

## New Spectrophotometric Method for the Estimation of Isosorbide Mononitrate in Bulk and Tablet Formulation

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### ABSTRACT

A validated simple, accurate and rapid spectrophotometric method was developed for the estimation of isosorbide mononitrate in bulk and tablet formulation. The method is based on the reduction of nitrate group of isosorbide mononitrate to nitrite ion by zinc/sodium chloride. Sulphanilamide undergoes diazotization with nitrite ion formed during reduction process in presence of hydrochloric acid. The diazonium salt is then coupled with N-(1-naphthyl) ethylene-diamine dihydrochloride (NED) to form a coloured product which is measured at 540 nm. The method was optimised for the amount of reagents required. Under the optimized experimental condition, Beer's law is obeyed in the concentration range of 5-60  $\mu\text{g ml}^{-1}$ . The average percentage recovery of the drug for the proposed method was found to be in the range of 105.7-106.6% indicating no significant interference from tablet excipients.

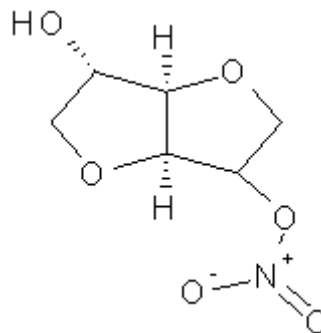
**Key words:** Isosorbide mononitrate, N-(1-naphthyl) ethylene-diamine dihydrochloride, spectrophotometry, sulphanilamide.

### INTRODUCTION

Isosorbide mononitrate (1,4,3,6-dianhydro-D-glucitol 5-nitrate)<sup>1</sup> is a cardiovascular drug classified under organic nitrates. Isosorbide mononitrate is a drug used principally in the treatment of angina pectoris caused by coronary artery disease. It acts by dilating the blood vessels so as to reduce the blood pressure<sup>2-3</sup> and increase the supply of blood and oxygen to the heart while reducing its workload<sup>4</sup>.

Isosorbide mononitrate is used for the prophylactic treatment of angina pectoris that is taken in order to prevent or at least reduce the

occurrence of angina. Research on isosorbide mononitrate as a cervical ripener to reduce time at hospital for birth is supportive<sup>2</sup>.



From literature survey few analytical methods were found reported for the estimation of isosorbide mononitrate, that includes HPLC, HPTLC<sup>1</sup>, differential pulse polarography<sup>4</sup> and liquid chromatography coupled with mass spectrometry<sup>5</sup>. Based on the chemistry of isosorbide mononitrate that forms nitrite it was proposed to develop simple and economical methods for routine development of isosorbide mononitrate.

A simple, sensitive accurate rapid and economical validated visible spectrophotometric method for the determination of isosorbide mononitrate is now described.

The nitrate group of isosorbide mononitrate was reduced to nitrite ion with zinc/sodium chloride. The nitrite formed on reduction shows diazotisation reaction with sulphanilamide in presence of hydrochloric acid, which was then coupled with N-(1-naphthyl) ethylene-diamine dihydrochloride to produce an azo dye that has maximum absorbance at 540 nm (Fig 1). The proposed method was optimized.

## EXPERIMENTAL

### Apparatus

A Perkin Elmer lambda 25 UV-Visible spectrometer with matching quartz cells were used for the absorbance measurements.

### Reagents and standards

Reference standard sample of isosorbide mononitrate was kindly provided by Wallace Pharmaceuticals, Ponda, Goa, India. The commercial dosage form of isosorbide mononitrate Ismo (Abbott Healthcare Pvt Ltd, Baddi, India) was purchased from the local market.

All chemicals used were of analytical grade and distilled water was used in the preparation of all solutions. Standard solution of isosorbide mononitrate (1mg ml<sup>-1</sup>) was prepared in distilled water. Sulphanilamide (1% w/v) and N-(1-naphthyl) ethylenediamine dihydrochloride (1% w/v) was also prepared in distilled water.

Zinc/sodium chloride mixture was prepared by mixing 0.5 g of zinc with 100 g of sodium chloride.

### Procedure

#### Development of standard colour for isosorbide mononitrate

Volume of 10 ml of standard isosorbide mononitrate solution, 1mg ml<sup>-1</sup> was transferred in to a beaker containing conc. Hydrochloric acid and 1ml of zinc/sodium chloride granular mixture and was heated in a water bath for 30 min with occasional stirring, then the solution was filtered through a whatmann filter no. 42 and transferred to 100 ml standard flask and diluted up to the mark.

Aliquots of standard solution containing 5-60 µg ml<sup>-1</sup> of reduced nitrate were transferred into series of 10 ml standard flask and further mixed with 2 ml each of 1% w/v sulphanilamide solution and shaken for 5 min for completion of diazotization reaction. Further 2 ml each of N-(1-naphthyl) ethylenediamine dihydrochloride (1% w/v) was added to form an azo dye and then the volume was made up to 10ml with water. The absorbance of the coloured azo dye was measured at 540 nm against reagent blank and the standard calibration plot was recorded (Fig 1).

#### Determination of isosorbide mononitrate in marketed tablet dosage formulation

Ten tablets were weighed and grounded. Powder equivalent to 25 mg of Isosorbide mononitrate was weighed accurately and dissolved in 10 ml distilled water. The resulting solution was filtered through whatmann no.42 paper. Then the filtrate was diluted to 25 ml with water. The procedure given under Development of standard colour was then followed for development of colour.

#### Optimization of reagent volumes and conditions

The volume of reagent concentrations required for obtaining maximum absorbance for the solutions has been optimized.

Further, with optimized conditions, the proposed method has been validated for linearity, accuracy, precision, sensitivity, reproducibility and stability of colour. Recovery studies were carried out by mixing standard solutions of the drug at 3 different levels with previously analyzed tablet samples of isosorbide mononitrate. The results of the validation study and recovery study is presented in Table I and Table II, respectively.

## RESULTS AND DISCUSSION

Zinc is one of the most frequently used reducing agent for the reduction of nitrate into nitrite ion, while sodium chloride is used to assist the reduction process<sup>7-10</sup>. Isosorbide mononitrate possesses a nitrate group which on treatment with Zinc/sodium chloride yields a nitrite ion. The nitrite ion thus obtained undergoes diazotization reaction with sulphanilamide in presence of HCl and then subsequently coupling with N-(1-naphthyl)

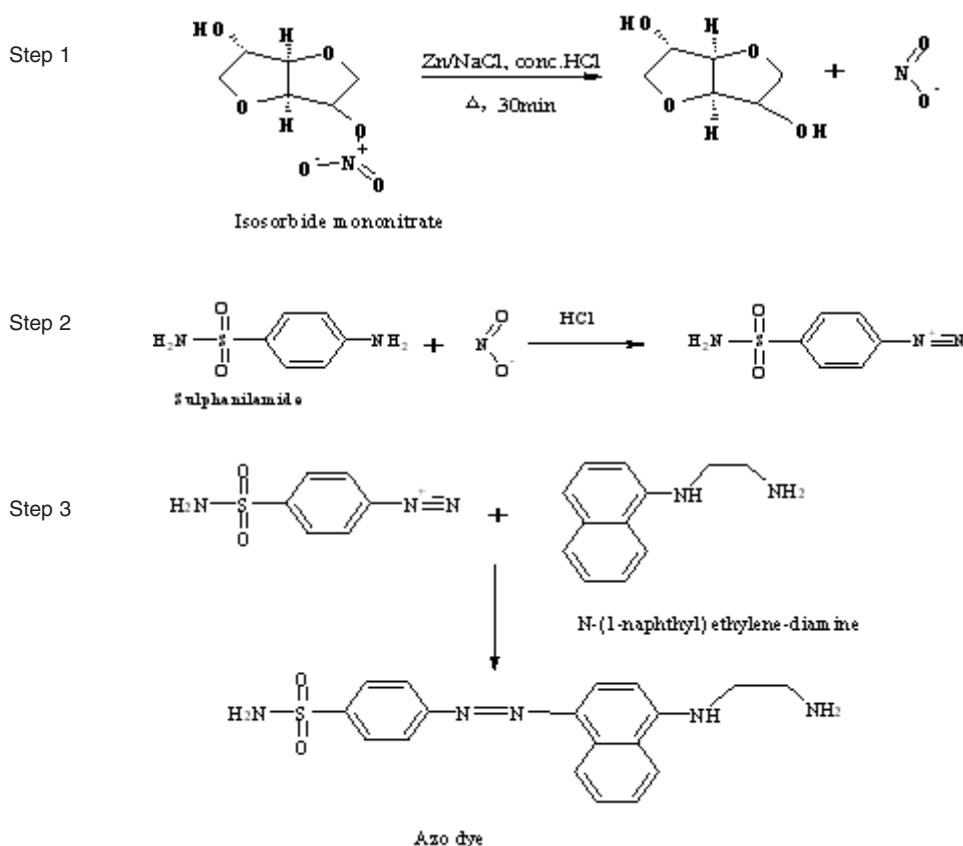
Table 1: Validation Parameters

Parameters	Observations
Linearity	5 – 60 $\mu\text{g mL}^{-1}$
Precision	1.06% RSD
Sandell's sensitivity	$3.6 \times 10^{-3} \mu\text{g cm}^{-2}$
Equation of linearity graph	$0.0037X - 0.0104$
Slope:	0.003
Intercept:	0.0104
Stability of Colour	>24 hours

Table 2: Recovery Study of Isosorbide mononitrate from Tablet samples

Concentration of sample ( $\text{mcg ml}^{-1}$ )	Concentration of standard added ( $\text{mcg/ml}$ )	Absorbance 540 $\text{nm}^*$	Concentration from graph	% deviation
	7.5	0.0631	18.5	105.7
10	10	0.0726	21	105
	12.5	0.0821	24	106.6

(\*Average of three determinations)



Scheme 1: Reaction sequence for the Formation of coloured product

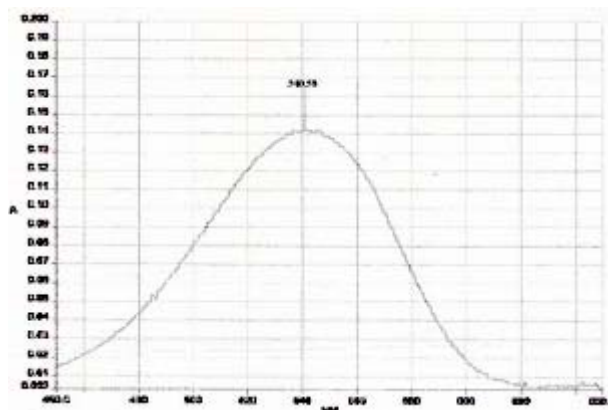


Fig. 1: UV spectrum of coloured azo dye

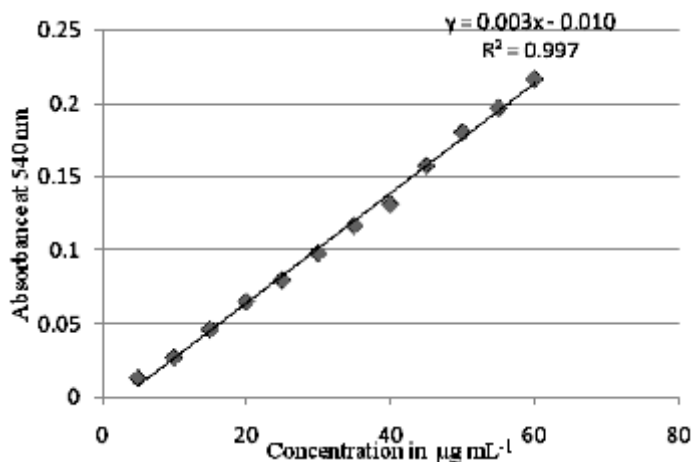


Fig. 2: Standard Calibration Plot

ethylenediamine dihydrochloride to give a purplish-pink or violet coloured product that absorbs maximum at 540 nm. The reaction sequence is shown in Scheme.

A linear relationship was observed within isosorbide mononitrate concentration range 5-60  $\mu\text{g mL}^{-1}$  as shown in Fig. 2. The precision was high as found from RSD of 1.06%. The sensitivity of method was found to be satisfactory as found from sandell's sensitivity which was calculated from  $3.6 \times 10^{-3} \mu\text{g cm}^{-2}$ . The colour was found to be stable for more than 24 hours and the accuracy of the method was determined where by standard sample of isosorbide mononitrate was added to previously analysed sample of isosorbide mononitrate at 3

different levels (75-125%). Recovery ranging from 105-106.6% was observed.

## CONCLUSION

The proposed method is based on the reduction of nitrate group of isosorbide mononitrate into nitrite ion, diazotisation of sulphanilamide with liberated nitrite in acid medium and subsequently coupling with NED. The reagents provide a simple, sensitive and accurate method for the spectrophotometric determination of isosorbide mononitrate. The developed method does not involve any stringent reaction conditions and offers the advantages of colour stability for more than 24 hours.

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