefor Isosorbide Dinitrate

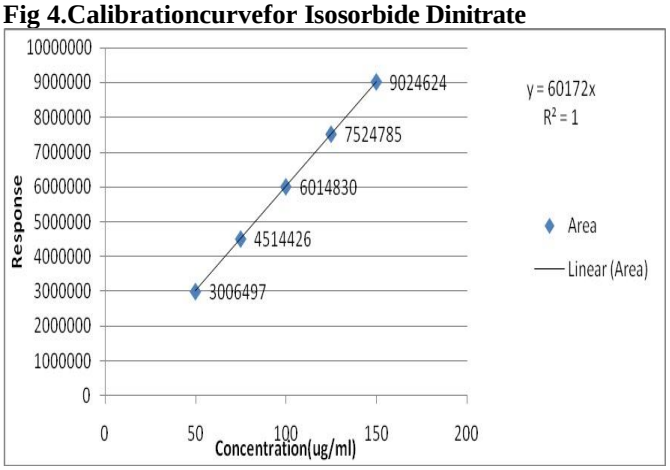
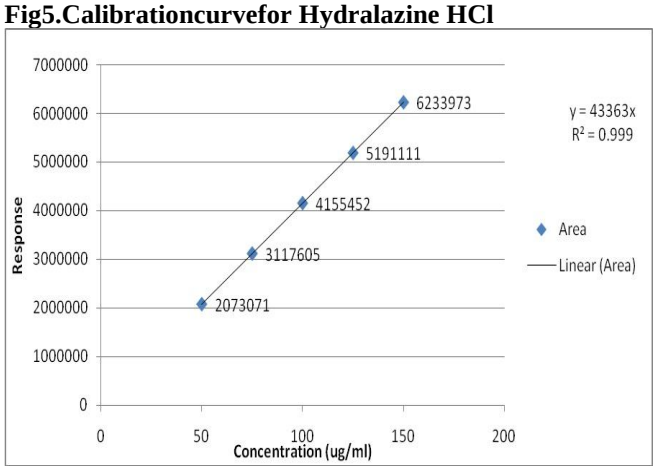


Fig5.Calibrationcurvefor Hydralazine HCl



RESULTS AND DISCUSSION

Several mobile phase compositions were tried to resolve the peak of Isosorbide Dinitrate and Hydralazine HCl. The mobile phase containing buffer 6.5 (potassium dihydrogen phosphate) : Acetonitrile in proportion of 70:30v/v was found ideal to resolve the peak of Isosorbide Dinitrate and Hydralazine HCl. Retention time of Isosorbide Dinitrate and Hydralazine HCL were 3.6 and 2.7 min respectively (Figure 2&3). Result of assay is shown in Table-1. The proposed method was found to be linear in concentration range 50-150µg/ml for Isosorbide Dinitrate and 50-150µg/ml for Hydralazine HCl. The data was shown in Table-2 and Figure 4&5. System suitability parameters were evaluated and results shown in (Table-3), which were with in acceptance criteria. The mean percentage recovery for Isosorbide Dinitrate and Hydralazine HCl was found to be 99.99% and 99.99% respectively, which are well within the limit and hence the method was found to be accurate (Table-4). LOD and LOQ values were 2.9538µg/mL and 9.846 µg/mL for Isosorbide Dinitrate and 2.880µg/mL and 9.600µg/mL for Hydralazine HCl (Table-5). Results of precision were showninthe (Table-6). The robustness of the method was investigated by varying experimental conditions such as changes in flow rate and temperature. The result obtained implies method is robust for routine qualitative analysis (Table-7).

CONCLUSION

The proposed RP-HPLC method was validated as per International Conference on Harmonization (ICH) guidelines, and found to be applicable for routine quality control analysis for the simultaneous estimation of Isosorbide Dinitrate and Hydralazine HCl using isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The proposed method is highly sensitive, reproducible, reliable, rapid and specific.

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